AUGMENTATION OF BRAIN FUNCTION: FACTS, FICTION AND CONTROVERSY

VOLUME II: NEUROSTIMULATION AND PHARMACOLOGICAL APPROACHES

EDITED BY: Ioan Opris, Manuel F. Casanova and Mikhail Lebedev

PUBLISHED IN: Frontiers in Systems Neuroscience and Frontiers in Neuroscience

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-615-4
DOI 10.3389/978-2-88945-615-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

AUGMENTATION OF BRAIN FUNCTION: FACTS, FICTION AND CONTROVERSY

VOLUME II: NEUROSTIMULATION AND PHARMACOLOGICAL APPROACHES

Topic Editors:
Ioan Opris, University of Miami, United States
Manuel F. Casanova, University of South Carolina, United States
Mikhail Lebedev, Duke University Durham, United States

The Volume II is entitled "Neurostimulation and pharmacological approaches". This volume describes augmentation approaches, where improvements in brain functions are achieved by modulation of brain circuits with electrical or optical stimulation, or pharmacological agents. Activation of brain circuits with electrical currents is a conventional approach that includes such methods as (i) intracortical microstimulation (ICMS), (ii) transcranial direct current stimulation (tDCS), and (iii) transcranial magnetic stimulation (TMS). tDCS and TMS are often regarded as noninvasive methods. Yet, they may induce long-lasting plastic changes in the brain. This is why some authors consider the term "noninvasive" misleading when used to describe these and other techniques, such as stimulation with transcranial lasers. The volume further discusses the potential of neurostimulation as a research tool in the studies of perception, cognition and behavior. Additionally, a notion is expressed that brain augmentation with stimulation cannot be described as a net zero sum proposition, where brain resources are reallocated in such a way that gains in one function are balanced by costs elsewhere. In recent years, optogenetic methods have received an increased attention, and several articles in Volume II cover different aspects of this technique. While new optogenetic methods are being developed, the classical electrical stimulation has already been utilized in many clinically relevant applications, like the vestibular implant and tactile neuroprosthesis that utilizes ICMS. As a peculiar usage of neurostimulation and pharmacological methods, Volume II includes several articles on augmented memory. Memory prostheses are a popular recent development in the stimulation-based BMIs. For example, in a hippocampal memory prosthesis, memory content is extracted from hippocampal activity using a multiple-input, multiple-output non-linear dynamical model. As to the pharmacological approaches to augmenting memory and cognition, the pros and cons of using nootropic drugs are discussed.

Citation: Opris, I., Casanova, M. F., Lebedev, M., eds (2018). Augmentation of Brain Function: Facts, Fiction and Controversy. Volume II: Neurostimulation and Pharmacological Approaches. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-615-4

Table of Contents

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 26 September 2014
doi: 10.3389/fnsys.2014.00182

A practical application of text mining to literature on cognitive rehabilitation and enhancement through neurostimulation

Puiu F. Balan[1]*, Annelies Gerits[1] and Wim Vanduffel[1,2,3]

[1] *Laboratory for Neuro-and Psychophysiology, Katholieke Universiteit Leuven Medical School, Leuven, Belgium*
[2] *Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, USA*
[3] *Department of Radiology, Harvard Medical School, Charlestown, MA, USA*

Edited by:
Ioan Opris, Wake Forest University, USA

Reviewed by:
Estate M. Sokhadze, University of Louisville, USA
Mikhail Lebedev, Duke University, USA

****Correspondence:***
Puiu F. Balan, Laboratory for Neuro-and Psychophysiology, KU Leuven Medical School, Herestraat 49, Bus 1021, Leuven 3000, Belgium
e-mail: puiu.balan@med.kuleuven.be

The exponential growth in publications represents a major challenge for researchers. Many scientific domains, including neuroscience, are not yet fully engaged in exploiting large bodies of publications. In this paper, we promote the idea to partially automate the processing of scientific documents, specifically using text mining (TM), to efficiently review big corpora of publications. The "cognitive advantage" given by TM is mainly related to the automatic extraction of relevant trends from corpora of literature, otherwise impossible to analyze in short periods of time. Specifically, the benefits of TM are increased speed, quality and reproducibility of text processing, boosted by rapid updates of the results. First, we selected a set of TM-tools that allow user-friendly approaches of the scientific literature, and which could serve as a guide for researchers willing to incorporate TM in their work. Second, we used these TM-tools to obtain basic insights into the relevant literature on cognitive rehabilitation (CR) and cognitive enhancement (CE) using transcranial magnetic stimulation (TMS). TM readily extracted the diversity of TMS applications in CR and CE from vast corpora of publications, automatically retrieving trends already described in published reviews. TMS emerged as one of the important non-invasive tools that can both improve cognitive and motor functions in numerous neurological diseases and induce modulations/enhancements of many fundamental brain functions. TM also revealed trends in big corpora of publications by extracting occurrence frequency and relationships of particular subtopics. Moreover, we showed that CR and CE share research topics, both aiming to increase the brain's capacity to process information, thus supporting their integration in a larger perspective. Methodologically, despite limitations of a simple user-friendly approach, TM served well the reviewing process.

Keywords: text mining, transcranial magnetic stimulation, cognitive, rehabilitation, enhancement

INTRODUCTION

Gathering accurate and reliable information from web repositories became increasingly complex because of the exponential growth in the number of publications. For example, a PubMed search retrieved 9407 papers including 1172 reviews for TMS in "All Fields," and the ratio became 8186/988 when the filter "[Title/Abstract]" was applied. Reading without some selection criteria becomes challenging. Even when selectively focusing on specific topics in a review, this increases the chances to miss trends shown only by huge bodies of literature. Thus, when processing vast corpora of publications, we are facing challenges that require automated solutions. One of the most promising approaches to alleviate these problems is to assist the human operator with computers running artificial intelligence applications. Here, we selected one of these applications, text mining (TM), and showed that TM will enable us to efficiently deal with huge amounts of information from the TMS-related literature.

Our approach was also motivated by the fact that, neuroscience has to make efforts to integrate data mining and TM when dealing with huge and diverse experimental datasets (Akil et al., 2011) and text documents. TM is able to catch the complexity of all relevant studies in an efficient manner. Statistical and natural language processing (NLP) procedures to "mine" the literature have been developed to address big data general problems (Dias et al., 2011). Here, we used a practical approach to promote TM as a tool for the reviewing process. Specifically, we selected a set of TM-tools that allowed user-friendly approaches to reveal relevant outcomes in large corpora of publications. Our intention was to use TM-tools that are not too demanding on programming skills, required knowledge and training period. Therefore, the example set of TM-tools could serve as an attractive guide map for researchers willing to incorporate TM in their work.

Next, we demonstrate the use of TM-tools in gaining basic insights into the relevant literature on cognitive rehabilitation

(CR) and cognitive enhancement (CE) using transcranial magnetic stimulation (TMS). TMS is a valuable non-invasive perturbation method used to address fundamental and clinical neuroscience questions, both in human and animal models. Cognitive rehabilitation (CR-TMS) and cognitive enhancement (CE-TMS) are two important TMS applications. Indeed, TMS is establishing itself as major tool used in rehabilitation improving a wide variety of impaired mental functions (Miniussi and Rossini, 2011; Kammer and Spitzer, 2012; Vicario and Nitsche, 2013). Moreover, recent studies reported TMS-induced enhancements of normal brain functions (Brem et al., 2014; Luber and Lisanby, 2014).

The TM application to CR- and CE-TMS literature was focused on two main aspects. First, we aimed to show that TM could reveal the diversity of TMS applications in CR and CE, automatically retrieving trends already described in published reviews. Second, we specifically aimed to find trends only noticeable in big corpora of publications. The main feature of our findings is given by the statistical power of such analyses. Detailed analyses of the CR- and CE-TMS literature revealed relevant terms in the form of lists, topics and classes of terms associated with specific subtopics. It also showed relations between the relevant terms in the form of co-occurrences maps, groups of relevant terms with high probability co-occurrences and lists of relevant relational verbs. Moreover, the TM approach revealed conclusive sentences that appeared with a high probability. Finally, a large-scale corpora perspective showed that CR-TMS and CE-TMS share research topics allowing us to make inferences about their similarities. Although they start from specific states of the brain (impaired for CR and normal for CE), both aim to increase the brain's capacity to process information and to optimize adaptation. This unitary perspective is supported by fields that use TMS in diagnostic (TMS-DIAG) or in clinical and fundamental research (TMS-RES), which show that TMS is effective for changing and studying normal and abnormal brain processes. Accordingly, CR-TMS and CE-TMS also share research topics with these fields, showing their appurtenance to a larger context, which integrates diagnostic, fundamental research and fMRI studies.

TEXT MINING AS A METHOD TO PARTIALLY AUTOMATE THE REVIEWING OF BIG CORPORA OF PUBLICATIONS

Scholarly journals and data sources are increasingly available in electronic and Open Access form. Nonetheless, availability is not enough to extract specific information, mainly due to the abundance of information. TM comes with solutions for this problem offering automated methods to extract condensed information hidden within huge volumes of publications. TM can be achieved using several complementary approaches (Cohen and Hunter, 2008). Co-occurrence-based methods look for concepts that occur in the same unit of text (sentence/abstract) and posit a relationship between them. The statistical or machine learning systems rely on statistic properties of the text and work by building classifiers that operate on any level, from labeling part of speech to classifying full sentences or documents. The rule-based systems use knowledge about how language is structured and about how domain relevant things, facts and their relationships are stated in publications. The main areas/stages of TM are (Lourenco et al., 2009): Information Analysis that includes Information Retrieval and Information Extraction (e.g., Name Entity Recognition, Relationship Extraction, document classification and summarization); Information Synthesis that uses the databases generated by Information Extraction for answering simple questions, discovering new information and generating hypotheses.

A wide variety of publications and software tools approach TM at different levels of complexity, hampering the selection of relevant TM-tools. We chose a specific set of TM-tools, aiming to evaluate how they can help the TM-non-specialist accelerate the review of big corpora of literature, using the following criteria:

a. Allow a user-friendly approach by selecting TM-tools requiring medium investments in training, programming and specific knowledge.
b. Use free open-source software, selected based on features like: easiness of installation; quality of the documentation and support; accessible formats for input and output.
c. Use TM-tools with general functionalities like: allowance of document corpora; pre-processing the text; built-in biomedical Name Entity Recognition; queries to a document or corpora; support for ontologies and terminologies.

We used three groups of TM-tools that served different purposes:

I Basic resources that gave foundation to TM: the MeSH browser; the PubMed repository of publications; repositories of NLP resources (e.g., Neuroscience Information Framework, National Institute of Neurological Disorders and Stroke).
II TM-tools II (**Table 1**) that are web-based ready-to-use tools requiring no programming efforts and performing simple TM tasks (Lu, 2011).
III TM-tools III that were used in the final stage of the study optimized for the reviewed topics:

1. Statistical or machine-learning-based approaches: Mallet (McCallum, 2002), Text to Matrix Generator (TMG) (Zeimpekis and Gallopoulos, 2006) and Matlab applications for NLP.
2. TM-tools with predefined NLP processing stream: KH Coder (Higuchi, 2012).
3. Integrated environments for visual programming of NLP: VisualText (Meyers, 2003; Alfred et al., 2014).
4. Biomedical TM rule-based approaches using fully automated stages in text processing: Anote2 (Lourenco et al., 2009) and Biological Research Assistant for TM (BioRAT) (Corney et al., 2004).

A PRACTICAL APPLICATION OF THE TEXT MINING TO LITERATURE ON COGNITIVE REHABILITATION AND ENHANCEMENT THROUGH NEUROSTIMULATION

To show that TM enables us to efficiently deal with big corpora of publications and for publishing practical reasons, the TM application to CR- and CE-TMS literature was limited

Table 1 | TM-tools II presented in the order (indicated by numbers) of their use.

TM tool	INPUT				OUTPUT				Targeted processing	Topics						
	Query - T	Query – S	Predefined	Filters	Statistic	Corpus (C)	Terms (T)	Sentences (S)		CR-TMS	CE-TMS	TMS	CR	CE	TMS -	DIAG/RS TMS-fMRI
Mesh	1			1			1		Terms (T)							
PubMed	1			1		1			Search optimization; Corpus (C)							
MEDSUM	1			1	1		1		Statistic							
PubReMiner	1			1	1		1		Statistic							
LingerCat	1			1			1		T cloud; papers -T relationships							
Carrot	1			1		1	1		Clustering papers; Plot clusters							
Anne O'Tate	1			1	1		1		Clustering papers; statistic; Topics							
BioTextQuest	1			1		1	1		Clustering papers; Biomedical T							
askMEDLINE	1	1		1		1		1	Bottom Line summary of abstracts							
Ultimate Res. Assistant	1			1			1	1	Text summarization; Taxonomies; Mind maps							
Quertle	2	2		2		2	2	2	Ranking papers; Subject verb - object relationships							
GoPubMed	2			2	2	2	2	2	Clustering papers; Statistic							
PubMatrix	3		T				3		Matrix of terms co - occurrence							
Medline Ranker	3		C	3		3	3	3	Ranking papers; Evaluate ranking performance							
XplorMed			C			3	3	3	Clustering papers; T relationships							
Textpresso Neuroscience	4			4		4	4	4	Information Extraction; Transition to TM-tools III							

Gray scale qualitatively codes their weights in TM (the darker, the higher the importance). Each subgroup (1–4) includes kernels of TM-tools (circled red) and "targeted processing" (red text) that have the highest importance for our study. INPUT: query terms (T) = query using combinations of terms; query sentences (S) – query using free text or questions; predefined = fixed user-predefined input consisting of lists of terms or publications; filters = tuning the search using supplementary terms, constrains regarding the type/part of the publication to be processed etc. OUTPUT: Statistic about authors, journals, papers per year, topics; corpus (C) = retrieving sets of relevant papers; terms (T) = extracting lists of frequent and relevant terms and their relationships; sentences (S) = extracting relevant sentences and paper summaries. Targeted processing = processing from the TM-tool repertoire used for this review. The last 7 columns show topics whose corpora of publications were studied with TM (gray scale codes the weight of the approach). References: MEDSUM (Bridges-Webb, 1986), PubReMiner (Koster, 2008), LingerCat (Sarkar et al., 2009), Carrot2 (Carpineto et al., 2009), Anne O'Tate (Smalheiser et al., 2008), BioTextQuest (Iliopoulos et al., 2001), askMEDLINE (Fontelo et al., 2005), Ultimate Research Assistant (Hoskinson, 2005), Quertle (Giglia, 2011), GoPubMed (Doms and Schroeder, 2005), PubMatrix (Becker et al., 2003), Medline Ranker (Fontaine et al., 2009), XplorMed (Perez-Iratxeta et al., 2001), Textpresso for Neuroscience (Muller et al., 2008).

to few aspects. First, we aimed to show that TM could retrieve the diversity of TMS applications in vast corpora of publications about CR and CE (see Cognitive Rehabilitation and Enhancement Accomplished with TMS), automatically retrieving trends already described in published reviews (see Discussions and Conclusions). Second, we looked for trends noticeable only in big corpora of publications, relying on the statistical power of the analyses and including results like topics' occurrence frequency and relationships, relevant relational verbs, and high probability conclusive sentences (see Cognitive Rehabilitation and Enhancement Accomplished with TMS). Furthermore, we analyzed large context relationships between topics showing how CR- and CE-TMS integrate with diagnostic, fundamental research and fMRI studies (see A General Context).

Using TM to efficiently review corpora of publications requires roughly three stages: pre-TM-processing, TM-processing, and post-TM-processing. In this paper, we focused on the TM-processing by showing mainly the "raw" TM results. Accordingly, the seemingly redundant diversity of results is determined by our intention to illustrate few similar results given by different TM-tools.

We used a multi-stage and multi-tool approach ordered by the complexity in TM, which was gradually increased, starting with TM-tools II and continuing with TM-tools III. The same analysis was performed with few TM-tools (**Table 2**), which can be regarded as alternative solutions for the same problem. This helped us to cope with the limited perspective offered by different TM-tools, to perform comparisons and cross-validations, and to build synthetic results.

The main classes of TM processing on the selected corpora were:

– Statistics about the number of publications, authors, journals; thematic/MeSH headings division of the field; clustering

Table 2 | Number of publications retrieved from PubMed using search queries defined previously.

Search query	Publications per search filter:		
	TIAB	PBRK	TIABREV
CR-TMS	**4074**	**0.913**	**641**
CE-TMS	**181**	**0.956**	**41**
TMS	8386	0.909	1018
CR	4220	0.896	914
CE	1235	0.895	312
TMS-DIAG	212	0.994	68
TMS-RES	360	0.994	93
TMS-fMRI	875	0.906	211

PBRK, the probability of correct ranking of a random positive-negative pair of publications determined with Medline Ranker.

of publications; selection and ranking of relevant corpora of papers necessary for TM-tools III (all performed with TM-tools II).

– Extract relevant terms and their relationships, involving: categorization of key concepts; building correlation matrices for relevant terms and showing the most informative correlations; showing the topological maps of the main terms, using their relationships inferred from co-occurrence in the same text units.
– Retrieve automatically relevant sentences, study their probability of occurrence, and identify parts-of-sentence (e.g., predicates) relevant for deriving conclusions.
– Build a "map of science," which characterize large-scale relationships between multiple topics. Each topic-topic relationship was evaluated based on common relevant terms retrieved from each corpus, similar thematic clustering of the publications, common authors, journals and publications approaching both topics.

To create a larger context allowing a better understanding of CR- and CE-TMS, we selected topics like TMS, CR, CE, TMS-RES, TMS-DIAG, and TMS-fMRI. **Figure 1** presents a "qualitative hypothesis" about the topology of this context and we used TM to test it. Corpora of publications for each topic were retrieved using the following PubMed queries:

– For CR-TMS: ("transcranial magnetic stimulation") AND ("cognitive rehabilitation" OR "rehabilitation" OR "cognitive therapy" OR "therapy" OR "cognitive recovery" OR "recovery" OR "cognitive treatment" OR "treatment" OR "cognitive repair" OR "neurorehabilitation" OR "improvement" OR "decrease").
– For CE-TMS: ("transcranial magnetic stimulation") AND [("cognitive enhancement") OR ("cognitive augmentation") OR ("cognitive improvement") OR ("cognitive enrichment") OR ("cognitive amelioration") OR ("neuroenhancement")].
– For TMS: "transcranial magnetic stimulation."
– Queries for CR/CE include the second operand of the AND operator in the CR-TMS/CE-TMS queries.

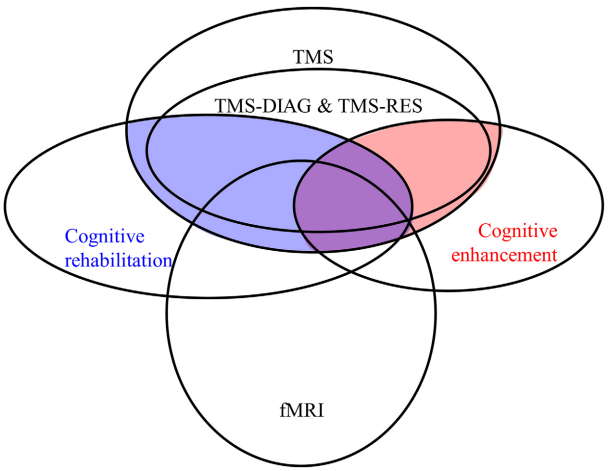

FIGURE 1 | Qualitative approximation of a larger context for CR- and CE-TMS. Topics are represented by ellipses with CR-TMS (filled blue) and CE-TMS (red) representing intersections of topics.

All queries were used separately with two filters ("[Title/Abstract]" (TIAB-filter) and "[Title/Abstract] AND Review" TIABREV-filter) creating two separate corpora of abstracts: TIAB-corpora (the main target for TM) and TIABREV-corpora (used for comparisons). Empty or less relevant abstracts were removed from the corpora. Corpora were also compared with local databases and missing publications were added manually.

Finally, the TM results were evaluated in few ways. First of all, we used TM-tools that are already tested and evaluated, building our results on this general basis. Second, we used the *post-hoc* judgment of the system outputs (Cohen and Hunter, 2008) in few stages, and compared: results of similar processing (e.g., term extraction) performed with different TM-tools using TIAB-corpora (see A Practical Application of the Text Mining to Literature on Cognitive Rehabilitation and Enhancement Through Neurostimulation); results of similar processing using TIAB-corpora vs. TIABREV-corpora (see A Practical Application of the Text Mining to Literature on Cognitive Rehabilitation and Enhancement Through Neurostimulation); TM-results vs. manual-curated results (see Discussions and Conclusions).

A GENERAL CONTEXT

We used TM-tools II to determine relationships that put CR- and CE-TMS topics in the same neighborhood on a "map of science." An outline of the main results includes:

1. MeSH headings mention TMS as a preferred term, defined by its use in brain mapping, neurophysiology and treatment of depression (replacing electroconvulsive therapy), while paired-pulse TMS, rTMS and single-pulse TMS are narrower concepts. The MeSH tree considers that TMS belongs to the fields of neurological diagnostic and therapeutic techniques. CR is related with MeSH headings like Cognitive Therapy, Rehabilitation and Treatment/Rehabilitation Outcome, and CE is related with Cognitive Therapy, Nootropic Agents and Cognitive Enhancers.

2. The number of the publications retrieved from PubMed (**Table 2**) for each topic.

It is noteworthy that a large number of reviews were written for each topic.

3. The number of publications per year evaluating the interest for each topic (**Figure 2**), which showed the following trends:

   – The interest for all topics increased in the last decades;
   – TMS generated numerous publications per year;
   – CR-TMS has a stronger representation than CE-TMS;
   – Across-topics perspectives (CR & CE; CR & CE-TMS; TMS-DIAG & RES) are less represented.

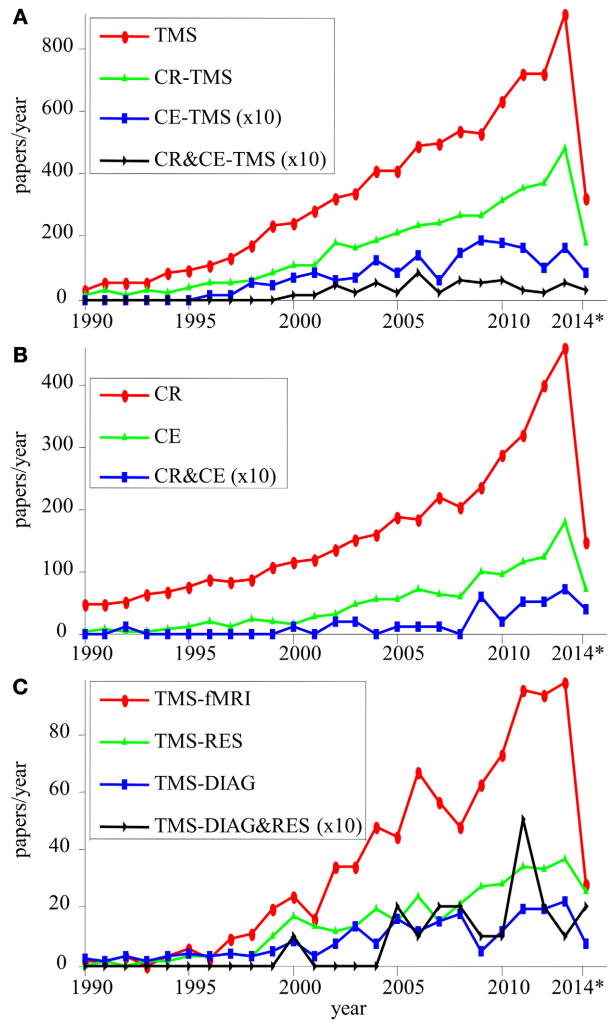

FIGURE 2 | Number of publication per year (2014*, only the first 3 months) for different topics: TMS, CR-TMS, and CE-TMS (A); CE and CR (B); applications of TMS in research (TMS-RES), diagnostic (TMS-DIAG) and concurrent TMS-fMRI (C). Results for topics represented by small numbers of publications are multiplied by 10 (×10). We show also results for conjunctions of topics: (CR&CE-TMS) **(A)**; (CR&CE) **(B)**; (TMS-DIAG&RES) **(C)**.

   – Fundamental research (e.g., TMS-RES, TMS-DIAG) is less represented than practical applications (e.g., CR-TMS).

4. Co-occurrence matrix for terms used to build the search queries, retrieved with PubMatrix (**Figures 3A,B**). We made the following observations:

   – TMS co-occurred very frequently (publications $\sim 10^3$) with terms like therapy, treatment, brain function, brain physiology, and diagnostic (mainly CR-TMS);
   – TMS co-occurred frequently (publications $\sim 10^2$) with rehabilitation, recovery, cognitive treatment, improvement, decrease, brain anatomy, brain performance, brain networks, psychology, brain mapping, MRI, mental disorder, mental disease and psychiatric disorder (mainly CR-TMS and CE-TMS);

5. Frequent terms co-occurring in different corpora (included between brackets):

   a. Among the first 10 MeSH headings, we mention:
      – [CR-TMS]: Motor Cortex/physiology, TMS/methods, Evoked Potentials, Treatment Outcome, Evoked Potentials, Electromyography, Functional Laterality, Stroke, Brain, Motor, (Major) Depressive Disorder;
      – [CE-TMS]: TMS/methods, Brain Mapping, Brain, Functional Laterality, Cognition, Psychomotor Performance, Reaction Time/physiology, Motor Cortex/physiology, Cognition Disorders, Prefrontal Cortex/physiology.
   b. Among the first 100 common MeSH headings (average frequency range [51, 2010]) for groups of topics (included between brackets), we mention:
      – [CR-TMS; CE-TMS; TMS; TMS-fMRI]: Brain, Cognition, Brain Mapping, Electromyography, Evoked Potentials, Motor Skills, MRI, Motor Cortex/physiology, Neural Inhibition, Neuronal Plasticity, Neuropsychological Tests, Parietal Lobe, Prefrontal Cortex, Psychomotor Performance, Reaction Time, TMS/methods, Treatment Outcome;
      – [CR-TMS; CR]: Brain, Chronic Disease, Cognition, (Major) Depressive Disorder, MRI, Motor Cortex, Neuropsychological Tests, Psychomotor Performance, Recovery Of Function, Schizophrenia, Severity Of Illness Index, Treatment Outcome, Neuronal Plasticity/physiology, Recovery of Function/physiology, Stroke;
      – [CE-TMS; CE]: Attention, Brain, Cognition, Learning, MRI, Memory, Neurons, Neuropsychological Tests, Psychomotor Performance, Reaction Time, Prefrontal Cortex, Treatment Outcome, Cognition Disorders/diagnosis and etiology.
   c. Other trends noticed using the TM-tools II:
      – Different groups of topics (e.g., [CR-TMS; CE-TMS]; [TMS; TMS-fMRI; TMS-RES; TMS-DIAG]) are connected as shown by common headings/terms;
      – CR-TMS related terms: motor cortex, treatment, excitability, facilitation, antidepressant, plasticity, MEP, stroke, Parkinson, severity of illness index, treatment outcome, (major) depressive disorder, schizophrenia,

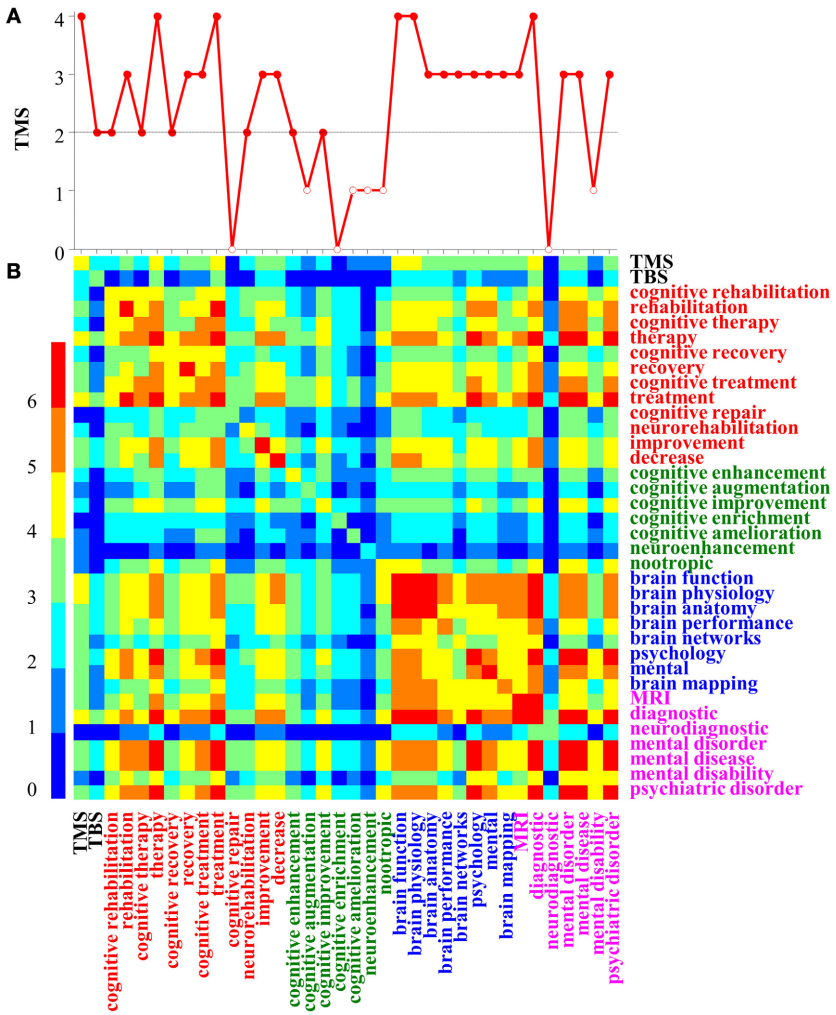

FIGURE 3 | Co-occurrence matrix built with PubMatrix. The matrix **(B)** represents the decimal logarithm of the number of publications retrieved from PubMed using queries combining all possible conjunctions of pairs of terms (e.g., TMS and neurorehabilitation), which label the lines and the rows of the matrix. Panel **(A)** represents the first line in the matrix, showing co-occurrences involving the term TMS. Different colors (see the left color-coding bar) represent different powers of 10. The color of the text marks terms associated dominantly with: TMS (black); CR-TMS (red); CE-TMS (green); TMS-RES (blue); TMS-DIAG (purple).

stroke/complications, neuronal plasticity, rehabilitation, cognition, recovery, parietal lobe, prefrontal cortex, psychomotor performance;
- CE-TMS related terms: prefrontal cortex, DLPFC, cognition, psychomotor performance, attention, learning, memory, performance, placebo, cognition disorders, severity of illness index, treatment outcome, neuroenhancement, Hebbian, neurofeedback, enhancement, improvement;
- [CR-TMS; CE-TMS] and [CR; CE] groups of topics have "mental diseases" and "treatments" as common subtopics, thus supporting the hypothesis that the relative improvement of the mental performance is a common aspect of their definitions.

6. The first 5 authors for different topics (all including Pascual-Leone A on the first place):
   - [CR-TMS]: Fregni F, Daskalakis ZJ, Fitzgerald PB, Rothwell JC;
   - [CE-TMS]: Walsh V, Fregni F, Cowey A, Miniussi C;
   - [TMS]: Rothwell JC, Hallett M, Cohen LG, Fitzgerald PB;

Some authors covered several of the selected topics (e.g., common authors for [CR-TMS; CE-TMS; TMS; TMS-fMRI]: Pascual-Leone A, Cohen LG, Fregni F, Lisanby SH, Miniussi C, Rothwell JC), and this is an indirect argument for potential connections between topics.

7. Finally, comparisons between corpora for different topics showed relevant numbers of common publications (**Figure 4**), thus emphasizing a unitary context. Consistently, clustering the publications for each topic using Carrot2 showed also different clusters sharing publications.

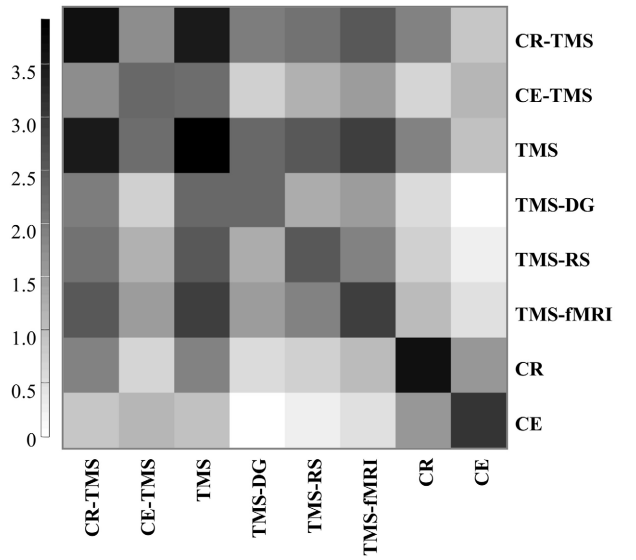

FIGURE 4 | Number of common publications (in decimal logarithmic scale) for pairs of corpora representing topics labeling the rows and the columns of the matrix. Different shades of gray (see the color-coding bar) represent different powers of 10.

COGNITIVE REHABILITATION AND ENHANCEMENT ACCOMPLISHED WITH TMS

First, we need to reiterate that the relevance of all the retrieved terms is based on the idea that words co-occurring frequently in the abstracts are related in ways intrinsically constrained by the topic of the abstracts. Moreover, our specific selection of publications guarantees that term like TMS (all protocols) are present in all abstracts included in corpora. Thus, TMS is strongly related with all frequent terms retrieved with different TM-tools. Specifically, if, for example, the frequent terms are treatment and depression, this means that TMS is used frequently in depression treatment.

We used TM-tools III to increase our insights in CR-TMS and CE-TMS literature, and selected the following groups of results:

1. Statistical or machine-learning-based NLP:

   a. Topic modeling with Mallet (500 iterations, 7 topics, topics proportion threshold 0.05, and removed the standard Mallet stop-words). Each topic is a set of terms used with high probability by the authors, thus reflecting a specific thinking pattern involving TMS. Without knowing anything about the meaning of the words in a text, topic modeling assumes that any piece of text is written by selecting words from possible topics. Thus, it becomes possible to mathematically decompose a text into probable topics from which the words originated.

Example topics separated in CR/CE related terms (brackets) and TMS related terms (double brackets):

a1. CR-TMS:

- [treatment, depression, clinical, therapy, disorder, major, antidepressant, electroconvulsive therapy], [transcranial, magnetic, stimulation, effective];
- [motor, stroke, patients, recovery, function, hemisphere, affected, rehabilitation, limb], [magnetic, transcranial, stimulation].

The [CR] kernel of 2 topics includes CE-related terms (e.g., [cortex, learn, activity, visual, memory, performance, area]).

a2. CE-TMS:

- [cortex, DLPFC, prefrontal, dorsolateral, memory, processing], [stimulation, applied, TMS, effect, frequency], significant;
- [cognitive, brain, healthy, functions, enhance, neuroenhancement, parietal, cognition], [stimulation, magnetic, TMS], studies.

The [CE] kernel of 3 topics includes CR-related terms (e.g., [improvement, depression, treatment, deprivation, sleep, occipital, functional, disease, fluoxetine]).

b. Term-related queries with TMG toolbox. Document retrieval relies often on matching terms from documents with those from queries. However, natural languages present some challenges (e.g., polysemy, synonymy) that render term-matching inaccurate. TMG is using latent semantic analysis to overcome these problems (Landauer et al., 1998), based on the application of singular value decomposition of a term-by-document matrix (TDM).

We performed the indexing for each corpus creating new TDMs using "common-words" stop-list, logarithmic local weights, "GfIdf" global term weighs, normalization of terms, removing of alpha-numerics and numbers. For dimensionality reduction and TDM best rank approximation, we selected the dimensionality via the use of profile likelihood (Zhu and Ghodsi, 2006). Example results:

b1. For CR-TMS, the TDM included 607 documents, 6387 terms with average 111 indexing terms/document for the query matrix, and the best rank approximation 360. Among the best retrieved terms (apparition frequency > 86; range [870, 896] terms), we mention: [stimulation, rTMS, treatment, TMS, brain, motor, depression, patients, stroke, cortical, therapy, clinical, effects, disorders, pain, cortex] in the first 20; [recovery, disorder, schizophrenia, tinnitus, excitability, cognitive, plasticity, antidepressant, rehabilitation, mechanisms, psychiatric, symptoms, aphasia, language, noninvasive, sham, EEG, Parkinson, resistant, human, induced, movement, neglect] in the next 80; [cerebral, epilepsy, deep, migraine, neural, bipolar, visual, OCD, seizures, acute, neurological, diagnosis, auditory, neuroimaging, reorganization, prefrontal, inhibition, improvement, mood, neuropsychiatric, neuromodulation] in the next 50 terms.

b2. For CE-TMS, the TDM included 174 documents, 3235 terms with average 117 indexing terms/document for the query matrix, and the best rank approximation 174. Among the best retrieved terms (apparition frequency > 37; range [776, 1010] terms), we mention: [TMS, rTMS, motor, cortex, brain, visual, memory, cognitive, performance, parietal, healthy, patients, learning, improvement, enhancement] in the first 20; [excitability, neural, DLPFC, facilitation, plasticity, attention, control, prefrontal, network, evoked, neuroscience, perception, EEG, mechanisms, modulation, semantic, training, frontal, FEF, treatment, fluoxetine, neuroenhancement, sleep, emotional, skill, inhibition, Parkinson] in the next 80; [fMRI, MEP, Wernicke, search, discrimination, pain, cognition, increased, encoding, oscillatory, language, psychiatric, decrease, experimental, stroke, illness, major, effective, resistant, improve] in the next 50 terms.

2. Processing performed with KH Coder:

   a. Determine the co-occurrence network for CR-TMS and CE-TMS corpora using 7 sets of coding rules. A coding rule is a list of terms connected with OR (|), AND (&) or NOT used to "focus" the TM of the corpora toward specific topics. We used coding rules (marked by brackets) that were common for CR-TMS and CE-TMS: [TMS] (transcranial & magnetic & stimulation | rTMS | TBS etc.); [mental disabilities] (depression | stroke | schizophrenia etc.); [brain functions] (memory | learning | attention etc.); [research methods] (fMRI | EEG etc.); [verbs-] describing negative effects of the stimulation (fail | worsen | damage | debilitate | miss etc.). To these we added a set of rules specific to CR-TMS/CE-TMS: [verbs+] describing positive effects for CR-TMS (rehabilitate | treat | recover | restore etc.) or CE-TMS (enhance | improve | augment | strengthen etc.); [CR-TMS-effects] (rehabilitation | cognitive & rehabilitation | cognitive & therapy | neurorehabilitation etc.); [CE-TMS-effects] (performance | enhancement | neuroenhancement | neuromodulation etc.). The co-occurrence networks revealed that the terms from the coding rules [TMS], [CR-TMS-effects], [mental disabilities] and [Verbs+] have the highest co-occurrence frequency in the CR-TMS corpus. The CE-TMS corpus is characterized by high co-occurrence frequency of the terms from the coding rules [TMS], [CE-TMS-effects] and [Verbs+].

   b. Determine the keyword-in-context (KWIC) collection for terms in both CR-TMS (**Table 3**) and CE-TMS (**Table 4**). The KWIC statistic revealed information about both the relevant terms co-occurring with specific query terms (e.g., TMS) and statistical regularities (e.g., probable word positions) about the way the scientists build their statements.

3. Summary of results obtained with VisualText, ANote2, BioRAT and our Matlab applications (MAPP). All TM-tools used similar resources, including: dictionaries (mental disabilities, brain anatomy, cognitive processes, built using Neuroscience Information Framework (NIF) resources; CR-TMS-effects, CE-TMS-effects and TMS, built using our term statistic, similar with KH Coder coding rules); ontologies (NIF gross-anatomy and NIF dysfunction; human disease and neuro-behavior ontology from The Open Biological and Biomedical Ontologies Foundry); lexical words (Mallet stop-words; verbs+ and verbs- for CR-TMS and CE-TMS, similar with KH Coder rules). Each dictionary/ontology could be considered a class of terms (indicated by brackets; e.g., [mental disabilities]).

The text processing relied on predefined streams of processing or on existing libraries of examples (e.g., TAIParse general text analyzer for VisualText). Our Matlab applications (MAPP) were used to handle the results, to perform relation extraction and to extract sentences with high probability of occurrence (low perplexity coefficients, PP).

We summarized here few results from the following processing: Evaluate term frequency and co-occurrences and perform Name Entity Recognition using lexical resources (ANote2,

Table 3 | KWIC examples for CR-TMS.

Word	Total	LT	RT	L5	L4	L3	L2	L1	KW	R1	R2	R3	R4	R5
Treatment	160	63	97	25	11	11	16	0	TMS	5	17	53	12	10
Depression	113	37	76	8	11	18	0	0	TMS	0	22	13	14	27
Alzheimer	100	55	45	20	13	19	3	0	TMS	2	12	10	5	16
Therapy	98	58	40	25	4	3	26	0	TMS	5	3	6	22	4
fMRI	79	57	22	14	6	19	18	0	TMS	0	3	8	0	11
Stroke	50	35	15	9	5	11	10	0	TMS	0	4	3	4	4
Disorder	38	27	11	9	3	2	13	0	TMS	0	0	6	4	1
Recovery	34	28	6	15	6	4	3	0	TMS	0	2	0	0	4
Schizophrenia	15	12	3	5	1	2	4	0	TMS	0	1	0	0	2
Parkinson	14	6	8	2	4	0	0	0	TMS	0	5	1	1	1
Improvement	13	5	8	3	0	1	1	0	TMS	0	0	2	1	5
Neurorehabilitation	20	14	6	7	0	1	6	0	TMS	0	2	1	1	2

Columns show the number of co-occurrences of the context word (left) at different positions left/right (L5-L1/R1-R5) in the sentence relative to the query keyword (KW) and their total (LT/RT).

Table 4 | KWIC examples for CE-TMS.

Word	Total	LT	RT	L5	L4	L3	L2	L1	KW	R1	R2	R3	R4	R5
Induce	29	6	23	2	1	3	0	0	TMS	3	15	1	2	2
Performance	24	12	12	5	4	3	0	0	TMS	0	1	7	2	2
EEG	18	5	13	2	0	1	2	0	TMS	0	11	1	1	0
Paired-pulse	17	16	1	0	1	2	0	13	TMS	0	0	0	0	1
Cognitive	16	4	12	2	0	2	0	0	TMS	1	2	6	2	1
Memory	13	9	4	2	3	3	1	0	TMS	0	1	1	2	0
Stimulation	13	5	8	1	2	0	2	0	TMS	2	0	3	2	1
fMRI	9	7	2	1	1	3	2	0	TMS	0	2	0	0	0
Enhance	8	3	5	1	1	1	0	0	TMS	1	2	0	2	0
Facilitate	7	1	6	1	0	0	0	0	TMS	1	1	0	3	1
Improve	6	1	5	0	0	1	0	0	TMS	1	3	0	0	1
Perception	5	3	2	0	2	1	0	0	TMS	0	0	0	0	2

MAPP); Relations Extraction (ANote2, MAPP); Extract relevant sentences (VisualText, BioRAT, MAPP). The selected results are:

a. Terms statistic from Name Entity Recognition applied to CR-TMS (ANote2; 27506 annotations):

   – Top terms (number of occurrences): treatment (1177), rTMS (1000), brain (982), TMS (1410), therapy (503), depression (456), stroke (420);
   – Classes of terms (class per document, number of occurrences for all terms of the class): [neuro-behavior ontology] (22.8, 14903), [CR-TMS-effects] (4.8, 3163), [TMS] (4.3, 2811), [mental disabilities] (3.2, 2073), [cognitive processes] (1.6, 1028), [CE-TMS-effects] (0.2, 137);
   – Examples from the detailed class statistic (ANote2, MAPP; parentheses indicate number of occurrences):
     - [TMS]: rTMS (1301), TMS (1466), TBS (32);
     - [CR-TMS-effects]: treatment (1177), decrease (27), inhibition (96), antidepressant (175), antipsychotic (23), neurorehabilitation (45), recovery (286), rehabilitation (171), therapy (503);
     - [mental disabilities]: ADHD (25), Alzheimer's (14), OCD (92), Parkinson (104), Tourette (9), anxiety (49), auditory hallucinations (24), bipolar depression (27), bipolar disorder (25), depression (462), epilepsy (77), major depressive disorder (111), neglect (68), psychiatric disorders (80), schizophrenia (158), seizures (69), stress (25), stroke (422), tinnitus (153);

b. Terms statistic from Name Entity Recognition applied to CE-TMS (ANote2; 7404 annotations):

   – Top terms (number of occurrences): TMS (727), rTMS (360), brain (207), cognitive (131), performance (130), facilitation (67);
   – Classes of terms (class per document, number of occurrences for all terms of the class): [neuro-behavior ontology] (22.97, 4111), [TMS] (6.23, 1116), [CE-TMS-effects] (3.70, 663), [cognitive processes] (3.49, 624), [mental disabilities] (0.35, 63), [CR-TMS-effects] (0.32, 57);
   – Examples from the detailed class statistic (ANote2, MAPP):
     - [TMS]: TMS (739), rTMS (365);
     - [CE-TMS-effects]: activation (28), cognitive (131), cognitive enhancement (19), enhancement (34), facilitation (67), improvement (29), neuroenhancement (27), performance (130), performance enhancement (8), rehabilitation (27), therapy (37);
     - [cognitive processes]: attention (53), cognition (25), learning (70), memory (85), working memory (46), perception (37), visual search (19), skill acquisition (12), consolidation (9), decision (14), emotion (8), encoding (23), language (21), movement (17), recognition (13), semantic processing (2), speech (7);

c. Relationship Extraction for CR-TMS (ANote2; 27506 annotations). From 6754 relations, 2163 were verb associated relations, and we found among the top 30 the following verbs: induced, related, controlled, based, treating, underlying, compared, modulate, affected, applied, provide, discuss, to study, has been used, combined, to treat, improving. The statistics showed positive (89.1%), negative (1.7%), conditional (9.2%) for the polarity of the relations, and one-one (11.8%), one-many (16.7%), many-one (13.4%), many-many (12.1%) for their cardinality.

d. Examples of terms from classes [TMS] (omitted, next) & [CR-TMS-effects] & [mental disabilities] co-occurring in the same sentence (MAPP; 1290 sentences; | = OR):

   – [treat] & [depression (24%)| disorder (12%)| tinnitus (4.3%)| schizophrenia (3%)| stress (1%)| pain (1%)| stroke (1%)| epilepsy (1%)| anxiety (1%)| seizures (0.2%)| neglect (0.1%)];
   – [therapy] & [depression (5%)| stroke (3%)| pain (1%)| epilepsy (0.4%)| seizures (0.5%)| schizophrenia (0.2%)| mood (0.2%)];
   – [improve] & [stroke (3%)| depression (0.6%)| tinnitus (0.3%)| reading (0.1%)| attention (0.1%)| schizophrenia (0.1%)];

e. Relationship Extraction for CE-TMS (ANote2; 7404 annotations). From 2025 relations, 903 were verbs associated relations, and we found among the top 30 the following verbs:

is, induced, applied, guided, learning, improved, increasing, enhancing, was applied, paired, stimulated, delivered, reduced, impaired, performed, encoding. The statistics showed positive (92.0%), negative (1.2%), conditional (6.8%) for the polarity of the relations, and one-one (14.0%), one-many (17.6%), many-one (14.5%), many-many (12.4%) for their cardinality.

f. Example of terms from classes [TMS] (omitted, next) & [CE-TMS-effects] & [cognitive processes] occurring in the same sentence (MAPP; 1591 sentences):

- [performance] & [memory (3.7%)| attention (1%)| language (1%)| motor (1%)| visual (1%)];
- [facilitation] & [motor (6%)| memory (2%)| linguistic (2%)| concept (1%)| control (1%)| knowledge (1%)| logical (1%)| awareness (1%)| visual (1%)];
- [improve] & [memory (1%)| visual (1%)| language (1%)];

g. Example of high probability sentences (PP, range [4, 99]) from the CR-TMS corpus including terms from classes [TMS] & [CR-TMS-effects] & [mental disabilities] (see d):

- "Daily rTMS improves mood in depression" (PP 13.0).
- "Excitatory rTMS induces improvements in chronic post-stroke aphasia" (PP 13.5).
- "Slow TMS can rapidly reduce resistant auditory hallucinations in schizophrenia" (PP 16.8).

Using VisualText we showed also that from 4085 sentences, 1417 include terms from [TMS] hierarchy, and 847 include terms from [TMS] & [CR-TMS-effects OR mental disabilities]. Examples from the last group:

- "TMS has been shown to be an effective treatment for mental illnesses including major depressive disorder."
- "...rTMS has been developed for the treatment of major depression and schizophrenia."

h. Example of high probability sentences (PP; range [3, 50]) from the CE-TMS corpus including terms from classes [TMS] & [CE-TMS-effects] & [cognitive processes] (see f):

- "...rTMS to left dorsal premotor cortex enhances motor consolidation of new skills" (PP 9).
- "rTMS over Wernicke's area leads to a brief facilitation of picture naming by shortening linguistic processing time" (PP 11).
- "...rTMS at alpha frequency can modulate short-term memory capacity by influencing the ability to suppress distracting information" (PP 15).

Using VisualText we showed that from 1008 sentences, 445 included terms from [TMS], and 238 include terms from [TMS] & [CE-TMS-effects OR cognitive processes]. Examples from the last group:

- "...rTMS of the DLPFC can affect the performance in an affective go-no-go task."
- "...here we report the ipsilateral enhancement of visual attention after rTMS of parietal cortex at parameters known to reduce cortical excitability."

k. Example of sentences extracted with BioRAT from the CR-TMS corpus using a specific rule, which indicate classes of terms that have to be found at specific locations (block) in the sentence (**Table 5**).

DISCUSSIONS AND CONCLUSIONS

We here used a set of selected TM-tools to obtain basic insights into the relevant literature on the CR- and CE-TMS. For obvious reasons, we limited this application to few simple aspects. First, we showed that TM could retrieve from vast corpora of publications the diversity of TMS applications in CR and CE, automatically extracting trends already described in published reviews. Second, we searched for trends noticeable only in big corpora of publications.

Along this exercise, we attempted to validate our results in different ways. For example, we compared similar results obtained using different TM-tools applied to different corpora (TIAB-corpora or TIABREV-corpora). Relevant and common aspects, synthesized in unique results per type of analysis and topics were shown in the paper. Finally, we compared TM to human curation efforts. Accordingly, we selected for human curation 30 of the top ranked (with Medline Ranker) publications from

Table 5 | Example sentences retrieved with BioRAT using a specific rule.

Blocks of the rule						Context
<LOOKUP: TMS>	**<MACRO: WORD>**	**<MACRO: WORD?>**	**<LOOKUP: CR-TMS-effects>**	**<MACRO: WORD>**	**<LOOKUP: mental disabilities>**	
rTMS	for	–	treatment	of	depression	"... the first cases report of using rTMS for the treatment of depression ..."
TMS	for	–	treatment	of	obsessive compulsive disorder	"... TMS for the treatment of obsessive compulsive disorder ..."

"< >" delimitate a block of the rule; LOOKUP, looking for a term included in a class ([TMS], [CR-TMS-effects] or [mental disabilities]); MACRO WORD, any word; "?", the block is optional.

the TIABREV-corpora, separately for CR-TMS and CR-TMS. We performed a selective manual curation aimed to retrieve relevant terms co-occurring with TMS (all types of protocol) in the abstracts, which belong to the following classes: mental functions, healthy or impaired, modulated by TMS; mental disabilities treated with TMS; rehabilitation or enhancement effects of TMS. We also searched for relationships between classes of relevant terms and conclusive sentences summarizing research results.

Very briefly, the manual curation gave the following perspective over the main topics:

a. CR-TMS. TMS is continuously establishing itself as one of the "tools of the trade" in psychiatric therapeutic practice (Kammer and Spitzer, 2012) improving mental functions in: Parkinson's disease (Pascual-Leone et al., 1994), aphasia (Medina et al., 2012), motor control after stroke (Takeuchi et al., 2005), epilepsy (Nitsche and Paulus, 2009), depression (Lisanby et al., 2009; Conforto et al., 2014), schizophrenia (Levkovitz et al., 2011; Kammer and Spitzer, 2012), autism (Krause et al., 2012), chronic migraine (Conforto et al., 2014), dyslexia (Costanzo et al., 2013), neglect (Fasotti and Van Kessel, 2013), obsessive-compulsive disorder (OCD) (Mantovani et al., 2013), chronic pain (Moreno-Duarte et al., 2014), and social anxiety disorder (Paes et al., 2013). The TMS therapy applied to younger patients (children and adolescents) improves cognitive functions (Vicario and Nitsche, 2013) in: stroke affecting the motor cortex (Kirton et al., 2008), epilepsy (Fregni et al., 2005), ADHD (Weaver et al., 2012), Tourette syndrome (Le et al., 2013), autism (Baruth et al., 2010), treatment-resistant depression (Bloch et al., 2008), and medication-resistant schizophrenia (Jardri et al., 2012).
b. CE-TMS. CE is defined as any augmentation of core information processing systems in the brain underlying perception, attention, conceptualization, memory, reasoning and motor performance (Sandberg and Bostrom, 2006; Luber and Lisanby, 2014). Studies reported TMS-induced modulations and enhancements of brain functioning and neural processing involved in: language comprehension (Floel et al., 2008), learning and memory (Vicario et al., 2013), cortical plasticity improving learning (Vallence and Ridding, 2014), motor memory (Butefisch et al., 2004), working memory (Gaudeau-Bosma et al., 2013), memory (Gagnon et al., 2011; Blumenfeld et al., 2014), phonological memory (Kirschen et al., 2006), perception (Hamilton et al., 2013), perceptual discrimination (Luber and Lisanby, 2014), eye movements and visual search, (Gerits et al., 2011; Luber and Lisanby, 2014), attention (Cooper et al., 2004; Lee et al., 2013), reward behavior (Stanford et al., 2013), analogic reasoning (Boroojerdi et al., 2001), motor learning (Luber and Lisanby, 2014), consolidation of new skills (Boyd and Linsdell, 2009), visual awareness (Grosbras and Paus, 2003), activity of specific frequencies supporting functions of the brain (Rahnev, 2013), and Pavlovian conditioning (Luber et al., 2007).

CR-TMS and CE-TMS used various TMS paradigms, including single-pulse, theta-burst, paired-pulse, and trains of rTMS at both low and high frequencies (Luber and Lisanby, 2014).

Comparisons with the manual curation showed that the TM-tools were also able to extract:

– All the relevant terms for CR-TMS and CE-TMS in the form of: lists; topics; classes of terms associated with specific subtopics (e.g., mental disabilities, cognitive processes).
– Relations between relevant terms in the form of: co-occurrences maps (**Figure 3**); groups of relevant terms with high probabilities co-occurrences; KWIC (**Tables 3**, **4**); lists of relevant relational verbs.
– High probability and relevance conclusive sentences (see examples and **Table 5**). We studied also structural statistical regularities in both conclusive sentences and abstracts shown by: the relative position in the sentence for groups of relevant terms (**Tables 3–5**); combinations of relevant terms with high probability occurrence; the occurrence frequency for conclusive sentences.

In addition, the TM approach has clear advantages emerging from the statistical properties of big corpora. Accordingly, the tirade (terms, terms-relationships, sentences) gained statistical strength, enabling us to quantify the frequency of a term or occurrence probabilities for specific relationships between terms or for conclusive sentences. For example, the hierarchy of the top terms for the CR-TMS-corpus includes TMS, treatment, rTMS, brain, therapy, depression, and stroke. We also found hierarchies for classes of terms like [CR-TMS-effects] (e.g., treatment, therapy, recovery, antidepressant, rehabilitation, neurorehabilitation) and [mental disabilities] (e.g., depression, stroke, schizophrenia, tinnitus, major depressive disorder, Parkinson, OCD, epilepsy, seizures, neglect, anxiety, ADHD, stress, Alzheimer's). For the CE-TMS-corpus the top terms are TMS, rTMS, brain, cognitive, performance, and facilitation. We also added hierarchies for classes of terms like [CE-TMS-effects] (e.g., performance enhancement, improvement, facilitation, neuromodulation, neurostimulation, therapy, neuroenhancement, rehabilitation, CE) and [cognitive processes] (e.g., memory, learning, attention, working memory, perception, language skill acquisition, decision, emotion, speech, semantic processing).

The relevance of all the retrieved terms and of their relationships is based on the idea that words co-occurring frequently in the abstracts are related in specific ways intrinsically constrained by the (TMS-related) topic of the abstracts. Thus, TMS is strongly related with all frequent terms retrieved with different TM-tools. Although in a relatively crude form, determined by our intention to show "raw" TM results, our study is showing that TMS emerged as one of the important non-invasive tools that can both improve cognitive and motor functions in numerous neurological diseases and induce enhancements of many fundamental brain functions.

We were able to characterize topics considering their dynamic relationships, trends in research and the interest shown by the scientific community. For example, CR-TMS and CE-TMS share studies (**Figure 4**), being an argument for their similarity. The reviewed topics share also publications with other

fields suggesting their appurtenance to a larger context, which integrates diagnostic, fundamental research and fMRI studies. TMS can be used both to investigate and to modify brain physiology and performance in healthy and diseased subjects (Vicario and Nitsche, 2013).

Methodologically speaking, we conclude that TM was helpful in getting an overall perspective on a huge corpus of literature with some level of detail, intentionally limited to handle complexity. Richer information can be extracted using more complex TM methods focused on narrower topics, but this requires extensive training and knowledge.

A decision factor to use TM relates to how profitable and how difficult the tools may be. The study aimed to address these simple issues in a pragmatic way. First and foremost, we argue that TM-tools may become a basic component in the methodological library. Unfortunately, it is equally clear that TM is a difficult task. With this in mind, we aimed to evaluate relatively immediate advantages of a user-friendly TM approach, based on easy-to-use TM-tools applied to CR- and CE-TMS corpora of abstracts. The hierarchical structure of our example set of TM-tools could serve as a guide for researchers aiming to use TM. Accordingly, for a rapid enrichment of the PubMed search, TM-tools II could be used, with special considerations for Carrot2, PubReMiner, Quertle, Medline Ranker, and Textpresso for Neuroscience. All TM-tools III could help a more elaborate TM without a considerable increase in demands to the user. For complex studies combining multiple aspects of the "mining," we recommend systems like Knime, RapidMiner, and Taverna.

ACKNOWLEDGMENT

This work was supported by IUAP 7-11, PF10/008, FWO-K714811, and G.083111.

REFERENCES

Akil, H., Martone, M. E., and Van Essen, D. C. (2011). Challenges and opportunities in mining neuroscience data. *Science* 331, 708–712. doi: 10.1126/science.1199305

Alfred, R., Razali, M., Alias, S., and On, C. (2014). "A visualization approach to automatic text documents categorization based on HAC," in *The 8th International Conference on Knowledge Management in Organizations, Social and Big Data Computing for Knowledge Management, KMO 2013*, eds L. Uden, L. S. L. Wang, J. M. Corchado Rodríguez, H.-C. Yang, and I. H. Ting (Kaohsiung: Springer), 273–284. doi: 10.1007/978-94-007-7287-8_22

Baruth, J. M., Casanova, M. F., El-Baz, A., Horrell, T., Mathai, G., Sears, L., et al. (2010). Low-frequency repetitive transcranial magnetic stimulation (rTMS) modulates evoked-gamma frequency oscillations in autism spectrum disorder (ASD). *J. Neurother.* 14, 179–194. doi: 10.1080/10874208.2010.501500

Becker, K. G., Hosack, D. A., Dennis, G. Jr., Lempicki, R. A., Bright, T. J., Cheadle, C., et al. (2003). PubMatrix: a tool for multiplex literature mining. *BMC Bioinform.* 4:61. doi: 10.1186/1471-2105-4-61

Bloch, Y., Grisaru, N., Harel, E. V., Beitler, G., Faivel, N., Ratzoni, G., et al. (2008). Repetitive transcranial magnetic stimulation in the treatment of depression in adolescents: an open-label study. *J. ECT* 24, 156–159. doi: 10.1097/YCT.0b013e318156aa49

Blumenfeld, R. S., Lee, T. G., and D'Esposito, M. (2014). The effects of lateral prefrontal transcranial magnetic stimulation on item memory encoding. *Neuropsychologia* 53, 197–202. doi: 10.1016/j.neuropsychologia.2013.11.021

Boroojerdi, B., Phipps, M., Kopylev, L., Wharton, C. M., Cohen, L. G., and Grafman, J. (2001). Enhancing analogic reasoning with rTMS over the left prefrontal cortex. *Neurology* 56, 526–528 doi: 10.1212/WNL.56.4.526

Boyd, L. A., and Linsdell, M. A. (2009). Excitatory repetitive transcranial magnetic stimulation to left dorsal premotor cortex enhances motor consolidation of new skills. *BMC Neurosci.* 10:72. doi: 10.1186/1471-2202-10-72

Brem, A. K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *Neuroimage* 85 Pt 3, 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Bridges-Webb, C. (1986). A computer summary for general practice medical records: MEDSUM. *J. Fam. Pract.* 23, 389–392

Butefisch, C. M., Khurana, V., Kopylev, L., and Cohen, L. G. (2004). Enhancing encoding of a motor memory in the primary motor cortex by cortical stimulation. *J. Neurophysiol.* 91, 2110–2116. doi: 10.1152/jn.01038.2003

Carpineto, C., Osinski, S., Romano, G., and Weiss, D. (2009). A survey of Web clustering engines. *ACM Comput. Surv.* 41, 1–38. doi: 10.1145/1541880.1541884

Cohen, K. B., and Hunter, L. (2008). Getting started in text mining. *PLoS Comput. Biol.* 4:e20. doi: 10.1371/journal.pcbi.0040020

Conforto, A. B., Amaro, E. Jr., Goncalves, A. L., Mercante, J. P., Guendler, V. Z., Ferreira, J. R., et al. (2014). Randomized, proof-of-principle clinical trial of active transcranial magnetic stimulation in chronic migraine. *Cephalalgia* 34, 464–472. doi: 10.1177/0333102413515340

Cooper, A. C., Humphreys, G. W., Hulleman, J., Praamstra, P., and Georgeson, M. (2004). Transcranial magnetic stimulation to right parietal cortex modifies the attentional blink. *Exp. Brain Res.* 155, 24–29. doi: 10.1007/s00221-003-1697-9

Corney, D. P., Buxton, B. F., Langdon, W. B., and Jones, D. T. (2004). BioRAT: extracting biological information from full-length papers. *Bioinformatics* 20, 3206–3213. doi: 10.1093/bioinformatics/bth386

Costanzo, F., Menghini, D., Caltagirone, C., Oliveri, M., and Vicari, S. (2013). How to improve reading skills in dyslexics: the effect of high frequency rTMS. *Neuropsychologia* 51, 2953–2959. doi: 10.1016/j.neuropsychologia.2013.10.018

Dias, A. M., Mansur, C. G., Myczkowski, M., and Marcolin, M. (2011). Whole field tendencies in transcranial magnetic stimulation: a systematic review with data and text mining. *Asian J. Psychiatr.* 4, 107–112. doi: 10.1016/j.ajp.2011.03.003

Doms, A., and Schroeder, M. (2005). GoPubMed: exploring PubMed with the Gene Ontology. *Nucleic Acids Res.* 33, W783–W786. doi: 10.1093/nar/gki470

Fasotti, L., and Van Kessel, M. (2013). Novel insights in the rehabilitation of neglect. *Front. Hum. Neurosci.* 7:780. doi: 10.3389/fnhum.2013.00780

Floel, A., Rosser, N., Michka, O., Knecht, S., and Breitenstein, C. (2008). Noninvasive brain stimulation improves language learning. *J. Cogn. Neurosci.* 20, 1415–1422. doi: 10.1162/jocn.2008.20098

Fontaine, J.-F., Barbosa-Silva, A., Schaefer, M., Huska, M. R., Muro, E. M., and Andrade-Navarro, M. A. (2009). MedlineRanker: flexible ranking of biomedical literature. *Nucleic Acids Res.* 37, W141–W146. doi: 10.1093/nar/gkp353

Fontelo, P., Liu, F., and Ackerman, M. (2005). askMEDLINE: a free-text, natural language query tool for MEDLINE/PubMed. *BMC Med. Inform. Decis. Mak.* 5:5. doi: 10.1186/1472-6947-5-5

Fregni, F., Thome-Souza, S., Bermpohl, F., Marcolin, M. A., Herzog, A., Pascual-Leone, A., et al. (2005). Antiepileptic effects of repetitive transcranial magnetic stimulation in patients with cortical malformations: an EEG and clinical study. *Stereotact. Funct. Neurosurg.* 83, 57–62. doi: 10.1159/000086674

Gagnon, G., Schneider, C., Grondin, S., and Blanchet, S. (2011). Enhancement of episodic memory in young and healthy adults: a paired-pulse TMS study on encoding and retrieval performance. *Neurosci. Lett.* 488, 138–142. doi: 10.1016/j.neulet.2010.11.016

Gaudeau-Bosma, C., Moulier, V., Allard, A. C., Sidhoumi, D., Bouaziz, N., Braha, S., et al. (2013). Effect of two weeks of rTMS on brain activity in healthy subjects during an n-back task: a randomized double blind study. *Brain Stimul.* 6, 569–575. doi: 10.1016/j.brs.2012.10.009

Gerits, A., Ruff, C., Guipponi, O., Wenderoth, N., Driver, J., and Vanduffel, W. (2011). Transcranial magnetic stimulation of macaque frontal eye fields decreases saccadic reaction time. *Exp. Brain Res.* 212, 143–152. doi: 10.1007/s00221-011-2710-3

Giglia, E. (2011). Quertle and KNALIJ: searching PubMed has never been so easy and effective. *Eur. J. Phys. Rehabil. Med.* 47, 687–690

Grosbras, M. H., and Paus, T. (2003). Transcranial magnetic stimulation of the human frontal eye field facilitates visual awareness. *Eur. J. Neurosci.* 18, 3121–3126. doi: 10.1111/j.1460-9568.2003.03055.x

Hamilton, R. H., Wiener, M., Drebing, D. E., and Coslett, H. B. (2013). Gone in a flash: manipulation of audiovisual temporal integration using transcranial magnetic stimulation. *Front. Psychol.* 4:571. doi: 10.3389/fpsyg.2013.00571

Higuchi, K. (2012). "The internet in newspaper articles and people's minds: a corpus-based exploratory approach to social consciousness in japan," in *The 4th International Conference on Corpus* (Linguistics), 115–116.

Hoskinson, A. (2005). Creating the ultimate research assistant. *Computer* 38, 97–99. doi: 10.1109/MC.2005.375

Iliopoulos, I., Enright, A. J., and Ouzounis, C. A. (2001). Textquest: document clustering of Medline abstracts for concept discovery in molecular biology. *Biocomputing* 2001, 384–395. doi: 10.1142/9789814447362_0038

Jardri, R., Bubrovszky, M., Demeulemeester, M., Poulet, E., Januel, D., Cohen, D., et al. (2012). Repetitive transcranial magnetic stimulation to treat early-onset auditory hallucinations. *J. Am. Acad. Child Adolesc. Psychiatry* 51, 947–949. doi: 10.1016/j.jaac.2012.06.010

Kammer, T., and Spitzer, M. (2012). Brain stimulation in psychiatry: methods and magnets, patients and parameters. *Curr. Opin. Psychiatry* 25, 535–541. doi: 10.1097/YCO.0b013e328358df8c

Kirschen, M. P., Davis-Ratner, M. S., Jerde, T. E., Schraedley-Desmond, P., and Desmond, J. E. (2006). Enhancement of phonological memory following transcranial magnetic stimulation (TMS). *Behav. Neurol.* 17, 187–194 doi: 10.1155/2006/469132

Kirton, A., Chen, R., Friefeld, S., Gunraj, C., Pontigon, A. M., and Deveber, G. (2008). Contralesional repetitive transcranial magnetic stimulation for chronic hemiparesis in subcortical paediatric stroke: a randomised trial. *Lancet Neurol.* 7, 507–513. doi: 10.1016/S1474-4422(08)70096-6

Koster, J. (2008). *PubMed. PubReMiner*. Available online at: http://hgserver2.amc.nl/cgi-bin/miner/miner2.cgi

Krause, L., Enticott, P. G., Zangen, A., and Fitzgerald, P. B. (2012). The role of medial prefrontal cortex in theory of mind: a deep rTMS study. *Behav. Brain Res.* 228, 87–90. doi: 10.1016/j.bbr.2011.11.037

Landauer, T. K., Foltz, P. W., and Laham, D. (1998). An introduction to latent semantic analysis. *Discourse Process.* 25, 259–284. doi: 10.1080/01638539809545028

Le, K., Liu, L., Sun, M., Hu, L., and Xiao, N. (2013). Transcranial magnetic stimulation at 1 Hertz improves clinical symptoms in children with Tourette syndrome for at least 6 months. *J. Clin. Neurosci.* 20, 257–262. doi: 10.1016/j.jocn.2012.01.049

Lee, J., Ku, J., Han, K., Park, J., Lee, H., Kim, K. R., et al. (2013). rTMS over bilateral inferior parietal cortex induces decrement of spatial sustained attention. *Front. Hum. Neurosci.* 7:26. doi: 10.3389/fnhum.2013.00026

Levkovitz, Y., Rabany, L., Harel, E. V., and Zangen, A. (2011). Deep transcranial magnetic stimulation add-on for treatment of negative symptoms and cognitive deficits of schizophrenia: a feasibility study. *Int. J. Neuropsychopharmacol.* 14, 991–996. doi: 10.1017/S1461145711000642

Lisanby, S., Husain, M., Rosenquist, P., Maixner, D., Gutierrez, R., Krystal, A., et al. (2009). Daily left prefrontal repetitive transcranial magnetic stimulation in the acute treatment of major depression: clinical predictors of outcome in a multisite, randomized controlled clinical trial. *Neuropsychopharmacology* 34, 522–534 doi: 10.1038/npp.2008.118

Lourenco, A., Carreira, R., Carneiro, S., Maia, P., Glez-Pena, D., Fdez-Riverola, F., et al. (2009). @Note: a workbench for biomedical text mining. *J. Biomed. Inform.* 42, 710–720. doi: 10.1016/j.jbi.2009.04.002

Lu, Z. (2011). PubMed and beyond: a survey of web tools for searching biomedical literature. *Database (Oxford)* 2011:baq036. doi: 10.1093/database/baq036

Luber, B., Balsam, P., Nguyen, T., Gross, M., and Lisanby, S. H. (2007). Classical conditioned learning using transcranial magnetic stimulation. *Exp. Brain Res.* 183, 361–369. doi: 10.1007/s00221-007-1052-7

Luber, B., and Lisanby, S. H. (2014). Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* 85(Pt 3), 961–970. doi: 10.1016/j.neuroimage.2013.06.007

Mantovani, A., Rossi, S., Bassi, B. D., Simpson, H. B., Fallon, B. A., and Lisanby, S. H. (2013). Modulation of motor cortex excitability in obsessive-compulsive disorder: an exploratory study on the relations of neurophysiology measures with clinical outcome. *Psychiatry Res.* 210, 1026–1032. doi: 10.1016/j.psychres.2013.08.054

McCallum, A. K. (2002). *MALLET: A Machine Learning for Language Toolkit*. Available online at: http://mallet.cs.umass.edu

Medina, J., Norise, C., Faseyitan, O., Coslett, H. B., Turkeltaub, P. E., and Hamilton, R. H. (2012). Finding the right words: transcranial magnetic stimulation improves discourse productivity in non-fluent aphasia after stroke. *Aphasiology* 26, 1153–1168. doi: 10.1080/02687038.2012.710316

Meyers, A. (2003). *Multi-Pass Multi-Strategy Nlp*. Available online at: http://www.textanalysis.com/tai-multi2003.pdf

Miniussi, C., and Rossini, P. M. (2011). Transcranial magnetic stimulation in cognitive rehabilitation. *Neuropsychol. Rehabil.* 21, 579–601. doi: 10.1080/09602011.2011.562689

Moreno-Duarte, I., Morse, L. R., Alam, M., Bikson, M., Zafonte, R., and Fregni, F. (2014). Targeted therapies using electrical and magnetic neural stimulation for the treatment of chronic pain in spinal cord injury. *Neuroimage* 85 Pt 3, 1003–1013. doi: 10.1016/j.neuroimage.2013.05.097

Muller, H. M., Rangarajan, A., Teal, T. K., and Sternberg, P. W. (2008). Textpresso for neuroscience: searching the full text of thousands of neuroscience research papers. *Neuroinformatics* 6, 195–204. doi: 10.1007/s12021-008-9031-0

Nitsche, M. A., and Paulus, W. (2009). Noninvasive brain stimulation protocols in the treatment of epilepsy: current state and perspectives. *Neurotherapeutics* 6, 244–250. doi: 10.1016/j.nurt.2009.01.003

Paes, F., Baczynski, T., Novaes, F., Marinho, T., Arias-Carrion, O., Budde, H., et al. (2013). Repetitive transcranial magnetic stimulation (rTMS) to treat social anxiety disorder: case reports and a review of the literature. *Clin. Pract. Epidemiol. Ment. Health* 9, 180–188. doi: 10.2174/1745017901309010180

Pascual-Leone, A., Valls-Sole, J., Brasil-Neto, J. P., Cammarota, A., Grafman, J., and Hallett, M. (1994). Akinesia in Parkinson's disease. II. Effects of subthreshold repetitive transcranial motor cortex stimulation. *Neurology* 44, 892–898 doi: 10.1212/WNL.44.5.892

Perez-Iratxeta, C., Bork, P., and Andrade, M. A. (2001). XplorMed: a tool for exploring MEDLINE abstracts. *Trends Biochem. Sci.* 26, 573–575 doi: 10.1016/S0968-0004(01)01926-0

Rahnev, D. (2013). Entrainment of neural activity using transcranial magnetic stimulation. *J. Neurosci.* 33, 11325–11326. doi: 10.1523/JNEUROSCI.2012-13.2013

Sandberg, A., and Bostrom, N. (2006). Converging cognitive enhancements. *Ann. N.Y. Acad. Sci.* 1093, 201–227. doi: 10.1196/annals.1382.015

Sarkar, I. N., Schenk, R., Miller, H., and Norton, C. N. (2009). LigerCat: using "MeSH Clouds" from journal, article, or gene citations to facilitate the identification of relevant biomedical literature. *AMIA Annu. Symp. Proc.* 2009, 563–567.

Smalheiser, N. R., Zhou, W., and Torvik, V. I. (2008). Anne O'Tate: a tool to support user-driven summarization, drill-down and browsing of PubMed search results. *J. Biomed. Discov. Collab.* 3:2. doi: 10.1186/1747-5333-3-2

Stanford, A. D., Luber, B., Unger, L., Cycowicz, Y. M., Malaspina, D., and Lisanby, S. H. (2013). Single pulse TMS differentially modulates reward behavior. *Neuropsychologia* 51, 3041–3047. doi: 10.1016/j.neuropsychologia.2013.09.016

Takeuchi, N., Chuma, T., Matsuo, Y., Watanabe, I., and Ikoma, K. (2005). Repetitive transcranial magnetic stimulation of contralesional primary motor cortex improves hand function after stroke. *Stroke* 36, 2681–2686. doi: 10.1161/01.STR.0000189658.51972.34

Vallence, A. M., and Ridding, M. C. (2014). Non-invasive induction of plasticity in the human cortex: uses and limitations. *Cortex* 58C, 261–271. doi: 10.1016/j.cortex.2013.12.006

Vicario, C. M., Candidi, M., and Aglioti, S. M. (2013). Cortico-spinal embodiment of newly acquired, action-related semantic associations. *Brain Stimul.* 6, 952–958. doi: 10.1016/j.brs.2013.05.010

Vicario, C. M., and Nitsche, M. A. (2013). Non-invasive brain stimulation for the treatment of brain diseases in childhood and adolescence: state of the art, current limits and future challenges. *Front. Syst. Neurosci.* 7:94. doi: 10.3389/fnsys.2013.00094

Weaver, L., Rostain, A. L., Mace, W., Akhtar, U., Moss, E., and O'Reardon, J. P. (2012). Transcranial magnetic stimulation (TMS) in the treatment of attention-deficit/hyperactivity disorder in adolescents and young adults: a pilot study. *J. ECT* 28, 98–103. doi: 10.1097/YCT.0b013e31824532c8

Zeimpekis, D., and Gallopoulos, E. (2006). "TMG: a MATLAB toolbox for generating term-document matrices from text collections," in *Grouping Multidimensional Data: Recent Advances in Clustering*, eds J. Kogan, C. Nicholas, and M. Teboulle (Berlin, Heidelberg: Springer), 187–210.

Zhu, M., and Ghodsi, A. (2006). Automatic dimensionality selection from the scree plot via the use of profile likelihood. *Comput. Stat. Data Anal.* 51, 918–930 doi: 10.1016/j.csda.2005.09.010

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 May 2014; accepted: 08 September 2014; published online: 26 September 2014.

Citation: Balan PF, Gerits A and Vanduffel W (2014) A practical application of text mining to literature on cognitive rehabilitation and enhancement through neurostimulation. Front. Syst. Neurosci. ***8****:182. doi: 10.3389/fnsys.2014.00182*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 18 December 2015
doi: 10.3389/fnins.2015.00478

Functional Electrical Stimulation Alters the Postural Component of Locomotor Activity in Healthy Humans

Vera Talis[1]*, *Yves Ballay*[2], *Alexander Grishin*[1] and *Thierry Pozzo*[2, 3, 4]

[1] Institute for Information Transmission Problems, Moscow, Russia, [2] Institut National de la Santé et de la Recherche Médicale, U1093, Cognition Action Plasticité Sensorimotrice, Dijon, France, [3] Department of Robotics, Brain and Cognitive Sciences, Istituto Italiano di Tecnologia, Genova, Italy, [4] Université de Bourgogne, UFR STAPS (Sciences du Sport), Dijon, France

Knowledge of the effects of Functional Electrical Stimulation (FES) of different intensity on postural stability during walking in healthy subjects is necessary before these relationships in patients with postural disorders can be assessed and understood. We examined healthy subjects in Control group walking on a treadmill for 40 min and in FES group—provided with 30 min of stimulation, which intensity increased every 10 min. The main difference between Control and FES group was the progressive increase of trunk oscillations in sagittal, frontal, and horizontal planes and an increase of relative stance duration in parallel with FES intensity increase. Both Control and FES groups exhibited shank elevation angle increase as an after-effect. It is concluded, that high intensity FES significantly changes the postural component of locomotor activity, but the fatigue signs afterwards were not FES specific.

Keywords: FES, postural stability, locomotion, healthy subjects

OPEN ACCESS

Edited by:
Mikhail Lebedev,
Duke University, USA

Reviewed by:
Hari S. Sharma,
Uppsala University, Sweden
Victor Manuel Pulgar,
Wake Forest School of Medicine, USA

***Correspondence:**
Vera Talis
talis@iitp.ru

Specialty section:
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

Received: *21 September 2015*
Accepted: *30 November 2015*
Published: *18 December 2015*

Citation:
Talis V, Ballay Y, Grishin A and Pozzo T (2015) Functional Electrical Stimulation Alters the Postural Component of Locomotor Activity in Healthy Humans. Front. Neurosci. 9:478. doi: 10.3389/fnins.2015.00478

INTRODUCTION

Human walking is characterized by the repetitive motion of limb segments aimed to propel the body forward. Joint movement is highly synchronized and muscle activity displays a typical periodical pattern (Bernstein, 1990; Perry, 1992). These rhythmic locomotor movements are produced by a brainstem-spinal central pattern generator (CPG) that is activated by descending command signals (Grillner, 1981; Grasso et al., 1998; Selionov et al., 2009). Any unpredicted disturbance during walking, such as stumbling (Eng et al., 1994; Schilling et al., 2000), could be compensated through sensory feedback. In the experimental environment, the kinematics of walking have been shown to be well preserved even under the conditions of body weight unloading (Ivanenko et al., 2002), additional loading of the legs (Smith and Martin, 2007), and during "split-belt" locomotion (Jensen et al., 1998). As an example, Ivanenko et al. (2002) has shown that dynamical change, such as an artificial decrease of foot pressure, resulted in weak changes in the coordination of segments.

A specific type of gait disturbance is the direct stimulation of muscles at the time of muscle action during the step cycle. Such a periodical stimulation could be implemented by means of electrical or vibratory intervention. Ivanenko et al. (2000) analyzed the effects of phasic leg muscle vibration on human locomotion, and have shown that the vibration of hamstring muscles produced an increase

of walking speed, while the vibration of other leg muscles did not. Based on the well-known fact of position and movement illusions caused by muscle vibration in an upright standing position (Lackner and Levine, 1979; Talis and Solopova, 2000), Ivanenko and colleagues explained that hamstring vibration induced a rise in speed through the modification of kinesthetic information about joint angels consistent with the lengthening of the hamstring muscle.

In this paper, we studied the effects of electrical phasic stimulation of leg muscles (Functional Electrical Stimulation, FES) during walking. In contrast to vibratory stimulation, electrical stimulation, directly affecting the relation between motor command and force output, ensures an almost immediate muscle contraction. This increases the precision of the application of the stimulus and thus allows the stimulation of several muscles in an alternative manner through each gait cycle. The force of the muscle response to the electrical stimulation can be increased to up to 70% of maximum voluntary contraction MVC (DeVahl, 1992) [vibration elicited not more than 30% of MVC of the stimulated muscle (Matthews, 1966)]. Walking speed increase with regular use of FES in spinal cord injury (Ladouceur and Barbeau, 2000; Pomeroy et al., 2006) and stroke patients (Lindquist et al., 2007) was reported. In was shown, that this gait speed advantage lasted from weeks to a month in stroke (Bogataj et al., 1995; Alon and Ring, 2003; Daly et al., 2011) and spinal cord injury patients (Ladouceur and Barbeau, 2000; for reviews see Barbeau et al., 2002). At the same time, the origin of the retention of an increased walking speed without continued FES, meaning the long lasting effect of FES-training, called the therapeutic effect of FES-assisted walking, is unclear and even discounted (see for instance the discussion about implantable electrode technology, as a possible future of electrical stimulation in Burridge and Hughes, 2010). For instance, post-stroke gait speed increase due to FES might be because walking rate reduction was the primary reason for FES use (Taylor et al., 1999).

The rationale of the present study was to study the kinematics of healthy subjects during treadmill locomotion and compare the results of two groups, one of which was simultaneously provided with FES (FES group), and the other was not (Control group). A short account of some of the present findings was published as an abstract (Talis et al., 2011). The collected data of FES-assisted walking in healthy subjects could be implemented in the pathology of FES-assisted walking.

MATERIALS AND METHODS

Subjects

Eight healthy subjects participated in the FES group [seven males and one female, between 25 and 49 year of age, 74 ± 11 (SD) kg, 1.76 ± 0.1 m] and eight subjects in the Control group (six males and two females, between 20 and 49 year of age, 73 ± 11 kg, 1.74 ± 0.09 m, five of them from the FES group). None of the subjects had any history of neurological disease or vestibular impairment. The experiments conformed to the Declaration of Helsinki and written informed consent was obtained from all the participants according to the protocol of the Ethics Committee of the Universite de Bourgogne.

Experimental Setup and Stimulation Techniques

The subjects walked on a treadmill at individually adjusted speed of about 0.7 m/s with their shoes on. Subjects from the FES group have eight bipolar stimulation surface electrodes (5×5 and 5×10 cm) placed bilaterally on four muscles [Tibialis Anterior (TA), Gastrocnemius Medialis (GM), Quadriceps (Q), and Biceps Femoris (BF) of both legs] with the negative electrode over a motor point (DeVahl, 1992). Electrical stimulus consisted of repetitive trains of rectangular pulses with 65 mA amplitude at 65 Hz. A custom-made eight-channel stimulator delivered the desired stimulation train, triggered by the signal of the right knee goniometer in such a way, that the timing of the stimulation sequence corresponded to the timing of the activation sequence of these muscles during normal gait (Perry, 1992).

Data Recording

Body kinematics was recorded by means of the ELITE system (BTS, Italy). Nine 120-Hz TV cameras were spaced around a treadmill in a $4 \times 4 \times 2$ m acquisition volume. Hemispherical reflective markers of 15-mm diameter attached to the skin overlying the following body landmarks for the two hemibodies: laterally on the fifth metatarsophalangeal joint (MTP), lateral malleolus (MAL), lateral tibial tables (KN), greater trochanters (GT), anterior-superior iliac spines (IS), and gleno-humeral-joints (GH).

Pain during FES-assisted walking was registered by means of a 10-cm analog pain scale, while a value close to 10 means higher pain. The pain scale was presented to the subject three times (Stim1, Stim2, and Stim3).

Experimental Protocol

Before data collection, the subject had 5–7 s to reach steady motion on the treadmill. Data collections (3 min each) were performed five times during treadmill walking: before FES (Before), three times during FES (Stim1, Stim2, and Stim3) and after FES (After). The same protocol of data collection was used in the Control group where participants walked for 40 min on the treadmill without FES.

In the FES group, the experimenter increased the intensity of stimulation every 10 min (muscle by muscle, in the range 0–250 μs of impulse duration under the verbal control of the subject—every time up to the tolerant level of pain intensity). Each increase was performed during the first minute of each 3-min interval of data collection.

Data Analysis

The spatial coordinates of each marker were recorded, the body being represented as an interconnected chain of rigid segments. Kinematics data were filtered with a low-pass zero-phase shift Butterworth filter with a 5 Hz cut-off frequency. Stride length, walking frequency and velocity were estimated using the body mid-point (average of left and right GT, IS coordinates). This

resulting point provides a good estimation of the center of mass (Courtine and Schieppati, 2003).

The elevation angle of each segment in the sagittal plane corresponds to the angle between the projected segment and the vertical and were computed as:

$$\theta_{i\_sagittal} = \tan^{-1}[(X_{\mathrm{id}} - X_{\mathrm{ip}})/(Y_{\mathrm{id}} - Y_{\mathrm{ip}})],$$

where X and Y designate the coordinates of the proximal (p) and distal (d) markers for the *ith* frame of the acquisition. The elevation angle in the sagittal plane of the thigh (GT-KN), shank (KN-MAL) and foot (MAL-MTP) segments were calculated.

The elevation angle of the trunk in the sagittal, frontal, and horizontal planes corresponds to:

$$\theta_{trank\_sagittal} = \tan^{-1}\left\{\frac{\left(\frac{(X_{\mathrm{IS\_left}}+X_{\mathrm{IS\_right}})}{2} - \frac{(X_{\mathrm{GH\_left}}+X_{\mathrm{GH\_right}})}{2}\right)}{\left(\frac{(Y_{\mathrm{IS\_left}}+Y_{\mathrm{IS\_right}})}{2} - \frac{(Y_{\mathrm{GH\_left}}+Y_{\mathrm{GH\_right}})}{2}\right)}\right\}$$

and was calculated for each frame of the acquisition (Laroche et al., 2007). The trunk elevation angle in the frontal plane was computed with the same equation in the ZY plane and in the horizontal—in the XZ plane. Each trial was separated into gait cycles using the elevation angle of the lower limb axis (the line joining the MAL and GT), as described in Borghese et al. (1996). Stance phases were computed using the limb axis as described in Ivanenko et al. (2002) and was expressed in percentage of the gait cycle.

The coefficient of variation (CV) was calculated for each elevation angle to represent data variability and was calculated as the standard deviation divided by mean values across all steps of each subject during each 3 min of data collections (Bacarin et al., 2009).

Statistics

Mean and Descriptive statistics included means and the SE of the mean. Paired *t*-test and ANOVA were used when appropriate to compare means. In particular, to evaluate the effects of FES on the amplitude of trunk oscillations and spatio-temporal parameters, the two-way ANOVA with first factor "FES" (Before, Stim1, Stim2, Stim3 and After) and the second factor "group"(FES, Control) was used. When significant effects were found, *post-hoc* Tukey's testing was conducted to identify the loci of these effects. The level of statistical significance was set at 0.05.

RESULTS

General Gait Parameters

Figure 1 shows the mean values (over all trials and subjects) of relative stance duration, stride length, walking velocity and step frequency in the Control and FES groups. FES significantly affects stance time: in the FES group, the relative duration of a stance during Stim3 conditions was at average 65.29 ± 0.36% of the cycle; that is a larger value than that of any of the Control group. Note, that stride length was about the same in the FES and Control groups for these conditions (0.60 ± 0.02 m and 0.59 ± 0.03 m in the FES and Control groups, correspondingly). Statistical analyses revealed significant interaction between groups and conditions for relative duration of the stance [ANOVA, $F_{(4,\ 28)} = 6.21$, $p = 0.001$]. *Post-hoc* tests showed that the relative duration of the stance in the FES group was significantly larger in the Stim3 than in the Before condition. **Figure 1** also shows that control subjects exhibited the tendency of the well-known monotonic relationship of stride length and step frequency with speed increase (see Bernstein, 1935; Grillner, 1981; Winter and Scott, 1991).

Trunk Oscillations

Figure 2 shows mean individual (**Figure 2A**) and group mean data (**Figure 2B**) of trunk oscillations in sagittal (Pitch), frontal (Roll), and horizontal (Yaw) planes. Group mean data shows that before stimulation trunk oscillations were not different in Control and FES group (mean trunk oscillation in Before condition was in saggital plane 3.26 ± 0.14° and 3.45 ± 0.15°, in frontal plane 4.0 ± 0.55°, and 4.15 ± 0.55° and in horizontal plane 15.60 ± 0.57° and 13.78 ± 0.85° for FES and Control, respectively). Electrical stimulation of leg muscle significantly affects the amplitude of trunk oscillation: these amplitudes progressively increase in the FES group [ANOVA $F_{(4,\ 56)} = 12.28$, $p = 0.0002$; $F = 6.48$, $p = 0.0002$; $F = 5.98$, $p = 0.0004$ for Pitch, Roll and Yaw, correspondingly].

Limb Elevation Angels in Sagittal Plane

Figure 3 shows the profile of mean individual data (**Figure 3A**) and group mean data (**Figure 3B**) of limb elevation angels in sagittal plane. Due to stimulation, the profile of limb elevation angels didn't change, but the mean individual amplitude of foot angels decreased. Mean group data presented on **Figure 3B** also shows this tendency for distal joints, but these changes didn't approach the level of significance due to high variability of limb elevation angels due to FES. The CV of foot angle increased from 0.03 at Before up to 0.07 at Stim2 and 0.08 at Stim3 conditions (t-test, $p < 0.01$).

Perceptual Effects

All subjects in FES group reported the instability increase due to FES and the fatigue sensation afterwards. There were subjective reports during FES such as: "The locomotion is not free, my legs are out of my control, I feel my ankle joint blocked, "freezing," muscles are fatigued, I have sensation as walking in flippers." After the end of FES, several subjects sensed that the treadmill decelerated, and locomotion seemed "unusually light." Actually, some participants approached the forward part of the treadmill belt during FES. Subjective pain rating increased up to 8 cm in the Stim 3 condition (**Table 1**).

After-Effect

At the end of 40 min locomotion limb elevation angels appeared slightly increased in both groups in comparison with Before (**Figure 3B**). This increase approached the level of significance for shank angle in both groups (t-test, $p < 0.05$).

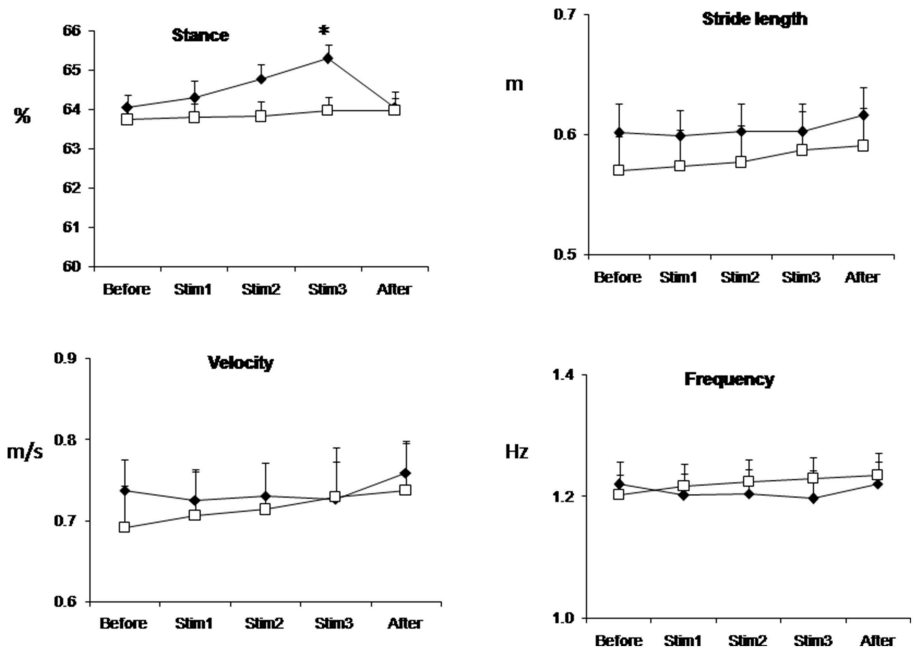

FIGURE 1 | Stance duration, stride length, velocity, and frequency of gait averaged across all trials and subjects in FES (black diamond) and Control (white square) groups. Values are mean ± SE. *$p < 0.05$.

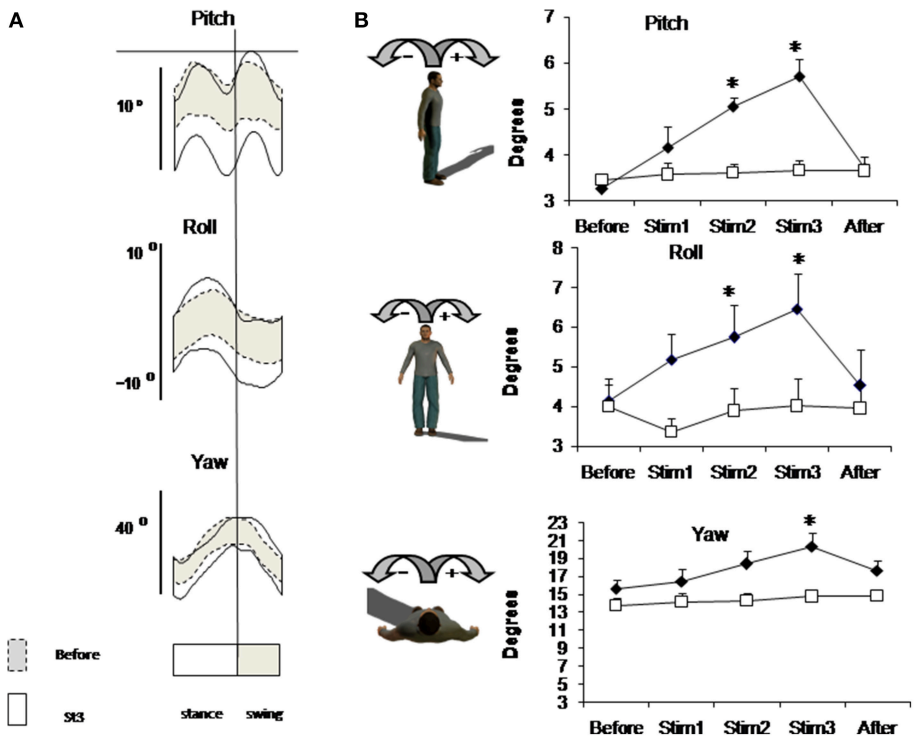

FIGURE 2 | Trunk oscillation evoked by FES. (A) Trunk motion (±SD value) in one representative subject from FES group averaged across 3-min intervals of Before (dashed lines) and Stim3 (solid lines) periods. **(B)** Peak-to-peak amplitudes of trunk oscillations averaged across FES (black diamond) and across Control (white square) groups (mean ± SE). Asterisks indicate statistical difference in Stim condition in comparison with Before condition in FES group, *$p < 0.05$.

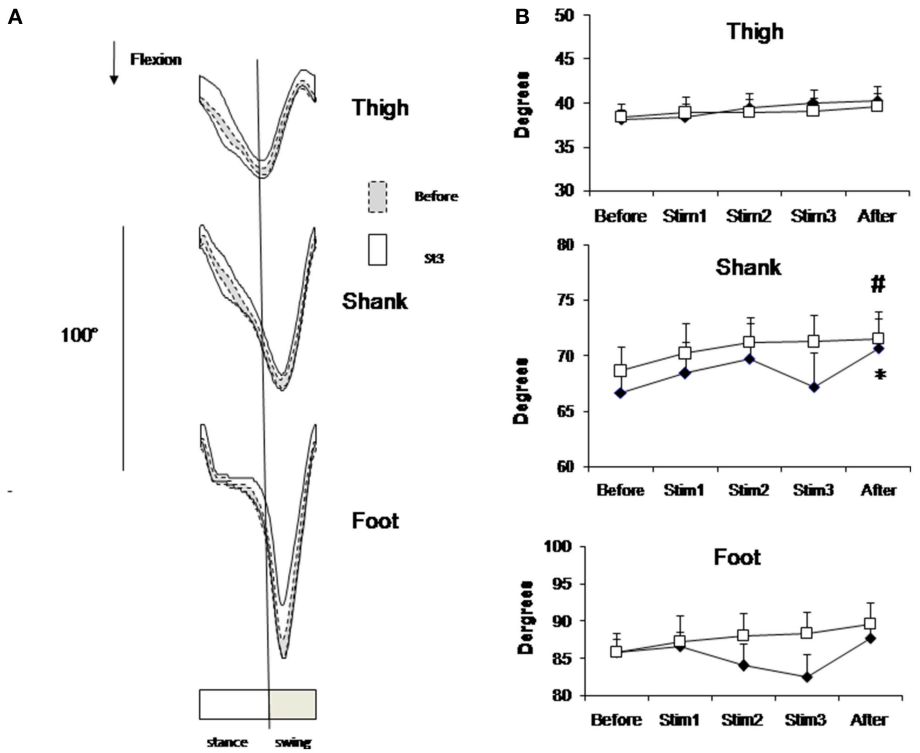

FIGURE 3 | Elevation angels in sagittal plane. (A) Segment elevation angels (±SD value) in one representative subject from FES group averaged across 3-min intervals of Before (dashed lines) Stim3 (solid lines) periods. **(B)** Peak-to-peak amplitudes of elevation angels averaged across FES (black diamond) and Control (white square) groups (mean ± SE) Asterisk (*) indicates statistical difference in the condition in comparison with Before condition in FES group; sharp (#) indicates statistical difference—in the condition in comparison with Before condition in Control group ($p < 0.05$).

TABLE 1 | Stimulation intensity (μs) of four muscles and the severity of pain for one typical subject in Stim1, Stim2 and Stim3 conditions.

Pain (cm)	Intensity of stimulation (duration of stimulation train, μs)			
	TA	GM	RF	B
5.5	60.6	82.9	92.4	92.4
7.5	143.4	162.6	162.6	130.9
8.5	226.3	239.1	258.2	334.7

DISCUSSION

In this paper, we analyzed the effects of FES on the kinematics of a healthy subjects' locomotion. The most remarkable changes caused by FES are the increase of body oscillation, accompanied by an increase in stance time duration.

Postural Instability

Although the mechanical reason of increased trunk pitch could be the excessive contraction of quadriceps muscle, the increased trunk oscillation both in sagittal, frontal and horizontal planes indicates the decrease of locomotion stability and movement disturbance caused by FES. The disruption of the normal gait by FES manifests itself as an increase in stance time, providing an increased support time—another index of instability (Bernstein, 1935; Kirtley, 2006). The fact that these parameters returned to the norm immediately after FES are in line with this explanation. Pain, especially due to strong stimulation of the tibialis muscle could also distort the sensory feedback from muscle and skin receptors and there by the descending control from the CNS.

Comparing FES-assisted and non-assisted walking of one incomplete spinal injuries patient Ladouceur and Barbeau (2000) have also found FES-induced decrease of ankle plantar flexion by 5.6°, which is similar to our data of FES-assisted walking (**Figure 3A**). The tendency of ankle and shank joints to flex less in the swing phase during intensive FES is similar to the effect of under-flexion of the knee during fatigued walking (Bernstein, 1935). However, these effects could be of a different origin: in our study the TA muscle being stimulated along the swing phase is more pain sensitive (**Table 1**). To avoid pain, the subject could aim to dorsiflex the TA muscle less than would be seen in a natural gait pattern so as to decrease the TA stimulation time and thus the amount of pain. This, in turn, resulted in excessive dorsiflexion of the ankle, which leads to a decrease of push of force.

Clinical Application

FES is a commonly used clinical tool to improve walking ability due to its simplicity, low-cost, and strong muscle response,

however, muscle fatigue is a major limiting factor in FES applications (Kralj et al., 1988; Karu et al., 1995). FES is shown to have a more significant effect in comparison with physiotherapy in walking speed increase during and after FES-assisted locomotor training (see review by Taylor et al., 2013). This notion is supported by the study of Khaslavskaia et al. (2002), which has shown that the changes in healthy participants in the TA MEP during locomotion were seen over 20 min following the cessation of the stimulation of the common peroneal nerve. It is now widely accepted, that FES improves ankle dorsiflexion in the long-term perspective, increasing the corticospinal excitability and that FES-assisted training "facilitates motor relearning" in patients (Ladouceur and Barbeau, 2000; Alon and Ring, 2003; Lindquist et al., 2007; Barrett et al., 2009; Daly et al., 2011). In the present study, intensive phasic electrical stimulation was applied to both ankles and hip antagonist muscles, and similarly to leg orthosis in neurological subjects. Similarly to clinical settings, the timing of stimulation was on-line controlled trough the feedback from the current knee joint angle in each stride (this way, in clinical practice, the pathological walking is aimed to be adjusted to the typical normal walking, then the "re-education" of pathological walking is expected). Our results indicate that, in healthy subjects, the postural component of locomotor activity was changed due to strong electrical stimulation of leg muscles, while the rhythmic component remains intact. It could be speculated, that the artificial nature of muscle contractions during FES-assisted walking in healthy subjects transfers the locomotor activity from involuntary to a more voluntary controlled movement. It could be concluded, that the functional role of FES for patients is the "adaption training" rather than "re-education" of pathological walking into the typical "normal" walking.

ACKNOWLEDGMENTS

Granted by Conseil Reginal de Bourgogne, France. RFBR grant #14-04-00950.

REFERENCES

Alon, G., and Ring, H. (2003). Gait and hand function enhancement following training with a multi-segment hybrid-orthosis stimulation system in stroke patients. *J. Stroke Cerebrovasc. Dis.* 12, 209–216. doi: 10.1016/S1052-3057(03)00076-4

Bacarin, T. A., Sacco, I. C., and Hennig, E. M. (2009). Plantar pressure distribution patterns during gait in diabetic neuropathy patients with a history of foot ulcers. *Clinics (Sao Paulo)* 64, 113–120. doi: 10.1590/S1807-59322009000200008

Barbeau, H., Ladouceur, M., Mirbagheri, M. M., and Kearney, R. E. (2002). The effect of locomotor training combined with functional electrical stimulation in chronic spinal cord injured subjects: walking and reflex studies. *Brain Res. Rev.* 40, 274–291. doi: 10.1016/S0165-0173(02)00210-2

Barrett, C. L., Mann, G. E., Taylor, P. N., and Strike, P. (2009). A randomized trial to investigate the effects of functional electrical stimulation and therapeutic exercise on walking performance for people with multiple sclerosis. *Mult. Scler.* 15, 493–504. doi: 10.1177/1352458508101320

Bernstein, N. A. (1935). *Biodynamic of Locomotion.* Moscow: VIEM.

Bernstein, N. A. (1990). *Movement Physiology and Activity. In the Series Classics of Science*. Moscow: Nauka.

Bogataj, U. L., Gros, N., Kljajic, M., Acimovic, R., and Malezic, M. (1995). The rehabilitation of gait in patients with hemiplegia: a comparison between conventional therapy and multichannel functional electrical stimulation therapy. *Phys. Ther.* 75, 490–502.

Borghese, N. A., Bianchi, L., and Lacquaniti, F. (1996). Kinematic determinants of human locomotion. *J. Physiol.* 494, 863–879. doi: 10.1113/jphysiol.1996.sp021539

Burridge, J. H., and Hughes, A. M. (2010). Potential for new technologies in clinical practice. *Curr. Opin. Neurol.* 23, 671–677. doi: 10.1097/WCO.0b013e3283402af5

Courtine, G., and Schieppati, M. (2003). Human walking along a curved path. I. Body trajectory, segment orientation and the effect of vision. *Eur. J. Neurosci.* 18, 177–190. doi: 10.1046/j.1460-9568.2003.02736.x

Daly, J. J., Zimbelman, J., Roenigk, K. L., McCabe, J. P., Rogers, J. M., Butler, K., et al. (2011). Recovery of coordinated gait: randomized controlled stroke trial of functional electrical stimulation (FES) versus no FES, with weight-supported treadmill and over-ground training. *Neurorehabil. Neural Repair* 25, 588–596. doi: 10.1177/1545968311400092

DeVahl, J. (1992). "Neuromuscular electrical stimulation (NMES) in rehabilitation," in *Electrotherapy in Rehabilitation*, ed M. R. Gersh (Philadelphia, PA: F.A. Davis Company), 227.

Eng, J. J., Winter, D. A., and Patla, A. E. (1994). Strategies for recovery from a trip in early and late swing during human walking. *Exp. Brain Res.* 102, 339–349. doi: 10.1007/BF00227520

Grasso, R., Bianchi, L., and Lacquaniti, F. (1998). Motor patterns for human gait: backward versus forward locomotion. *J. Neurophysiol.* 80, 1868–1885.

Grillner, S. (1981). "Control of locomotion in bipeds, tetra pods, and fish," in *Handbook of Physiology, Sec 1, The Nervous System II Motor Control*, ed V. B. Brooks (Bethesda: Waverly), 1179–1236.

Ivanenko, Y. P., Grasso, R., and Lacquaniti, F. (2000). Influence of leg muscle vibration on human walking. *J. Neurophysiol.* 84, 1737–1747.

Ivanenko, Y. P., Grasso, R., Macellari, V., and Lacquaniti, F. (2002). Control of foot trajectory in human locomotion: role of ground contact forces in simulated reduced gravity. *J. Neurophysiol.* 87, 3070–3089.

Jensen, L., Prokop, T., and Dietz, V. (1998). Adaptation effects during human split-belt walking: influence of afferent input. *Exp. Brain Res.* 118, 126–130. doi: 10.1007/s002210050262

Karu, Z. Z., Durfee, W. K., and Barzilai, A. M. (1995). Reducing muscle fatigue in FES applications by stimulating with N-let pulse trains. *IEEE Trans. Biomed. Eng.* 42, 809–817. doi: 10.1109/10.398642

Khaslavskaia, S., Ladouceur, M., and Sinkjaer, T. (2002). Increase in tibialis anterior motor cortex excitability following repetitive electrical stimulation on the common peroneal nerve. *Exp. Brain Res.* 145, 309–315. doi: 10.1007/s00221-002-1094-9

Kirtley, C. (2006). *Clinical Gait Analysis*. Churchill Livingstone: Elsevier.

Kralj, A., Bajd, T., and Turk, R. (1988). Enhancement of gait restoration in spinal injured patients by functional electrical stimulation. *Clin. Orthop. Relat. Res.* 233, 34–43.

Lackner, J. R., and Levine, M. S. (1979). Changes in apparent body orientation and sensory localization induced by vibration of postural muscles: vibratory myesthetic illusions. *Aviat. Space Environ. Med.* 50, 346–354.

Ladouceur, M., and Barbeau, H. (2000). Functional electrical stimulation-assisted walking for persons with incomplete spinal injuries: changes in the kinematics and physiological cost of overground walking. *Scand. J. Rehab. Med.* 32, 72–79. doi: 10.1080/003655000750045587

Laroche, D., Ornetti, P., Thomas, E., Ballay, Y., Maillefert, J. F., and Pozzo, T. (2007). Kinematic adaptation of locomotor pattern in rheumatoid arthritis patients with forefoot impairment. *Exp. Brain Res.* 176, 85–97. doi: 10.1007/s00221-006-0597-1

Lindquist, A. R., Prado, C. L., Barros, R. M., Mattioli, R., da Costa, P. H., and Salvini, T. F. (2007). Gait training combining partial body-weight support, a treadmill, and functional electrical stimulation: effects on poststroke gait. *Phys. Ther.* 87, 1144–1154. doi: 10.2522/ptj.20050384

Matthews, P. B. C. (1966). Reflex activation on the soleus muscle of the decerebrate cat by vibration. *Nature* 209, 204–205. doi: 10.1038/209204b0

Perry, J. (1992). *Gait Analysis: Normal and Pathological Function.* Thorofare, NJ: Slack.

Pomeroy, V. M., King, L., Pollock, A., Baily-Hallam, A., and Langhorne, P. (2006). Electrostimulation for promoting recovery of movement or functional ability after stroke. *Cochrane Database Syst. Rev.* 19:CD003241. doi: 10.1002/14651858.CD003241.pub2

Schillings, A. M., van Wezel, B. M., Mulder, T., and Duysens, J. (2000). Muscular responses and movement strategies during stumbling over obstacles. *J. Neurophysiol.* 83, 2093–2102.

Selionov, V. A., Ivanenko, Y. P., Solopova, I. A., and Gurfinkel, V. S. (2009). Tonic central and sensory stimuli facilitate involuntary air-stepping in humans. *J. Neurophysiol.* 101, 2847–2858. doi: 10.1152/jn.90895.2008

Smith, J. D., and Martin, P. E. (2007). Walking patterns change rapidly following asymmetrical lower extremity loading. *Hum. Mov. Sci.* 26, 412–425. doi: 10.1016/j.humov.2006.12.001

Talis, V., Ballay, Y., Grishin, A., Alexandrov, A., and Pozzo, T. (2011). "Influence of FES on healthy subject locomotion," in *Proceeding of 2nd Annual Conference of the UKRI-IFESS*, University of Dublin, Dublin, Ireland, March 10–11, 30.

Talis, V. L., and Solopova, I. A. (2000). Vibration-induced postural reaction continues after the contact with additional support. *Motor Control* 4, 407–419.

Taylor, P., Humphreys, L., and Swain, I. (2013). The long-term cost-effectiveness of the use of Functional Electrical Stimulation for the correction of dropped foot due to upper motor neuron lesion. *J. Rehabil. Med.*. 45, 154–160. doi: 10.2340/16501977-1090

Taylor, P. N., Burridge, J. H., Dunkerley, A. L., Wood, D. E., Norton, J. A., Singleton, C., et al. (1999). Clinical use of the Odstock dropped foot stimulator: its effect on the speed and effort of walking. *Arch. Phys. Med. Rehabil.* 80, 1577–1583. doi: 10.1016/S0003-9993(99)90333-7

Winter, D. A., and Scott, S. H. (1991). Technique for interpretation of electromyography for concentric and eccentric contractions in gait. *J. Electromyogr. Kinesiol.* 1, 263–269. doi: 10.1016/1050-6411(91)90013-U

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 24 February 2014
doi: 10.3389/fnsys.2014.00025

Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation

Beatrix Krause* and Roi Cohen Kadosh

Department of Experimental Psychology, University of Oxford, Oxford, UK

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Peter B. Reiner, University of British Columbia, Canada
Marom Bikson, The City College of New York of The City University of New York, USA

****Correspondence:***
Beatrix Krause, Department of Experimental Psychology, University of Oxford, Tinbergen Building, 9 South Parks Road, Oxford, OX1 3UD, UK
e-mail: beatrix.krause@psy.ox.ac.uk

A current issue in the research of augmentation of brain functions using transcranial electrical stimulation (tES) is the diversity and inconsistency in outcome results. Similar studies often report different results, depending on the parameters and tasks used. Such inconsistencies have led to significant doubts about the efficacy of the method in the broader scientific community, despite its promising potential for patient recovery and treatment. Evidence on the large variability in individual cortical excitability and response to tES suggests that stimulation may affect individuals differently, depending on the subject's age, gender, brain state, hormonal levels, and pre-existing regional excitability. Certain factors might even lead to the reversal of polarity-dependent effects, and therefore have crucial implications for neurorehabilitation and cognitive enhancement. Research paradigms may have to be refined in the future to avoid the confounding effects of such factors.

Keywords: inhibition, excitation, transcranial electrical stimulation, individual, responsive, efficacy

INTRODUCTION

Transcranial electrical stimulation (tES) in its various forms (anodal vs. cathodal transcranial direct current stimulation (tDCS); transcranial random noise stimulation (tRNS); and transcranial alternating current stimulation (tACS)) has become a highly popular research tool to enhance a wide range of typical, as well as atypical cognitive patterns of behavior (Miniussi et al., 2008; Brasil-Neto, 2012; Cohen Kadosh, 2013; Krause and Cohen Kadosh, 2013). A multitude of studies include healthy participants, patients with behavioral and neuropsychiatric disorders, as well as brain damage or neurological conditions. The external modulation of cortical excitability aims to induce beneficial changes in cortical efficiency and functioning, and thereby enhance plasticity, which subsequently improves the outcome of the training or testing variable in question. The enhancement of synaptic plasticity in the stimulated area is thought to increase the area's processing efficiency, which supports learning and/or recovery (Cramer et al., 2011). However, the diversity of different interactions between deficits and functional systems in different individual populations, as well as the resulting potential individual differences in tES effects has not been disentangled yet. In fact, differences at the individual level of regional brain function and anatomy may lead to profoundly different outcomes. The current idea is that excitatory tES methods, such as anodal tDCS and tRNS enhance cortical excitability (Nitsche and Paulus, 2001; Terney et al., 2008), whereas cathodal tDCS decreases cortical excitation (Nitsche et al., 2003). However, while this might be a generally accepted idea, the real pattern seems more complex.

We have previously suggested that the optimal balance between cortical excitation and inhibition (E/I balance) differs between individual brain areas and subjects and therefore the application of, for instance, anodal tDCS may lead to fundamentally different results in an individual with high regional excitability (i.e., anodal tDCS will lead to overexcitation and non-optimal performance), whereas the same stimulation in a different brain regions with different E/I levels, or in an individual with lower excitation may be more beneficial (Krause et al., 2013). The optimal excitability level would then be at the tip of an inverted-U shaped function of excitation/inhibition and behavior. In line with this hypothesis, researchers have now discovered that experimental populations tDCS research can almost be split into two separate groups: responders and non-responders (López Alonso et al., 2014). The study was based on a previous study that used transcranial magnetic stimulation (TMS) to the motor cortex to investigate cortical excitability in the form of motor evoked potentials (MEPs) in a sample of 56 individuals at various different time points (Hamada et al., 2013). Instead of averaging across the whole group, as is most commonly done in brain stimulation research, Hamada et al. (2013) tracked the patterns of MEP amplitudes per subject across the eight time points. The result was that the individual responses were highly variable and when averaging across all subjects, the average result was nearly zero. More recently, López Alonso et al. (2014) observed a similar pattern using tDCS. A cluster analysis pointed to a subset of 55% of the subjects that not only failed to show an increase, but instead even a slight decrease in TMS-elicited MEP amplitudes in response to the stimulation, which was significantly

distinguishable from the 45% of subjects that showed the expected increase. Similarly, another study has shown that increasing the level of excitation by increasing stimulation intensities of tRNS and tACS can reverse excitation to inhibition (Moliadze et al., 2012). There are methods to assess changes in cortical excitability, such as magnetic resonance spectroscopy (MRS) or TMS, but there are also methods to affect inhibition and excitation. For example, paired associative stimulation (PAS), deep brain stimulation (DBS) and direct current stimulation (DCS) in rodents, can improve the interpretation of data. In humans, MRS is an especially valuable research technique in this regard, as it is a noninvasive *in vivo* magnetic resonance imaging (MRI) method that reliably assesses total concentrations of GABA and glutamate in a predefined voxel (Mekle et al., 2009), typically between $1.5 \times 1.5 \times 1.5$ cm^3 and $3 \times 3 \times 3$ cm^3, which makes it possible to estimate E/I in the to-be-stimulated brain area. Such findings are highly relevant to the field of tES research and we will later discuss a variety of influences on cortical excitability that may be responsible for the drastic individual differences in responses to the stimulation previously described. The identification of such confounding variables may improve tES research and analysis strategies in the future and allow for better controlled design and application of tES in research and potentially in the future also in clinical settings.

NEUROTRANSMITTER BALANCES

The brain's main excitatory and inhibitory neurotransmitters, glutamate and GABA, respectively, are strongly involved in learning and experience-dependent plasticity (Trepel and Racine, 2000; Ge and Dani, 2005). For example, regional GABA levels decrease with learning in the domain associated with the stimulated brain region (e.g., motor learning in M1) (Floyer-Lea et al., 2006). Moreover, the higher the observed learning increment, the steeper the GABA decrease in response to anodal tDCS (Stagg et al., 2011). Such a reduction can in turn facilitate long-term potentiation (LTP), which allows for cortical reorganization (Hess and Donoghue, 1994) and the authors suggest that the responsiveness of an individual's regional GABA system to the stimulation is related to their learning capacity.

Assuming that an increase in cortical excitability is beneficial for learning, we should also be aware of some of its negative consequences. Overexcitation of the cortex (i.e., the excessive release of glutamate), leads to excitotoxicity and cell death (Faden et al., 1989; Belousov, 2012). Excessive GABAergic inhibition, however, prevents LTP and reduces neuronal output (Mcdonnell et al., 2007). Enhanced inhibition is therefore associated with higher network stability but also reduced cortical plasticity (Hess and Donoghue, 1996). Accordingly, a fine balance in the interaction between excitation and inhibition is required to optimize the efficiency of information transfer in the brain (Turrigiano and Nelson, 2000; Bavelier et al., 2010). For tES application, this means that there is a certain dose-response relationship that interacts with pre-existing baseline levels that are currently unknown to the experimenter. Besides other confounding factors in tES research that we will discuss later, this interaction could explain the observed individual differences in experimental outcomes and the large variability in the current literature.

So far, researchers have mainly been concerned with extreme abnormalities in excitability (for instance epileptic patterns of brain activity), and have used it as an exclusion criterion for tES experiments. It is important to note that other neurotransmitter systems also interact with cortical excitability and therefore abnormal neurotransmission in those may equally moderate the effects of tES and potentially the subjects' health. For instance, elevations in extracellular serotonin are associated with increased excitability induced by anodal, and surprisingly also by cathodal tDCS (Nitsche et al., 2009). Therefore, to avoid these confounds in experimental work, we generally recommend to exclude individuals with psychological or psychiatric problems, as well as individuals taking medication that influences hormone or neurotransmitter systems.

Individual differences in pre-existing neurotransmitter levels and in cortical efficiency are also reflected in brain activity, as measured by functional magnetic resonance imaging (fMRI), such that baseline levels of glutamate and GABA are associated with regional activity levels. For example, GABA concentrations measured by MRS correlate positively with γ oscillation frequency, which reflects inhibitory activity, and are inversely related with functional activity in the cortex (Muthukumaraswamy et al., 2009). This means that inhibition can be expressed in the strength of γ oscillations measured by electroencephalography (EEG) or magnetoencephalography (MEG), and that the task-related blood-oxygenated level dependent (BOLD) response decreases with elevated inhibition. Similarly, the baseline GABA concentration predicts the properties of the activation-dependent hemodynamic response function (HRF), such that higher baseline inhibition is related to lower activity (Muthukumaraswamy et al., 2012). Furthermore, task-dependent activity in several different cortical and subcortical areas is associated with glutamate levels in the brain area in question but also in remote areas that are heavily connected. However, the direction of the relationship between activity (low vs. high) and task demands is modulated by pre-existing glutamate levels (low vs. high) (Falkenberg et al., 2012).

These results demonstrate that common findings in brain activation studies can be reasonably well explained by local concentrations of baseline glutamate and GABA levels. Moreover, individual differences in pre-existing neurotransmitter levels cause research subjects to respond differently to external modulation of E/I. For example, a subject with high initial inhibition may never reach a similar level of regional cortical plasticity as a subject with low inhibition. In turn, with two different groups of individuals showing opposing effects in response to tES, the outcome effect will be reduced, or even regress the mean of the whole sample towards zero.

CURRENT APPLICATION

Given the large number of options available in the selection of tES parameters, the effects on the individual subject's cortical excitability and tissue may be very specific and extremely variable across a whole sample. For instance, there are sharp contrasts in outcomes observed using different current strengths, such that

1 and 2 mA of A-tDCS achieve different outcomes on cognitive tasks. One study reports reduced reaction times with prolonged but not shorter stimulation periods at 2 mA, whereas reaction times increased with longer stimulation times at 1 mA (Teo et al., 2011). Reaction times were therefore similarly low under short periods of 1 mA and longer periods of 2 mA. Similarly, 2 mA of C-tDCS over the motor cortex can even flip the intended inhibitory effect on MEPs achieved at 1 mA into cortical facilitation (Batsikadze et al., 2013). Such reversal effects suggest that more (in terms of both intensity and duration) is not necessarily better and there is a fine line between the optimal and accidentally impairing current application.

Several different variations of tES are available, whereby the underlying neurobiological mechanisms are better understood for some than for others. For instance, the user can decide whether to excite a region in one hemisphere and inhibit the same region in the other, or he can place one of the electrodes on an area with minimal or no interference (e.g., the vertex, forehead, cheek or arm). It is currently unclear, which option is ideal for which purpose (for a first step see Moliadze et al., 2010). One of the major reasons for this lies in the principle of interhemispheric inhibition. The two hemispheres work in concert to produce behavioral output and damage to an area in one hemisphere may unleash unprecedented activation of the same area in the other hemisphere (Cramer et al., 1997; Zimmerman and Hummel, in press). In the presence of brain damage or dysfunction for example, the contralateral hemisphere often tries to compensate for the loss and therefore responds with atypical patterns of activity (see Johnston, 2009). The particular pattern is known to the tES user, however, such that the prediction of tES effects is specific to the choice of parameters.

The effect of left anodal tDCS (cathode attached to the forehead) on resting state activity in a prefrontal network indeed demonstrated increases in functional connectivity to the same area in the right hemisphere, whereas connectivity to other areas within the same hemisphere was reduced (Park et al., 2013). The authors hypothesized that the behavioral results found in cognitive tES studies may be based on the changes in interhemispheric connectivity and that different placements of the cathode may have caused fundamentally different results. Such effects may be similar or different for different brain areas and between the two hemispheres. Additionally, different subject populations might respond differently to such effects, depending on the pre-existing interhemispheric connectivity patterns (e.g., see for elderly Cabeza, 2002). Given this fact, the question about the optimal stimulation method for a given purpose is critical.

In tACS the current alternates between the cathode and the anode at a fixed frequency (Zaghi et al., 2010) and it is known to modulate brain oscillations. Its beneficial effect on cognition or behavior has not yet been fully established, and has even been found to impair perceptual processes in certain cases (Brignani et al., 2013). From DBS we know that the stimulation frequency also leads to fundamental differences in the effectiveness of the treatment of for instance motor disorders, such as Parkinson's disease (PD) (see McConnell et al., 2012). Despite the fact that DBS (with its implanted electrodes) has a different mechanism of action, it demonstrates how varying the stimulation parameters can successfully direct the output effects of a stimulated cortical network. There is now also first evidence for the effectiveness of tACS in modulating Parkinson-related brain oscillations (Brittain et al., 2013). Similarly, the effect of the different stimulation frequencies used in tACS often depends on external factors, such as lighting conditions within the testing room (Kanai et al., 2008). In this case the same stimulation parameters in a well-lit room may differ from the ones in a room under darker conditions during perceptual processing. The authors point to an interaction between ongoing cortical oscillations in the cortex and the applied current frequency. Accordingly, the subject's current cortical excitability will interact with the stimulation. Where tACS has a variety of different frequencies that can be freely chosen (e.g., α, β and γ waves) tRNS also has different frequency settings, that are mostly split into full-spectrum, high-frequency (Hf-) and low-frequency (Lf-) tRNS. Under certain conditions, tACS has been shown to induce stronger excitability increases than full-spectrum tRNS (Moliadze et al., 2012), but when tRNS conditions are directly compared, Hf-tRNS induces stronger excitability than Lf-tRNS (Terney et al., 2008). Again, it is important to note that there are few experiments available that compare different parameters of tES within the same study and often the outcome measure is an excitability variable (mostly in the motor domain), rather than a cognitive or behavioral outcome. This means that the current knowledge might be restricted to very specific experimental conditions and it is unknown whether these effects are generalizable across domains and testing conditions.

There is a large variety of tES applications with its excitatory vs. inhibitory modulation (anodal and cathodal tDCS), excitation through noise induction (tRNS) and the modulation of cortical oscillations (tACS), as well as possible parameters including frequency range, current strength and electrode positioning interact with ongoing regional excitability of the cortex. The problem is that the experimenter is usually unaware of the excitability levels and these might differ under different experimental conditions and might be particularly sensitive in perceptual domains.

THE RESEARCH DESIGN

In addition to individual differences in biological substrates, variations in study design can have a striking impact on the outcomes of tES studies. For example, daily tDCS leads to greater excitability changes than second daily application (Alonzo et al., 2012) such that a more sensitive neural system may accumulate higher excitability over several sessions. In addition, it is crucial to assess long-term effects of improvements and potentially impairments, as these eventually determine the success of the intervention. Some have already demonstrated long-term positive effects (e.g., Reis et al., 2009; Cohen Kadosh et al., 2010b; Snowball et al., 2013), whereas most studies do not perform such follow-up testing. Another question is at what point in time an improvement in the testing variable will be visible. Many studies test and evaluate performance during the stimulation (e.g., Bolognini et al., 2010; Weiss and Lavidor, 2012), whereas others also compare pre- and post-measures (e.g., Dockery et al., 2009; Snowball et al., 2013). The quality of the effect may be different in such cases and before tES

can be applied in clinical settings, the evolution of performance change should be monitored to find the optimal time for training assessments.

THE INITIAL BRAIN STATE

An important but hard to control factor in research is the brain state of the individual subject. Silvanto et al. (2007) point out the importance of subject factors, such as fatigue and wakefulness, attention, intoxication and the habituation to the presented task material. These and others can be potential confounders that can even flip polarity-dependent effects of tES into the opposite polarity. For example, state-dependent effects associated with baseline brain activity can be related to resting α-band power, which has been showed to modulate the threshold for excitability probed by TMS (Romei et al., 2008). Similarly, using tACS with its ability to entrain cortical oscillations, the stimulation frequency has been showed to interact with ongoing brain activity. The highest increase in motor cortical excitability at rest was achieved using β-tACS (20 Hz), whereas the highest excitability levels during motor imagery were observed during θ-tACS (5 Hz) (Feurra et al., 2013). The authors attribute the effect of θ-tACS to the underlying use of working memory processing during the imagery task, whereas β stimulation is thought to correspond to the natural cortical response during rest.

Using a neural adaptation paradigm, Silvanto et al. demonstrated that less active neuronal populations respond more strongly to TMS than more active ones (Silvanto et al., 2008). Further studies extended this finding to high-level cognition and the parietal lobes (Cohen Kadosh et al., 2010a). Furthermore, the experimental manipulation of cortical excitability responds in a similar way, such that preconditioning the cortex with A-tDCS causes repetitive TMS (rTMS) to be inhibitory, whereas C-tDCS preconditioning reverses subsequent rTMS effects to cortical excitation (Lang et al., 2004; Siebner et al., 2004). The effects of the initial brain state are even visible across different tasks. Motor cortical excitability could be reduced by A-tDCS, and increased by C-tDCS to M1 during a cognitive task, compared to the same stimulation during rest (Antal et al., 2007). However, cortical excitability was reduced by both A-tDCS and C-tDCS when engaging in a motor task, compared to during rest. In this study, A-tDCS therefore only increased cortical excitability during rest, but flipped the effect to inhibition during cognitive, and even more so during motor engagement. In contrast, C-tDCS led to a slight excitability decrease at rest but a sharp decrease during motor processing, while it increased excitability during cognitive processing. The authors concluded that areas that are not involved in the cognitive task at hand become deactivated, while the reduction in excitability during the motor task is more likely to be associated with muscle fatigue. It is therefore apparent that ongoing neuronal activation interacts with different types of stimulation to modulate cortical excitability and behavioral responses. The effects of experimentally uncontrolled influences on the brain seem so profound that they have the potential to even flip intended inhibition to excitation (and *vice versa*) and are therefore not consistent with the current idea of polarity-specific tDCS. Currently, such baseline cortical activity factors are unknown variables in tES research. Experimental instructions and procedures may bias the brain state of subjects to respond to the stimulation in a certain way, confounding the desired outcome.

THE INDIVIDUAL BRAIN

The situation is further complicated by individual variations in head and tissue morphology. Different head sizes and tissue thicknesses might cause different current distributions and require different current strengths to achieve the same current flow (Bikson et al., 2012). For example, depending on where on the head the electrodes are placed, the stimulation can be more focal than in other configurations, which may be related to the orientation of neurons and the current flow applied and how the current propagates along the tissue connections (Neuling et al., 2012). Individual morphologies of cortical gyri and sulci also affect the pattern of the current flow (Datta et al., 2012). The same stimulation design can therefore lead to large differences in the induced current and the resulting electric field, due to brain and body morphological differences (Datta et al., 2012; Truong et al., 2013). The resulting individual differences in the strength of the induced electric field effects on neuronal activity and E/I will therefore be fundamentally different. As observed in experiments applying different intensities of current (e.g., Batsikadze et al., 2013), an intended excitation can flip to inhibition in some subjects but not in others. This in turn will affect both physiological and behavioral effects negatively.

Furthermore, depending on the task applied under stimulation (especially using cognitive tasks), subjects may recruit different brain regions for the same task depending on their stage of brain and cognitive development and due to the individual strategy use (Rivera et al., 2005). The consequence of this is that one might stimulate an area that is currently not involved in the processing of the task (i.e., the "wrong" area for the task at hand based on previous fMRI studies in different populations), which will eventually not benefit the individual's abilities and add further noise to the experimental results.

Similarly, the interaction between brain areas might differ across individuals, e.g., due to differences in the strength and efficiency of network connections. Since tES has been found to affect whole networks rather than just the stimulated region in isolation (Keeser et al., 2011; Zheng et al., 2011), it is possible that by enhancing brain functioning at one point in the network, subsequent network areas might be negatively affected, such that the outcome is disadvantageous (Brem et al., 2014). For example, the increased cortical excitability may lead to reduced inhibition to a subsequent area, such that this area will produce excessive output and impair behavioral functioning. It has indeed been found that stimulation of frontal areas can improve certain cognitive aspects while interfering with others, while stimulating parietal areas reverses the pattern (Iuculano and Cohen Kadosh, 2013). Considering this possibility, individuals with certain neurological vulnerabilities, young and old individuals, as well as patients with brain abnormalities or damage can be expected to respond differently to the same type of stimulation. For instance, the behavioral effects of tES on elderly compared to younger participants seem to be reversed and hemisphere-dependent (Ross et al., 2010, 2011). The anticipation of tES effects should therefore never

be generalized from one group to another, but should instead carefully explored to prevent a null result, or even an accidental induction of cognitive impairment.

THE DEVELOPING AND AGING BRAIN

The brain is not static. It changes continuously across the lifetime and, along with these changes, occur changes in behavior and how the individual responds to stimuli in the environment. During development, a certain relative balance between excitation and inhibition defining functional properties of the cortex is established and eventually maintained throughout later stages of life (Turrigiano and Nelson, 2004). However, evidence from animal research suggests that early experiences shape the coupling of excitatory and inhibitory neural activity and thereby affect cortical plasticity. Still, the initial interactive activity is subject to change and refinement across the course of postnatal development (Dorrn et al., 2010) and E/I balances may therefore guide the timing of developmental critical periods of plasticity for experience-dependent learning (Hensch and Bilimoria, 2012). Early experiences and E/I interactions may therefore determine the later responsiveness to tES.

Research using TMS demonstrated how age-related differences in cortical excitability affect the speed of signal transduction in motor pathways. Motor responses are slowed in elderly compared to younger adults (Smith et al., 2009), which is associated with age-related changes in intracortical inhibition. This leads to a decline in the functional modulation of corticospinal activity (Fujiyama et al., 2012). Similarly, the aging individual also has to face cognitive slowing, which has been associated with a weakening of white matter connections between different cortical areas but also a decline in structural gray matter, whereby the pace of change differs by the structure or area (Raz et al., 2005; Jackson et al., 2012).

Along with such changes in brain structure, regional neurotransmitter balances change as well, affecting experience-dependent plasticity (Hess and Donoghue, 1996). For example, GABAergic receptor distribution changes from early childhood to the early teenager years and then again during older age (Pinto et al., 2010). More specifically, there is an age-related decline in GABA levels that can be observed in the elderly brain using MRS (Gao et al., 2013). Glutamate availability has also been found to decrease in the aging brain in rodents (Mcentee and Crook, 1993). An observed loss in NMDA receptors (Wenk et al., 1991) (in rats and monkeys) may be responsible for such changes and may also impact the capacity to form LTP. With such changes and shifts in E/I, experience-dependent plasticity in the cortex changes across the lifetime (Hensch et al., 1998; for a comprehensive review on the role of spike timing-dependent plasticity in plasticity, see Caporale and Dan, 2008). Interestingly, the artificial reduction of GABAergic signaling can restore some of the age-related decline in learning in rodents (Lasarge et al., 2009). Therefore, the external modulation of GABA using tES may also lead to beneficial behavioral effects in the elderly but it is unclear how the type and dosage of the stimulation affects elderly individuals differently from younger age groups. The evidence on regional GABA and glutamate concentrations, as well as on the effects of tES in elderly populations is currently extremely scarce.

In summary, along with the continuous brain development and age-related changes in structure and function, we can expect changes in E/I balance across the lifespan, which are currently under-investigated. The interactions between tES and E/I balance are therefore even less predictable than in healthy young adults. This is due to the fact that the research on *in vivo* assessments of GABA and glutamate in developing and aging human samples and the use of tES in these groups is still in its infancy and there is little available evidence at this point.

CIRCADIAN RHYTHM

Circadian influences, such as sleep and time of the day have been found to affect cortical excitability, such that TMS-probed intracortical inhibition was found to decrease throughout the day (Lang et al., 2011). Moreover, with more time staying awake, especially after sleep deprivation, motor cortical excitability gradually increases along with an increase in EEG θ waves, which is commonly observed with prolonged wakefulness (Huber et al., 2013). This sleep-dependent increase in cortical excitability has critical implications for subjects' sleeping patterns prior to stimulation. Sleep deprivation may enhance the risk for seizure activity, especially in combination with repeated sessions of tES, which by itself increases excitability (Alonzo et al., 2012). Such combination could accumulate cortical excitability to potentially harmful levels in susceptible participants. A variety of different psychological and neuropsychiatric disorders involve abnormal circadian rhythms or deficient sleep patterns and some can already be distinguished on the basis of MRS-measured GABA and/or glutamate concentrations at a group level (e.g., Goto et al., 2009; Yoon et al., 2010; Rojas et al., 2013). Careful screening procedures should therefore be applied to monitor potential pre-existing abnormalities in E/I.

HORMONAL LEVELS

Another source of variation is related to hormonal levels, which fluctuate substantially more in women than men, such that some studies exclude females completely from their research on cortical excitability to reduce the noise (e.g., Alonzo et al., 2012). Two main phases in the menstrual cycle can be distinguished: the follicular phase, characterized by rising levels of estrogen and low levels of progesterone, and the luteal phase, which starts with ovulation and is associated with moderate levels of estrogen and high levels of progesterone. Cortical inhibition, as probed by TMS and measured by MEPs measured is enhanced and simultaneously excitability reduced, during periods of higher progesterone levels (i.e., the luteal phase) (Smith et al., 1999). Furthermore, cortical excitation is relatively low during the first half of the follicular phase (including the menstruation period), which is characterized by low levels of both progesterone and estradiol in particular, but then excitability increases in the second half of the follicular phase, when progesterone is still low but estradiol peaks (Smith et al., 2002). Excitation is then decreased again, and inhibition increased during the luteal phase, with rising progesterone and estradiol levels. Progesterone therefore seems to drive the increase in cortical inhibition, whereas estradiol

increases excitability. This is supported by a study using MRS to assess GABA concentrations in the primary visual cortex, which appeared lower during the luteal than the follicular phase in healthy women and GABA was inversely related with both estradiol and progesterone levels (Epperson et al., 2002). However, since the study did not concurrently measure levels of glutamate, no inferences can be made on the E/I balance, as those might counteract or dominate GABA levels differentially during different phases of the cycle. Moreover, in the study that measured cortical excitability using TMS (Smith et al., 2002), the follicular phase was subdivided into an early and a late phase due to the peaking levels of estradiol in the second half, whereas the MRS study investigated the follicular phase as a whole, which may have failed to capture the measurement during the rising levels of estradiol. Instead, the researchers subdivided the luteal phase into early and late, the two halves of which show little difference in their respective estradiol and progesterone levels (Epperson et al., 2002). This might additionally have affected the interpretation of GABA levels throughout the cycle. For future studies it will be useful to subdivide both phases equally and inspect all four time points. In a different study, glutamate concentrations in the medial frontal cortex were found to be significantly lower during the luteal than the follicular phase (Batra et al., 2008). Since both studies investigated different brain areas, it is hard to draw conclusions but it is likely that assessments of the ratio of glutamate and GABA will confirm the findings of previous TMS studies that relative inhibition is increased during the luteal phase, and reduced during the follicular phase (see **Figure 1** for a summary of the current results in relationship with estrogens and progesterone).

For a more complete picture of global brain excitability, Harada and associates investigated three different brain areas (left frontal cortex, lentiform nuclei and cingulate cortex) using MRS and found that only in the lentiform nuclei and the left frontal cortex GABA levels were decreased during the luteal compared to the follicular phase but not in the anterior cingulate cortex (Harada et al., 2011). This means that E/I might additionally depend on the interaction between hormonal fluctuations and local brain regions. To make things more complex, smoking is another possible noise variable in E/I balance, as GABA levels have been found to differ across the menstrual cycle. Specifically, GABA is higher during the follicular than the luteal phase in nonsmoking women (Epperson et al., 2005). Furthermore, there was no difference between smoking and non-smoking men and their GABA levels were similar to women in the luteal phase. In contrast, smoking women in the follicular phase showed slightly but not significantly reduced levels of GABA, compared to all other groups. No differences in GABA concentrations were found after 48 h of abstinence in the smokers. Despite the fact that an individual's lifestyle may affect E/I and induce additional noise into tES experiments, long term smoking behavior in this case might even stabilize GABA concentrations (although causal inferences from these study results are not possible).

A rapid change in E/I balance due to hormonal fluctuations has been associated with neurological conditions, which already serve as exclusion criteria and/or control variables in tES research. These are for instance migraine, epileptic seizures, but also premenstrual mood disorders (i.e., during periods of high cortical inhibition) (for a more detailed discussion on biological alterations, see Finocchi and Ferrari, 2011). Despite the increasing evidence on excitability differences due to hormonal changes, this type of information is not by itself informative about the efficiency of the information transfer in the brain and the degree of capacity for plastic changes.

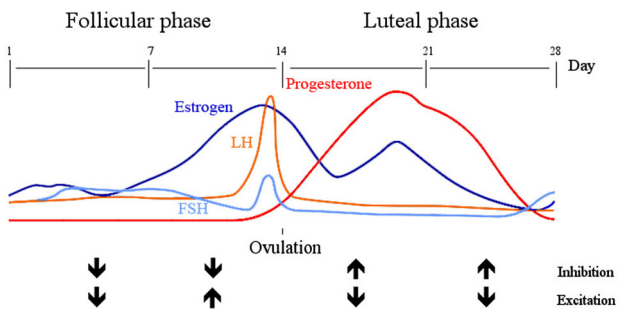

FIGURE 1 | Inhibition and excitation across the menstrual cycle. The early follicular phase (day 1 –the start of menstruation—until day 7) is characterized by low levels of estrogen and progesterone, during which both inhibition and excitation are low. With an increase in estrogen from day 7–14, luteinizing hormone (LH) and follicular stimulating hormone (FSH) peak and stimulate ovulation on day 14. During this late follicular phase inhibition is low and excitation high, which reverses in the following 14 days (early luteal phase). Estrogen, in particular estradiol is now moderately high but progesterone peaks.

DRIVING AND PREDICTING PLASTICITY

By transiently enhancing plasticity, we attempt to induce favorable long-lasting changes that allow for increased experience-dependent learning. However, there is also an optimal balance between plasticity (i.e., the flexibility of synaptic connections to change according to experience) and stability (keeping the system balanced). Critical periods of development are characterized by enhanced levels of brain plasticity because learning during such periods is crucial to shape the cortical networks for later efficient processing (stability). Stability is necessary to make the network cost-efficient in terms of energy expenditure and thereby provide constant and predictable patterns for the preferred output (Knudsen, 2004). Strengthening pathways in the "wrong" way during such a period of high plasticity levels can cause unwanted and irreversible (stable) changes during cortical development (Knudsen, 2004). Therefore, caution is needed when artificially enhancing plasticity, as behavioral changes in the wrong direction may be difficult to reverse.

Stability, in terms of long-term adaptation to plasticity-induced training effects may therefore be more crucial to tES than previously anticipated. This means that we should not aim for the maximum cortical excitability, but instead for the *optimal balance* between plasticity and stability in terms of E/I. Not only does the E/I balance determine the flexibility of a network and hence the functioning of the brain area (Knudsen, 2004; Murphy et al., 2005), but additional compensatory mechanisms also regulate the net output of the system. While Hebbian learning drives experience-dependent plasticity, compensatory mechanisms are

required to maintain the stability in a homeostatic way and thereby maintain a fixed set point of firing rates (Turrigiano and Nelson, 2004). This process is called homeostatic plasticity. The previously discussed flip in excitatory and inhibitory effects after polarity-dependent tDCS is another example of homeostatic plasticity. Some authors have suggested that the cortex uses this flip to maintain a functionally useful range of cortical excitability, which subsequently affects the potential for plasticity (Lang et al., 2004; Siebner et al., 2004). They explain that pre-existing excitation and inhibition determine the net effect of TMS and whether its action is excitatory or inhibitory. Such pre-existing E/I levels may be directly related to differences in the state of the brain (e.g., alertness, attention or familiarity with a task and neuronal populations), and thereby interact with the applied stimulation (Silvanto et al., 2007). Therefore, in the presence of elevated levels of excitation, TMS or tES effects may be incapable of inducing further excitation and instead may suppress cortical activity. Neurorehabilitation would therefore be naturally limited.

Given a history of successful cognitive and clinically symptomatic improvements achieved by tES, the question is how strongly these homeostatic mechanisms are affected by the stimulation. This question has been neglected in tES research so far and is worth serious investigation. If tES is affected by homeostatic plasticity, there might be an upper level of improvement we cannot exceed and the question is whether excessive stimulation will lead to a ceiling effect or whether it reverses the effects to the worse. The latter possibility would reflect our previous hypothesis of an inverted-U shape of cortical excitability and behavioral outcome (Krause et al., 2013). Beyond a certain point the stimulation will start causing impairments rather than further improvements. Another question is if homeostatic set points differ across individuals and whether the range of possible plastic changes inducible by tES is similar in each individual. Furthermore, we also have to take into account that this set point may change with development and aging. Dose-response experiments monitoring changes, especially impairments in behavioral and cognitive outcomes, are therefore of high importance (e.g., Teo et al., 2011; Moliadze et al., 2012; Brignani et al., 2013).

In practice, these compensatory mechanisms are likely to occur over an extended period of time and may therefore not be immediately measurable in changes on behavioral tasks or symptom outcomes used during experiments. However, changes induced by homeostatic plasticity can even counteract Hebbian learning effects (Turrigiano and Nelson, 2004), which may moderate the effects of tES greatly. This again has important implications for rehabilitation, especially in epilepsy, where an unintended increase in excitability may have severe health effects.

HOW TO DEAL WITH THE VARIABILITY

The wide variety of options for tES parameters paired with the multitude of individual differences in pre-existing neurotransmitter levels makes the evaluation of tES research results as a whole difficult. Guleyupoglu et al. (2013) stress the importance of current dose for the outcome of the study. For example, they define tES dosage in terms of the parameters of the electrodes, including the size, number, shape, position and composition, as well as the waveform in terms of intensity and the general form of the waves administered, the pulse shape (wherever relevant), amplitude, width, polarity and repetition frequency of the current waves, the number of sessions and the inter-session interval. For research purposes, as well as for the generalization of results it is important to always report the exact parameters used, as the current flow and the resulting induced electric fields depends on these parameters (Peterchev et al., 2012). In severe clinical cases when the financial means are available, electrode shapes and sizes can even be custom designed to better control and enhance tES effects but this method requires structural brain scans (MRI) and neuronavigation for the production and fitting of the stimulation (Tecchio et al., 2013). Individualized tES with more focal effects (high-definition tDCS) may become more feasible in the future, with the development of more automated and less time-consuming methods for the prediction of current flow (Datta et al., 2012; Huang et al., 2012; Edwards et al., 2013).

One possible solution to specifically target the individual regional E/I balance is to assess GABA and glutamate levels in the voxels of interest using MRS and thereby determine the directionality of the current polarity/parameters to optimize E/I. Similarly, tACS has been shown to have particularly long-lasting effects on cortical excitability and cognition when the stimulation frequency is tuned to the endogenous cortical firing frequency, as assessed by EEG (Neuling et al., 2013). This way, tES applications can be individually tailored. However, the high costs of neuroimaging may not always be feasible and more knowledge of the ideal balance required. Many of the current experiments exclude women due to the hormonal fluctuations and the influence on cortical excitation but if we want to eventually affect brain and behavior of the general population using tES, we must explore the relationship between the menstrual cycle and E/I further. The same applies to developing and aging populations. In the case of hormonal influences, blood measures of hormone levels may give indications of relative cortical excitability levels in women. However, more evidence is required to substantiate the evidence on the relationship between E/I and hormonal interactions.

Another solution, which might be a somewhat crude indicator, is individual variability in behavior. If E/I is related to brain oscillations and metabolic responses, which again are associated with behavioral response patterns, behavioral performance may distinguish at least extremely elevated or reduced levels of E/I. While behavioral performance does not capture the entire E/I variance or other factors that we mentioned here, it may still serve as a useful additional controlling factor for the variety of influential factors discussed here.

DISCUSSION

Evidence stemming from noninvasive brain stimulation studies suggests that there are separate subgroups of experimental subjects that differentially respond to stimulation. Specifically, up to half of them respond with reductions in excitability in response to the stimulation, whereas the other half responds, as expected, with increases in measures of excitability (Wassermann, 2002; Hamada

et al., 2008; López Alonso et al., 2014). Such subgroups may have diminished many of the expected beneficial effects of tES in the past and identification of the type of responder before the application of tES would substantially help the outcome analysis and interpretation of tES effects. In order to achieve successful clinical intervention, this information is crucial for the user. We believe that the biological determinants of the subject response outcome depend on neurotransmitter balances, in particular glutamate and GABA, as their interaction defines the E/I balance in the area that is to be stimulated. The baseline in E/I balance might be differently skewed in each individual, such that some start off with higher relative excitation, whereas others have relatively low regional excitability. This will subsequently have differential effects on the capacity for the induction of plastic changes and therefore lead to different outcomes in experiments where each subject receives the same treatment. This would explain why in some cases up to half the sample responds to the enhanced excitability, whereas the same enhancement is disadvantageous in others (López Alonso et al., 2014). As Pavlov hypothesized more than 50 years ago, different personalities underlie different ratios between excitation and inhibition and therefore produce different behavioral outcomes (for a discussion, see Strelau, 1997). How these differences in cortical E/I balance arise is currently unknown, but it has been found that siblings show similar responses to brain stimulation, which suggests that there is either a heritable factor biasing the E/I balance, or that stimulation responses are similar in siblings due to some common morphological properties of their skulls and cortices (Wassermann, 2002).

For reasons of baseline neurotransmitter system activity, tES might lead to different results in different individuals. This can be due to the discussed factors and potential pre-existing vulnerabilities, as well as structural differences in cortical gray and white matter, skull and tissue thickness. The effects of tES can therefore be binary (effect vs. no effect), or they can show in varying degrees of the effect or even in negative effects. The outcome of the result does not always allow for inferences on the underlying mechanism, such that the uncertainty in current research interpretations is concerning. The degree of the response to tES may also vary with time of the day, the time point of menstrual cycle in women, environmental testing conditions, and general preexisting levels of neurotransmitter balances in the brain. These may further be influenced by medication or lifestyle preferences, such as smoking (as well as other methods of intoxication) or sleep patterns.

We would also like to note that our view implies that polarity-dependent effects of tES are not always straightforward and predictable. The common view that A-tDCS is generally excitatory and C-tDCS inhibitory has been challenged, as discussed here, and can be seen as a relatively crude average outcome. For example, the polarity-specific effect of tDCS depends on the organization, morphology and orientation of cortical neurons to the incoming current (Bikson et al., 2004; Kabakov et al., 2012; Rahman et al., 2013). Due to the curvature of an axon, there is always a combination between excitatory and slightly stronger inhibitory activity, such that the sum between these determines the net output (Kabakov et al., 2012).

For these reasons, it is possible that the biological processes underlying tES effects are even more complex than we suggest here. Despite the extensive research on inhibitory and excitatory effects of the current, the effects may not solely be explained by E/I. For instance, a subtle change in excitation and inhibition induced by weak current can alter network dynamics by simply changing the pattern of E/I. These changes are non-linear and the size of the effect depends on the current state of the network, affecting the rate and timing of neuronal firing (Reato et al., 2010). This means that even small simultaneous changes in the levels of excitation and inhibition can lead to a different outcome within the dynamics of the network.

Cellular tissue studies can provide more direct evidence for our idea in the future. However, investigating cellular E/I interactions in human higher-level cognition is currently not feasible. In order to fully understand the effects of tES on plasticity-behavior relationships, we have to understand the resulting formation of LTP and long-term depression (LTD) by the actions of NMDA and AMPA receptor activity (Huganir and Nicoll, 2013). Examining individual differences at the receptor level in humans is *in vivo* unfortunately is not feasible at the moment. It is also important to note that there is quite a leap from the interpretation of current effects at the receptor level in cell tissue and human behavioral studies. The bridge between molecular and human research consists of computational modeling of the current flow through different tissue configurations (for a comprehensive review see e.g., Bikson et al., 2012), which is a useful tool to understand current effects. In order to understand and predict the exact effects of tES (with its different types and parameters) we need to understand the cellular, broader cortical (e.g., regional interactions between brain areas) and behavioral effects of tES and how these are linked together. This link may be far simpler for lower-level skills, such as motor or perceptual brain functions, but more complex for higher-order cognitive abilities, including attention, working memory and arithmetic.

A potential problem with the majority of the evidence from tES research on cortical excitability is that it is all performed in the motor domain and may therefore not be generalizable to other behavioral and cognitive domains. For instance, the motor cortex may respond in fundamentally different patterns to other areas (e.g., in the prefrontal cortex). For example the observed reduction in inhibition associated with anodal tDCS and motor learning (Floyer-Lea et al., 2006; Stagg et al., 2009) stands in sharp contrast to the beneficial effect of cathodal inhibition on certain frontal cognitive functions (Weiss and Lavidor, 2012). In some domains cortical inhibition appears to be more beneficial than in others, especially considering cognitive functions involving attentional focus or the inhibition of irrelevant material, such as in the latter case. Therefore, one should be cautious about generalizing across domains.

MRS can be used to quantify concentrations of glutamate and GABA in different areas. As a measure of E/I balance, these quantifications are already used to distinguish healthy from psychiatric populations (e.g., Eichler and Meier, 2008; Yoon et al., 2010; Kubas et al., 2012). The addition of this neuroimaging technique or similarly EEG/MEG to measure oscillations, tES

studies for the predetermination of E/I levels in potential subject pools would be very cost intensive. Nevertheless, we believe that this might clarify tES results and foster our understanding of both functional brain neurochemistry and tES methodology in the future.

CONCLUSION

Here we summarized the most well-known currently unpredictable factors influencing cortical excitability and plasticity and how the interaction of individual differences with the available multitude of stimulation parameters may influence the effects of noninvasive, plasticity-inducing electrical stimulation at the individual level. We conclude that the simple perception of tES polarity-specific neuronal modulation is an oversimplification of the complex effects and that the effects are currently far less predictable than assumed in the majority of the scientific community. We suggest that tES effects are moderated by pre-existing baseline E/I. Imbalances in E/I can be found in clinical or neuropsychiatric populations, during hormonal fluctuations (especially in women) and caused by interactions with the baseline neuronal activity, external influences, such as smoking or medication use, developmentally and age-related changes in E/I across the lifespan. Additional factors include individual differences in skull and cortical morphology, circadian influences that are currently not clarified, such as time of day or sleep deprivation, interactions with other neurotransmitter systems, and differential effects of tES due to unusual use of strategies in e.g., cognition. In addition, the current state of brain functioning and previous experiences can influence, or even flip polarity-dependent tDCS effects. For future research, it is of particular importance that scientists are aware of such variations and that they select their desired research populations with care in regard to potential unwanted noise in the data, and/or in some extreme cases the potential increase in seizure risk. These could be achieved by taking some of these factors into account, pre-assessing E/I levels or activation patterns using neuroimaging methods, in certain cases TMS, or at least behavioral patterns of performance.

ACKNOWLEDGMENTS

We would like to thank Michael Clayton for his comments. Beatrix Krause is supported by the Economic and Social Research Council (ESRC), the German Academic Exchange Service (DAAD), and the Studienstiftung des Deutschen Volkes. Roi Cohen Kadosh is a Wellcome Trust Career Development Fellow (0883781). Roi Cohen Kadosh filed a patent for an apparatus for improving and/or maintaining numerical ability.

REFERENCES

Alonzo, A., Brassil, J., Taylor, J. L., Martin, D., and Loo, C. K. (2012). Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation. *Brain Stimul.* 5, 208–213. doi: 10.1016/j.brs.2011.04.006

Antal, A., Terney, D., Poreisz, C., and Paulus, W. (2007). Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. *Eur. J. Neurosci.* 26, 2687–2691. doi: 10.1111/j.1460-9568.2007.05896.x

Batra, N. A., Seres-Mailo, J., Hanstock, C., Seres, P., Khudabux, J., Bellavance, F., et al. (2008). Proton magnetic resonance spectroscopy measurement of brain glutamate levels in premenstrual dysphoric disorder. *Biol. Psychiatry* 63, 1178–1184. doi: 10.1016/j.biopsych.2007.10.007

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., and Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bavelier, D., Levi, D. M., Li, R. W., Dan, Y., and Hensch, T. K. (2010). Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *J. Neurosci.* 30, 14964–14971. doi: 10.1523/jneurosci.4812-10.2010

Belousov, A. B. (2012). Novel model for the mechanisms of glutamate-dependent excitotoxicity: role of neuronal gap junctions. *Brain Res.* 1487, 123–130. doi: 10.1016/j.brainres.2012.05.063

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J. Physiol.* 557, 175–190. doi: 10.1113/jphysiol.2003.055772

Bikson, M., Rahman, A., and Datta, A. (2012). Computational models of transcranial direct current stimulation. *Clin. EEG Neurosci.* 43, 176–183. doi: 10.1177/1550059412445138

Bolognini, N., Fregni, F., Casati, C., Olgiati, E., and Vallar, G. (2010). Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. *Brain Res.* 1349, 76–89. doi: 10.1016/j.brainres.2010.06.053

Brasil-Neto, J. P. (2012). Learning, memory and transcranial direct current stimulation. *Front. Psychiatry* 3:80. doi: 10.3389/fpsyt.2012.00080

Brem, A.-K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *Neuroimage* 85(Pt. 3), 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Brignani, D., Ruzzoli, M., Mauri, P., and Miniussi, C. (2013). Is transcranial alternating current stimulation effective in modulating brain oscillations? *PLoS One* 8:e56589. doi: 10.1371/journal.pone.0056589

Brittain, J. S., Probert-Smith, P., Aziz, T. Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. *Curr. Biol.* 23, 436–440. doi: 10.1016/j.cub.2013.01.068

Cabeza, R. (2002). Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol. Aging* 17, 85–100. doi: 10.1037//0882-7974.17.1.85

Caporale, N., and Dan, Y. (2008). Spike timing-dependent plasticity: a Hebbian learning rule. *Annu. Rev. Neurosci.* 31, 25–46. doi: 10.1146/annurev.neuro.31.060407.125639

Cohen Kadosh, R. (2013). Using transcranial electrical stimulation to enhance cognitive functions in the typical and atypical brain. *Transl. Neurosci.* 4, 20–33. doi: 10.2478/s13380-013-0104-7

Cohen Kadosh, R., Muggleton, N., Silvanto, J., and Walsh, V. (2010a). Double dissociation of format-dependent and number-specific neurons in human parietal cortex. *Cereb. Cortex* 20, 2166–2171. doi: 10.1093/cercor/bhp273

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R., and Walsh, V. (2010b). Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Curr. Biol.* 20, 2016–2020. doi: 10.1016/j.cub.2010.10.007

Cramer, S. C., Nelles, G., Benson, R. R., Kaplan, J. D., Parker, R. A., Kwong, K. K., et al. (1997). A functional MRI study of subjects recovered from hemiparetic stroke. *Stroke* 28, 2518–2527. doi: 10.1161/01.str.28.12.2518

Cramer, S. C., Sur, M., Dobkin, B. H., O'brien, C., Sanger, T. D., Trojanowski, J. Q., et al. (2011). Harnessing neuroplasticity for clinical applications. *Brain* 134, 1591–1609. doi: 10.1093/brain/awr039

Datta, A., Truong, D., Minhas, P., Parra, L. C., and Bikson, M. (2012). Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. *Front. Psychiatry* 3:91. doi: 10.3389/fpsyt.2012.00091

Dockery, C. A., Hueckel-Weng, R., Birbaumer, N., and Plewnia, C. (2009). Enhancement of planning ability by transcranial direct current stimulation. *J. Neurosci.* 29, 7271–7277. doi: 10.1523/jneurosci.0065-09.2009

Dorrn, A. L., Yuan, K., Barker, A. J., Schreiner, C. E., and Froemke, R. C. (2010). Developmental sensory experience balances cortical excitation and inhibition. *Nature* 465, 932–936. doi: 10.1038/nature09119

Edwards, D., Cortes, M., Datta, A., Minhas, P., Wassermann, E. M., and Bikson, M. (2013). Physiological and modeling evidence for focal transcranial electrical brain stimulation in humans: a basis for high-definition tDCS. *Neuroimage* 74, 266–275. doi: 10.1016/j.neuroimage.2013.01.042

Eichler, S. A., and Meier, J. C. (2008). E-I balance and human diseases - from molecules to networking. *Front. Mol. Neurosci.* 1:2. doi: 10.3389/neuro.02.002.2008

Epperson, C. N., Haga, K., Mason, G. F., Sellers, E., Gueorguieva, R., Zhang, W., et al. (2002). Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: a proton magnetic resonance spectroscopy study. *Arch. Gen. Psychiatry* 59, 851–858. doi: 10.1001/archpsyc.59.9.851

Epperson, C. N., O'malley, S., Czarkowski, K. A., Gueorguieva, R., Jatlow, P., Sanacora, G., et al. (2005). Sex, GABA and nicotine: the impact of smoking on cortical GABA levels across the menstrual cycle as measured with proton magnetic resonance spectroscopy. *Biol. Psychiatry* 57, 44–48. doi: 10.1016/j.biopsych.2004.09.021

Faden, A. I., Demediuk, P., Panter, S. S., and Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* 244, 798–800. doi: 10.1126/science.2567056

Falkenberg, L. E., Westerhausen, R., Specht, K., and Hugdahl, K. (2012). Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. *Proc. Natl. Acad. Sci. U S A* 109, 5069–5073. doi: 10.1073/pnas.1115628109

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., and Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J. Neurosci.* 33, 17483–17489. doi: 10.1523/jneurosci.1414-13.2013

Finocchi, C., and Ferrari, M. (2011). Female reproductive steroids and neuronal excitability. *Neurol. Sci.* 32(Suppl. 1), S31–S35. doi: 10.1007/s10072-011-0532-5

Floyer-Lea, A., Wylezinska, M., Kincses, T., and Matthews, P. M. (2006). Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. *J. Neurophysiol.* 95, 1639–1644. doi: 10.1152/jn.00346.2005

Fujiyama, H., Hinder, M. R., Schmidt, M. W., Tandonnet, C., Garry, M. I., and Summers, J. J. (2012). Age-related differences in corticomotor excitability and inhibitory processes during a visuomotor RT task. *J. Cogn. Neurosci.* 24, 1253–1263. doi: 10.1162/jocn_a_00201

Gao, F., Edden, R. A., Li, M., Puts, N. A., Wang, G., Liu, C., et al. (2013). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *Neuroimage* 78, 75–82. doi: 10.1016/j.neuroimage.2013.04.012

Ge, S., and Dani, J. A. (2005). Nicotinic acetylcholine receptors at glutamate synapses facilitate long-term depression or potentiation. *J. Neurosci.* 25, 6084–6091. doi: 10.1523/jneurosci.0542-05.2005

Goto, N., Yoshimura, R., Moriya, J., Kakeda, S., Ueda, N., Ikenouchi-Sugita, A., et al. (2009). Reduction of brain gamma-aminobutyric acid (GABA) concentrations in early-stage schizophrenia patients: 3T Proton MRS study. *Schizophr. Res.* 112, 192–193. doi: 10.1016/j.schres.2009.04.026

Guleyupoglu, B., Schestatsky, P., Edwards, D., Fregni, F., and Bikson, M. (2013). Classification of methods in transcranial Electrical Stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. *J. Neurosci. Methods* 219, 297–311. doi: 10.1016/j.jneumeth.2013.07.016

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., and Rothwell, J. C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cereb. Cortex* 23, 1593–1605. doi: 10.1093/cercor/bhs147

Hamada, M., Ugawa, Y., and Tsuji, S. (2008). High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. *Mov. Disord.* 23, 1524–1531. doi: 10.1002/mds.22168

Harada, M., Kubo, H., Nose, A., Nishitani, H., and Matsuda, T. (2011). Measurement of variation in the human cerebral GABA level by in vivo MEGA-editing proton MR spectroscopy using a clinical 3 T instrument and its dependence on brain region and the female menstrual cycle. *Hum. Brain Mapp.* 32, 828–833. doi: 10.1002/hbm.21086

Hensch, T. K., and Bilimoria, P. M. (2012). Re-opening windows: manipulating critical periods for brain development. *Cerebrum* 2012:11.

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S., and Kash, S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282, 1504–1508. doi: 10.1126/science.282.5393.1504

Hess, G., and Donoghue, J. P. (1994). Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. *J. Neurophysiol.* 71, 2543–2547.

Hess, G., and Donoghue, J. P. (1996). Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. *Acta Neurobiol. Exp. (Wars)* 56, 397–405.

Huang, Y., Su, Y., Rorden, C., Dmochowski, J., Datta, A., and Parra, L. C. (2012). An automated method for high-definition transcranial direct current stimulation modeling. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2012, 5376–5379. doi: 10.1109/EMBC.2012.6347209

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A. G., et al. (2013). Human cortical excitability increases with time awake. *Cereb. Cortex* 23, 332–338. doi: 10.1093/cercor/bhs014

Huganir, R. L., and Nicoll, R. A. (2013). AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80, 704–717. doi: 10.1016/j.neuron.2013.10.025

Iuculano, T., and Cohen Kadosh, R. (2013). The mental cost of cognitive enhancement. *J. Neurosci.* 33, 4482–4486. doi: 10.1523/jneurosci.4927-12.2013

Jackson, J. D., Balota, D. A., Duchek, J. M., and Head, D. (2012). White matter integrity and reaction time intraindividual variability in healthy aging and early-stage Alzheimer disease. *Neuropsychologia* 50, 357–366. doi: 10.1016/j.neuropsychologia.2011.11.024

Johnston, M. V. (2009). Plasticity in the developing brain: implications for rehabilitation. *Dev. Disabil. Res. Rev.* 15, 94–101. doi: 10.1002/ddrr.64

Kabakov, A. Y., Muller, P. A., Pascual-Leone, A., Jensen, F. E., and Rotenberg, A. (2012). Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. *J. Neurophysiol.* 107, 1881–1889. doi: 10.1152/jn.00715.2011

Kanai, R., Chaieb, L., Antal, A., Walsh, V., and Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Curr. Biol.* 18, 1839–1843. doi: 10.1016/j.cub.2008.10.027

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., et al. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J. Neurosci.* 31, 15284–15293. doi: 10.1523/jneurosci.0542-11.2011

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *J. Cogn. Neurosci.* 16, 1412–1425. doi: 10.1162/0898929042304796

Krause, B., and Cohen Kadosh, R. (2013). Can transcranial electrical stimulation improve learning difficulties in atypical brain development? A future possibility for cognitive training. *Dev. Cogn. Neurosci.* 6, 176–194. doi: 10.1016/j.dcn.2013.04.001

Krause, B., Marquez-Ruiz, J., and Cohen Kadosh, R. (2013). The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? *Front. Hum. Neurosci.* 7:602. doi: 10.3389/fnhum.2013.00602

Kubas, B., Kulak, W., Sobaniec, W., Tarasow, E., Lebkowska, U., and Walecki, J. (2012). Metabolite alterations in autistic children: a 1H MR spectroscopy study. *Adv. Med. Sci.* 57, 152–156. doi: 10.2478/v10039-012-0014-x

Lang, N., Rothkegel, H., Reiber, H., Hasan, A., Sueske, E., Tergau, F., et al. (2011). Circadian modulation of GABA-mediated cortical inhibition. *Cereb. Cortex* 21, 2299–2306. doi: 10.1093/cercor/bhr003

Lang, N., Siebner, H. R., Ernst, D., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. *Biol. Psychiatry* 56, 634–639. doi: 10.1016/j.biopsych.2004.07.017

Lasarge, C. L., Banuelos, C., Mayse, J. D., and Bizon, J. L. (2009). Blockade of GABA(B) receptors completely reverses age-related learning impairment. *Neuroscience* 164, 941–947. doi: 10.1016/j.neuroscience.2009.08.055

López Alonso, V., Cheeran, B., Río-Rodríguez, D., and Fernandez-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul.* (in press).

McConnell, G. C., So, R. Q., Hilliard, J. D., Lopomo, P., and Grill, W. M. (2012). Effective deep brain stimulation suppresses low-frequency network oscillations in the basal ganglia by regularizing neural firing patterns. *J. Neurosci.* 32, 15657–15668. doi: 10.1523/jneurosci.2824-12.2012

Mcdonnell, M. N., Orekhov, Y., and Ziemann, U. (2007). Suppression of LTP-like plasticity in human motor cortex by the GABAB receptor agonist baclofen. *Exp. Brain Res.* 180, 181–186. doi: 10.1007/s00221-006-0849-0

Mcentee, W. J., and Crook, T. H. (1993). Glutamate: its role in learning, memory and the aging brain. *Psychopharmacology (Berl)* 111, 391–401. doi: 10.1007/bf02253527

Mekle, R., Mlynarik, V., Gambarota, G., Hergt, M., Krueger, G., and Gruetter, R. (2009). MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magn. Reson. Med.* 61, 1279–1285. doi: 10.1002/mrm.21961

Miniussi, C., Cappa, S. F., Cohen, L. G., Floel, A., Fregni, F., Nitsche, M. A., et al. (2008). Efficacy of repetitive transcranial magnetic stimulation/transcranial direct current stimulation in cognitive neurorehabilitation. *Brain Stimul.* 1, 326–336. doi: 10.1016/j.brs.2008.07.002

Moliadze, V., Antal, A., and Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clin. Neurophysiol.* 121, 2165–2171. doi: 10.1016/j.clinph.2010.04.033

Moliadze, V., Atalay, D., Antal, A., and Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimul.* 5, 505–511. doi: 10.1016/j.brs.2011.11.004

Murphy, K. M., Beston, B. R., Boley, P. M., and Jones, D. G. (2005). Development of human visual cortex: a balance between excitatory and inhibitory plasticity mechanisms. *Dev. Psychobiol.* 46, 209–221. doi: 10.1002/dev.20053

Muthukumaraswamy, S. D., Edden, R. A., Jones, D. K., Swettenham, J. B., and Singh, K. D. (2009). Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to visual stimulation in humans. *Proc. Natl. Acad. Sci. U S A* 106, 8356–8361. doi: 10.1073/pnas.0900728106

Muthukumaraswamy, S. D., Evans, C. J., Edden, R. A., Wise, R. G., and Singh, K. D. (2012). Individual variability in the shape and amplitude of the BOLD-HRF correlates with endogenous GABAergic inhibition. *Hum. Brain Mapp.* 33, 455–465. doi: 10.1002/hbm.21223

Neuling, T., Rach, S., and Herrmann, C. S. (2013). Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Front. Hum. Neurosci.* 7:161. doi: 10.3389/fnhum.2013.00161

Neuling, T., Wagner, S., Wolters, C. H., Zaehle, T., and Herrmann, C. S. (2012). Finite-element model predicts current density distribution for clinical applications of tDCS and tACS. *Front. Psychiatry* 3:83. doi: 10.3389/fpsyt.2012.00083

Nitsche, M. A., Kuo, M. F., Karrasch, R., Wachter, B., Liebetanz, D., and Paulus, W. (2009). Serotonin affects transcranial direct current-induced neuroplasticity in humans. *Biol. Psychiatry* 66, 503–508. doi: 10.1016/j.biopsych.2009.03.022

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., and Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin. Neurophysiol.* 114, 600–604. doi: 10.1016/s1388-2457(02)00412-1

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 57, 1899–1901. doi: 10.1212/wnl.57.10.1899

Park, C. H., Chang, W. H., Park, J. Y., Shin, Y. I., Kim, S. T., and Kim, Y. H. (2013). Transcranial direct current stimulation increases resting state interhemispheric connectivity. *Neurosci. Lett.* 539, 7–10. doi: 10.1016/j.neulet.2013.01.047

Peterchev, A. V., Wagner, T. A., Miranda, P. C., Nitsche, M. A., Paulus, W., Lisanby, S. H., et al. (2012). Fundamentals of transcranial electric and magnetic stimulation dose: definition, selection and reporting practices. *Brain Stimul.* 5, 435–453. doi: 10.1016/j.brs.2011.10.001

Pinto, J. G., Hornby, K. R., Jones, D. G., and Murphy, K. M. (2010). Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. *Front. Cell. Neurosci.* 4:16. doi: 10.3389/fncel.2010.00016

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *J. Physiol.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., et al. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15, 1676–1689. doi: 10.1093/cercor/bhi044

Reato, D., Rahman, A., Bikson, M., and Parra, L. C. (2010). Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *J. Neurosci.* 30, 15067–15079. doi: 10.1523/jneurosci.2059-10.2010

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., et al. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc. Natl. Acad. Sci. U S A* 106, 1590–1595. doi: 10.1073/pnas.0805413106

Rivera, S. M., Reiss, A. L., Eckert, M. A., and Menon, V. (2005). Developmental changes in mental arithmetic: evidence for increased functional specialization in the left inferior parietal cortex. *Cereb. Cortex* 15, 1779–1790. doi: 10.1093/cercor/bhi055

Rojas, D. C., Singel, D., Steinmetz, S., Hepburn, S., and Brown, M. S. (2013). Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. *Neuroimage* 86, 28–34. doi: 10.1016/j.neuroimage.2013.01.045

Romei, V., Rihs, T., Brodbeck, V., and Thut, G. (2008). Resting electroencephalogram alpha-power over posterior sites indexes baseline visual cortex excitability. *Neuroreport* 19, 203–208. doi: 10.1097/wnr.0b013e3282f454c4

Ross, L. A., Mccoy, D., Wolk, D. A., Coslett, H. B., and Olson, I. R. (2010). Improved proper name recall by electrical stimulation of the anterior temporal lobes. *Neuropsychologia* 48, 3671–3674. doi: 10.1016/j.neuropsychologia.2010.07.024

Ross, L. A., Mccoy, D., Wolk, D. A., Coslett, H. B., and Olson, I. R. (2011). Improved proper name recall in aging after electrical stimulation of the anterior temporal lobes. *Front. Aging Neurosci.* 3:16. doi: 10.3389/fnagi.2011.00016

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J. Neurosci.* 24, 3379–3385. doi: 10.1523/jneurosci.5316-03.2004

Silvanto, J., Muggleton, N. G., Cowey, A., and Walsh, V. (2007). Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. *Eur. J. Neurosci.* 25, 1874–1881. doi: 10.1111/j.1460-9568.2007.05440.x

Silvanto, J., Muggleton, N., and Walsh, V. (2008). State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* 12, 447–454. doi: 10.1016/j.tics.2008.09.004

Smith, M. J., Adams, L. F., Schmidt, P. J., Rubinow, D. R., and Wassermann, E. M. (2002). Effects of ovarian hormones on human cortical excitability. *Ann. Neurol.* 51, 599–603. doi: 10.1002/ana.10180

Smith, M. J., Keel, J. C., Greenberg, B. D., Adams, L. F., Schmidt, P. J., Rubinow, D. A., et al. (1999). Menstrual cycle effects on cortical excitability. *Neurology* 53, 2069–2072. doi: 10.1212/wnl.53.9.2069

Smith, A. E., Ridding, M. C., Higgins, R. D., Wittert, G. A., and Pitcher, J. B. (2009). Age-related changes in short-latency motor cortex inhibition. *Exp. Brain Res.* 198, 489–500. doi: 10.1007/s00221-009-1945-8

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Stagg, C. J., Bachtiar, V., and Johansen-Berg, H. (2011). The role of GABA in human motor learning. *Curr. Biol.* 21, 480–484. doi: 10.1016/j.cub.2011.01.069

Stagg, C. J., Best, J. G., Stephenson, M. C., O'shea, J., Wylezinska, M., Kincses, Z. T., et al. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J. Neurosci.* 29, 5202–5206. doi: 10.1523/jneurosci.4432-08.2009

Strelau, J. (1997). The contribution of pavlov's typology of CNS properties to personality research. *Eur. Psychol.* 2, 125–138. doi: 10.1027/1016-9040.2.2.125

Tecchio, F., Cancelli, A., Cottone, C., Tomasevic, L., Devigus, B., Zito, G., et al. (2013). Regional personalized electrodes to select transcranial current stimulation target. *Front. Hum. Neurosci.* 7:131. doi: 10.3389/fnhum.2013.00131

Teo, F., Hoy, K. E., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Investigating the role of current strength in tDCS modulation of working memory performance in healthy controls. *Front. Psychiatry* 2:45. doi: 10.3389/fpsyt.2011.00045

Terney, D., Chaieb, L., Moliadze, V., Antal, A., and Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *J. Neurosci.* 28, 14147–14155. doi: 10.1523/JNEUROSCI.4248-08.2008

Trepel, C., and Racine, R. J. (2000). GABAergic modulation of neocortical long-term potentiation in the freely moving rat. *Synapse* 35, 120–128. doi: 10.1002/(sici)1098-2396(200002)35:2<120::aid-syn4>3.0.co;2-6

Truong, D. Q., Magerowski, G., Blackburn, G. L., Bikson, M., and Alonso-Alonso, M. (2013). Computational modeling of transcranial direct current stimulation (tDCS) in obesity: impact of head fat and dose guidelines. *Neuroimage Clin.* 2, 759–766. doi: 10.1016/j.nicl.2013.05.011

Turrigiano, G. G., and Nelson, S. B. (2000). Hebb and homeostasis in neuronal plasticity. *Curr. Opin. Neurobiol.* 10, 358–364. doi: 10.1016/s0959-4388(00)00091-x

Turrigiano, G. G., and Nelson, S. B. (2004). Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* 5, 97–107. doi: 10.1038/nrn1327

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin. Neurophysiol.* 113, 1165–1171. doi: 10.1016/s1388-2457(02)00144-x

Weiss, M., and Lavidor, M. (2012). When less is more: evidence for a facilitative cathodal tDCS effect in attentional abilities. *J. Cogn. Neurosci.* 24, 1826–1833. doi: 10.1162/jocn_a_00248

Wenk, G. L., Walker, L. C., Price, D. L., and Cork, L. C. (1991). Loss of NMDA, but not GABA-A, binding in the brains of aged rats and monkeys. *Neurobiol. Aging* 12, 93–98. doi: 10.1016/0197-4580(91)90047-n

Yoon, J. H., Maddock, R. J., Rokem, A., Silver, M. A., Minzenberg, M. J., Ragland, J. D., et al. (2010). GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. *J. Neurosci.* 30, 3777–3781. doi: 10.1523/JNEUROSCI.6158-09.2010

Zaghi, S., Acar, M., Hultgren, B., Boggio, P. S., and Fregni, F. (2010). Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. *Neuroscientist* 16, 285–307. doi: 10.1177/1073858409336227

Zheng, X., Alsop, D. C., and Schlaug, G. (2011). Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. *Neuroimage* 58, 26–33. doi: 10.1016/j.neuroimage.2011.06.018

Zimmerman, M., and Hummel, F. (in press). "Brain stimulation and its role in neurological diseases," in *The Stimulated Brain*, ed K. Cohen Kadosh (Amsterdam: Elsevier).

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 November 2013; accepted: 05 February 2014; published online: 24 February 2014.

*Citation: Krause B and Cohen Kadosh R (2014) Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation. Front. Syst. Neurosci. **8**:25. doi: 10.3389/fnsys.2014.00025*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 24 January 2014
doi: 10.3389/fnsys.2014.00002

Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be)

Jared C. Horvath *, Olivia Carter and Jason D. Forte

Psychological Sciences, University of Melbourne, Melbourne, VIC, Australia

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Carmelo M. Vicario, University of Queensland, Australia
Nick J. Davis, Swansea University, UK

****Correspondence:***
Jared C. Horvath, Psychological Sciences, University of Melbourne, Redmond Barry Bldg. 613, Melbourne, VIC 3010, Australia
e-mail: jch155@mail.harvard.edu

Transcranial Direct Current Stimulation (tDCS) is a neuromodulatory device often publicized for its ability to enhance cognitive and behavioral performance. These enhancement claims, however, are predicated upon electrophysiological evidence and descriptions which are far from conclusive. In fact, a review of the literature reveals a number of important experimental and technical issues inherent with this device that are simply not being discussed in any meaningful manner. In this paper, we will consider five of these topics. The first, *inter-subject variability*, explores the extensive between- and within-group differences found within the tDCS literature and highlights the need to properly examine stimulatory response at the individual level. The second, *intra-subject reliability*, reviews the lack of data concerning tDCS response reliability over time and emphasizes the importance of this knowledge for appropriate stimulatory application. The third, *sham stimulation and blinding*, draws attention to the importance (yet relative lack) of proper control and blinding practices in the tDCS literature. The fourth, *motor and cognitive interference,* highlights the often overlooked body of research that suggests typical behaviors and cognitions undertaken during or following tDCS can impair or abolish the effects of stimulation. Finally, the fifth, *electric current influences*, underscores several largely ignored variables (such as hair thickness and electrode attachments methods) influential to tDCS electric current density and flow. Through this paper, we hope to increase awareness and start an ongoing dialog of these important issues which speak to the efficacy, reliability, and mechanistic foundations of tDCS.

Keywords: transcranial direct current stimulation (tDCS), variability, reliability, efficacy, mechanisms of action

INTRODUCTION

Transcranial direct current stimulation (tDCS) is currently being promoted as a cheap and effective tool to enhance cognitive and behavioral function. A recent surge of public interest in this device (evidenced by several tDCS devices appearing on the public market) has doubtless been driven by the belief that these enhancement claims are robust, reliable, and well elucidated. However, the research exploring the efficacy of tDCS is far from conclusive.

It's commonly assumed that tDCS shifts the resting membrane potential and synaptic strength of neurons in a predictable and consistent manner. More specifically, hypo-polarization of neurons under the anodal electrode is believed to increase the likelihood of their firing, whilst hyper-polarization of neurons under the cathodal electrode is believed to decrease the likelihood of their firing [for an in depth mechanistic overview, see, Stagg and Nitsche (2011)]. Similar to efficacy, however, a close inspection of the literature reveals short-comings of the anode excite/cathode inhibit model.

In response to efficacy and mechanistic uncertainties, many practitioners focus on the manipulation of three adjustable tDCS parameters: current density, electrode position, and stimulation duration. Whereas these three variables certainly play a large role in tDCS outcomes, there are a number of equally important issues relevant to both efficacy and mechanism that simply are not being discussed in any meaningful manner.

In this paper, we will explore five notable indicators and/or sources of inconsistency associated with the use of tDCS in the current literature: inter-subject variability, intra-subject reliability, lack of effective sham and blinding protocols, motor and cognitive interference, and electric current influences. Throughout this piece, we will draw examples solely from studies which explore the effects of tDCS over the motor cortex on MEP amplitude in healthy populations. We have chosen to do this for two reasons: first, MEP amplitude modulation is easily the most explored and reliably demonstrated outcome measure in the tDCS in the literature. Second, as the majority of neurophysiologic, clinical, and behavioral claims cite this work as mechanistically foundational, any issues apparent in this literature will necessarily be applicable to and concern any other outcome measure utilized.

INTER-SUBJECT VARIABILITY

tDCS must demonstrate similar (or comparable) effects across a range of people before it can be meaningfully applied in healthy and/or clinical populations. However, a survey of the literature reveals extensive between- and within-group variation suggestive of an inconsistent effect between individuals.

As an example of large between-group variation, Fricke et al. (2011) recently reported data from two different groups that underwent an identical stimulation protocol (0.0286 mA/cm^2 current density; anode M1/cathode contralateral orbit montage; 5 min duration). Whereas one group demonstrated an average MEP amplitude enhancement of 93.2% in the 5 min following tDCS, the second group demonstrated an average MEP amplitude enhancement of only 9.2%: a between-group difference of 913%. Similarly, in two different studies from 2004 using identical stimulation protocols (0.0286 mA/cm^2 current density; cathode M1/anode contralateral orbit montage; 9 min duration), Nitsche et al. reported 30 min group MEP amplitude inhibitions of 42.9% (Nitsche et al., 2004a) and 20.0% (Nitsche et al., 2004b): a difference of 110%. Even more variable, these researchers has reported group MEP amplitude enhancements following identical stimulation protocols (0.0286 mA/cm^2 current density; anode M1/cathode contralateral orbit montage; 13 min duration) ranging from 54.4% (Nitsche et al., 2003a) to 19.3% (Nitsche et al., 2009); a difference of 184%.

Specific examples of within-group variability (beyond common deviation and/or error measures) are harder to come by as very few studies include individual data with their reports. However, of the few that have, the results are illuminating. For instance, following 9 min of anodal stimulation (0.0286 mA/cm^2 current density; M1/contralateral orbit montage), Nitsche and Paulus (2001) reported one subject who demonstrated an incredible 295% increase in MEP amplitude and a second who demonstrated a weak 5% increase. More recently, following 20 min of anodal stimulation (0.06 mA/cm^2 current density; M1/Contralateral orbit montage), Tremblay et al. (2013) reported one subject who demonstrated a 251% increase in MEP amplitude and a second who demonstrated a 41% *decrease* (see also, Roche et al., 2011).

One potential explanation for this extreme between- and within-group variability is the difficulty in properly and reliably targeting TMS pulses during lengthy protocols (Herwig et al., 2001; Sparing et al., 2008; Ahdab et al., 2010). Although modern MRI guided neuronavigation systems can be used to ensure accurate coil positioning across time, many tDCS studies have not utilized (or do not report utilizing) these systems. As such, it is possible subtle variation in coil placement and orientation with time may influence response variation.

A second potential explanation for this extreme between- and within-group variability is that tDCS generates differential response at the individual level which is masked by group averaging. An individual's unique neurophysiology, anatomy, and psychology may influence his/her response to tDCS. In fact, recent modeling work suggests parameters such as skull thickness, subcutaneous fat levels, cerebrospinal fluid density, and cortical surface topography can greatly influence current flow and density patterns during stimulation (Datta et al., 2012; Truong et al., 2013). As such, elucidation of individual and environmental influences on tDCS is necessary and may only be possible by looking at response characteristics (and related correlative factors) at the individual level.

INTRA-SUBJECT RELIABILITY

Beyond individual response patterns, it must be demonstrated that people respond in a similar and predictable manner to repeated sessions of tDCS before this tool can be meaningfully applied. Unfortunately, to our knowledge, response *reliability* at the level of the individual has not been explored (or, at least, reported) in the literature to date.

Of the (only) four studies which have explored group effects of tDCS on MEP amplitude in healthy populations across multiple days, two suggest response patterns may be reliable and replicable. Alonzo et al. (2012) explored the effects of anodal stimulation (0.0571 mA/cm^2 current density; M1/contralateral orbit montage; 20 min duration) on MEP amplitude over the course of 5 days (Monday–Friday). Although these researchers reported variable baseline levels across the week, the ratio of pre- to post-stimulation group average MEP amplitudes did not significantly change from day-to-day. Using a similar protocol, Gálvez et al. (2013) reported similar findings: namely, whereas baseline levels changed throughout the week, the group averaged after-effects of daily stimulation did not significantly vary across 5 days.

Interestingly, the remaining two studies to explore group effects of tDCS on MEP amplitude in healthy populations across multiple days suggest response patterns may be unreliable and unpredictable. Monte-Silva et al. have twice looked at the effects of two sessions of tDCS on MEP amplitude with a 24 h block between sessions (Monte-Silva et al., 2010, 2012). In the first study (0.0286 mA/cm^2 current density; cathodal M1/anodal contralateral orbit montage; 9 min duration), these researchers reported significantly reduced modulation of MEP amplitude following the second session of stimulation. More concerning, in the second study (0.0286 mA/cm^2 current density; anodal M1/cathodal contralateral orbit montage; 13 min duration) these researchers reported not only a significant reduction in MEP amplitude modulation following the second session of stimulation, but also a reverse in modulation direction (inhibition rather than excitation following anodal stimulation) and unpredictable timing effects.

Considerably more data investigating effects across time is required before concluding tDCS is a reliable device. As individual response reliability is explored, however, it will be important to remember that intra-subject variability may not, in itself, suggest tDCS is unreliable. It is likely that circadian, metabolic, and hormonal cycles will differentially impact response. In fact, several researchers have already shown that stages of the menstrual cycle and cortisol levels impact plastic response to varied TMS protocols (Smith et al., 1999; Inghilleri et al., 2004; Sale et al., 2008, 2010). In addition, proper and reliable TMS coil positioning during lengthy protocols may also impact response variability (see above). With this in mind, it will certainly be informative to identify the factors that might influence unique tDCS response and whether these factor, themselves, modulate response in a reliable and predictable manner.

SHAM STIMULATION AND BLINDING

If the dichometric anode excite/cathode inhibit mechanism of tDCS is valid, then comparing the polarities to each other makes determining the true effect of each extremely difficult (as one can never be certain the exact contribution of each polarity

to the overall difference). Although practitioners aware of this comparative shortcoming extol the use of various control stimulation procedures (such as *sham* or *off-target active* stimulation), these procedures have not always proven effective or reliable across varied tDCS protocols (Ambrus et al., 2012; Brunoni et al., 2013; Davis et al., 2013; Palm et al., 2013). In addition, not nearly as many researchers have utilized control conditions as one might expect. In fact, of the 80 studies published to date exploring the effect of 0.0286 mA/cm^2 tDCS current density with an M1/Orbit electrode placement on MEP modulation (the most utilized protocol in the literature), only 10 have compared results to a control condition (**Table 1**). This means 87.5% of the studies examining the foundational claim upon which the modern tDCS field is built have not utilized a proper control condition.

Comparing each polarity to its own baseline level (rather than the opposing polarity) does little to address the underlying issues inherent with sham-less protocols. It is commonly acknowledged that MEP amplitude is naturally an extremely variable measure (in fact, Valls-Sole recently pointed out, "The amplitude of MEP to single pulse TMS is not usually employed as a measure of functional relevance because of its large variability and dependence on many technical factors"; p. 9, *in press*). As such, it can be assumed there will *always* be some shift away from baseline levels, regardless of intervention (or lack thereof). Accordingly, the utilization of a control condition to differentiate between natural fluctuation and tDCS engendered effects is imperative.

As O'Connell et al. (2012) recently pointed out, tDCS blinding (especially when sham stimulation is being utilized) is of utmost importance yet incredibly difficult to achieve. In fact, these authors reported that, during stimulation using a high current density (0.0571 mA/cm^2), neither the practitioner nor participant was effectively blinded. Beyond this, observable vasodilation (typically over the right orbit) makes practitioner blinding difficult at any current density (see, Palm et al., 2013). Finally, clear sensorial differences between active and sham stimulation (primarily reported as itching, tingling, and/or burning) make blinding participants who undergo multiple conditions difficult at any current density (Davis et al., 2013). As with any scientific study, ineffective blinding may lead to a number of undesirable confounds, including expectation effects, on-the-fly protocol adjustments, and reporting/assessment biases.

In order to elucidate the effects of varied tDCS paradigms, it is essential to continue to amend current and create novel, more effective control conditions. In addition, until such time as more reliable control protocols are developed, it may be beneficial to test for and report blinding procedures and efficacy (or lack-thereof).

MOTOR AND COGNITIVE INTERFERENCE

Several lines of research suggest that any active motor and/or cognitive activity undertaken during or following tDCS can negatively interfere with or altogether abolish the effects of stimulation. The failure of many practitioners to take account of and further characterize this evidence is concerning.

Quartarone et al. (2004) were the first to report evidence of this interference effect. This group explored MEP amplitude modulation following 5 min of tDCS during motor imagery (0.0286 mA/cm^2 current density; M1/contralateral orbit montage). Whereas imagery (undertaken following stimulation) appeared to prolong the effects of cathodal stimulation, it abolished the effects of anodal stimulation. Despite this important finding (that the act of *thinking* about motor movement could potentially eliminate tDCS efficacy), this paper went largely ignored and is rarely cited.

Several additional studies have confirmed this interference effect. For instance, Antal et al. (2007) reported that a cognitive task (a combined mathematics, language, geography, and history questionnaire) undertaken during stimulation abolished the effects of both anodal and cathodal stimulation on MEP amplitude modulation (0.0286 mA/cm^2 current density; M1/contralateral orbit montage; 10 min duration). In addition, a simple motor task (pushing around a ball) undertaken during stimulation led to an equivalent decrease in MEP amplitude following both anodal and cathodal stimulation. This equivalent drop suggests the motor activity (perhaps due to fatigue of the target muscle) abolished the effect of stimulation as well. More recently, Miyaguchi et al. (2013) reported that anodal tDCS (0.0571 mA/cm^2 current density; bilateral M1 montage; 10 min duration) delivered with a concurrent non-exhaustive active or passive motor task (self initiated or machine initiated finger abduction-adduction) led to an equivalent MEP amplitude reduction as did undertaking the active motor task alone (without stimulation). Again, this suggests the motor task abolished the effect of stimulation (see also, Thirugnanasambandam et al., 2011).

Secondary evidence for an interference effect can be seen in the often reported diminished tDCS MEP amplitude modulation in voluntarily contracted muscles (common in non-hand targets) compared to resting muscles. For instance, in the 40 min following cathodal stimulation (0.0286 mA/cm^2 current density; M1/contralateral orbit montage; 15 min duration), Bradnam et al. (2010) reported an average compound MEP decrease of 15.3% in the right infraspinitus (shoulder) when the muscle was at rest, and an *increase* of 1.3% when the muscle was active. Similarly, in the 60 min following anodal stimulation (0.0571 mA/cm^2 density; M1/contralateral orbit montage; 10 min duration), Jeffery et al. (2007) reported an average MEP amplitude increase of 34.8% in the right tibialis anterior (leg) when the muscle was at rest, and an increase of only 25% when the muscle was activated. Again, these results suggest that motor activity undertaken immediately following stimulation can significantly reduce or eliminate the modulatory effects of tDCS.

These findings suggest that relatively simple and difficult to control for thoughts and/or behaviors may eliminate tDCS efficacy. Clinically, tDCS is often used as an adjunct to physical rehabilitation following stroke (for review, Johansson, 2011). If the aforementioned studies are correct, combining tDCS with motor training may eliminate any desired tDCS effect. This holds true for healthy populations as well. Oftentimes, during long-duration, off-line stimulatory protocols, participants are instructed to simply relax during tDCS. This relaxation can take

Table 1 | Studies exploring the effects of 0.0286 mA/cm^2 current density, M1/Contralateral Orbit tDCS montage on TMS elicited MEP amplitude of intrinsic hand muscles at rest in healthy participants.

Study	*N*	tDCS duration	Anode	Cathode	Control
Nitsche and Paulus, 2000 (*x4*)	10 and 9 (*x1*)/12 (*x2*)	4 s (*x1*)/5 min (*x3*)	**X** (*x4*)	**X** (*x4*)	–
Nitsche and Paulus, 2001 (*x5*)	12 (*x5*)	5, 7, 9, 11, and 13 min (*x1*)	**X** (*x5*)	–	–
Liebetanz et al., 2002	11	5 min	**X**	**X**	–
Nitsche et al., 2003a (*x3*)	12 (*x1*)/10 (*x2*)	4 s (*x1*)/9–13 min (*x2*)	**X** (*x3*)	**X** (*x3*)	–
Nitsche et al., 2003b (*x3*)	12 (*x3*)	5, 7, and 9 min (*x1*)	–	**X** (*x3*)	–
Lang et al., 2004a	8	10 min	**X**	**X**	–
Lang et al., 2004b (*x2*)	5 and 10 (*x1*)	10 min (*x2*)	**X** (*x2*)	**X** (*x2*)	**X** (*x1*)
Siebner et al., 2004 (*x2*)	5 and 8 (*x1*)	10 min (*x2*)	**X** (*x2*)	**X** (*x2*)	**X** (*x1*)
Nitsche et al., 2004a (*x4*)	6 (*x3*)/12 (*x1*)	4 s (*x1*)/7, 9–13 min (*x1*)	**X** (*x4*)	**X** (*x4*)	–
Nitsche et al., 2004b (*x3*)	12, 9, and 10 (*x1*)	4 s (*x1*)/5, 9–11 min (*x1*)	**X** (*x3*)	**X** (*x3*)	–
Nitsche et al., 2004c	12	9 min C/13 min A	**X**	**X**	–
Quartarone et al., 2004 (*x2*)	7 and 21 (*x1*)	5 min (*x2*)	**X** (*x1*)	**X** (*x1*)	–
Quartarone et al., 2005	8	10 min	**X**	**X**	**X**
Nitsche et al., 2006	12	9 min C/13 min A	**X**	**X**	–
Power et al., 2006	10	10 min	**X**	**X**	**X**
Nitsche et al., 2007a (*x8*)	12 (*x8*)	4 s (*x3*), 7 min (*x3*)10 min (*x2*)	**X** (*x8*)	**X** (*x8*)	–
Nitsche et al., 2007b	12	7 min	**X**	**X**	–
Kuo et al., 2007	7	9 min C/13 min A	**X**	**X**	–
Antal et al., 2007	12	10 min	**X**	**X**	–
Boros et al., 2008	17	13 min	**X**	–	–
Kuo et al., 2008	7	9 min C/13 min A	**X**	**X**	–
Nitsche et al., 2009	12	9 min C/13 min A	**X**	**X**	–
Monte-Silva et al., 2009 (*x4*)	12 (*x4*)	9 min C/13 min A (*x4*)	**X** (*x4*)	**X** (*x4*)	–
Monte-Silva et al., 2010 (*x2*)	12 (*x2*)	9 and 18 min (*x1*)	–	**X** (*x2*)	–
Bradnam et al., 2011	18	15 min	–	**X**	**X**
Fricke et al., 2011 (*x4*)	8–12 (*x4*)	5 min (*x2*), 7 and 10 min (*x1*)	**X** (*x4*)	**X** (*x4*)	–
List et al., 2011	12	10 min	–	**X**	–
McCambridge et al., 2011	7 Active/5 Sham	10 min	–	**X**	**X**
Munneke et al., 2011 (*x3*)	10 (*x3*)	7, 11, and 15 min (*x1*)	–	**X** (*x3*)	–
Scelzo et al., 2011	12	13 min	**X**	**X**	–
Thirugnanasambandam et al., 2011	16	20 min	**X**	**X**	–
Di Lazzaro et al., 2012	30	20 min	–	**X**	–
Hasan et al., 2012	18	9 min	–	**X**	–
Schade et al., 2012 (*x2*)	8 (*x2*)	5 min (*x2*)	**X** (*x2*)	**X** (*x2*)	–
Suzuki et al., 2012	9	10 min	**X**	**X**	**X**
Monte-Silva et al., 2012 (*x2*)	15 (*x2*)	13 and 26 min (*x1*)	**X** (*x2*)	–	–
Hasan et al., 2013	20	9 min	–	**X**	–
Batsikadze et al., 2013 (*x2*)	9 and 8 (*x1*)	20 min (*x2*)	–	**X** (*x1*)	**X** (*x1*)
Schabrun et al., 2012 (*x3*)	21 A, 9 C, 13 s	20 min (*x3*)	**X** (*x1*)	**X** (*x1*)	**X** (*x1*)
Simis et al., 2013	11	20 min	**X**	–	**X**
	TOTAL		62	67	10

A sham condition from Quartarone et al. (2004) was excluded due to reporting MEP modulation during motor imagery only (not at rest). One study from Monte-Silva et al., 2009 was excluded due to presenting replication data. A, Anode; C, Cathode; S, Sham.

Studies include the placebo condition/s in any drug or device interaction studies.

the form of reading, texting, surfing the internet, doing homework, etc. Is it possible these seemingly innocuous activities are enough to negate or otherwise interfere with the effects of tDCS as well? Additionally, in experiments utilizing MRI to explore the effects of tDCS, stimulation is often given outside the scanning room (although several tDCS devices are now MR compatible). During these protocols, participants must walk back to the scanning room and re-enter the scanner following stimulation: a series of non-trivial motor actions which may, again, interfere with or abolish any tDCS effects.

If it's possible the effects of tDCS are too weak to manifest during typical human behavior, this is important to determine before more effort and funding are expended utilizing inappropriate protocols. Until such a time as this issue is clearly resolved, it is important practitioners minimize motor and cognitive activity during and immediately following tDCS and during any proceeding procedure (including TMS and/or MRI).

ELECTRIC CURRENT INFLUENCES

There are a number of variables which may influence current density and flow to a great extent that have simply not been discussed in the literature to date. Although, as noted above, countless papers have discussed optimal electrode positioning, current density, and stimulation duration for specific outcomes (for discussion, Paulus, 2011), these discussions never seem to evolve past these three parameters.

One variable which may impact current density and flow (but which has yet to be discussed in the literature) is hair thickness. Simply put: hair is not a conductor—it is an insulator. Measurements suggest dry hair (<7% 25% H_20 content) has a resistivity of approximately $3 \times 10^{12}\Omega$/cm whilst wet hair (25% H_20 content) has a resistivity of approximately $6 \times 10^{6}\Omega$/cm (Feughelman, 1997). To put that into perspective, skin (the contact surface in many tDCS modeling studies) has a resistivity of approximately 2.15 $\times 10^{-2}\Omega$/cm (see, Miranda et al., 2006; Wagner et al., 2007: Ω = Ohm: Note—lower resistivity values equate to higher conductance).

To combat this, practitioners often utilize large amounts of saline to saturate dense hair. Unfortunately, saline spread or dripping at the level of the scalp can guide current flow in undesirable and unpredictable directions. This fact can be easily demonstrated. First, place two saline soaked tDCS sponge electrodes on an easily accessible area of skin (such as the forearms or quadriceps). Next, place a piece of thick, non-conductive plastic under one of the electrodes to ensure no contact is made between the sponge and the skin. Under this set-up, you should be *un*able to complete the electric circuit. Now, cut a small hole in the plastic barrier (exposing the skin underneath), fill the small hole with saline, and run a continuous stream of saline between the hole and the sponge atop the plastic. Under this new set-up, you should be able to complete the electric circuit quite easily, regardless of how far away from the sponge you have made the small hole (if you are having trouble running a stream of saline between the electrode and the hole, you can substitute a thicker conductive gel: **Figure 1**).

This demonstration reveals that, even when there is no direct electrode/scalp contact (as may occur in participants with thick hair), excess saline can be used to bridge the tDCS current. However, when this is done, the precise location of the electric current entrance and/or exit points on the scalp will be largely unknown and unpredictable. In addition, when the electric current follows saline to the scalp, the current density also becomes largely unknown and unpredictable (as the number and size of contact points at the scalp becomes uncertain).

Sweat is a second often-ignored variable that may impact electric current dynamics. Because sweat increases skin conductivity (for review, Dawson et al., 2001), the amount of sweat on a participants scalp may influence current flow in important ways. It's possible that as salts, oils, and electrolytes accumulate in pores on the scalp, the skin will generate enough conductivity to ensure little or no current enters the cortex. However, aside from washing each subject's hair and ensuring a temperature controlled testing environment, how can this be accounted and/or controlled?

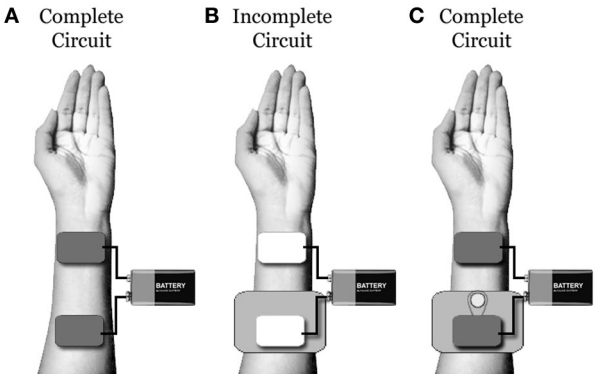

FIGURE 1 | (A) Under typical conditions, one will be able to build an electric circuit using two saline soaked tDCS sponges placed on a piece of clean skin. (B) When a piece of non-conductive plastic is placed underneath one electrode, the circuit will be broken. **(C)** When a small hole is cut in the plastic and a small stream of saline is used to connect the skin under the hole to the electrode, the circuit will be re-built. This demonstrates that, even if a tDCS electrode is not in contact with the scalp, excessive saline can be used to bridge between the electrode and the skin. Unfortunately, with excessive saline, determining the location of the circuit connection and electrical density at this point is extremely difficult.

Finally, the means by which electrodes are held in place at the scalp may also influence electric current dynamics. For instance, several contemporary tDCS sponge electrodes include plastic rings at the corners (presumably anchor the electrode in place). Unfortunately, unless specifically manufactured, most plastics are non-conductive. Whether or not the plastic used in these electrodes has been produced to conduct electricity is uncertain, although vasodilation patterns following stimulation with these electrodes (which typically reveals no dilatory response underneath the plastic rings themselves) suggests they are not. This may impact current density and flow in unpredictable and uncontrollable ways. In addition, many practitioners hold sponge electrodes in place using rubber straps which are narrower than the electrodes themselves. With these straps, centralized pressure can cause the periphery of the electrodes to "flare" upwards reducing contact area (and, by extension, increasing current density). Given the apparent variability seen within and between individuals (outlined above), it is important to properly consider the influence these (and other) factors may be having on response characteristics.

CONCLUSION

Recently, several practitioners have noted concerns about modern tDCS conceptions and mechanistic models (Bikson, 2013; Paulus et al., 2013). In addition, a number of studies have also begun to explore response variation in response to adjustments

in current density, electrode position, and/or stimulation duration (Im et al., 2008; Bikson et al., 2010; Bastani and Jaberzadeh, 2013a,b). Although doubtless important, this work does not address the larger foundational issues raised in this paper.

Although we have chosen to focus on tDCS, many of the issues examined in this paper are applicable to other non-invasive modulatory tools; such as transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS). These devices are often modified tDCS devices and the protocols utilized by each are often modeled after modern tDCS protocols. Because of this, although there is not enough data in the literature to confidently discuss response variability and reliability, issues of blinding, interference, and electric current influences are highly relevant to these novel tools.

If the field of tDCS is to avoid becoming a footnote in the annals of neuroscientific research, it is time to collectively acknowledge there are shortcomings in our current understanding of this device, its functional parameters, its general efficacy, and its reliability. Rather than seeing the aforementioned issues as a detriment to the field, we should use them to guide future research and exploration. For instance, acknowledging variability can encourage us to explore individual response patterns (and correlate these with related secondary measures to tease-out possible state-dependency effects). Acknowledging the lack of effective sham and blinding techniques can encourage us to develop better more effective devices. Acknowledging the interference effect of motor and/or cognitive activity can inspire us to devise more comprehensive protocols. It is hoped that increased awareness and open discussion of these important issues will lead to a more rigorous and accurate foundation upon which tDCS can be developed into the future.

REFERENCES

Ahdab, R., Ayache, S. S., Brugieres, P., Goujon, C., and Lefaucheur, J. P. (2010). Comparison of "standard" and "navigated" procedures of TMS coil positioning over motor, premotor and prefrontal targets in patients with chronic pain and depression. *Clin. Neurophysiol.* 40, 27–36. doi: 10.1016/j.neucli.2010.01.001

Alonzo, A., Brassil, J., Taylor, J. L., Martin, D., and Loo, C. K. (2012). Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation. *Brain Stimul.* 5, 208–213. doi: 10.1016/j.brs.2011.04.006

Ambrus, G. G., Al-Moyed, H., Chaieb, L., Sarp, L., Antal, A., and Paulus, W. (2012). The fade-in–short stimulation–fade out approach to sham tDCS–reliable at 1 mA for naive and experienced subjects, but not investigators. *Brain Stimul.* 5, 499–504. doi: 10.1016/j.brs.2011.12.001

Antal, A., Terney, D., Poreisz, C., and Paulus, W. (2007). Towards unravelling task−related modulations of neuroplastic changes induced in the human motor cortex. *Eur. J. Neurosci.* 26, 2687–2691. doi: 10.1111/j.1460-9568.2007.05896.x

Bastani, A., and Jaberzadeh, S. (2013a). a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. *Brain Stimul.* 6, 932–937. doi: 10.1016/j.brs.2013.04.005

Bastani, A., and Jaberzadeh, S. (2013b). Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation. *PLoS ONE* 8:e72254. doi: 10.1371/journal.pone.0072254

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., and Nitsche, M. A. (2013). Partially non−linear stimulation intensity−dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bikson, M. (2013). Origins of specificity during tDCS: anatomical, activity-selective, and input-bias mechanisms. *Front. Hum. Neurosci.* 7:688. doi: 10.3389/fnhum.2013.00688

Bikson, M., Datta, A., Rahman, A., and Scaturro, J. (2010). Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. *Clin. Neurophysiol.* 121, 1976. doi: 10.1016/j.clinph.2010.05.020

Boros, K., Poreisz, C., Münchau, A., Paulus, W., and Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *Eur. J. Neurosci.* 27, 1292–1300. doi: 10.1111/j.1460-9568.2008.06090.x

Bradnam, L. V., Stinear, C. M., and Byblow, W. D. (2011). Cathodal transcranial direct current stimulation suppresses ipsilateral projections to presumed propriospinal neurons of the proximal upper limb. *J. Neurophysiol.* 105, 2582–2589. doi: 10.1152/jn.01084.2010

Bradnam, L. V., Stinear, C. M., Lewis, G. N., and Byblow, W. D. (2010). Task-dependent modulation of inputs to proximal upper limb following transcranial direct current stimulation of primary motor cortex. *J. Neurophysiol.* 103, 2382–2389. doi: 10.1152/jn.01046.2009

Brunoni, A. R., Schestatsky, P., Lotufo, P. A., Benseñor, I. M., and Fregni, F. (2013). Comparison of blinding effectiveness between sham tDCS and placebo sertraline in a 6-week major depression randomized clinical trial. *Clin. Neurophysiol.* doi: 10.1016/j.clinph.2013.07.020. [Epub ahead of print].

Datta, A., Truong, D., Minhas, P., Parra, L. C., and Bikson, M. (2012). Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. *Front. Psychiatry* 3:91. doi: 10.3389/fpsyt.2012.00091

Davis, N. J., Gold, E., Pascual-Leone, A., and Bracewell, R. M. (2013). Challenges of proper placebo control for non-invasive brain stimulation in clinical and experimental applications. *Eur. J. Neurosci.* 38, 2973–2977. doi: 10.1111/ejn.12307

Dawson, M. E., Schell, A. M., and Filion, D. L. (2001). "The electrodermal system," in *Handbook of Psycholphysiology*, eds J. T. Cacioppo, L. G. Tassinary, and G. G. Berntson (Cambridge, UK: Cambridge University Press), 159–181.

Di Lazzaro, V., Manganelli, F., Dileone, M., Notturno, F., Esposito, M., Capasso, M., et al. (2012). The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex. *J. Neural Transm.* 119, 1499–1506. doi: 10.1007/s00702-012-0845-4

Feughelman, M. (1997). *Mechanical Properties and Structure of Alpha-Keratin Fibres: Wool, Human Hair, and Related Fibres*. Sydney, NSW: University of New South Wales Press.

Fricke, K., Seeber, A. A., Thirugnanasambandam, N., Paulus, W., Nitsche, M. A., and Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *J. Neurophysiol.* 105, 1141–1149. doi: 10.1152/jn.00608.2009

Gálvez, V., Alonzo, A., Martin, D., and Loo, C. K. (2013). Transcranial direct current stimulation treatment protocols: should stimulus intensity be constant or incremental over multiple sessions. *Int. J. Neuropsychopharmacol.* 16, 13–21. doi: 10.1017/S1461145712000041

Hasan, A., Aborowa, R., Nitsche, M. A., Marshall, L., Schmitt, A., Gruber, O., et al. (2012). Abnormal bihemispheric responses in schizophrenia patients following cathodal transcranial direct stimulation. *Eur. Arch. Psychiatry Clin. Neurosci.* 262, 415–423. doi: 10.1007/s00406-012-0298-7

Hasan, A., Misewitsch, K., Nitsche, M. A., Gruber, O., Padberg, F., Falkai, P., et al. (2013). Impaired motor cortex responses in non-psychotic first-degree relatives of schizophrenia patients: a cathodal tDCS pilot study. *Brain Stimul.* 6, 821–829. doi: 10.1016/j.brs.2013.03.001

Herwig, U., Padberg, F., Unger, J., Spitzer, M., and Schönfeldt-Lecuona, C. (2001). Transcranial magnetic stimulation in therapy studies: examination of the reliability of "standard" coil positioning by neuronavigation. *Biol. Psychiatry* 50, 58–61. doi: 10.1016/S0006-3223(01)01153-2

Im, C. H., Jung, H. H., Choi, J. D., Lee, S. Y., and Jung, K. Y. (2008). Determination of optimal electrode positions for transcranial direct current stimulation (tDCS). *Phys. Med. Biol.* 53, N219. doi: 10.1088/0031-9155/53/11/N03

Inghilleri, M., Conte, A., Curra, A., Frasca, V., Lorenzano, C., and Berardelli, A. (2004). Ovarian hormones and cortical excitability. An rTMS study in humans. *Clin. Neurophysiol.* 115, 1063–1068. doi: 10.1016/j.clinph.2003.12.003

Jeffery, D. T., Norton, J. A., Roy, F. D., and Gorassini, M. A. (2007). Effects of transcranial direct current stimulation on the excitability of the leg motor cortex. *Exp. Brain Res.* 182, 281–287. doi: 10.1007/s00221-007-1093-y

Johansson, B. B. (2011). Current trends in stroke rehabilitation. A review with focus on brain plasticity. *Acta Neurol. Scand.* 123, 147–159. doi: 10.1111/j.1600-0404.2010.01417.x

Kuo, M. F., Grosch, J., Fregni, F., Paulus, W., and Nitsche, M. A. (2007). Focusing effect of acetylcholine on neuroplasticity in the human motor cortex. *J. Neurosci.* 27, 14442–14447. doi: 10.1523/JNEUROSCI.4104-07.2007

Kuo, M. F., Paulus, W., and Nitsche, M. A. (2008). Boosting focally-induced brain plasticity by dopamine. *Cereb. Cortex* 18, 648–651. doi: 10.1093/cercor/bhm098

Lang, N., Nitsche, M. A., Paulus, W., Rothwell, J. C., and Lemon, R. N. (2004a). Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Exp. Brain Res.* 156, 439–443. doi: 10.1007/s00221-003-1800-2

Lang, N., Siebner, H. R., Ernst, D., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004b). Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. *Biol. Psychiatry* 56, 634–639. doi: 10.1016/j.biopsych.2004.07.017

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC−stimulation−induced after−effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

List, J., Duning, T., Meinzer, M., Kürten, J., Schirmacher, A., Deppe, M., et al. (2011). Enhanced rapid-onset cortical plasticity in CADASIL as a possible mechanism of preserved cognition. *Cereb. Cortex* 21, 2774–2787. doi: 10.1093/cercor/bhr071

McCambridge, A. B., Bradnam, L. V., Stinear, C. M., and Byblow, W. D. (2011). Cathodal transcranial direct current stimulation of the primary motor cortex improves selective muscle activation in the ipsilateral arm. *J. Neurophysiol.* 105, 2937–2942. doi: 10.1152/jn.00171.2011

Miranda, P. C., Lomarev, M., and Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clin. Neurophysiol.* 117, 1623–1629. doi: 10.1016/j.clinph.2006.04.009

Miyaguchi, S., Onishi, H., Kojima, S., Sugawara, K., Tsubaki, A., Kirimoto, H., et al. (2013). Corticomotor excitability induced by anodal transcranial direct current stimulation with and without non-exhaustive movement. *Brain Res.* 1529, 83–91.

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., et al. (2012). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul.* 6, 424–432. doi: 10.1016/j.brs.2012.04.011

Monte-Silva, K., Kuo, M. F., Liebetanz, D., Paulus, W., and Nitsche, M. A. (2010). Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). *J. Neurophysiol.* 103, 1735–1740. doi: 10.1152/jn.00924.2009

Monte-Silva, K., Kuo, M. F., Thirugnanasambandam, N., Liebetanz, D., Paulus, W., and Nitsche, M. A. (2009). Dose-dependent inverted U-shaped effect of dopamine (D2-like) receptor activation on focal and nonfocal plasticity in humans. *J. Neurosci.* 29, 6124–6131. doi: 10.1523/JNEUROSCI.0728-09.2009

Munneke, M. A., Stegeman, D. F., Hengeveld, Y. A., Rongen, J. J., Schelhaas, H. J., and Zwarts, M. J. (2011). Transcranial direct current stimulation does not modulate motor cortex excitability in patients with amyotrophic lateral sclerosis. *Muscle Nerve* 44, 109–114. doi: 10.1002/mus.22012

Nitsche, M. A., Doemkes, S., Karakoese, T., Antal, A., Liebetanz, D., Lang, N., et al. (2007a). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *J. Neurophysiol.* 97, 3109–3117. doi: 10.1152/jn.01312.2006

Nitsche, M. A., Roth, A., Kuo, M. F., Fischer, A. K., Liebetanz, D., Lang, N., et al. (2007b). Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. *J. Neurosci.* 27, 3807–3812. doi: 10.1523/JNEUROSCI.5348-06.2007

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003a). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J. Physiol.* 553, 293–301. doi: 10.1113/jphysiol.2003.049916

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., and Paulus, W. (2003b). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin. Neurophysiol.* 114, 600–604. doi: 10.1016/S1388-2457(02)00412-1

Nitsche, M. A., Grundey, J., Liebetanz, D., Lang, N., Tergau, F., and Paulus, W. (2004a). Catecholaminergic consolidation of motor cortical neuroplasticity in humans. *Cereb. Cortex* 14, 1240–1245. doi: 10.1093/cercor/bhh085

Nitsche, M. A., Liebetanz, D., Schlitterlau, A., Henschke, U., Fricke, K., Frommann, K., et al. (2004b). GABAergic modulation of DC stimulation−induced motor cortex excitability shifts in humans. *Eur. J. Neurosci.* 19, 2720–2726. doi: 10.1111/j.0953-816X.2004.03398.x

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., and Paulus, W. (2004c). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology* 29, 1573. doi: 10.1038/sj.npp.1300517

Nitsche, M. A., Kuo, M. F., Karrasch, R., Wächter, B., Liebetanz, D., and Paulus, W. (2009). Serotonin affects transcranial direct current–induced neuroplasticity in humans. *Biol. Psychiatry* 66, 503–508. doi: 10.1016/j.biopsych.2009.03.022

Nitsche, M. A., Lampe, C., Antal, A., Liebetanz, D., Lang, N., Tergau, F., et al. (2006). Dopaminergic modulation of long−lasting direct current−induced cortical excitability changes in the human motor cortex. *Eur. J. Neurosci.* 23, 1651–1657. doi: 10.1111/j.1460-9568.2006.04676.x

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 57, 1899–1901. doi: 10.1212/WNL.57.10.1899

O'Connell, N. E., Cossar, J., Marston, L., Wand, B. M., Bunce, D., Moseley, G. L., et al. (2012). Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2 mA. *PLoS ONE* 7:e47514. doi: 10.1371/journal.pone.0047514

Palm, U., Reisinger, E., Keeser, D., Kuo, M. F., Pogarell, O., Leicht, G., et al. (2013). Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. *Brain Stimul.* 6, 690–695. doi: 10.1016/j.brs.2013.01.005

Paulus, W. (2011). Transcranial electrical stimulation (tES–tDCS; tRNS, tACS) methods. *Neuropsychol. Rehabil.* 21, 602–617. doi: 10.1080/09602011.2011.557292

Paulus, W., Peterchev, A. V., and Ridding, M. (2013). "Transcranial electric and magnetic stimulation: technique and paradigms," in *Brain Stimulation E-Book: Handbook of Clinical Neurology*, Vol. 116, Series eds M. J. Aminoff, F. Boller, and D. F. Swaab (Amsterdam, ND: Elsevier B.V.), 329.

Power, H. A., Norton, J. A., Porter, C. L., Doyle, Z., Hui, I., and Chan, K. M. (2006). Transcranial direct current stimulation of the primary motor cortex affects cortical drive to human musculature as assessed by intermuscular coherence. *J. Physiol.* 577, 795–803. doi: 10.1113/jphysiol.2006.116939

Quartarone, A., Morgante, F., Bagnato, S., Rizzo, V., Sant'Angelo, A., Aiello, E., et al. (2004). Long lasting effects of transcranial direct current stimulation on motor imagery. *Neuroreport* 15, 1287–1291. doi: 10.1097/01.wnr.0000127637.22805.7c

Quartarone, A., Rizzo, V., Bagnato, S., Morgante, F., Sant'Angelo, A., Romano, M., et al. (2005). Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia. *Brain* 128, 1943–1950. doi: 10.1093/brain/awh527

Roche, N., Lackmy, A., Achache, V., Bussel, B., and Katz, R. (2011). Effects of anodal transcranial direct current stimulation over the leg motor area on lumbar spinal network excitability in healthy subjects. *J. Physiol.* 589, 2813–2826. doi: 10.1113/jphysiol.2011.205161

Sale, M. V., Ridding, M. C., and Nordstrom, M. A. (2008). Cortisol inhibits neuroplasticity induction in human motor cortex. *J. Neurosci.* 28, 8285–8293. doi: 10.1523/JNEUROSCI.1963-08.2008

Sale, M. V., Ridding, M. C., and Nordstrom, M. A. (2010). Circadian modulation off neuroplasticity in humans and potential therapeutic implications. *Rev. Neurosci.* 21, 55–66. doi: 10.1515/REVNEURO.2010.21.1.55

Scelzo, E., Giannicola, G., Rosa, M., Ciocca, M., Ardolino, G., Cogiamanian, F., et al. (2011). Increased short latency afferent inhibition after anodal transcranial direct current stimulation. *Neurosci. Lett.* 498, 167–170. doi: 10.1016/j.neulet.2011.05.007

Schabrun, S. M., Chipchase, L. S., Zipf, N., Thickbroom, G. W., and Hodges, P. W. (2012). Interaction between simultaneously applied neuromodulatory interventions in humans. *Brain Stimul.* 6, 624–630. doi: 10.1016/j.brs.2012.09.009

Schade, S., Moliadze, V., Paulus, W., and Antal, A. (2012). Modulating neuronal excitability in the motor cortex with tDCS shows moderate hemispheric asymmetry due to subjects' handedness: a pilot study. *Restor. Neurol. Neurosci.* 30, 191–198. doi: 10.3233/RNN-2012-110175

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J. Neurosci.* 24, 3379–3385. doi: 10.1523/JNEUROSCI.5316-03.2004

Simis, M., Adeyemo, B. O., Medeiros, L. F., Miraval, F., Gagliardi, R. J., and Fregni, F. (2013). Motor cortex-induced plasticity by noninvasive brain stimulation: a comparison between transcranial direct current stimulation and transcranial magnetic stimulation. *Neuroreport* 24, 973–975. doi: 10.1097/WNR.0000000000000021

Smith, M. J., Keel, J. C., Greenberg, B. D., Adams, L. F., Schmidt, P. J., Rubinow, D. A., et al. (1999). Menstrual cycle effects on cortical excitability. *Neurology* 53, 2069–2069. doi: 10.1212/WNL.53.9.2069

Sparing, R., Buelte, D., Meister, I. G., Pauš, T., and Fink, G. R. (2008). Transcranial magnetic stimulation and the challenge of coil placement: a comparison of conventional and stereotaxic neuronavigational strategies. *Hum. Brain Mapp.* 29, 82–96. doi: 10.1002/hbm.20360

Stagg, C. J., and Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *Neuroscientist* 17, 37–53. doi: 10.1177/1073858410386614

Suzuki, K., Fujiwara, T., Tanaka, N., Tsuji, T., Masakado, Y., Hase, K., et al. (2012). Comparison of the after-effects of transcranial direct current stimulation over the motor cortex in patients with stroke and healthy volunteers. *Int. J. Neurosci.* 122, 675–681. doi: 10.3109/00207454.2012.707715

Thirugnanasambandam, N., Sparing, R., Dafotakis, M., Meister, I. G., Paulus, W., Nitsche, M. A., et al. (2011). Isometric contraction interferes with transcranial direct current stimulation (tDCS) induced plasticity–evidence of state-dependent neuromodulation in human motor cortex. *Restor. Neurol. Neurosci.* 29, 311–320. doi: 10.3233/RNN-2011-0601

Tremblay, S., Beaulé, V., Lepage, J. F., and Théoret, H. (2013). Anodal transcranial direct current stimulation modulates GABAB-related intracortical inhibition in the M1 of healthy individuals. *Neuroreport* 24, 46–50. doi: 10.1097/WNR.0b013e32835c36b8

Truong, D. Q., Magerowski, G., Blackburn, G. L., Bikson, M., and Alonso-Alonso, M. (2013). Computational modeling of transcranial direct current stimulation (tDCS) in obesity: impact of head fat and dose guidelines. *Neuroimage Clin.* 2, 759–766. doi: 10.1016/j.nicl.2013.05.011

Wagner, T., Fregni, F., Fecteau, S., Grodzinsky, A., Zahn, M., and Pascual-Leone, A. (2007). Transcranial direct current stimulation: a computer-based human model study. *Neuroimage* 35, 1113–1124. doi: 10.1016/j.neuroimage.2007.01.027

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 December 2013; accepted: 08 January 2014; published online: 24 January 2014.

Citation: Horvath JC, Carter O and Forte JD (2014) Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). Front. Syst. Neurosci. ***8****:2. doi: 10.3389/fnsys.2014.00002*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

TECHNOLOGY REPORT
published: 09 March 2015
doi: 10.3389/fnsys.2015.00027

Wearable functional near infrared spectroscopy (fNIRS) and transcranial direct current stimulation (tDCS): expanding vistas for neurocognitive augmentation

Ryan McKendrick[1], *Raja Parasuraman*[1] *and Hasan Ayaz*[2]*

[1] Center of Excellence in Neuroergonomics, Technology, and Cognition (CENTEC), George Mason University, Fairfax, VA, USA, [2] School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

Contemporary studies with transcranial direct current stimulation (tDCS) provide a growing base of evidence for enhancing cognition through the non-invasive delivery of weak electric currents to the brain. The main effect of tDCS is to modulate cortical excitability depending on the polarity of the applied current. However, the underlying mechanism of neuromodulation is not well understood. A new generation of functional near infrared spectroscopy (fNIRS) systems is described that are miniaturized, portable, and include wearable sensors. These developments provide an opportunity to couple fNIRS with tDCS, consistent with a neuroergonomics approach for joint neuroimaging and neurostimulation investigations of cognition in complex tasks and in naturalistic conditions. The effects of tDCS on complex task performance and the use of fNIRS for monitoring cognitive workload during task performance are described. Also explained is how fNIRS + tDCS can be used simultaneously for assessing spatial working memory. Mobile optical brain imaging is a promising neuroimaging tool that has the potential to complement tDCS for realistic applications in natural settings.

Keywords: HDtDCS, fNIRS, neuroergonomics, DLPFC, spatial working memory

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Andrea Kübler, University of Würzburg, Germany
Makii Muthalib, Universite de Montpellier 1, France

***Correspondence:**
Hasan Ayaz, School of Biomedical Engineering, Science and Health Systems, Drexel University, 3508 Market Street, Monell Suite 101, Philadelphia, PA 19104, USA
hasan.ayaz@drexel.edu

Received: *01 November 2014*
Accepted: *14 February 2015*
Published: *09 March 2015*

Citation:
McKendrick R, Parasuraman R and Ayaz H (2015) Wearable functional near infrared spectroscopy (fNIRS) and transcranial direct current stimulation (tDCS): expanding vistas for neurocognitive augmentation. Front. Syst. Neurosci. 9:27. doi: 10.3389/fnsys.2015.00027

Introduction

The rediscovery, over a decade ago (Nitsche and Paulus, 2000), of transcranial brain stimulation has led to a proliferation of research on brain and cognitive augmentation, both in healthy adults and in patients with neurological or psychiatric disease (Clark and Parasuraman, 2014). Augmentation refers to the improvement of cognitive functioning through task performance, or reversal of cognitive deficits that are normal consequences of performance in healthy adults (e.g., fatigue, stress) or those related to brain disorders. Ayaz et al., 2006; Hunter et al., 2013). Although the initial motivating rationale for the use of techniques such as transcranial Direct Current Stimulation (tDCS) was to develop alternative therapies for the treatment of neuropsychiatric diseases, augmentation effects were also seen in the healthy participants (Clark and Parasuraman, 2014; Flöel, 2014). These findings led to the current interest in developing methods of neurocognitive enhancement for healthy adults, for example to enhance human performance in complex tasks (such as air traffic control) or to accelerate skill acquisition in tasks (such as piloting unmanned vehicles) that typically require many hours or days of practice to master

(Coffman et al., 2014; Parasuraman and Mckinley, 2014). Contemporary tDCS studies have provided a growing base of evidence for enhancing cognition through the non-invasive delivery of weak electric currents to the brain (Coffman et al., 2014). The main effect of tDCS is to modulate cortical excitability, depending on the polarity of the applied current. However, the underlying mechanism for the neuromodulation, such as how it is induced, how longs it persists, and the ways in which such modulation translate into improvement in performance are still not well understood and are currently the object of much research interest. Combining tDCS with multimodal neuroimaging techniques can enhance knowledge of its neuromodulatory effects in the brain (Hunter et al., 2013).

Traditional neuroimaging modalities such as functional magnetic resonance imaging (fMRI) have also been successfully utilized for studying cognition and understanding the neural mechanisms that contribute to the acquisition, development, and use of cognitive skills in artificial, controlled and stand-alone settings. These can be referred as read-only settings where functional neuroimaging is used to record brain activation and hence the flow of information is from brain to a computer. Moreover, modulation of neural signals can also be achieved through a neurofeedback training where a computer presents some derivative of the acquired brain signal in real-time back to user in visual or auditory form to establish the feedback loop (Hanslmayr et al., 2005; Gruzelier, 2009; Miller et al., 2010; Slagter et al., 2011; Ninaus et al., 2013). Neurofeedback training aims to allow volitional control of specific brain activity and has been extensively used in clinical neurorehabilitation or brain disorders such as attention-deficit hyperactivity, autism, epilepsy and mood disorders (Lubar et al., 1995; Hoffman et al., 1996; Raymond et al., 2005; Angelakis et al., 2007; Kouijzer et al., 2009; Lim et al., 2012; Heinrich et al., 2014). Neurofeedback training has been shown to enhance performance in cognitive tasks (Angelakis et al., 2007; Gruzelier, 2009) however, in this manuscript, we focused on tDCS based neuromodulation as it does not require training and has been utilized for human computer interaction applications (Clark and Parasuraman, 2014). Neuroimaging methods based on the MRI technique, such as functional MRI, resting state functional connectivity, and diffusion tensor analysis, have provided important information on the gray matter, white matter, and brain connectivity changes that accompany skill acquisition (Lewis et al., 2009; Lövdén et al., 2010; Voss et al., 2012; Strenziok et al., 2014), thus setting the stage for the development of theories of neuroplasticity, specifically for functional reorganization of neural networks and adaptation. For a review, see Elbert and Rockstroh (2004). However, some limitations of MRI are its requirement for participant immobility and its high operational cost. These factors have stimulated a need for lower-cost neuroimaging techniques that are portable and can be used in freely moving participants performing everyday tasks (Gramann et al., 2011, 2014). Among these are electroencephalography (EEG) and functional near infrared spectroscopy (fNIRS), both of which can be used for mobile brain imaging (Makeig et al., 2009; Gramann et al., 2011; Mehta and Parasuraman, 2013). The use of mobile brain imaging and stimulation techniques also falls within the field of neuroergonomics, defined as the study of the human brain in relation to performance at work and everyday settings (Parasuraman, 2003, 2011; Parasuraman and Rizzo, 2007). The main goal of neuroergonomics is to advance knowledge of brain functions in complex tasks and naturalistic work settings.

Overview of Paper

The neuroergonomic approach has been considerably facilitated by the recent rise of development of portable and wearable neuroimaging devices, including EEG and fNIRS (Gramann et al., 2014). In this paper we review the potential uses of joint fNIRS and tDCS and describe wireless and battery operated fNIRS sensors (Ayaz et al., 2013) that provide new opportunities for brain and cognitive augmentation. We first briefly describe tDCS studies for enhancing skill acquisition in complex cognitive tasks. It is particularly important to assess and measure operator mental workload in situations where performance failures could result in catastrophic losses (e.g., military command and control, air traffic control, etc.). Improving operators' cognitive abilities (such as working memory or attention) would help improve overall safety and productivity in such systems. Next we review tDCS studies that have targeted and assessed human operator performance. We then describe how fNIRS can be used to monitor brain dynamics during cognitive tasks, with a focus on evaluating effects on cognitive load. As a wearable and continuous monitoring sensor, fNIRS provides a safe and practical approach for monitoring brain activity in natural environments. We review studies that demonstrate task load related activity in the fNIRS signal. Next, we examine the combined use of fNIRS and tDCS for monitoring and enhancement of spatial working memory. Moreover, fNIRS + tDCS can realize new applications that were not possible before, such as "read-write" Brain Computer Interfaces (BCI) which can acquire (read) brain signals and also provide feedback directly to the brain (write) through stimulation. In general, optical brain imaging techniques such as fNIRS are a promising neuroimaging method and as the instruments continue to evolve, have the potential to become a complementary tool to tDCS for neuroergonomic applications in complex work tasks and in natural settings.

Effects of tDCS on Complex Task Performance

Many noninvasive brain stimulation techniques for enhancing neurocognitive function exist, including transcranial magnetic stimulation (TMS) and tDCS (Clark and Parasuraman, 2014). In TMS an electric current is transiently passed through a magnetic coil positioned over the participant's scalp over a brain region of interest. This creates a changing magnetic field that passes through the skull and induces current flow in the underlying cortical tissue sufficient to alter neural firing (Walsh and Pascual-Leone, 2005). tDCS involves application of a weak direct current (DC) electric current (1–2 mA) with electrodes attached to the scalp. A positive polarity (anode) is typically used

to facilitate neuronal firing whereas a negative polarity (cathode) is used to inhibit neuronal firing. Application of tDCS is safe for experimental use in healthy participants for up to 30 min of stimulation (Bikson et al., 2009).

Understanding the mechanisms by which the tDCS modulations are induced and persist is still an open question. Initially, it was thought that application of weak DC current increases the resting neuronal membrane potential and thus lowers the threshold for firing of neurons (Bindman et al., 1964), but subsequent work suggests that other mechanisms are probably involved, such as dynamic modulation of synaptic efficacy (Rahman et al., 2013) and changes in neurotransmitter concentrations (Clark et al., 2011). Pharmacological tDCS studies also suggest neuronal membrane depolarisation during anodal stimulation may be responsible for the after-effects on cortical excitability (Liebetanz et al., 2002).

tDCS can be applied to better understand brain mechanisms and their relation to cognitive processes, although tDCS is not as focal in activating or inhibiting brain regions in comparison to TMS given the diffusivity of current flow for anode over region of interest, extra-cephalic cathode montages ("ring" montages; anode over region of interest encircled by multiple cathodes provide more focal stimulation but not to the level implemented by TMS (Datta et al., 2009)). Recent tDCS studies have allowed researchers to make inferences regarding the neural basis of learning, memory, perception, and motor actions (Filmer et al., 2014). The study by Holland et al. (2011) investigated language function of healthy participants and aimed to help develop the approach for potential clinical deployment for rehabilitation of brain-damaged patients. Authors utilized fMRI to monitor and localize the effects of tDCS stimulation concurrently. Left frontal anodal tDCS was used during an overt picture-naming task and results provided important evidence of contribution of the left inferior frontal cortex in the naming task and identified Broca's area for tDCS based rehabilitation (Holland et al., 2011).

Another example is a study by Clarke et al. (2014) in which the role of dorsolateral prefrontal cortex in attention bias modification (ABM) was investigated. The exaggerated attention to mildly threatening conditions is defined as the attention bias to threat and has been reliably observed across a range of anxiety and mood disorders. Reducing attention to threat in high anxiety patients has been demonstrated to also reduce anxiety symptoms, and thus suggests the promise of treatment of anxiety pathology. The authors utilized tDCS to isolate and test fMRI findings reported earlier by Browning et al. (2010) which implicated lateral prefrontal cortex in inhibitory control of attention in relation to threatening information. This study by Clarke et al. (2014) demonstrated the complementary nature of neuroimaging and neurostimulation (as the finding verified the functional MRI results of Browning et al. (2010)); and, highlights the potential power of joint neuroimaging and neurostimulation for novel interventions while establishing a broad neurocognitive framework. Below we further examine such joint investigations by combining tDCS and fNIRS.

Many studies have found that stimulation of different brain regions with tDCS can enhance performance of basic cognitive tasks that recruit the corresponding brain regions. For example, stimulating the dorso-lateral prefrontal cortex (DLPFC), which has been shown in neuroimaging studies to be involved in working memory, accordingly enhances performance on working memory tasks (Fregni et al., 2005). Beyond working memory, tDCS has also been found to enhance learning and performance on a wide variety of perceptual, cognitive, and motor tasks (for reviews, see Jacobson et al. (2012) and Coffman et al. (2014)). Here we provide a few examples of the effects of tDCS on more complex tasks representative of work settings.

One example involves surveillance and security operations, as in threat detection (Parasuraman and Galster, 2013). Accurate and timely detection of obscured or concealed objects, or the actions and movements of other people, is a critical need in many such work environments, both in the military and in civilian organizations. Skill in such threat detection tasks typically develops only after extensive training lasting many days. Can the development of expertise be speeded up with tDCS? Recent studies provide a positive answer (Clark et al., 2012; Falcone et al., 2012). These studies involved use of a complex task requiring participants to watch videos of naturalistic scenes containing movements of soldiers and civilians. Still images were extracted from the videos and manipulated so that half were targets, defined as concealed objects (e.g., bombs), people engaging in threatening activity (e.g., snipers), and so on, whereas the same scene without the threat was a non-target. An fMRI study was first conducted to determine optimal sites for application of tDCS (Clark et al., 2012). A total of 104 participants volunteered for the study and were imaged as novices. A subset, 13 participants performed the task during fMRI data collection to identify the brain networks supporting the identification of concealed objects and changes with learning. The results indicated that the right inferior frontal gyrus was the major locus of a distributed brain network that mediated acquisition of the threat detection task and so was chosen as the optimal stimulation site.

Falcone et al. (2012) examined whether tDCS applied to this location enhanced perceptual sensitivity in threat detection. Participants were given four training blocks of and were required to indicate whether a threat was present or absent. Two test blocks were given before training and were similar to training blocks, except that no feedback was given after each response. Anodal tDCS was applied to the electrode site F10 in the EEG system, over the right sphenoid bone, corresponding to an area overlying the inferior frontal gyrus. Although this is not as precise as subject-fMRI guided location selection, anatomical landmarks using international 10–20 system provided a viable solution which was confirmed by the results of the study. The cathode was placed on the contralateral (left) upper arm. Participants were randomly assigned to either active (2 mA current) or sham stimulation (0.1 mA) for a total of 30 min during the first two training blocks.

Compared to the 0.1 mA sham stimulation control, 2 mA stimulation increased perceptual sensitivity in detecting targets and accelerated learning. Performance was near chance ($d' = 0$) in both groups at the beginning of training. However, skill

acquisition with tDCS was both rapid and extensive: On completion of training, participants in the active stimulation group had more than double the d' of the control group. There were no group or training effects on the response bias measure β, indicating that tDCS improved the actual efficiency of threat detection. Furthermore, threat detection sensitivity remained at a high level immediately after training and, more importantly, 24 h later. This last finding bodes well for the use of tDCS as a training method with potentially lasting effects in naturalistic work tasks.

A second example involves intelligence analysis, McKinley et al. (2013) trained image analysts to find and correctly identify ground targets, such as tanks and surface-to-air missile launchers, in synthetic aperture radar imagery. Stimulation of the right frontal cortex, using the same anodal F10 scalp location (cathode on the contralateral bicep) as in the previously described study of Falcone et al. (2012), significantly improved object recognition learning rates. During the first phase of training, one group was given active tDCS for 30 min; another, sham tDCS (active tDCS for 30 s); and a third group, no tDCS. Participants were then given a second round of training with the stimulation conditions reversed (i.e., the active tDCS group switched to sham tDCS, whereas the sham tDCS group received active tDCS in the second round). Both groups experienced larger increases in target acquisition accuracy when given active tDCS when compared to sham or no stimulation in either session. The image analysis task also included a change detection task in both training sessions. After the target image was complete, one of the targets (randomly assigned) changed in orientation, position, target type, or disappeared completely. Change detection performance was improved only when tDCS was applied in the second session. Thus, tDCS aided in change detection only after the analyst gained some experience with the images and target types. A similar finding was reported by Coffman et al. (2012), who found that tDCS had a larger effect on threat detection for images that had been viewed previously. These findings may reflect tDCS-induced plasticity changes in the brain networks responsible for object encoding and retrieval.

These are just two examples of the effectiveness of tDCS as a neuroergonomic tool for accelerating skill acquisition in complex, work-relevant tasks. Other examples are reviewed by Parasuraman and Mckinley (2014). Prior neuroimaging evidence suggests that such performance gains probably resulted from activation of specific brain networks associated with the relevant cognitive functions. However, direct evidence of modulation of brain dynamics would provide stronger evidence for such an association. Below, we examine how the combined use of fNIRS and tDCS can help in this endeavor. We begin, however, with a brief overview of the use of fNIRS alone in studies of cognitive workload.

Using fNIRS to Monitor the Relationship of Cognitive Workload and Brain Dynamics

fNIRS provides an attractive method for continuous monitoring of brain dynamics in both seated or mobile participants. fNIRS is safe, highly portable, user-friendly and relatively inexpensive, with rapid application times and near-zero run-time costs (Villringer and Chance, 1997; Ferrari and Quaresima, 2012). The most commonly used form of fNIRS uses infrared light, introduced at the scalp, to measure changes in blood oxygenation as oxy-hemoglobin converts to deoxy-hemoglobin during neural activity, i.e., the cerebral hemodynamic response. fNIRS uses specific wavelengths of light to provide measures of cerebral oxygenated and deoxygenated hemoglobin that are correlated with the fMRI BOLD signal (Cui et al., 2011; Sato et al., 2013). Below we briefly review fNIRS studies of cognitive workload.

For objective measures of cognitive workload in naturalistic environments, fNIRS offers a number of advantages over other measurement techniques such as fMRI. In particular, the high operational costs of fMRI makes long-duration or longitudinal (e.g., training) studies impractical. Cost is less of an issue with fNIRS as the systems themselves are less expensive and once purchased require no extra costs to run. fNIRS also does not require the participant to be immobile and the use of wireless fNIRS allows for imaging brain dynamics during tasks that require a participant to move regularly, as in motor and other physical tasks (Mehta and Parasuraman, 2014) and in naturalistic settings (Ayaz et al., 2013). fNIRS also offers a compromise between the spatial resolution of fMRI and temporal resolution of EEG. The superior spatial resolution (localization of activation) of fNIRS relative to EEG allows for greater accuracy in identifying specific brain regions responding to changes in workload. The superior temporal resolution (higher sampling rate) of fNIRS relative to fMRI affords improved statistical power when analyzing changes in the shape of the hemodynamic response.

fNIRS has proven beneficial for measuring workload in a number of complex tasks. Examples include supervisory control, natural orifice surgery simulations, and driving. In a study of air traffic controllers, Ayaz et al. (2012) found that as the number of supervised aircraft increased there was an increase in cerebral oxygenation (oxygenated hemoglobin minus deoxygenated hemoglobin) in the left medial/orbito frontal cortex. The relationship was linear and corresponded with increased oxygenation observed in the same sample during a multi-load N-back working memory task (Ayaz et al., 2012). Similarly during natural orifice translumenal endoscopic surgery (NOTES) simulation experienced surgeons familiar with NOTES showed increases in oxygenated hemoglobin in bilateral ventral lateral prefrontal cortex (VLPFC) when the simulation required a more difficult navigation path through an orifice (James et al., 2011).

fNIRS measurement of mental workload has also been used within the context of driving. In two separate studies while individuals drove on a closed road it was observed that deceleration increased oxygenated hemoglobin in regions involved in eye movements and optic flow (Yoshino et al., 2013a,b). The results indicated that deceleration is more cognitively taxing on visual processing than acceleration or constant velocity driving. Increases in oxygenated hemoglobin in bilateral VLPFC during U-turns was also observed (Yoshino et al., 2013b), suggesting the need for increased executive control relative to acceleration, deceleration, and constant velocity driving. Other recent studies also demonstrated the potential of

fNIRS for assessment of cognitive workload (Abibullaev and An, 2012; Naseer and Keum-Shik, 2013; Afergan et al., 2014; Bogler et al., 2014; Derosière et al., 2014; Herff et al., 2014; Schudlo and Chau, 2014; Solovey et al., 2015).

Although a linear relationship between task workload and hemodynamics has often been observed (Ayaz et al., 2012; Fishburn et al., 2014) where the difficulty of the task at hand does not exceed the cognitive capacity of participant, whereas when cognitive capacity is exceeded the observed effects on hemodynamics conform to the shape reported by the Yerkes--Dodson law (Yerkes and Dodson, 1908). On a supervisory control task a negative quadratic relationship (inverted U) between workload and DLPFC activation was found (Durantin et al., 2014). Individuals were asked to control remotely operated vehicles as they navigated through an airspace while avoiding no fly zones. Workload was manipulated by altering crosswinds, vehicle inertia and memory load regarding supervisory control. It was also noted that there was a strong correlation between increased DLPFC activation in the highest workload condition and performance. This actually suggests that workload alone does not have a quadratic relationship with functional hemodynamics, but instead once mental overload is reached functional activation decreases. Evidence from two other studies supports this claim. Yamauchi et al. (2013) had participants play a modified version of "rock, paper, scissors" against a computer, with the objective to actually lose each hand. The computer presented one of the three hands and the participant had to choose the losing hand. Workload was manipulated by decreasing the inter stimulus interval (ISI). Furthermore these decreases were adapted to each participants minimum effective ISI. When workload was manipulated as a function of an individual's maximum workload, only linear increases in oxygenated hemoglobin were observed in left lateral prefrontal cortex, premotor cortex and supplementary motor area (Yamauchi et al., 2013).

Similarly in a dual-working memory training study when task memory load increased as a function of participant's skill acquisition, a strong linear increase in total hemoglobin after an initial decrease in activation occurred while participants adapted to the task. However a different group of participants had their task memory load yoked to the performance of the other group, and they showed a negative quadratic relationship between memory load and total hemoglobin (McKendrick et al., 2014). Taken together these findings suggest that the presence of a negative quadratic slope during fNIRS monitoring of workload dynamics is indicative of task overload. This trend can be used to assess the points at which overload occurs for individuals, or as a means of ensuring that tests of workload only include load up to an individual's maximum effective capability. This can be used to optimize operator work periods, introducing adaptive automation (Byrne and Parasuraman, 1996), or delegation of tasks to other operators as methods of optimizing operator efficiency and system performance. It is also apparent that an individual's maximum effective workload can change with task training. Therefore this trend may occur concurrently with an individual's skill acquisition and non-linear components should be included within statistical models of workload dynamics to observe and utilize this quadratic trend.

The changes in oxygenated and deoxygenated hemoglobin representative of mental workload may not only arise from cognitive work. Both physical and emotional work can affect and potentially invalidate measures of cognitive workload. Submaximal physical effort can reduce mental performance, furthermore increasing submaximal physical effort has similar effects on oxygenated and deoxygenated hemoglobin as moving from a single cognitive task to a dual cognitive task (Mandrick et al., 2013). Mental and physical work to exhaustion may also cause cognitive interference resulting in decreased oxygenated and increased deoxygenated hemoglobin in prefrontal cortex (Mehta and Parasuraman, 2013).

Monitoring the Effects of tDCS on Brain Dynamics Using fNIRS

There is now considerable evidence that tDCS can boost brain plasticity processes and accelerate skill acquisition in complex cognitive tasks (Clark and Parasuraman, 2014). Less well known, however, is the neural changes that make such performance gains possible. There are only a few investigations of simultaneous neuroimaging and stimulation studies, such as using fMRI (Alon et al., 2011; Antal et al., 2011; Holland et al., 2011; Kwon and Jang, 2011). However, the electric current flow of tDCS can create confounds in simultaneous fMRI echo-planar imaging (Antal et al., 2014). For a review of neuroimaging artifacts and limitations during simultaneous tDCS and fMRI, see Saiote et al. (2013) and Antal et al. (2014). Hence, a neuroimaging tool is needed that is inherently independent of electrical stimulation. As an optical imaging technique fNIRS provides one such neuroimaging approach.

Combining fNIRS with tDCS can provide some insights for understanding brain plasticity associated with skill acquisition. An initial basic research direction for joint use of fNIRS and tDCS is for understating how tDCS effects the brain in both animal models (Han et al., 2014) and human studies (Merzagora et al., 2010; Khan et al., 2013; Muthalib et al., 2013; Ishikuro et al., 2014; Jones et al., 2015). Merzagora et al. (2010) reported on the anterior prefrontal cortex effects of tDCS before and after stimulation using a prefrontal sensor pad based fNIRS measurement. Results indicated that fNIRS successfully captured the activation changes induced by the tDCS stimulation. Khan et al. (2013) compared altered hemodynamic patterns in the sensorimotor cortex in response to bi-hemispheric tDCS polarities and their relationship to muscle activity and motor task performance. Muthalib et al. (2013) utilized anodal tDCS based motor cortex stimulation to study neuromuscular fatigue and task failure related prefrontal cortex activation measured by fNIRS. Ishikuro et al. (2014) studied the relationship between frontal and sensorimotor cortices and motor learning of tasks used in rehabilitation. Healthy participants performed the task using a whole head fNIRS system. The neuroimaging session was used to identify relevant brain area (anterior dorsomedial prefrontal cortex) for stimulation in a separate experiment. Participants performed the same task with and without tDCS. Authors reported significant effects of tDCS and improvement in performance with stimulation. Jones et al. (2015) investigated

the role of motivation (incentives) and tDCS in improving performance for both high and low working memory capacity participants. Authors used fNIRS to assess the cortical effects and brain activity changes.due to tDCS stimulation. The underlying motivation of such joint stimulation and neuroimaging studies is to extend boundaries of knowledge on brain-behavior relationships and translate the acquired knowledge for potential clinical and neuroergonomic applications.

Combined tDCS-fNIRS: Neuroergonomics Pilot Study

We illustrate the utility of the combination of simultaneous tDCS and fNIRS techniques in a study examining the effects of tDCS on spatial working memory. The task involved recalling the location of 5–7 randomly spaced black disks on a computer display after a short retention period. Each trial began with 15 s of fixation followed by a 1 s presentation of 5–7 randomly spaced black disks. A 4 s random noise mask was displayed after the presentation of the stimulus, after which participants were instructed to respond and recall the number and positions of the stimulus. A more complete description of the task is presented in McKendrick et al. (2014).

Participants received a block of baseline trials, followed by two blocks of sham stimulation, one block of 1 mA stimulation using a high-density tDCS montage, and a final block of continued stimulation monitoring, with each block consisting of 33 trials. Participants were fitted with an elastomere cap with high density tDCS (HD-tDCS) electrode holders positioned at F2 and F10 in the 10–20 EEG system. A Ag/AgCl sintered ring electrode was placed in each holder along with electroconductive gel to conduct the current to the scalp (See Villamar et al., 2013 for a more detailed description of the Soterix HD-tDCS system). An fNIR Devices Model 1100 NIRS imaging device sensor that has 16 optodes (10 photodetectors and 4 light emitters each using 730 nm and 850 nm wavelengths of light) was attached to the forehead for monitoring changes in frontal oxygenated and deoxygenated hemoglobin (for a more complete description of the fNIR Devices Model 1100 NIRS see Ayaz et al., 2012).

A pilot study using this task had identified a region of right VLPFC that showed an increase in activity during the task period relative to fixation (McKendrick et al., 2014). This region also showed a correlation between increased performance and increased neural efficiency (increased performance negatively modulated increases in activity amplitude). As such this region was selected for stimulation via HDtDCS and is an example of fNIRS guided tDCS. Using the modeling software HD-explore (Soterix Medical), we constructed a montage that elicited maximum current flow to right VLPFC (**Figure 1**) by placing the anode at F10 and cathode at F2.

The current density of this montage is considerably higher than that traditionally observed with two electrode montages using saline soaked sponges. For this reason twice during the study participants were asked to report the current severity of sensations such as heat, tingling, and itching. No participants were removed from the study due to reports of severe sensations.

Linear mixed effects models were used to assess changes in task performance as a function of time and stimulus condition. Analysis was performed in R with package "LME4" and function "lmer". Model selection and control for over fitting were done with AIC and BIC log-likelihood weighting functions with BIC taking precedence if the two weighting functions selected a different model.

Raw NIRS time series data were low pass filtered and corrected for motion artifacts, after which relative concentrations of oxygenated and deoxygenated hemoglobin were calculated with the modified Beer Lambert law, with the first 10 s of fixation for a given block of trials used as the NIRS baseline. Oxygenated and deoxygenated hemoglobin time series for each optode were analyzed with linear mixed effects regression. Orthogonal regressors were constructed with boxcar time series representing hypotheses of interest and convolved with a canonical hemodynamic response function. The following regressors were constructed and entered into a design matrix: (1) increased activity during the task period relative to fixation; (2) correlation between task activity amplitude and performance; (3) increased task amplitude during stimulation relative to baseline; (4) increased task amplitude during stimulation relative to sham; (5) correlation between task performance and increased task activity during stimulation relative to baseline; and (6) correlation between task performance and increased task activity during stimulation relative to sham. Fixed effects were composed of the full design matrix of regressors and random effects were selected via the same methods used for behavioral model selection. Multiple comparisons were corrected for using the Hochberg false discovery rate correction. Final effects of increases or decreases in activation were determined by comparing the sign of beta coefficients for significant changes in oxygenated and deoxygenated hemoglobin. Opposite signs of oxygenated and deoxygenated hemoglobin, where the beta coefficient of oxygenated hemoglobin was positive were interpreted as increases in activity; where the coefficient of oxygenated hemoglobin was negative were interpreted as decreases in activity.

The most parsimonious behavioral model specified fixed effects of a linear and quadratic effect of experimental block, and random effects of participant intercept uncorrelated with experimental block. There was a significant linear effect of block ($b = -0.38$, SE = 0.16, $p < 0.05$). This suggests that increased time on the task lead to a decrement in performance. However there was also a significant quadratic effect of block ($b = 0.06$, SE = 0.02, $p < 0.05$). This positive quadratic effect counteracted the decline in performance following the first three blocks. The uptrend in performance also corresponds with the time at which participants began receiving stimulation, and continues even after stimulation was removed.

In the NIRS data there were a number of optodes that showed significant effects in oxygenated and deoxygenated hemoglobin for the design matrix regressors we constructed; however for the sake of brevity and clarity only effects where oxygenated hemoglobin and deoxygenated hemoglobin had opposite beta coefficients are reported. Visualization of brain activation patterns are described elsewhere (Ayaz et al., 2006) and more

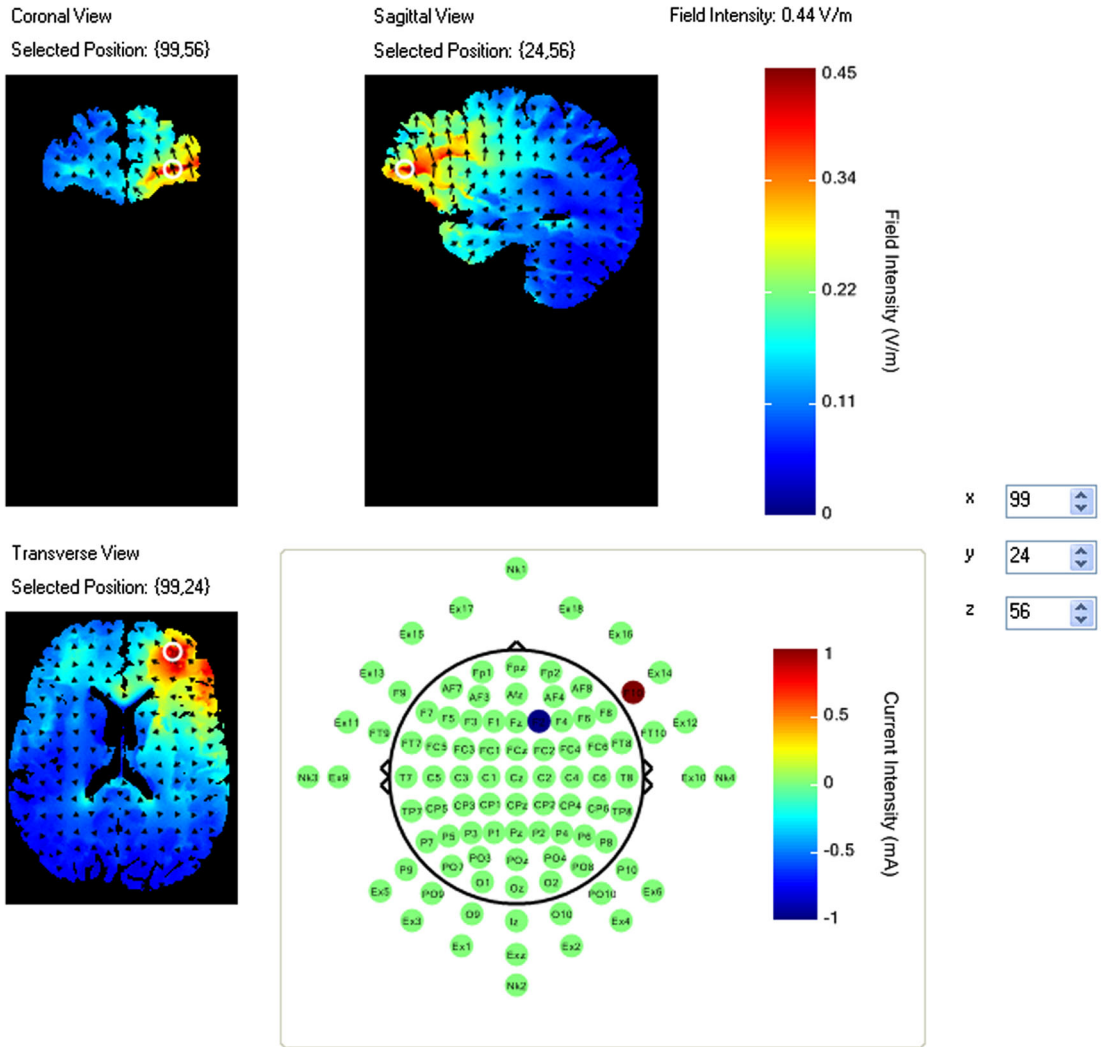

FIGURE 1 | Current flow model of tDCS montage (F10 anode, F2 cathode), field intensity of 0.44 V/m represented at white ring in coronal, sagittal and transverse views. Arrows represent direction of current flow.

information on placement of optodes, see Ayaz et al. (2012). Final models for each optode consisted of the full design matrix for fixed effects and random effects were participant intercept uncorrelated with time. There were no significant activation changes comparing task period to fixation, however optode 16 in coherence with our pilot findings showed a relationship between activity amplitude during the task period and subsequent performance (Oxy $b = 0.019$, SE = 0.008, $p < 0.05$, Deoxy $b = -0.008$, SE = 0.004, $p < 0.05$) (**Figure 2**).

Optodes 1, 3, 5, 6, 7, 11, 12, and 13 showed evidence of increased activation during the task period for stimulation blocks relative to the base line block. However these same regions and optode 14 showed a decrease in activation during the task period when comparing the stimulation blocks to the sham blocks (**Figure 3**).

We also observed that reduced positive activity in optodes 1 and 13 were associated with higher performance between the stimulation and baseline trials. Finally greater reduced activity in optodes 11 (Oxy $b = -0.058$, SE = 0.011, $p < 0.001$, Deoxy $b = 0.022$, SE = 0.005, $p < 0.001$) and 15 (Oxy $b = -0.118$, SE = 0.014, $p < 0.001$, Deoxy $b = 0.043$, SE = 0.009, $p < 0.001$) was associated with improved performance between the stimulation and sham trials, this was also accompanied by less negative activity in optode 6 (Oxy $b = 0.027$, SE = 0.012, $p < 0.05$, Deoxy $b = -0.017$, SE = 0.007, $p < 0.05$) correlating with improved performance (**Figure 4**).

1 mA of DC was applied with anode at F10 and cathode at F2 while participants performed a spatial memory task while being concurrently monitored with fNIRS. Task performance declined rapidly following baseline, possibly reflecting changes in vigilance or fatigue. However this decrement was overcome and almost eliminated following HDtDCS stimulation. The stimulation also had a number of effects on hemodynamic

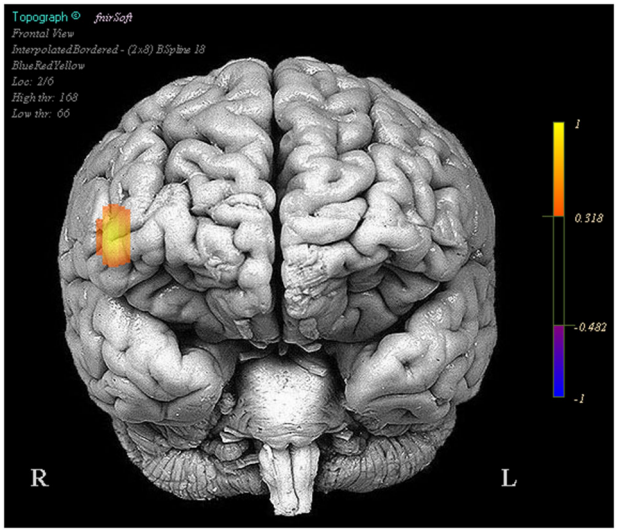

FIGURE 2 | Regions in which effects represent a correlation between increased activity and increased task performance. Legend represents the presence and direction of the effect, not *p* or *t* values.

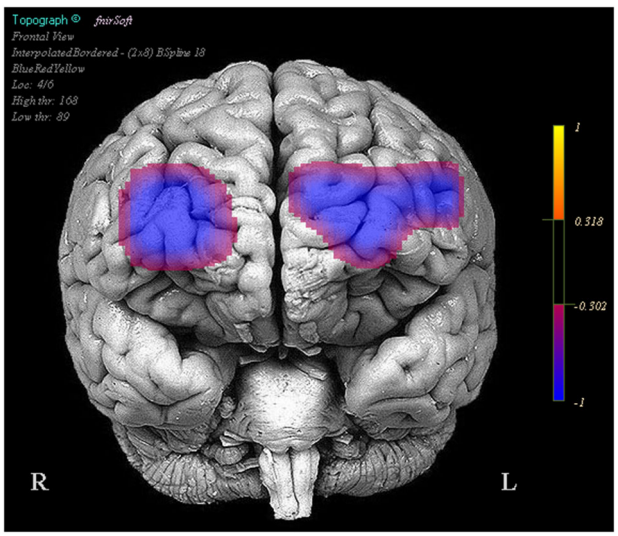

FIGURE 3 | Regions in which effects represent a decrease in activity during stimulation trials relative to the sham trials. Legend represents the presence and direction of the effect, not *p* or *t* values.

correlates of neural activity and their relationship to task performance. Specifically stimulation reduced the activity in bilateral prefrontal cortex, however most of these changes were unrelated to the effect of tDCS on task performance. Only continued decreased activity in right dorsal medial (optode 11), and right dorsolateral PFC (optode 15) were associated with the increase in performance experienced as participants shifted from the sham blocks to the stimulation blocks. This is particularly interesting as the cathode was placed directly above right dorsomedial PFC at the sight associated with performance recovery. Furthermore it is interesting to note that the region

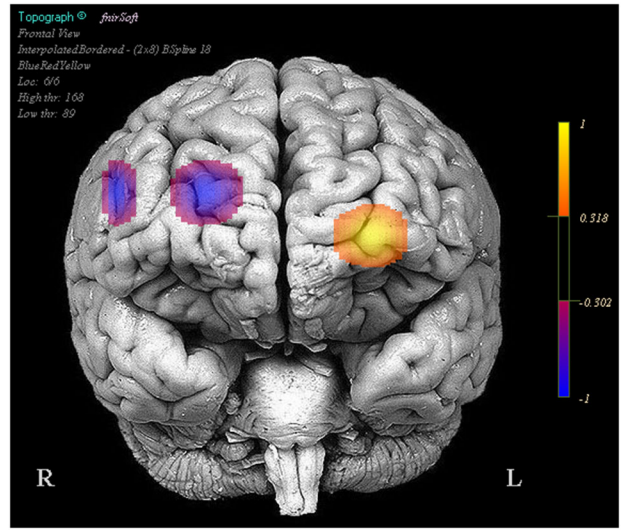

FIGURE 4 | Regions in which effects represent a correlation between activity and increases in performance in stimulation trials relative to the sham trials. Legend represents the presence and direction of the effect, not *p* or *t* values.

selected for modulation via our model of current flow was not actually modulated by stimulation, however its activity was still consistently associated with task performance. Taken together these results suggest that tDCS can modulate the neural activity of specific brain regions near the site of stimulation, however current models and protocol for determining tDCS montages are lacking, as it appears there are intimate interactions between stimulation montage, task and underlying hemodynamics that are complex. Additional joint tDCS and fNIRS studies are needed to further unravel these complexities and to better define the pattern of cortical excitation induced by tDCS during the performance of cognitive tasks.

Wireless Brain Imaging With tDCS

Significant progress has been made over the last decades in understanding the brain physiology and neural dynamics related to cognitive processes and behavior. However traditional neuroimaging tools such as fMRI severely restrict subject movements due to the inherent imaging operation (Makeig et al., 2009). Such technical limitations require brain imaging in more artificial settings separated from dynamic and multi-faceted natural environment (Gramann et al., 2014). To be able to capture brain dynamics related to natural cognition, mobile brain imaging systems are needed to operate in complex and partially unpredictable environments, consistent with mobile brain/body imaging (MoBI) and neuroergonomics approaches (Gramann et al., 2011; Parasuraman, 2011), A new generation of portable brain sensing technologies of EEG and fNIRS have begun to overcome the limitations of traditional neuroimaging through untethered measurements and wearable sensors (Liao et al., 2012; Ayaz et al., 2013; De Vos et al., 2014; Stopczynski et al., 2014; Mihajlovic et al., 2015). For a review of commercial

available mobile EEG systems see (Mihajlovic et al., 2015) and recent studies demonstrated combined EEG and tDCS (Faria et al., 2012; Schestatsky et al., 2013; Mangia et al., 2014) as well as the combined fNIRS and tDCS studies (Khan et al., 2013; Ishikuro et al., 2014; Jones et al., 2015). However, joint use of EEG and tDCS is prone to artifacts, requires additional effort (such as extra reference electrodes and processing) to control and isolate the electrical fields to prevent contamination. Since fNIRS is optical (no electrical interference) and fNIRS sensor usually has an opening directly over the measurement area (light source and detectors are positioned around the measurement area, see **Figure 6**) there's a natural opportunity for integration. Potential applications of portable fNIRS were reviewed recently for neuroergonomics (Ayaz et al., 2013) and economics research (Kopton and Kenning, 2014). These developments have provided an opportunity for coupling mobile brain imaging sensors with wireless tDCS for monitoring and modulating brain activity in ecologically valid natural environments.

Recent comprehensive reviews on fNIRS technology (Ferrari and Quaresima, 2012) confirm that the vast majority of instrumentation development has been conducted on continuous wave (CW) type fNIRS. CW systems have a limitation in terms of their information content (i.e., it measures only changes of oxy and deoxy-Hb) compared to frequency and time-resolved fNIRS systems. However, CW fNIRS is also most appropriate for miniaturization and portable system development, because the signal type and acquisition timing requirements are less demanding.

The development of wearable and low cost fNIRS systems began in 1990s and by Chance et al. (1997) specifically for prefrontal cortex brain hemodynamics and muscle measurements. These systems, were later further developed into the portable systems at Drexel University for functional brain imaging using both desktop and miniaturized wireless versions (Ayaz et al., 2013) as well as breast tumor scanning (Sao et al., 2003), chronic wound monitoring (Weingarten et al., 2008) and brain hematoma scanning (Ayaz et al., 2011a).

fNIRS based wireless brain imaging systems have also been of interest and Hoshi (2003) reported use of one the earliest for assessing regional blood flow related to emotion in children. The system had one detector and two light sources, providing two optodes overall. Participants were carrying the equipment and transmitter in a backpack. Later, Yurtsever et al. (2006) reported a pocket PC integrated system that reduced the overall size considerably by using off-the-shelf embedded system as computational platform and featured up to 48 channels (16 optodes). Also, Muehlemann et al. (2008) described an *in vivo* measurement system that featured wireless data transfer with up to 32 channels and high sampling rate to reach fast optical signal. Holper et al. (2010) used that system for virtual reality based neurorehabilitation approach during observation and motor imagery tasks. Also, an EEG integrated prototype has been used in epilepsy research (Safaie et al., 2013). More recently, Muthalib et al. (2014) presented an HD-tDCS EEG/fNIRS capable experiment setup that was used for studying both electrophysiological and hemodynamic components of the modulation of cortical sensorimotor networks.

In previous work we have reported a custom miniaturized system (Rodriguez and Pourrezaei, 2011) that can be used for general purpose functional neuroimaging studies of prefrontal cortex (Ayaz et al., 2013). The device is a smart-phone size unit that can be carried in hand (See **Figure 5**), and drive up to 5 optodes (15 channels) at 4 Hz sampling rate. The system interfaces and transmits data wirelessly to a PC that runs the COBI Studio (Ayaz et al., 2011b). The implemented system is depicted in **Figure 5** below. The main advantage is further miniaturization of the hardware unit and hence no need for backpack, subjects can carry the system in their pocket or hand allowing more freedom in experimental design.

Integration of fNIRS sensors with tDCS also shows promise given that optical brain imaging is not influenced by electrical stimulation. Simultaneous use with fiber based fNIRS sensors is less of an issue as placement of fibers that run perpendicular to scalp leaves much space for other types of sensors. However, such sensors require laser light sources and larger hardware equipment, which are not as portable as LED based systems. Hence further miniaturization and customization of LED based sensor pads is needed. **Figure 6** below depicts a miniaturized prototype fNIRS sensor pad that is compatible with the fNIRS wireless unit described above (Ayaz et al., 2013) and a similar configuration was already tested with tDCS (Rodriguez and Pourrezaei, 2011). The combined tDCS fNIRS in that study was constructed by first molding the insulated fNIRS PCB in a skin safe silicon cast which was designed to hold tDCS electrodes in standard size ($2'' \times 3.5''$) acting as a sleeve to the electrode. Systemic performance tests with varying power and gain parameters indicated that undesired interference is not introduced by the tDCS stimulation and that the fNIR sensor performs as expected. Similarly, there are also prototype fNIRS sensors that are already integrated with EEG electrodes for hybrid measurements (Lareau et al., 2011; Leamy et al., 2011; Safaie et al., 2013).

Since the fNIRS sensor positioning of the light source and detectors are around the measurement area (which is in between the light source and detector as illustrated in **Figure 6**) and not directly on top of the measurement area, combining with tDCS is feasible and practical from a hardware development perspective. Moreover recent developments in tDCS systems provide multi-channel tDCS systems that allow independent control of individual electrode currents, such as the HDtDCS systems developed by Soterix Medical and the wireless tDCS system Starstim (by NE Electrics) enabling potential ambulatory experimental protocols.

Future Directions

This paper reviews the potential joint use and future convergence of two technologies for neuroimaging and neurostimulation, fNIRS and tDCS, and how the two can be synergistically used together to enhance our current understanding of brain dynamics. Both technologies have complimentary capabilities, and both are built wearable and wireless that allow for application in natural environments and real world settings. Future neuroergonomics applications could range from

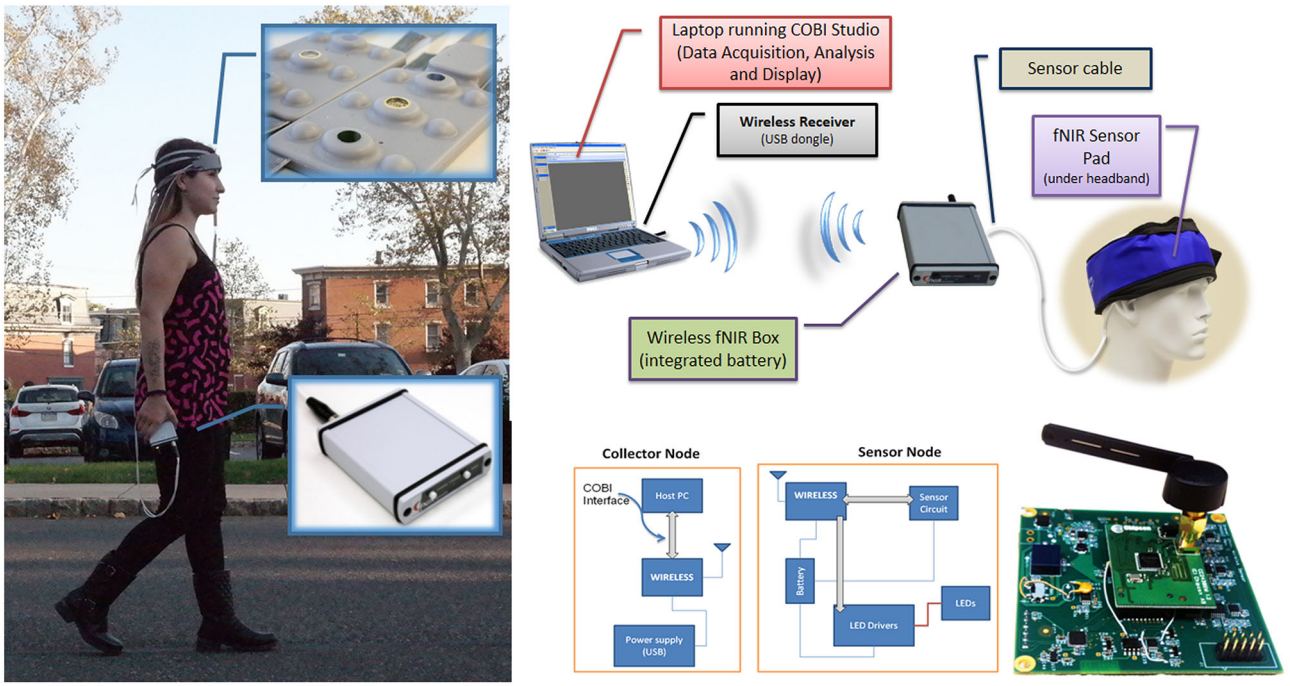

FIGURE 5 | Wireless fNIRS System. (left) Battery operated and wireless unit allows untethered outdoor measurement (right, up). Block diagram of the overall system (right, bottom), Building blocks and circuit representation (Ayaz et al., 2013).

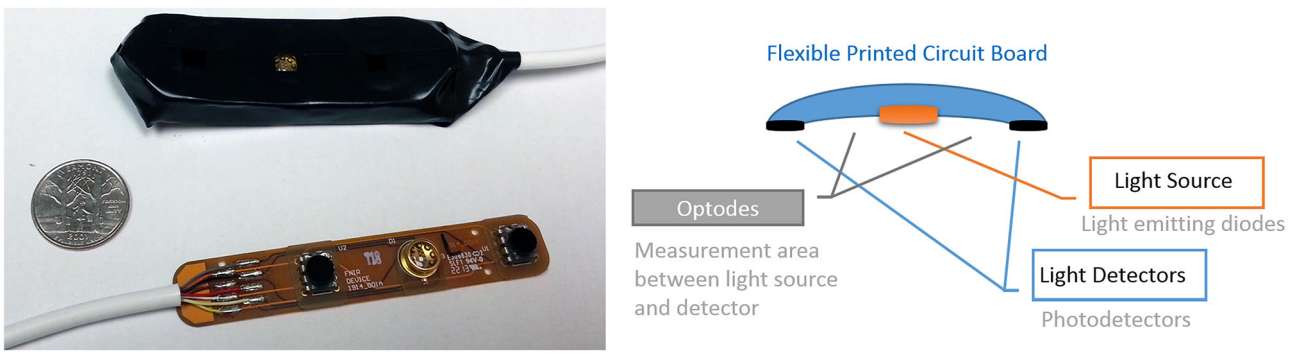

FIGURE 6 | Miniaturized and scalable fNIRS sensor pad with 2 optodes can be integrated with electrodes. (left) prototype sensor pad circuit board and covered with foam enclosure. A U.S. quarter is included for size. (right) The 2 optodes sensor pad parts.

enhanced/accelerated learning and training of complex human-machine systems to optimization of task load for improved safety and productivity.

Also, joint use of tDCS and fNIRS could enable new unique applications such as read-write BCI. A BCI is defined as a system that captures and transforms signals originating from the human brain into commands that can control external applications or instruments. In its most general form BCI provides a route for neural output that does not involve the neuromuscular system (Wolpaw et al., 2002; Lebedev, 2014). BCI systems have a wide range of potential applications, including rehabilitation and assistive use for severely paralyzed patients to help them communicate and interact with their environments, as well as monitoring brain activity for assessment of mental state or intervention in various psychiatric conditions and/or to augment the interactivity of healthy individuals.

Current noninvasive BCI systems are read-only as they capture brain activity and produce output/action for user. However, future portable and noninvasive BCI systems can also write to brain for direct communication and bypassing the peripheral nervous system and enhancing the brains' sensory input mechanism. Earlier studies in animal models achieved meaningful sensorimotor information in real time

using invasive intracortical microstimulation to deliver sensory feedback signals in rats (Pais-Vieira et al., 2013) and monkeys (O'Doherty et al., 2011). This concept has been tested on humans recently after lab prototypes and demonstrations indicated feasibility and Grau et al. (2014) published their approach for Brain to Brain Communication which was made possible with dual use of noninvasive neuroimaging and neurostimulation. In the study, authors utilized EEG for capturing voluntary motor imagery related activations which were relayed as light perception to second brain by stimulating occipital lobe via TMS. Practical brain to brain communication would have profound impact on how we communicate and work, and as a portable system, tDCS is the natural candidate for closing the loop for future portable BCI systems.

As the potential use of future BCI systems has implications from individual to society at large, ethical aspects have also been a focus of discussion as part of the rising field of neuroethics (Illes and Bird, 2006; Haselager et al., 2009; Schermer, 2009; Clausen, 2011; Nijboer et al., 2011; Vlek et al., 2012). One of the immediate concerns is related to "treatment vs. research" which is related to the decision of using new systems on clinical and vulnerable populations such as locked-in patients. As in all new medical technologies, clinical utility and benefit vs. the risk (e.g., when using invasive neuroimaging or burden of engaging with the system) has to be evaluated with due process (informed consent) (Vlek et al., 2012). Also, privacy has been a core concern (Nijboer et al., 2011; Fairclough, 2014) and mostly attributed to keeping ones' physiological signals private. With the influence of contemporary science-fiction, write-only or read-write BCI have often been considered akin to mind control. Writing to the brain has been used here in terms of modification/modulation of brain signals and is a physiological effect with immediate clinical uses (e.g., Parkinson treatment with deep brain stimulation). Current concepts of write-only or read-write BCI can only operate with the user's consent and engagement. And, the design of future BCI systems should be informed by neuroethics considerations from personal to societal perspectives. For a discussion of the near and long-term issues please see recent reviews by Clausen (2011), Nijboer et al. (2011), Vlek et al. (2012) and Attiah and Farah (2014).

Another interesting future direction could be the unification of neuroimaging and neurostimulation technology by using near infrared light. A novel integration of optics and genetics is the emerging field of optogenetics which uses light to control neurons that have been genetically modified to be sensitive to light (Deisseroth, 2011). Optogenetics studies has been exponentially growing to observe and perturb neural mechanisms from single cell level to animal brain models. The requirement of genetically encoded, protein-based probes to achieve experimental manipulation is a major limitation for human studies. Optical stimulation with near infrared lasers that are low powered but have high energy density could be a solution (Wells et al., 2005a,b; Shapiro et al., 2012). A recent study suggest that such lasers could be utilized to excite cells by changing their electrical capacitance (Shapiro et al., 2012). Although light sources for such lasers would be different for reading and writing, having a unified/fused wearable pad that can both record and stimulate brain activity could enable new applications in natural environments. In summary, the simultaneous use of tDCS and fNIRS, the development of wireless, portable fNIRS systems, and the potential development of optical systems for both stimulation and neuroimaging are opening up new vistas for neurocognitive augmentation, with exciting new clinical and neuroergonomic applications.

Disclosure

fNIR Devices, LLC manufactures the optical brain imaging instrument and licensed IP and know-how from Drexel University. H. Ayaz was involved in the technology development and thus offered a minor share in the new startup firm fNIR Devices, LLC.

References

Abibullaev, B., and An, J. (2012). Classification of frontal cortex haemodynamic responses during cognitive tasks using wavelet transforms and machine learning algorithms. *Med. Eng. Phys.* 34, 1394–1410. doi: 10.1016/j.medengphy.2012.01.002

Afergan, D., Peck, E. M., Solovey, E. T., Jenkins, A., Hincks, S. W., Brown, E. T., et al. (2014). "Dynamic difficulty using brain metrics of workload," in *Proc. ACM CHI 2014 Human Factors in Computing Systems Conference* (Toronto, Canada: ACM Press).

Alon, G., Roys, S., Gullapalli, R., and Greenspan, J. (2011). Non-invasive electrical stimulation of the brain (ESB) modifies the resting-state network connectivity of the primary motor cortex: a proof of concept fMRI study. *Brain Res.* 1403, 37–44. doi: 10.1016/j.brainres.2011.06.013

Angelakis, E., Stathopoulou, S., Frymiare, J. L., Green, D. L., Lubar, J. F., and Kounios, J. (2007). EEG neurofeedback: a brief overview and an example of peak alpha frequency training for cognitive enhancement in the elderly. *Clin. Neuropsychol.* 21, 110–129. doi: 10.1080/13854040600744839

Antal, A., Bikson, M., Datta, A., Lafon, B., Dechent, P., Parra, L. C., et al. (2014). Imaging artifacts induced by electrical stimulation during conventional fMRI of the brain. *Neuroimage* 85(Pt. 3), 1040–1047. doi: 10.1016/j.neuroimage.2012.10.026

Antal, A., Polania, R., Schmidt-Samoa, C., Dechent, P., and Paulus, W. (2011). Transcranial direct current stimulation over the primary motor cortex during fMRI. *Neuroimage* 55, 590–596. doi: 10.1016/j.neuroimage.2010.11.085

Attiah, M. A., and Farah, M. J. (2014). Minds and motherboards and money: futurism and realism in the neuroethics of BCI technologies. *Front. Syst. Neurosci.* 8:86. doi: 10.3389/fnsys.2014.00086

Ayaz, H., Ben Dor, B., Solt, D., and Onaral, B. (2011a). Infrascanner: cost effective, mobile medical imaging system for detecting hemotomas. *J. Med. Device.* 5:027540. doi: 10.1115/1.3591407

Ayaz, H., Izzetoglu, M., Platek, S. M., Bunce, S., Izzetoglu, K., Pourrezaei, K., et al. (2006). Registering fNIR data to brain surface image using MRI templates. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 1, 2671–2674. doi: 10.1109/iembs.2006.260835

Ayaz, H., Onaral, B., Izzetoglu, K., Shewokis, P. A., McKendrick, R., and Parasuraman, R. (2013). Continuous monitoring of brain dynamics with functional near infrared spectroscopy as a tool for neuroergonomic research: empirical examples and a technological development. *Front. Hum. Neurosci.* 7:871. doi: 10.3389/fnhum.2013.00871

Ayaz, H., Shewokis, P. A., Bunce, S., Izzetoglu, K., Willems, B., and Onaral, B. (2012). Optical brain monitoring for operator training and mental workload assessment. *Neuroimage* 59, 36–47. doi: 10.1016/j.neuroimage.2011.06.023

Ayaz, H., Shewokis, P. A., Curtin, A., Izzetoglu, M., Izzetoglu, K., and Onaral, B. (2011b). Using MazeSuite and functional near infrared spectroscopy to study learning in spatial navigation. *J. Vis. Exp.* 56:3443. doi: 10.3791/3443

Bikson, M., Datta, A., and Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clin. Neurophysiol.* 120, 1033–1034. doi: 10.1016/j.clinph.2009.03.018

Bindman, L. J., Lippold, O., and Redfearn, J. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382. doi: 10.1113/jphysiol.1964.sp007425

Bogler, C., Mehnert, J., Steinbrink, J., and Haynes, J. D. (2014). Decoding vigilance with NIRS. *PLoS One* 9:e101729. doi: 10.1371/journal.pone.0101729

Browning, M., Holmes, E. A., Murphy, S. E., Goodwin, G. M., and Harmer, C. J. (2010). Lateral prefrontal cortex mediates the cognitive modification of attentional bias. *Biol. Psychiatry* 67, 919–925. doi: 10.1016/j.biopsych.2009.10.031

Byrne, E. A., and Parasuraman, R. (1996). Psychophysiology and adaptive automation. *Biol. Psychol.* 42, 249–268. doi: 10.1016/0301-0511(95)05161-9

Chance, B., Luo, Q., Nioka, S., Alsop, D. C., and Detre, J. A. (1997). Optical investigations of physiology: a study of intrinsic and extrinsic biomedical contrast. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 352, 707–716. doi: 10.1098/rstb.1997.0053

Clark, V. P., Coffman, B. A., Mayer, A. R., Weisend, M. P., Lane, T. D., Calhoun, V. D., et al. (2012). TDCS guided using fMRI significantly accelerates learning to identify concealed objects. *Neuroimage* 59, 117–128. doi: 10.1016/j.neuroimage.2010.11.036

Clark, V. P., Coffman, B. A., Trumbo, M. C., and Gasparovic, C. (2011). Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: A^1 H magnetic resonance spectroscopy study. *Neurosci. Lett.* 500, 67–71. doi: 10.1016/j.neulet.2011.05.244

Clark, V. P., and Parasuraman, R. (2014). Neuroenhancement: enhancing brain and mind in health and in disease. *Neuroimage* 85, 889–894. doi: 10.1016/j.neuroimage.2013.08.071

Clarke, P. J., Browning, M., Hammond, G., Notebaert, L., and MacLeod, C. (2014). The causal role of the dorsolateral prefrontal cortex in the modification of attentional bias: evidence from transcranial direct current stimulation. *Biol. Psychiatry* 76, 946–952. doi: 10.1016/j.biopsych.2014.03.003

Clausen, J. (2011). Conceptual and ethical issues with brain-hardware interfaces. *Curr. Opin. Psychiatry* 24, 495–501. doi: 10.1097/YCO.0b013e32834bb8ca

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85, 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Coffman, B. A., Trumbo, M., Flores, R., Garcia, C. M., van der Merwe, A., Wassermann, E. M., et al. (2012). Impact of tDCS on performance and learning of target detection: interaction with stimulus characteristics and experimental design. *Neuropsychologia* 50, 1594–1602. doi: 10.1016/j.neuropsychologia.2012.03.012

Cui, X., Bray, S., Bryant, D. M., Glover, G. H., and Reiss, A. L. (2011). A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. *Neuroimage* 54, 2808–2821. doi: 10.1016/j.neuroimage.2010.10.069

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., and Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul.* 2, 201–207.e1. doi: 10.1016/j.brs.2009.03.005

Deisseroth, K. (2011). Optogenetics. *Nat. Methods* 8, 26–29. doi: 10.1038/nmeth.f.324

Derosière, G., Dalhoumi, S., Perrey, S., Dray, G., and Ward, T. (2014). Towards a near infrared spectroscopy-based estimation of operator attentional state. *PLoS One* 9:e92045. doi: 10.1371/journal.pone.0092045

De Vos, M., Kroesen, M., Emkes, R., and Debener, S. (2014). P300 speller BCI with a mobile EEG system: comparison to a traditional amplifier. *J. Neural Eng.* 11:036008. doi: 10.1088/1741-2560/11/3/036008

Durantin, G., Gagnon, J.-F., Tremblay, S., and Dehais, F. (2014). Using near infrared spectroscopy and heart rate variability to detect mental overload. *Behav. Brain Res.* 259, 16–23. doi: 10.1016/j.bbr.2013.10.042

Elbert, T., and Rockstroh, B. (2004). Reorganization of human cerebral cortex: the range of changes following use and injury. *Neuroscientist* 10, 129–141. doi: 10.1177/1073858403262111

Fairclough, S. (2014). Physiological data must remain confidential. *Nature* 505:263. doi: 10.1038/505263a

Falcone, B., Coffman, B. A., Clark, V. P., and Parasuraman, R. (2012). Transcranial direct current stimulation augments perceptual sensitivity and 24-hour retention in a complex threat detection task. *PLoS One* 7:e34993. doi: 10.1371/journal.pone.0034993

Faria, P., Fregni, F., Sebastião, F., Dias, A. I., and Leal, A. (2012). Feasibility of focal transcranial DC polarization with simultaneous EEG recording: preliminary assessment in healthy subjects and human epilepsy. *Epilepsy Behav.* 25, 417–425. doi: 10.1016/j.yebeh.2012.06.027

Ferrari, M., and Quaresima, V. (2012). A brief review on the history of human functional near-infrared spectroscopy (fNIRS) development and fields of application. *Neuroimage* 63, 921–935. doi: 10.1016/j.neuroimage.2012.03.049

Filmer, H. L., Dux, P. E., and Mattingley, J. B. (2014). Applications of transcranial direct current stimulation for understanding brain function. *Trends Neurosci.* 37, 742–753. doi: 10.1016/j.tins.2014.08.003

Fishburn, F. A., Norr, M. E., Medvedev, A. V., and Vaidya, C. J. (2014). Sensitivity of fNIRS to cognitive state and load. *Front. Hum. Neurosci.* 8:76. doi: 10.3389/fnhum.2014.00076

Flöel, A. (2014). tDCS-enhanced motor and cognitive function in neurological diseases. *Neuroimage* 85(Pt. 3), 934–947. doi: 10.1016/j.neuroimage.2013.05.098

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp. Brain Res.* 166, 23–30. doi: 10.1007/s00221-005-2334-6

Gramann, K., Gwin, J. T., Ferris, D. P., Oie, K., Jung, T.-P., Lin, C.-T., et al. (2011). Cognition in action: imaging brain/body dynamics in mobile humans. *Rev. Neurosci.* 22, 593–608. doi: 10.1515/RNS.2011.047

Gramann, K., Jung, T. P., Ferris, D. P., Lin, C. T., and Makeig, S. (2014). Toward a new cognitive neuroscience: modeling natural brain dynamics. *Front. Hum. Neurosci.* 8:444. doi: 10.3389/fnhum.2014.00444

Grau, C., Ginhoux, R., Riera, A., Nguyen, T. L., Chauvat, H., Berg, M., et al. (2014). Conscious brain-to-brain communication in humans using non-invasive technologies. *PLoS One* 9:e105225. doi: 10.1371/journal.pone.0105225

Gruzelier, J. (2009). A theory of alpha/theta neurofeedback, creative performance enhancement, long distance functional connectivity and psychological integration. *Cogn. Process.* 10, S101–S109. doi: 10.1007/s10339-008-0248-5

Han, C.-H., Song, H., Kang, Y.-G., Kim, B.-M., and Im, C.-H. (2014). Hemodynamic responses in rat brain during transcranial direct current stimulation: a functional near-infrared spectroscopy study. *Biomed. Opt. Express.* 5, 1812–1821. doi: 10.1364/BOE.5.001812

Hanslmayr, S., Sauseng, P., Doppelmayr, M., Schabus, M., and Klimesch, W. (2005). Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects. *Appl. Psychophysiol. Biofeedback* 30, 1–10. doi: 10.1007/s10484-005-2169-8

Haselager, P., Vlek, R., Hill, J., and Nijboer, F. (2009). A note on ethical aspects of BCI. *Neural Netw.* 22, 1352–1357. doi: 10.1016/j.neunet.2009.06.046

Heinrich, H., Busch, K., Studer, P., Erbe, K., Moll, G. H., and Kratz, O. (2014). EEG spectral analysis of attention in ADHD: implications for neurofeedback training? *Front. Hum. Neurosci.* 8:611. doi: 10.3389/fnhum.2014.00611

Herff, C., Heger, D., Fortmann, O., Hennrich, J., Putze, F., and Schultz, T. (2014). Mental workload during n-back task—quantified in the prefrontal cortex using fNIRS. *Front. Hum. Neurosci.* 7:935. doi: 10.3389/fnhum.2013.00935

Hoffman, D., Stockdale, S., and Van Egren, L. (1996). EEG neurofeedback in the treatment of mild traumatic brain injury. *Clin. Electroencephalogr.* 27, 105.

Holland, R., Leff, A. P., Josephs, O., Galea, J. M., Desikan, M., Price, C. J., et al. (2011). Speech facilitation by left inferior frontal cortex stimulation. *Curr. Biol.* 21, 1403–1407. doi: 10.1016/j.cub.2011.07.021

Holper, L., Muehlemann, T., Scholkmann, F., Eng, K., Kiper, D., and Wolf, M. (2010). Testing the potential of a virtual reality neurorehabilitation

system during performance of observation, imagery and imitation of motor actions recorded by wireless functional near-infrared spectroscopy (fNIRS). *J. Neuroeng. Rehabil.* 7:57. doi: 10.1186/1743-0003-7-57

Hoshi, Y. (2003). Functional near-infrared optical imaging: utility and limitations in human brain mapping. *Psychophysiology* 40, 511–520. doi: 10.1111/1469-8986.00053

Hunter, M. A., Coffman, B. A., Trumbo, M. C., and Clark, V. P. (2013). Tracking the neuroplastic changes associated with transcranial direct current stimulation: a push for multimodal imaging. *Front. Hum. Neurosci.* 7:495. doi: 10.3389/fnhum.2013.00495

Illes, J., and Bird, S. J. (2006). Neuroethics: a modern context for ethics in neuroscience. *Trends Neurosci.* 29, 511–517. doi: 10.1016/j.tins.2006.07.002

Ishikuro, K., Urakawa, S., Takamoto, K., Ishikawa, A., Ono, T., and Nishijo, H. (2014). Cerebral functional imaging using near-infrared spectroscopy during repeated performances of motor rehabilitation tasks tested on healthy subjects. *Front. Hum. Neurosci.* 8:292. doi: 10.3389/fnhum.2014.00292

Jacobson, L., Koslowsky, M., and Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Exp. Brain Res.* 216, 1–10. doi: 10.1007/s00221-011-2891-9

James, D. R. C., Orihuela-Espina, F., Leff, D. R., Sodergren, M. H., Athanasiou, T., Darzi, A. W., et al. (2011). The ergonomics of natural orifice translumenal endoscopic surgery (NOTES) navigation in terms of performance, stress and cognitive behavior. *Surgery* 149, 525–533. doi: 10.1016/j.surg.2010.11.019

Jones, K. T., Gözenman, F., and Berryhill, M. E. (2015). The strategy and motivational influences on the beneficial effect of neurostimulation: a tDCS and fNIRS study. *Neuroimage* 105, 238–247. doi: 10.1016/j.neuroimage.2014.11.012

Khan, B., Hodics, T., Hervey, N., Kondraske, G., Stowe, A. M., and Alexandrakis, G. (2013). Functional near-infrared spectroscopy maps cortical plasticity underlying altered motor performance induced by transcranial direct current stimulation. *J. Biomed. Opt.* 18:116003. doi: 10.1117/1.JBO.18.11.116003

Kopton, I. M., and Kenning, P. (2014). Near-infrared spectroscopy (NIRS) as a new tool for neuroeconomic research. *Front. Hum. Neurosci.* 8:549. doi: 10.3389/fnhum.2014.00549

Kouijzer, M. E. J., de Moor, J. M. H., Gerrits, B. J. L., Congedo, M., and van Schie, H. T. (2009). Neurofeedback improves executive functioning in children with autism spectrum disorders. *Res. Autism Spectr. Disord.* 3, 145–162. doi: 10.1016/j.rasd.2008.05.001

Kwon, Y. H., and Jang, S. H. (2011). The enhanced cortical activation induced by transcranial direct current stimulation during hand movements. *Neurosci. Lett.* 492, 105–108. doi: 10.1016/j.neulet.2011.01.066

Lareau, E., Lesage, F., Pouliot, P., Nguyen, D., Le Lan, J., and Sawan, M. (2011). Multichannel wearable system dedicated for simultaneous electroencephalography/near-infrared spectroscopy real-time data acquisitions. *J. Biomed. Opt.* 16:096014. doi: 10.1117/1.3625575

Leamy, D., Collins, R., and Ward, T. (2011). "Combining fNIRS and EEG to improve motor cortex activity classification during an imagined movement-based task," in *Foundations of Augmented Cognition. Directing the Future of Adaptive Systems*, eds D. D. Schmorrow and C. M. Fidopiastis (Berlin Heidelberg: Springer), 177–185.

Lebedev, M. (2014). Brain-machine interfaces: an overview. *Transl. Neurosci.* 5, 99–110. doi: 10.2478/s13380-014-0212-z

Lewis, C. M., Baldassarre, A., Committeri, G., Romani, G. L., and Corbetta, M. (2009). Learning sculpts the spontaneous activity of the resting human brain. *Proc. Natl. Acad. Sci. U S A* 106, 17558–17563. doi: 10.1073/pnas.0902455106

Liao, L.-D., Lin, C.-T., McDowell, K., Wickenden, A. E., Gramann, K., Jung, T.-P., et al. (2012). Biosensor technologies for augmented brain-computer interfaces in the next decades. *Proc. IEEE* 100, 1553–1566. doi: 10.1109/jproc.2012.2184829

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

Lim, C. G., Lee, T. S., Guan, C., Fung, D. S., Zhao, Y., Teng, S. S., et al. (2012). A brain-computer interface based attention training program for treating attention deficit hyperactivity disorder. *PLoS One* 7:e46692. doi: 10.1371/journal.pone.0046692

Lövdén, M., Bodammer, N. C., Kühn, S., Kaufmann, J., Schütze, H., Tempelmann, C., et al. (2010). Experience-dependent plasticity of white-matter microstructure extends into old age. *Neuropsychologia* 48, 3878–3883. doi: 10.1016/j.neuropsychologia.2010.08.026

Lubar, J. F., Swartwood, M. O., Swartwood, J. N., and O'Donnell, P. H. (1995). Evaluation of the effectiveness of EEG neurofeedback training for ADHD in a clinical setting as measured by changes in TOVA scores, behavioral ratings and WISC-R performance. *Biofeedback Self Regul.* 20, 83–99. doi: 10.1007/bf01712768

Makeig, S., Gramann, K., Jung, T.-P., Sejnowski, T. J., and Poizner, H. (2009). Linking brain, mind and behavior. *Int. J. Psychophysiol.* 73, 95–100. doi: 10.1016/j.ijpsycho.2008.11.008

Mandrick, K., Derosiere, G., Dray, G., Coulon, D., Micallef, J.-P., and Perrey, S. (2013). Prefrontal cortex activity during motor tasks with additional mental load requiring attentional demand: a near-infrared spectroscopy study. *Neurosci. Res.* 76, 156–162. doi: 10.1016/j.neures.2013.04.006

Mangia, A. L., Pirini, M., and Cappello, A. (2014). Transcranial direct current stimulation and power spectral parameters: a tDCS/EEG co-registration study. *Front. Hum. Neurosci.* 8:601. doi: 10.3389/fnhum.2014.00601

McKendrick, R., Ayaz, H., Olmstead, R., and Parasuraman, R. (2014). Enhancing dual-task performance with verbal and spatial working memory training: continuous monitoring of cerebral hemodynamics with NIRS. *Neuroimage* 85(Pt. 3), 1014–1026. doi: 10.1016/j.neuroimage.2013.05.103

McKinley, R. A., McIntire, L., Bridges, N., Goodyear, C., and Weisend, M. P. (2013). Acceleration of image analyst training with transcranial direct current stimulation. *Behav. Neurosci.* 127, 936–946. doi: 10.1037/a0034975

Mehta, R. K., and Parasuraman, R. (2013). Neuroergonomics: a review of applications to physical and cognitive work. *Front. Hum. Neurosci.* 7:889. doi: 10.3389/fnhum.2013.00889

Mehta, R. K., and Parasuraman, R. (2014). Effects of mental fatigue on the development of physical fatigue: a neuroergonomic approach. *Hum. Factors* 56, 645–656. doi: 10.1177/0018720813507279

Merzagora, A. C., Foffani, G., Panyavin, I., Mordillo-Mateos, L., Aguilar, J., Onaral, B., et al. (2010). Prefrontal hemodynamic changes produced by anodal direct current stimulation. *Neuroimage* 49, 2304–2310. doi: 10.1016/j.neuroimage.2009.10.044

Mihajlovic, V., Grundlehner, B., Vullers, R., and Penders, J. (2015). Wearable, wireless EEG Solutions in daily life applications: what are we missing? *IEEE J. Biomed. Health Inform.* 19, 6–21. doi: 10.1109/JBHI.2014.2328317

Miller, K., Schalk, G., Fetz, E., den Nijs, M., Ojemann, J., and Rao, R. (2010). Cortical activity during motor execution, motor imagery and imagery-based online feedback. *Proc. Natl. Acad. Sci. U S A* 107, 4430–4435. doi: 10.1073/pnas.0913697107

Muehlemann, T., Haensse, D., and Wolf, M. (2008). Wireless miniaturized in-vivo near infrared imaging. *Opt. Express* 16, 10323–10330. doi: 10.1364/oe.16.010323

Muthalib, M., Dutta, A., Besson, P., Rothwell, J., Ward, T., and Perrey, S. (2014). "Comparison of online vs offline effects of HD-tDCS induced modulation of cortical sensorimotor networks using a combined fNIRS-EEG setup," in *Poster presented at the International Society on Oxygen Transport to Tissues (ISOTT) Conference* (London).

Muthalib, M., Kan, B., Nosaka, K., and Perrey, S. (2013). "Effects of transcranial direct current stimulation of the motor cortex on prefrontal cortex activation during a neuromuscular fatigue task: an fNIRS study," in *Oxygen Transport to Tissue XXXV*, eds S. Van Huffel G. Naulaers A. Caicedo D. F. Bruley and D. K. Harrison (New York: Springer), 73–79.

Naseer, N., and Keum-Shik, H. (2013). "Functional near-infrared spectroscopy based discrimination of mental counting and no-control state for development of a brain-computer interface," in *Engineering in Medicine and Biology Society (EMBC), 35th Annual International Conference of the IEEE*, 1780–1783.

Nijboer, F., Clausen, J., Allison, B. Z., and Haselager, P. (2011). The asilomar survey: stakeholders' opinions on ethical issues related to brain-computer interfacing. *Neuroethics* 6, 541–578. doi: 10.1007/s12152-011-9132-6

Ninaus, M., Kober, S. E., Witte, M., Koschutnig, K., Stangl, M., Neuper, C., et al. (2013). Neural substrates of cognitive control under the belief of getting neurofeedback training. *Front. Hum. Neurosci.* 7:914. doi: 10.3389/fnhum.2013.00914

Nitsche, M., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

O'Doherty, J. E., Lebedev, M. A., Ifft, P. J., Zhuang, K. Z., Shokur, S., Bleuler, H., et al. (2011). Active tactile exploration using a brain-machine-brain interface. *Nature* 479, 228–231. doi: 10.1038/nature10489

Pais-Vieira, M., Lebedev, M., Kunicki, C., Wang, J., and Nicolelis, M. A. L. (2013). A brain-to-brain interface for real-time sharing of sensorimotor information. *Sci. Rep.* 3:1319. doi: 10.1038/srep01319

Parasuraman, R. (2003). Neuroergonomics: research and practice. *Theor. Issues Ergon. Sci.* 4, 5–20. doi: 10.1080/14639220210199753

Parasuraman, R. (2011). Neuroergonomics brain, cognition and performance at work. *Curr. Dir. Psychol. Sci.* 20, 181–186. doi: 10.1177/0963721411409176

Parasuraman, R., and Galster, S. (2013). Sensing, assessing and augmenting threat detection: behavioral, neuroimaging and brain stimulation evidence for the critical role of attention. *Front. Hum. Neurosci.* 7:273. doi: 10.3389/fnhum.2013.00273

Parasuraman, R., and Mckinley, R. A. (2014). Using noninvasive brain stimulation to accelerate learning and enhance human performance. *Hum. Factors* 56, 816–824. doi: 10.1177/0018720814538815

Parasuraman, R., and Rizzo, M. (2007). *Neuroergonomics: The Brain at Work*. New York, NY: Oxford University Press.

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *J. Physiol.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Raymond, J., Varney, C., Parkinson, L. A., and Gruzelier, J. H. (2005). The effects of alpha/theta neurofeedback on personality and mood. *Brain Res. Cogn. Brain Res.* 23, 287–292. doi: 10.1016/j.cogbrainres.2004.10.023

Rodriguez, M., and Pourrezaei, K. (2011). *Development of a Versatile Wireless fNIR System.* Philadelphia, PA: Masters Degree, Drexel University.

Safaie, J., Grebe, R., Abrishami Moghaddam, H., and Wallois, F. (2013). Toward a fully integrated wireless wearable EEG-NIRS bimodal acquisition system. *J. Neural Eng.* 10:056001. doi: 10.1088/1741-2560/10/5/056001

Saiote, C., Turi, Z., Paulus, W., and Antal, A. (2013). Combining functional magnetic resonance imaging with transcranial electrical stimulation. *Front. Hum. Neurosci.* 7:435. doi: 10.3389/fnhum.2013.00435

Sao, V., Pourrezaei, K., Akin, A., and Ayaz, H. (2003). "Breast tumor imaging using NIR LED based handheld continuous-wave imager," in *Bioengineering Conference, IEEE 29th Annual, Proceedings of* (IEEE).

Sato, H., Yahata, N., Funane, T., Takizawa, R., Katura, T., Atsumori, H., et al. (2013). A NIRS-fMRI investigation of prefrontal cortex activity during a working memory task. *Neuroimage* 83, 158–173. doi: 10.1016/j.neuroimage.2013.06.043

Schermer, M. (2009). The mind and the machine. On the conceptual and moral implications of brain-machine interaction. *Nanoethics* 3, 217–230. doi: 10.1007/s11569-009-0076-9

Schestatsky, P., Morales-Quezada, L., and Fregni, F. (2013). Simultaneous EEG monitoring during transcranial direct current stimulation. *J. Vis. Exp.* 76:e50426. doi: 10.3791/50426

Schudlo, L. C., and Chau, T. (2014). Dynamic topographical pattern classification of multichannel prefrontal NIRS signals: II. Online differentiation of mental arithmetic and rest. *J. Neural Eng.* 11:016003. doi: 10.1088/1741-2560/11/1/016003

Shapiro, M. G., Homma, K., Villarreal, S., Richter, C.-P., and Bezanilla, F. (2012). Infrared light excites cells by changing their electrical capacitance. *Nat. Commun.* 3:736. doi: 10.1038/ncomms1742

Slagter, H. A., Davidson, R. J., and Lutz, A. (2011). Mental training as a tool in the neuroscientific study of brain and cognitive plasticity. *Front. Hum. Neurosci.* 5:17. doi: 10.3389/fnhum.2011.00017

Solovey, E. T., Afergan, D., Peck, E. M., Hincks, S. W., and Jacob, R. J. K. (2015). Designing implicit interfaces for physiological computing: guidelines and lessons learned using fNIRS. *ACM Trans. Comput. Hum. Interact.* 21, 1–27. doi: 10.1145/2687926

Stopczynski, A., Stahlhut, C., Petersen, M. K., Larsen, J. E., Jensen, C. F., Ivanova, M. G., et al. (2014). Smartphones as pocketable labs: visions for mobile brain imaging and neurofeedback. *Int. J. Psychophysiol.* 91, 54–66. doi: 10.1016/j.ijpsycho.2013.08.007

Strenziok, M., Parasuraman, R., Clarke, E., Cisler, D. S., Thompson, J. C., and Greenwood, P. M. (2014). Neurocognitive enhancement in older adults: comparison of three cognitive training tasks to test a hypothesis of training transfer in brain connectivity. *Neuroimage* 85, 1027–1039. doi: 10.1016/j.neuroimage.2013.07.069

Villamar, M. F., Volz, M. S., Bikson, M., Datta, A., Dasilva, A. F., and Fregni, F. (2013). Technique and considerations in the use of 4x1 ring high-definition transcranial direct current stimulation (HD-tDCS). *J. Vis. Exp.* e50309. doi: 10.3791/50309

Villringer, A., and Chance, B. (1997). Non-invasive optical spectroscopy and imaging of human brain function. *Trends Neurosci.* 20, 435–442. doi: 10.1016/s0166-2236(97)01132-6

Vlek, R. J., Steines, D., Szibbo, D., Kubler, A., Schneider, M. J., Haselager, P., et al. (2012). Ethical issues in brain-computer interface research, development and dissemination. *J. Neurol. Phys. Ther.* 36, 94–99. doi: 10.1097/npt.0b013e31825064cc

Voss, M. W., Prakash, R. S., Erickson, K. I., Boot, W. R., Basak, C., Neider, M. B., et al. (2012). Effects of training strategies implemented in a complex videogame on functional connectivity of attentional networks. *Neuroimage* 59, 138–148. doi: 10.1016/j.neuroimage.2011.03.052

Walsh, V., and Pascual-Leone, A. (2005). *Transcranial Magnetic Stimulation: A Neurochronometrics of Mind.* Cambridge, MA: Bradford.

Weingarten, M. S., Papazoglou, E. S., Zubkov, L., Zhu, L., Neidrauer, M., Savir, G., et al. (2008). Correlation of near infrared absorption and diffuse reflectance spectroscopy scattering with tissue neovascularization and collagen concentration in a diabetic rat wound healing model. *Wound Repair Regen.* 16, 234–242. doi: 10.1111/j.1524-475x.2008.00364.x

Wells, J., Kao, C., Jansen, E. D., Konrad, P., and Mahadevan-Jansen, A. (2005a). Application of infrared light for in vivo neural stimulation. *J. Biomed. Opt.* 10:064003. doi: 10.1117/1.2121772

Wells, J., Kao, C., Mariappan, K., Albea, J., Jansen, E. D., Konrad, P., et al. (2005b). Optical stimulation of neural tissue in vivo. *Opt. Lett.* 30, 504–506. doi: 10.1364/ol.30.000504

Wolpaw, J. R., Birbaumer, N., Mcfarland, D. J., Pfurtscheller, G., and Vaughan, T. M. (2002). Brain-computer interfaces for communication and control. *Clin. Neurophysiol.* 113, 767–791. doi: 10.1016/S1388-2457(02)00057-3

Yamauchi, Y., Kikuchi, S., Miwakeichi, F., Matsumoto, K., Nishida, M., Ishiguro, M., et al. (2013). Relation between parametric change of the workload and prefrontal cortex activity during a modified version of the 'rock, paper, scissors' task. *Neuropsychobiology* 68, 24–33. doi: 10.1159/000350948

Yerkes, R. M., and Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol. Psychol.* 18, 459–482. doi: 10.1002/cne.920180503

Yoshino, K., Oka, N., Yamamoto, K., Takahashi, H., and Kato, T. (2013a). Correlation of prefrontal cortical activation with changing vehicle speeds in actual driving: a vector-based functional near-infrared spectroscopy study. *Front. Hum. Neurosci.* 7:895. doi: 10.3389/fnhum.2013.00895

Yoshino, K., Oka, N., Yamamoto, K., Takahashi, H., and Kato, T. (2013b). Functional brain imaging using near-infrared spectroscopy during actual driving on an expressway. *Front. Hum. Neurosci.* 7:882. doi: 10.3389/fnhum.2013.00882

Yurtsever, G., Ayaz, H., Kepics, F., Onaral, B., and Pourrezaei, K. (2006). *Wireless, Continuous Wave Near Infrared Spectroscopy System for Monitoring Brain Activity.* Philadelphia, PA: Drexel University.

Conflict of Interest Statement: fNIR Devices, LLC manufactures the optical brain imaging instrument and licensed IP and know-how from Drexel University. H. Ayaz was involved in the technology development and thus offered a minor share in the new startup firm fNIR Devices, LLC. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 05 February 2015
doi: 10.3389/fnsys.2015.00003

Enhancing multiple object tracking performance with noninvasive brain stimulation: a causal role for the anterior intraparietal sulcus

Eric J. Blumberg *, Matthew S. Peterson and Raja Parasuraman

Arch Lab, Department of Psychology, George Mason University, Fairfax, VA, USA

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Shalini Narayana, University of Tennessee Health Science Center at Memphis, USA
Richard Andrew McKinley, Air Force Research Laboratory, USA
Ruyuan Zhang, University of Rochester, China

****Correspondence:***
Eric J. Blumberg, Arch Lab, Department of Psychology, George Mason University, David King Hall 2083, MSN 3F5, 4400 University Drive, Fairfax, VA 22030-4444, USA
e-mail: ericjoshua@gmail.com

Multiple object tracking (MOT) is a complex task recruiting a distributed network of brain regions. There are also marked individual differences in MOT performance. A positive causal relationship between the anterior intraparietal sulcus (AIPS), an integral region in the MOT attention network and inter-individual variation in MOT performance has not been previously established. The present study used transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, in order to examine such a causal link. Active anodal stimulation was applied to the right AIPS and the left dorsolateral prefrontal cortex (DLPFC) (and sham stimulation), an area associated with working memory (but not MOT) while participants completed a MOT task. Stimulation to the right AIPS significantly improved MOT accuracy more than the other two conditions. The results confirm a causal role of the AIPS in the MOT task and illustrate that tDCS has the ability to improve MOT performance.

Keywords: tDCS, brain stimulation, multiple object tracking, anterior intraparietal sulcus, spatial attention

INTRODUCTION

Multiple object tracking (MOT) is a dynamic, effortful task that assesses how many moving objects a person can attend to over a short period of time (Pylyshyn and Storm, 1988). In the traditional MOT paradigm, participants are presented with multiple objects (e.g., circles) on a monitor and are instructed to track a subset of those objects. The objects move independently and continuously around the screen, and after they stop, participants attempt to indicate which objects they had been tracking. Accuracy, or the proportion of correctly identified targets, is generally used to measure MOT performance.

MOT tasks have been used to evaluate attention capacity (Alvarez and Franconeri, 2007; Horowitz and Cohen, 2010), mechanisms of perceptual organization (Yantis, 1992; Scholl et al., 2001), and distributed attention (Sears and Pylyshyn, 2000). While MOT is a process-intensive task involving attention, object selection, object tracking, memory, and multiple types of eye movements, a number of studies (Pylyshyn and Storm, 1988; Cavanagh and Alvarez, 2005) have illustrated that on average, individuals have a tracking accuracy of 85% for two objects, and as the number of items to track increases, accuracy decreases sharply. In addition, there are marked inter-individual differences in MOT tracking capacity, reflecting inter-individual variation in spatial ability (Oksama and Hyönä, 2004). Given such variability, it is important to understand the underlying neural mechanisms involved in performance of dynamic attentional tasks such as the MOT.

Several researchers (Culham et al., 1998, 2001; Jovicich et al., 2001; Howe et al., 2009) have used functional magnetic resonance imaging (fMRI) to identify the brain areas associated with MOT. Given the number of perceptual and attentional processes involved in MOT, it is not surprising that the fMRI studies have implicated 12 unique brain areas recruited during MOT. Culham et al. (1998) concluded that 11 different brain areas were recruited during MOT whereas Jovicich et al. (2001) identified 12 areas, 9 of which were consistent with the previous work by Culham et al. These studies identified brain regions sensitive to attention, motion, and areas involved in eye movements. However, Howe et al. (2009) identified a number of issues with the previous fMRI and MOT studies, the greatest of which was that the studies did not correctly differentiate brain areas specifically related to tracking objects vs. attending to objects, a critical differentiation in analysis of MOT performance.

After controlling for the effects of attention, Howe et al. (2009) concluded that the frontal eye fields (FEF), anterior intraparietal sulcus (AIPS), the superior parietal lobule (SPL), posterior intraparietal sulcus (PIPS), and the human motion areas (MT+) were all consistently activated during MOT. The FEF and SPL are involved with the generation and execution of eye movements and spatial attention, processes clearly involved with visually tracking objects (Nobre et al., 1997; Donner et al., 2000). Area MT+ plays

a critical role in motion-based tasks, and might potentially be responsible for updating location information (d'Avossa et al., 2007). Recent evidence has suggested that the PIPS plays a role in attention to both stationary and moving objects, and may be responsible for managing pointers to the spatial locations of attended objects. PIPS and MT+ may also interact to support MOT with the PIPS involved in attending to the items and MT+ associated with updating of locations (Howe et al., 2009). The AIPS was identified to be active only when objects were moving, suggesting a dissociation between tracking moving objects and attending to stationary ones, and indicating that it plays a crucial role within the identified attention network. In addition, AIPS has been shown to be sensitive to tracking load, with greater activation associated with increased number of items to be tracked (Culham et al., 2001; Jovicich et al., 2001). Supporting this view, a lesion study conducted by Battelli et al. (2001) showed that individuals with a unilateral right parietal lesion were significantly worse at tracking objects in the contralateral field even when only one object was presented in each visual field. Furthermore, Battelli et al. (2009) provided initial evidence supporting the causal role of the AIPS in MOT performance by demonstrating that MOT performance was inhibited by transcranial magnetic stimulation (TMS) over the right and left intraparietal sulcus but not MT+.

A method that can provide evidence for a positive causal relationship between the AIPS and MOT is transcranial direct current stimulation (tDCS). It involves the application of small amounts of constant direct electric current (1–2 mA) with electrodes attached to the scalp. A positive polarity (anode) is typically used to stimulate neuronal function and enhance performance, while a negative polarity (cathode) is used to inhibit neuronal activity. The electric current is thought to affect the resting potential of cortical neurons (Bindman et al., 1964; Antal et al., 2001) and also synaptic efficacy (Rahman et al., 2013), which in turn increases their sensitivity, leading to an increased likelihood of firing while performing a task. (See Bikson et al., 2004, for a deeper explanation on the neural affects of tDCS). The standard current values for active stimulation conditions can fluctuate up to 2 mA while control/sham levels are either 0.1 mA or a 2 mA ramp-up and immediate ramp-down (Clark et al., 2012). No serious side effects have been associated with normal tDCS operations for 30 min or less of prolonged stimulation (Bikson et al., 2009).

Research by Andrews et al. (2011) has indicated that the effects of tDCS are not global, and only occur when administered in a specific manner: the stimulation must be applied so that stimulation targets areas that are involved in the task being trained on. tDCS is thought to facilitate changes in active neurons and pathways, and those pathways must be active while the stimulation is being administered in order to show a benefit. Through the excitatory (anodal) and inhibitory (cathodal) affects on cell membranes, tDCS can improve our understanding of brain function and its corresponding behavioral correlates.

The present study used tDCS to provide a unique approach to investigating the causal role of the AIPS and of evaluating the plasticity of MOT. To demonstrate that the effects of stimulation are focal rather than global in nature both a target and a control site for stimulation were chosen. As discussed previously, the right AIPS was chosen as the targeted experimental site for potential enhancement of MOT performance. The left dorsolateral prefrontal cortex (DLPFC) was chosen as a control stimulation site because previous fMRI studies have shown that it is minimally involved, if at all, in MOT performance (Howe et al., 2009). In contrast, data from Culham et al., 2001 suggests a right lateralized recruitment in frontal brain areas during MOT. Stimulation of this area may lead to inadvertent affects on MOT performance, and because we focused on the right AIPS in the present study, we used the left DLPFC for the control stimulation condition. In addition to the active stimulation control, we also included a sham stimulation of the left DLPFC (to control for a placebo effect). The sham condition is included to control for placebo effects as previous studies have failed to identify meaningful effects of sham stimulation on task performance (Berryhill et al., 2014). Participants were naïve as to the relationship between scalp location and its corresponding behavioral outcomes, making the sham stimulation location unimportant.

We hypothesized that tDCS would improve performance in the right AIPS stimulation condition and that there would be differences in performance between participants stimulated over the right AIPS compared to those stimulated over the left DLPFC in both active and sham conditions. To assess the possible interaction of stimulation with the processing demand associated with attentional tracking, we administered both a low and a high tracking load version of the MOT. We anticipated that an effect of tDCS would be greatest for the high tracking load and may even be absent in the low load condition due to ceiling effects for tasks already at or near ceiling in performance (Ball et al., 2007; Schmiedek et al., 2010; Jaeggi et al., 2011). In addition, previous researchers have suggested that tDCS may be more beneficial for novices/lower performers than for experts/higher performers (Bullard et al., 2011; Tseng et al., 2012; Blumberg et al., 2014; Foroughi et al., 2015) and that tDCS may be more effective in difficult tasks (Berryhill et al., 2014), suggesting that both task difficulty and individual abilities may play a critical role in the effectiveness of stimulation.

MATERIALS AND METHODS

PARTICIPANTS

Forty-eight undergraduates participated in the experiment (28 females) with an average age of 19 years (range from 18–32). Participants met the following conditions: (1) right handed; (2) normal or corrected to normal vision; and (3) English as a first language. Participants were randomly assigned to one of the three between-subject stimulation conditions while they performed the MOT task under two tracking loads. Sixteen subjects were assigned to each condition. Participants were given course credit for the their participation. All participants gave written informed consent to participate in a protocol approved by the George Mason Institutional Review Board.

TASK AND EQUIPMENT

MOT task

Participants engaged in a computer-based MOT task on a Dell 15" inch LCD monitor at a distance of 40 cm from the screen. The experimental stimuli consisted of eight green circles (two or four of the circles were targets). The circles were 1° of visual angle in size. Each trial consisted of three steps. The eight circles initially appeared as static images (no movement) while the target circles flashed for 1 s. Then the circles moved continuously and independently for 8 s, and could overlap as they traveled across the screen. The circles moved at a constant rate of 13°/sec and in constant directions (when they encountered the border of the screen they were redirected in another direction based upon the angle of impact with the border). After the circles stopped, participants selected the target circles with mouse clicks. The experimental sequence can be seen below in **Figure 1**. Participants tracked two circles (25% of all the circles) in the low load condition and four (50% of all the circles) in the high load condition.

tDCS

tDCS was applied using an ActivaDose II Iontophoresis Delivery Unit. Current was constantly supplied to two electrode pads with 11 cm^2 saline soaked sponges that were attached (with self-adhesive bandage strips) to the participant's scalp and shoulder. The anode was placed on the scalp while the cathode was placed on the contralateral upper arm, consistent with a non-cephalic montage (Falcone et al., 2012; McKinley et al., 2013). Subjects were randomly assigned to one of three stimulation conditions: AIPS active anodal stimulation, DLPFC active anodal stimulation, and DLPFC sham stimulation. In the AIPS experimental condition the anode was placed near CP4 in the 10–20 EEG system while the cathode was placed on the contralateral upper arm. We used Soterix Medical's HDExplore software to identify an appropriate montage to best target the AIPS. A standard adult male head was incorporated for the model; therefore, a single current flow model was identified and applied for all participants. We modeled a number of different montages before identifying scalp site CP4 as the site that best activated AIPS. In both the active and sham control conditions the anode was placed near electrode site F3 in the 10–20 EEG system with the cathode placed on the contralateral upper arm (right). F3 is a commonly used site when modulating the DLPFC (Coffman et al., 2014). Participants in both experimental conditions were given 2.0 mA of stimulation for 30 min. Participants in the sham condition received a 2.0 mA ramp-up and immediate ramp-down to 0 mA lasting 30 s. The brief amount of stimulation provided participants with the full sensation of tDCS.

DESIGN

A 3 × 2 mixed design was employed. The between-subjects variable (stimulation site) had the following levels: AIPS active, DLPFC active, and DLPFC sham. The within-subjects variable was tracking load (low or high). Each participant completed six blocks of 44 trials (three blocks during baseline testing and three blocks while stimulation was administered). The trials in each block were randomized with an equal representation of low and high tracking trials.

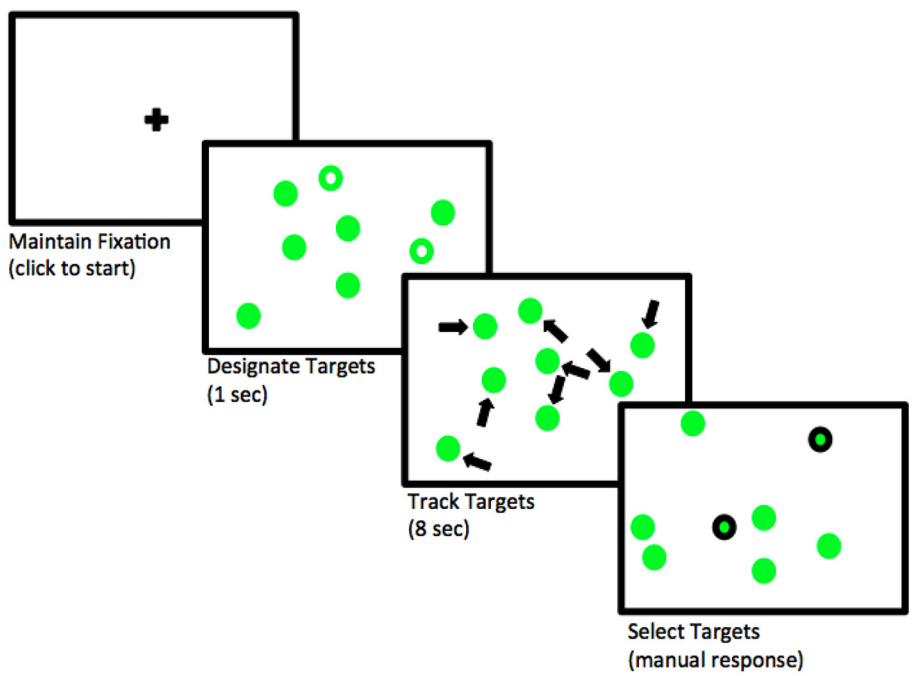

FIGURE 1 | Sequence of actions within the MOT task.

PROCEDURE

Upon arrival participants were asked to read and sign the informed consent form outlining the nature of the task and any risks/benefits they may receive for participating. The Snellen near-sightedness exam was administered to test vision (20–30 or better vision required). Participants were then instructed on how to perform the MOT task. Participants completed a baseline of three blocks of 44 trials to test their baseline performance. The three blocks were completed back-to-back without any breaks.

Following baseline testing, the experimenter prepared the tDCS setup. Participants were given the DCS Sensation Questionnaire (Scheldrup et al., 2014) at three time points throughout the stimulation subsession (approximately 1-, 10-, and 30 min post stimulation onset) measuring how much itching, heat/burning, and tingling the participant felt at that moment. Immediately following the first administration of the sensation questionnaire participants completed a demographic and video game questionnaire. After they finished the questionnaires they completed the second sensation questionnaire. Participants then completed the final three blocks of the MOT task (132 total trials) while tDCS was being administered. After completing the trials the tDCS unit was turned off and the electrodes were removed. Finally, each participant completed the third sensation questionnaire and then was debriefed about the experiment.

RESULTS

MOT ACCURACY

The primary goal of this experiment was to investigate whether a relatively short period of brain stimulation (30 min) could be used to improve MOT performance, thereby establishing a positive causal role of the right AIPS in MOT performance. The behavioral and dependent variable in the experiment was MOT accuracy. Accuracy was calculated by dividing the number of correctly identified targets by the total possible targets for each trial. Accuracy scores were then created for both the baseline and stimulation subsessions by averaging the accuracy scores across trials and blocks within each subsession. Separate accuracy scores were created for both load conditions leaving each participant with four different accuracy scores (see **Figure 2**).

BASELINE COMPARISON

We initially tested whether baseline performance across stimulation conditions was significantly different from one another. Two separate (low and high tracking loads) one-way analysis of variance (ANOVA) were conducted because we did not want a potential ceiling effect in the low tracking load condition (hypothesized *a priori*) to reduce the likelihood of finding an effect in the high tracking load condition. The ANOVA for the low tracking load condition failed to identify a significant main effect of stimulation condition ($F_{(2,45)} = 0.87$, $p > 0.20$). The ANOVA for the high tracking load condition also did not reveal a significant main effect of stimulation condition ($F_{(2,45)} = 1.34$, $p > 0.20$) suggesting that baseline performance was not significantly different across stimulation groups in either tracking load condition.

CONDITION SPECIFIC STIMULATION EFFECT

We then conducted a 2 × 2 × 3 mixed-design ANOVA with subsession and tracking load as the within-subjects factors, stimulation site as the between-subjects factor, and MOT accuracy as the independent variable. A self-assessment of first person shooter video game experience was initially included in the analysis as a covariate because prior research (Green and Bavelier, 2006) suggested it predicts MOT performance, however, this effect was not significant in the present study and was therefore removed from the subsequent analyses. The analysis revealed a 2-way interaction between tracking load and subsession ($F_{(1,45)} = 5.24$, $p < 0.05$, $\eta^2_{partial} = 0.10$), a main effect for subsession ($F_{(1,45)} = 351.14$, $p < 0.01$), and a main effect for tracking load ($F_{(1,45)} = 4.34$, $p < 0.05$). Tests of simple main effects for the two-way interaction using a Bonferroni correction ($\alpha = 0.05$) revealed that within the high tracking load condition, MOT performance was significantly greater in the stimulation subsession compared to baseline, ($F_{(1,45)} = 9.5$, $p < 0.01$, $\eta^2_{partial} = 0.17$). The three-way interaction was not significant ($p > 0.10$).

To better test our initial hypothesis about whether tDCS stimulation applied to the AIPS can improve MOT performance, a series of planned paired samples *t*-tests were conducted to identify if stimulation improved MOT accuracy beyond that of baseline. Six, separate paired samples *t*-test using a Ŝidák correction ($\alpha = 0.0063$, given six related tests) were conducted comparing each baseline score to its corresponding stimulation score (low and high tracking load for each stimulation site). A significant difference in performance was identified in the high tracking load condition between AIPS baseline ($M = 76.42\%$, $SE = 1.66$) and AIPS stimulation ($M = 72.54\%$, $SE = 1.86$), $t_{(15)} = 4.10$, $p = 0.00047$, $d = 1.03$ illustrating a 4% improvement in MOT accuracy, see **Figure 2**. No other *t*-test reached significance (largest $t = 1.0$; smallest $p = 0.33$). Given that stimulation did not affect performance in the low tracking load condition across any stimulation condition and we did not make any *a priori*

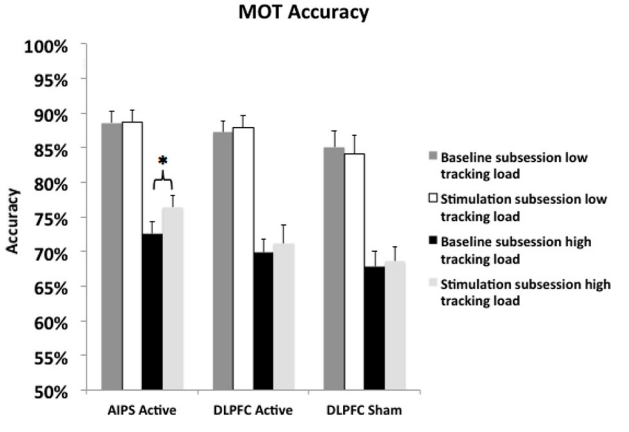

FIGURE 2 | MOT accuracy ± SEM (standard error of the mean) across stimulation sites broken down by time point (baseline and stimulation) and tracking load (low and high). * indicates significant difference.

predictions, the low tracking load condition was excluded from the following analyses.

COMPARISON OF STIMULATION EFFECTS ACROSS CONDITIONS

In addition to testing for changes in performance due to stimulation, we also examined whether stimulation led to group differences. Given the *a priori* hypothesis that in the high tracking load condition the AIPS stimulation condition would be significantly different from the two DLPFC control conditions, the DLPFC active (M = 71.16%, SE = 2.67) and DLPFC sham (M = 68.56%, SE = 2.12) groups were initially compared against one another to identify any differences. An independent samples t-test using a Ŝidák correction ($\alpha = 0.025$, given two related tests) was conducted to compare performance across the two control conditions in the stimulation subsession. The analysis did not reveal a significant difference between the two control groups, $t_{(30)} = 0.45$, $p = 0.41$. The two DLPFC control conditions were therefore collapsed into one control condition in the subsequent analysis, leaving two levels of the stimulation variable (AIPS active and DLPFC control).

We then tested if performance in the AIPS active and DLPFC control condition were significantly different from one another in the stimulation subsession, examining if AIPS stimulation led to better MOT performance compared to the DLPFC control. An independent samples t-test using a Ŝidák correction ($\alpha = 0.025$) was conducted to compare performance between the AIPS active (M = 76.42%, SE = 1.67) and DLPFC control (M = 69.86%, SE = 1.69) conditions. The analysis revealed a significant difference $t_{(46)} = 2.45$, $p = 0.009$, $d = 0.80$. The data suggests that in the high tracking load condition, right AIPS stimulation improved MOT accuracy significantly more than in the combined control condition, see **Figures 2**, **3**.

MOT CAPACITY

Due to the high accuracy scores in the low tracking load condition, an important question becomes: is accuracy a sensitive enough measure to detect performance changes close to ceiling? To answer this question, we non-linearly transformed the accuracy scores into capacity measures (k) according to Horowitz et al. (2007) and Scholl et al. (2001). The capacity measure did not lead to any significantly different outcomes compared to the accuracy measure, therefore, the analyses will not be included in this manuscript, see **Table 1** for means.

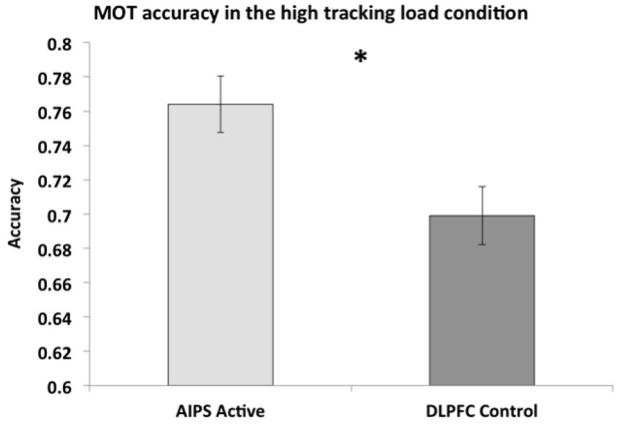

FIGURE 3 | MOT accuracy in the high tracking load condition + SEM (standard error of the mean). * indicates significant difference.

BASELINE VS. CHANGE IN MOT PERFORMANCE

We also examined whether baseline MOT accuracy predicted the amount of improvement exhibited in the right AIPS stimulation condition (high tracking load trials). To accomplish this we compared participants' baseline MOT accuracy to their change in MOT accuracy (stimulation minus baseline). The two scores were negatively correlated, $r_{(16)} = -0.45$, $p < 0.05$, see **Figure 4**. This significant association suggests that tDCS may be more beneficial to individuals with lower baseline MOT abilities. Note also, that all but two of the participants, irrespective of their baseline performance, showed improvement in MOT accuracy with tDCS.

RATE OF MOT IMPROVEMENT

Additionally, we examined the rate at which stimulation impacted MOT performance in the AIPS stimulation high tracking load condition. We ran a repeated measure ANOVA with block (only in the stimulation subsession) as the within-subjects factor. Block was not significant ($F_{(2,30)} = 0.04$, $p > 0.10$) indicating that stimulation led to an immediate boost in MOT performance that was sustained across the three blocks (see **Figure 5**—Blocks 4,5,6).

DISCUSSION

The present study evaluated the efficacy of using targeted non-invasive brain stimulation to improve understanding of the causal role of the right AIPS in MOT performance through improved learning and skill acquisition of MOT. tDCS was targeted to the right AIPS, a brain area that plays a unique and integral role for tracking multiple objects (Howe et al., 2009). Active anodal stimulation to the right AIPS improved MOT performance in the high tracking load condition but not in the low tracking load condition. Active and sham stimulation of the left DLPFC had no effect in either tracking load condition. This finding suggests that: (1) the right AIPS plays an active role in MOT; (2) modulation of this area by tDCS directly leads to changes in MOT performance; and (3) the effects of the tDCS were focal in nature and not a global enhancement due to stimulation of the entire cortex.

Right AIPS stimulation improved performance in the more difficult tracking load condition where participants' accuracy was relatively low (∼70%) whereas stimulation did not affect performance in the easier tracking load condition (85–90%) where participants were performing at or close to ceiling. These results are consistent with the *a priori* hypotheses and are in line with the previous literature that suggests that tDCS is more beneficial to novices (Bullard et al., 2011) and lower performers (Tseng et al., 2012; Blumberg et al., 2014; Foroughi et al., 2015) and may be more effective when paired with difficult tasks (Jones and Berryhill, 2012; Berryhill et al., 2014). Additionally, cognitive training is not beneficial for individuals already performing at

Table 1 | Means for both accuracy and capacity measure across stimulation condition, tracking load, and subsession.

		Low		High	
		Baseline	**Stimulation**	**Baseline**	**Stimulation**
Accuracy	AIPS Active	88.59	88.64	72.54	76.42
DLPFC Active		87.26	87.97	69.89	71.16
DLPFC Sham		85.09	84.14	67.80	68.56
Capacity	AIPS Active	1.77	1.77	2.88	3.04
DLPFC Active		1.74	1.76	2.77	2.82
DLPFC Sham		1.70	1.68	2.68	2.72

ceiling (Ball et al., 2007; Schmiedek et al., 2010; Jaeggi et al., 2011).

We also identified that in the high tracking load condition, the amount of improvement in MOT was negatively correlated with a participant's baseline MOT ability. On average, individuals with lower baseline MOT accuracies exhibited greater increases in accuracy compared to those with higher baseline abilities. Tseng et al. (2012) found a similar inverse effect in change-detection ability stimulating a posterior parietal location.

Additionally, we identified that stimulation had an immediate effect on MOT performance in the right AIPS high tracking load condition. Performances across the three blocks in the stimulation subsession were similar, illustrating a constant positive impact of stimulation. We believe this is due to the fact that MOT is a very simple task with little to no learning curve, therefore, stimulation immediately modulated the relationship between the right AIPS and MOT performance.

While research (Green and Bavelier, 2006; Boot et al., 2008) has previously shown that specific types of training such as playing action video games can improve MOT performance, this is the first study to show that brain stimulation can do so too, but in a much shorter time. If used as a tool for accelerated training, tDCS may offer a number of benefits compared to traditional training paradigms. Video game training can take extended periods of time (Green and Bavelier, 2006; Feng et al., 2007; Basak et al., 2008), whereas in this study, tDCS immediately improved spatial tracking performance. Also, specific subsets of the population cannot or do not enjoy playing video games because the games can be difficult to learn, can cause frustration, and can require fine motor control. On the other hand, tDCS requires little or no additional effort from the user apart from the task being performed, making it ideally suited to a larger segment of the population. tDCS focused on the right AIPS, a brain area integral to the attention network (Howe et al.,

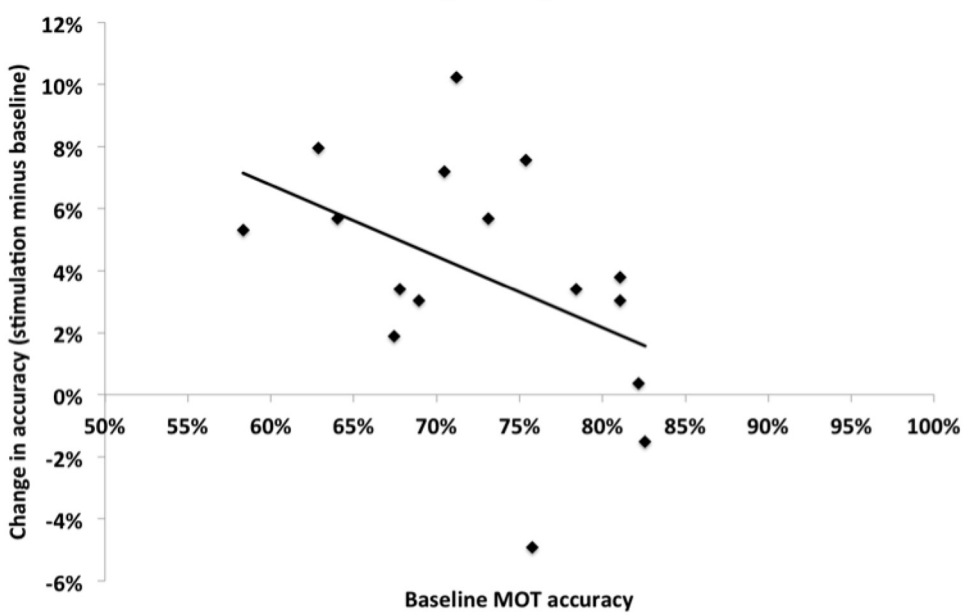

FIGURE 4 | Correlation between baseline MOT accuracy and the change in MOT accuracy within the AIPS high tracking load condition.

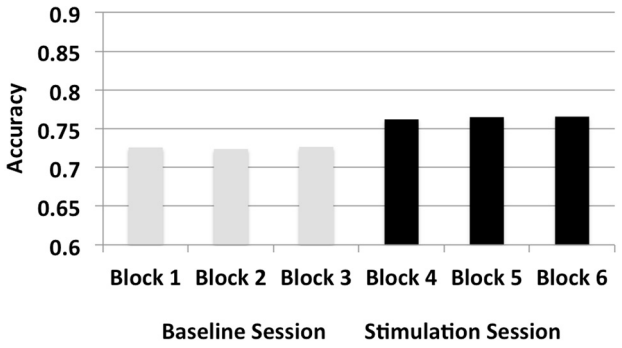

FIGURE 5 | Accuracy per block in the AIPS stimulation high tracking load condition, subsession differentiated by bar color.

2009) immediately improved MOT performance, this transient improvement was accomplished in a substantially shorter amount of time than through traditional training programs, however, the effects may not be as significant or as long lasting. While this study provides initial evidence that tDCS can rapidly improve MOT performance, further research should identify if these effects are transferable to other spatial tracking tasks in both basic and complex settings. Brain stimulation over the right AIPS offers a unique method to better understand the function of this area as it relates to MOT. This study adds to the existing literature that the right AIPS plays an active role in MOT and that the neural substrates recruited for MOT exhibit significant plasticity.

Our findings mirror previous tDCS studies that have found effects on perception, attention, and memory abilities (Coffman et al., 2014). For example, Clark et al. (2012) showed increased perceptual learning when tDCS electrodes were targeted to brain areas related to perceptual learning, with the benefit of tDCS being retained for at least 24 h (Falcone et al., 2012). Of particular note is the study by Moos et al. (2012), in which they observed that cathodal stimulation over the right AIPS increased top-down attentional selection. While they applied cathodal stimulation to the same area we did, the two studies measured different aspects of attentional selection. Additionally, TMS has previously been applied to support a causal mechanism between modulation of the IPS and MOT (Battelli et al., 2009). They inhibited IPS function, leading to decreased MOT accuracy. However, our study is the first to illustrate that the causal mechanism is in the positive direction as well. We used tDCS to illustrate the facilitative effects of AIPS stimulation on MOT ability, finding increased MOT accuracy. tDCS is also less invasive and more easily applied making it a more practical tool to accelerate MOT abilities.

This study had some limitations. Although current modeling was used to identify the electrode montage that would best lead to stimulation of AIPS, such modeling involves a number of assumptions that may not always be met, and modeling must be considered as a hypothesis to be tested rather than definitive. That the empirical evidence confirmed the hypothesis and showed that other stimulation sites did not lead to improvement in MOT performance is consistent with the predicted results. Furthermore, we did not directly measure cortical activation in AIPS or other parietal regions as a result of tDCS. However, previous research has illustrated that tDCS does affect neuronal firing (Radman et al., 2009). Additionally, the tDCS electrode montage used in the experiment may have resulted in stimulation of the posterior parietal cortex in general.

Another possible concern involves the baseline performance of the different stimulation/sham groups. While statistically significant differences between groups in MOT baseline performance were not found, one could argue that individuals in the AIPS condition were somewhat better to begin with, so that the difference identified in the stimulation subsession could just reflect these initial differences in performance and random variation or potentially a small tDCS effect. However, it would be highly unlikely that random noise would increase performance in individuals that were already performing at high levels and not for individuals performing poorly. Additionally, it is unlikely given that individuals with the lowest baseline MOT abilities saw the largest increases in MOT performance. If baseline averages were identical (decreasing initial AIPS accuracy) potentially more individuals would see greater benefits in their MOT performance. Task difficulty can also modulate the beneficial effects of tDCS (Jones and Berryhill, 2012; Berryhill et al., 2014). While AIPS stimulation significantly improved MOT accuracy in the four ball tracking condition (an amount at or close to our attention capacity) and not the two ball tracking condition, an even more difficult MOT condition (i.e., tracking six objects) may have resulted in larger effects. Therefore, the findings in this paper may be underestimating the beneficial effect of tDCS on MOT performance. Future research should investigate the limitations of applying tDCS to the intraparietal sulcus, how cathodal stimulation may affect spatial tracking performance, and how tDCS could be used in conjunction with other training programs to improve spatial tracking beyond that of just video game play or tDCS alone. Additionally, it will be very important to identify how long the tDCS effects last, especially if tDCS is used for long-term enhancement of spatial abilities.

The current study is the first to illustrate that brain stimulation can improve MOT accuracy. Stimulation to the AIPS, a central location in the attention network improved MOT accuracy while stimulation to the DLPFC did not. Accelerated training techniques like tDCS can be used to improve perceptual, attention, and memory training programs and to identify the causal relationships between brain and behavior.

ACKNOWLEDGMENTS

This research was supported by Air Force Office of Scientific Research Grant FA9550-10-1-0385 to RP and the Center of Excellence in Neuroergonomics, Technology, and Cognition (CENTEC). We would like to acknowledge Amanda Hanelli and Juliane Holmblad for helping with the experiment.

REFERENCES

Alvarez, G. A., and Franconeri, S. L. (2007). How many objects can you track?: evidence for a resource-limited attentive tracking mechanism. *J. Vis.* 7:14. doi: 10.1167/7.13.14

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimul.* 4, 84–89. doi: 10.1016/j.brs.2010.06.004

Antal, A., Nitsche, M. A., and Paulus, W. (2001). External modulation of visual perception in humans. *Neuroreport* 12, 3553–3555. doi: 10.1097/00001756-200111160-00036

Ball, K., Edwards, J. D., and Ross, L. A. (2007). The impact of speed of processing training on cognitive and everyday functions. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 62, 19–31. doi: 10.1093/geronb/62.special_issue_1.19

Basak, C., Boot, W. R., Voss, M. W., and Kramer, A. F. (2008). Can training in a real-time strategy video game attenuate cognitive decline in older adults?. *Psychol. Aging* 23, 765–777. doi: 10.1037/a0013494

Battelli, L., Alvarez, G. A., Carlson, T., and Pascual-Leone, A. (2009). The role of the parietal lobe in visual extinction studied with transcranial magnetic stimulation. *J. Cogn. Neurosci.* 21, 1946–1955. doi: 10.1162/jocn.2008.21149

Battelli, L., Cavanagh, P., Intriligator, J., Tramo, M. J., Hénaff, M. A., Michèl, F., et al. (2001). Unilateral right parietal damage leads to bilateral deficit for high-level motion. *Neuron* 32, 985–995. doi: 10.1016/s0896-6273(01)00536-0

Berryhill, M. E., Peterson, D. J., Jones, K. T., and Stephens, J. A. (2014). Hits and misses: leveraging tDCS to advance cognitive research. *Front. Psychol.* 5:800. doi: 10.3389/fpsyg.2014.00800

Bikson, M., Datta, A., and Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clin. Neurophysiol.* 120, 1033–1034. doi: 10.1016/j.clinph.2009.03.018

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J. Physiol.* 557, 175–190. doi: 10.1113/jphysiol.2003.055772

Bindman, L. J., Lippold, O. C. J., and Redfearn, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382. doi: 10.1113/jphysiol.1964.sp007425

Blumberg, E. J., Foroughi, C. K., Scheldrup, M. R., Peterson, M. S., Boehm-Davis, D. A., and Parasuraman, R. (2014). Reducing the disruptive effect of interruptions with noninvasive brain stimulation. *Hum. Factors*. doi: 10.1177/0018720814565189

Boot, W. R., Kramer, A. F., Simons, D. J., Fabiani, M., and Gratton, G. (2008). The effects of video game playing on attention, memory and executive control. *Acta Psychol. (Amst)* 129, 387–398. doi: 10.1016/j.actpsy.2008.09.005

Bullard, L. M., Browning, E. S., Clark, V. P., Coffman, B. A., Garcia, C. M., Jung, R. E., et al. (2011). Transcranial direct current stimulation's effect on novice versus experienced learning. *Exp. Brain Res.* 213, 9–14. doi: 10.1007/s00221-011-2764-2

Cavanagh, P., and Alvarez, G. A. (2005). Tracking multiple targets with multifocal attention. *Trends Cogn. Sci.* 9, 349–354. doi: 10.1016/j.tics.2005.05.009

Clark, V. P., Coffman, B. A., Mayer, A. R., Weisend, M. P., Lane, T. D., Calhoun, V. D., et al. (2012). tDCS guided using fMRI significantly accelerates learning to identify concealed objects. *Neuroimage* 59, 117–128. doi: 10.1016/j.neuroimage.2010.11.036

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85, 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Culham, J. C., Brandt, S. A., Cavanagh, P., Kanwisher, N. G., Dale, A. M., and Tootell, R. B. (1998). Cortical fMRI activation produced by attentive tracking of moving targets. *J. Neurophysiol.* 80, 2657–2670.

Culham, J. C., Cavanagh, P., and Kanwisher, N. G. (2001). Attention response functions: characterizing brain areas using fMRI activation during parametric variations of attentional load. *Neuron* 32, 737–745. doi: 10.1016/s0896-6273(01)00499-8

d'Avossa, G., Tosetti, M., Crespi, S., Biagi, L., Burr, D. C., and Morrone, M. C. (2007). Spatiotopic selectivity of BOLD responses to visual motion in human area MT. *Nat. Neurosci.* 10, 249–255. doi: 10.1038/nn1824

Donner, T., Kettermann, A., Diesch, E., Ostendorf, F., Villringer, A., and Brandt, S. A. (2000). Involvement of the human frontal eye field and multiple parietal areas in covert visual selection during conjunction search. *Eur. J. Neurosci.* 12, 3407–3414. doi: 10.1046/j.1460-9568.2000.00223.x

Falcone, B., Coffman, B. A., Clark, V. P., and Parasuraman, R. (2012). Transcranial direct current stimulation augments perceptual sensitivity and 24-hour retention in a complex threat detection task. *PLoS One* 7:e34993. doi: 10.1371/journal.pone.0034993

Feng, J., Spence, I., and Pratt, J. (2007). Playing an action video game reduces gender differences in spatial cognition. *Psychol. Sci.* 18, 850–855. doi: 10.1111/j.1467-9280.2007.01990.x

Foroughi, C. K., Blumberg, E. J., and Parasuraman, R. (2015). Activation and inhibition of posterior parietal cortex have bi-directional effects following interruptions. *Front. Syst. Neurosci.* 8:245. doi: 10.3389/fnsys.2014.00245

Green, C. S., and Bavelier, D. (2006). Enumeration versus multiple object tracking: the case of action video game players. *Cognition* 101, 217–245. doi: 10.1016/j.cognition.2005.10.004

Horowitz, T. S., and Cohen, M. A. (2010). Direction information in multiple object tracking is limited by a graded resource. *Atten. Percept. Psychophys.* 72, 1765–1775. doi: 10.3758/APP.72.7.1765

Horowitz, T. S., Klieger, S. B., Fencsik, D. E., Yang, K. K., Alvarez, G. A., and Wolfe, J. M. (2007). Tracking unique objects. *Percept. Psychophys.* 69, 172–184. doi: 10.3758/bf03193740

Howe, P. D., Horowitz, T. S., Morocz, I. A., Wolfe, J., and Livingstone, M. S. (2009). Using fMRI to distinguish components of the multiple object tracking task. *J. Vis.* 9:10. doi: 10.1167/9.4.10

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Shah, P. (2011). Short-and long-term benefits of cognitive training. *Proc. Natl. Acad. Sci. U S A* 108, 10081–10086. doi: 10.1073/pnas.1103228108

Jones, K. T., and Berryhill, M. E. (2012). Parietal contributions to visual working memory depend on task difficulty. *Front. Psychiatry* 3:81. doi: 10.3389/fpsyt.2012.00081

Jovicich, J., Peters, R. J., Koch, C., Braun, J., Chang, L., and Ernst, T. (2001). Brain areas specific for attentional load in a motion-tracking task. *J. Cogn. Neurosci.* 13, 1048–1058. doi: 10.1162/089892901753294347

McKinley, R. A., McIntire, L., Bridges, N., Goodyear, C., and Weisend, M. P. (2013). Acceleration of image analyst training with transcranial direct current stimulation. *Behav. Neurosci.* 127, 936–946. doi: 10.1037/a0034975

Moos, K., Vossel, S., Weidner, R., Sparing, R., and Fink, G. R. (2012). Modulation of top-down control of visual attention by cathodal tDCS over right IPS. *J. Neurosci.* 32, 16360–16368. doi: 10.1523/JNEUROSCI.6233-11.2012

Nobre, A. C., Sebestyen, G. N., Gitelman, D. R., Mesulam, M. M., Frackowiak, R. S., and Frith, C. D. (1997). Functional localization of the system for visuospatial attention using positron emission tomography. *Brain* 120, 515–533. doi: 10.1093/brain/120.3.515

Oksama, L., and Hyönä, J. (2004). Is multiple object tracking carried out automatically by an early vision mechanism independent of higher-order cognition? An individual difference approach. *Vis. Cogn.* 11, 631–671. doi: 10.1080/13506280344000473

Pylyshyn, Z. W., and Storm, R. W. (1988). Tracking multiple independent targets: evidence for a parallel tracking mechanism. *Spat. Vis.* 3, 179–197. doi: 10.1163/156856888x00122

Radman, T., Datta, A., Ramos, R. L., Brumberg, J. C., and Bikson, M. (2009). "One-dimensional representation of a neuron in a uniform electric field," in *Engineering in Medicine and Biology Society, 2009. EMBC 2009. Annual International Conference of the IEEE* (Minneapolis, MN: IEEE), 6481–6484.

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *J. Physiol.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Scheldrup, M., Greenwood, P. M., McKendrick, R., Strohl, J., Bikson, M., Alam, M., et al. (2014). Transcranial direct current stimulation facilitates cognitive multitask performance differentially depending on anode location and subtask. *Front. Hum. Neurosci.* 8:665. doi: 10.3389/fnhum.2014.00665

Schmiedek, F., Lövdén, M., and Lindenberger, U. (2010). Hundred days of cognitive training enhance broad cognitive abilities in adulthood: findings

from the COGITO study. *Front. Aging Neurosci.* 2:27. doi: 10.3389/fnagi.2010.00027

Scholl, B. J., Pylyshyn, Z. W., and Feldman, J. (2001). What is a visual object? Evidence from target merging in multiple object tracking. *Cognition* 80, 159–177. doi: 10.1016/s0010-0277(00)00157-8

Sears, C. R., and Pylyshyn, Z. W. (2000). Multiple object tracking and attentional processing. *Can. J. Exp. Psychol.* 54, 1–14. doi: 10.1037/h0087326

Tseng, P., Hsu, T. Y., Chang, C. F., Tzeng, O. J., Hung, D. L., Muggleton, N. G., et al. (2012). Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. *J. Neurosci.* 32, 10554–10561. doi: 10.1523/jneurosci.0362-12.2012

Yantis, S. (1992). Multielement visual tracking: attention and perceptual organization. *Cogn. Psychol.* 24, 295–340. doi: 10.1016/0010-0285(92)90010-y

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 November 2014; accepted: 09 January 2015; published online: 05 February 2015.

Citation: Blumberg EJ, Peterson MS and Parasuraman R (2015) Enhancing multiple object tracking performance with noninvasive brain stimulation: a causal role for the anterior intraparietal sulcus. Front. Syst. Neurosci. ***9****:3. doi: 10.3389/fnsys.2015.00003 This article was submitted to the journal Frontiers in Systems Neuroscience.*

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 12 January 2015
doi: 10.3389/fnsys.2014.00245

Activation and inhibition of posterior parietal cortex have bi-directional effects on spatial errors following interruptions

Cyrus K. Foroughi *†, Eric J. Blumberg † and Raja Parasuraman

Department of Psychology, Arch Lab, George Mason University, Fairfax, VA, USA

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Hugo Merchant, Universidad Nacional Autónoma de México, Mexico
Tal Makovski, The Open University of Israel, Israel
Vincent Clark, University of New Mexico, USA

****Correspondence:***
Cyrus K. Foroughi, Department of Psychology, Arch Lab, George Mason University, David King Hall 2084B, MSN 3F5, 4400 University Drive, Fairfax, VA 22030-4444, USA
e-mail: cyrus.foroughi@gmail.com

† These authors have contributed equally to this work.

Interruptions to ongoing mental activities are omnipresent in our modern digital world, but the brain networks involved in interrupted performance are not known, nor have the activation of those networks been modulated. Errors following interruptions reflect failures in spatial memory, whose maintenance is supported by a brain network including the right posterior parietal cortex (PPC). The present study therefore used bi-directional transcranial Direct Current Stimulation (tDCS) of right PPC to examine the neuromodulation of spatial errors following interruptions, as well as performance on another PPC-dependent task, mental rotation. Anodal stimulation significantly reduced the number of interruption-based errors and increased mental rotation accuracy whereas cathodal stimulation significantly increased errors and reduced mental rotation accuracy. The results provide evidence for a causal role of the PPC in the maintenance of spatial representations during interrupted task performance.

Keywords: tDCS, brain stimulation, spatial errors, interruptions, posterior parietal cortex, mental rotation, cognitive equalizing

INTRODUCTION

Interruptions to our ongoing mental activities are omnipresent in modern life—whether from cell phones, emails, navigation devices, alarms, etc. An observational study found that people are interrupted an average of 12 times per hour at work in our increasingly digital world (Cades et al., 2010), with such interruptions often leading to errors. Another study of nurses from two hospitals showed that interruptions increased both procedural (e.g., fail to check patient identification) and clinical judgment errors (e.g., give the wrong drug or wrong dose), with potentially life threatening consequences (Westbrook et al., 2010). Interruption-related errors are ubiquitous and appear to be unrelated to individual expertize (e.g., Dismukes et al., 2012; Prakash et al., 2014).

Ratwani and Trafton (2008) used eye-tracking to investigate visual search patterns of the resumption process in a simple data entry task following an interruption. The primary task required participants to place randomly generated numbers into one of fifteen different locations on a computer display following preset rules. The interruption task involved either solving math problems or performing mental rotation. Both interruption tasks impaired resumption accuracy; compared to a non-interrupted condition, individuals fixated on a location following an interruption that was further away from the correct location. However this effect was significantly larger when the interruption involved mental rotation, suggesting that the same visuo-spatial processes involved in mental rotation are important for the resumption process. Shen and Jiang (2006) also showed that an interruption involving a spatial search significantly decreased memory accuracy in a change detection search task. Both findings suggest that spatial representation may play an important role in guiding resumption after an interruption.

Despite the importance of interruptions in everyday life, the brain networks involved in interrupted performance are not known, nor have the activation of those networks been modulated. The present study used the latter strategy to better understand the neuromodulation of interruption performance. Active modulation of brain networks involved in spatial memory can provide direct evidence for the causal role of transient disruption of spatial representation in resumption performance following an interruption. There is considerable evidence that the posterior parietal cortex (PPC), and more specifically the intraparietal sulcus (IPS), is implicated in the maintenance of spatial representations (Cabeza and Nyberg, 2000; Cohen and Andersen, 2002; Jonides et al., 2005; Champod and Petrides, 2007).

These findings suggest that active stimulation or inhibition of the right PPC should respectively decrease or increase spatial errors during resumption after an interruption. We tested this hypothesis in the present study using transcranial Direct Current Stimulation (tDCS), which provides a method for non-invasive, bi-directional modulation of brain function (Nitsche and Paulus, 2000; Antal et al., 2001). The polarity of stimulation plays a critical role in how tDCS affects performance; typically anodal

(positive) stimulation over a particular cortical site increases cortical excitability and can improve performance (Cohen Kadosh et al., 2010; Coffman et al., 2014; Parasuraman and McKinley, 2014), whereas cathodal (negative) stimulation over the cortical area inhibits excitability and may lead to decrements in task performance (Bikson et al., 2004; Coffman et al., 2014). We therefore hypothesized that anodal stimulation of the right PPC would reduce spatial errors following an interruption, whereas cathodal stimulation of the same brain region would increase errors. For the primary task, we used the *Financial Management Task*, a complex computer-based task (Trafton et al., 2011; see **Figure 1**) commonly used in studies of interrupted task performance and the resumption process (Trafton et al., 2003; Brumby et al., 2013). The task requires participants to store information in memory and then place that information into different locations on the computer screen, either uninterrupted or following an interruption. The interruption task required participants to solve math problems.

Our main hypothesis was that compared to a sham (placebo) group, anodal stimulation of right PPC would reduce spatial errors following an interruption, whereas cathodal stimulation would increase errors. Additionally, as a manipulation check, we also used a mental rotation task, given the causal role of the PPC in mental rotation has been previously established in a repetitive transcranial magnetic stimulation (rTMS) study (Harris and Miniussi, 2003). We hypothesized that compared to a sham (placebo) group, anodal stimulation of the right PPC would improve mental rotation accuracy, whereas cathodal stimulation would decrease accuracy. A final hypothesis, based on previous findings (e.g., Blumberg et al., in press; Tseng et al., 2012), was that lower performing individuals (those with more interruption-related errors and lower mental rotation scores) receiving anodal stimulation of the right PPC would exhibit the greatest improvements in resumption performance compared to higher performing individuals.

METHODS

PARTICIPANTS

The George Mason University Institutional Review Board (IRB) approved this study. Forty-six right-handed students (M = 19.74 years, SD = 2.2, 35 females, 11 males) from George Mason University participated for course credit. One participant was excused from the study because of problems with the stimulation delivery device and the data were excluded from all analyses. Participants were randomly assigned to one of three groups: anodal stimulation (n = 15), cathodal stimulation (n = 15), and sham stimulation (n = 15) to the PPC. Sample size was determined based on effect sizes reported in previous modulation studies using tDCS over the PPC (e.g., Sparing et al., 2009; Stone and Tesche, 2009). Thus, the group size was set *a priori* at 15 resulting in a total sample size of 45.

TDCS

An ActivaDose II Iontophoresis Delivery Unit was used to deliver constant current via two electrode pads housing saline-soaked sponges with an 11 cm^2 contact area. One electrode was placed on the scalp (directly between sites CP4 and P4, identified as CPP4 of the 10–5 EEG Scalp Recording System; Oostenveld and Praamstra, 2001)—this is the same right parietal site previously found to decrease mental rotation performance in an rTMS study (Harris and Miniussi, 2003). The reference electrode was placed on the contralateral (left) upper arm. The electrodes were attached to each participant using velcro wraps. Participants received 2 mA of current for 30 min in the active stimulation group, an amount found to be safe in a number of previous studies (Coffman et al., 2014). Participants in the sham group received a 2 mA ramp up (30 s) and then immediate ramp down (6 s) of current, receiving the full 2 mA for a very short period of time (<5 s). This short stimulation duration (applied prior to the beginning of the experimental tasks) is enough to cause similar skin sensations compared to the active stimulation group, but is generally insufficient to produce lasting causal effects on cortical excitability (Coffman et al., 2014).

FINANCIAL MANAGEMENT TASK

The goal of this task was to successfully complete a client stock order as quickly and accurately as possible. To do this, participants first selected a stock order to buy or sell and then filled in twelve pieces of information relevant to that order. This information was placed, one component at a time, in one of twelve different boxes located throughout the computer screen. Importantly, participants had to place this information in order starting with the upper left box (labeled 1 in **Figure 1**), then the upper right box (labeled 2 in **Figure 1**), and so on, until all twelve pieces of information were correctly placed. If a participant went to the wrong box (i.e., made an error), the participant was unable to fill in the information. Instead, the box that the participant was supposed to go to would turn red. This indicated that an error was made and that the participant would need to place information in the red box before moving on.

Interruptions occurred randomly throughout the duration of the financial management task. The interruption task, which replaced the primary task screen, required participants to answer multiple choice addition (math) problems that were located on the bottom, center of the computer screen for the entire duration of the 15 s interruption (see **Figure 1**). Participants answered the problems at their own pace. Immediately following the interruption, the primary task screen reappeared and participants were able to continue the primary task. Importantly, when returning to the primary task following an interruption, all of the information that was on the screen before the interruption occurred was gone. Therefore, participants needed to remember where they left off to successfully re-engage the task without making an error (see Trafton et al., 2011 for more information about the Financial Management task).

MENTAL ROTATION TASK

The Vandenberg and Kuse Mental Rotation Test, Version C (MRT-C; Peters et al., 1995; Shepard and Metzler, 1971; Vandenberg and Kuse, 1978) was used to assess mental rotation ability. This version, unlike versions A and B, and most other MRT, rotates objects around both the vertical and horizontal axes, thereby increasing the difficulty of the test. The use of

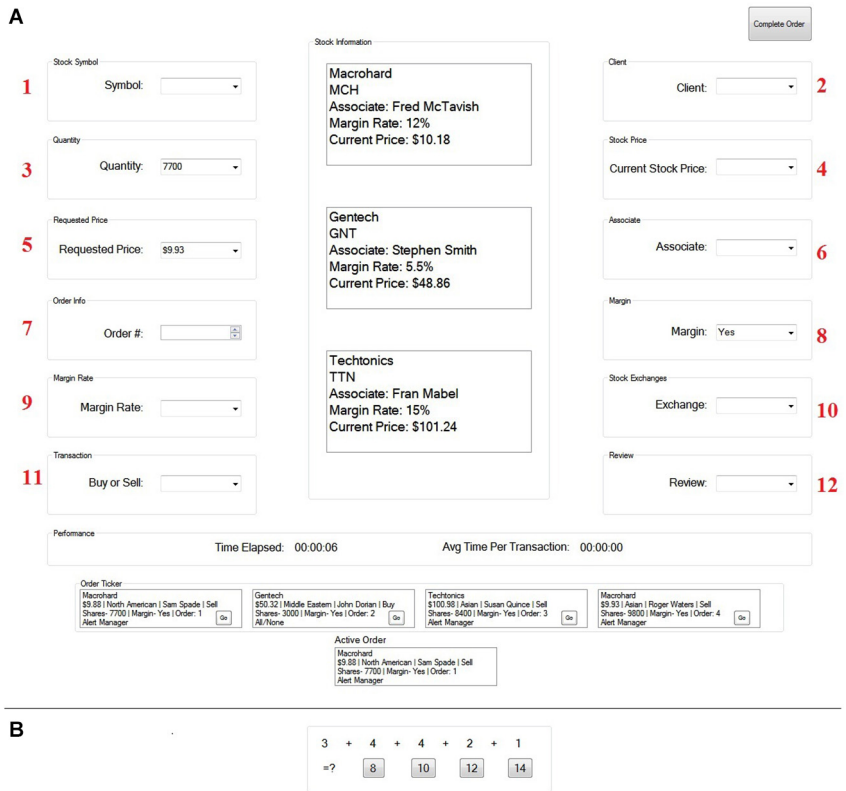

FIGURE 1 | The Financial Management Task (A) and the interruption task (B). The interruption task replaced the screen for the duration of each interruption.

this version of a mental rotation task made it less likely that individuals would be at ceiling levels of performance at baseline, thus allowing for assessment of potential improvement with anodal tDCS.

In this version, each question has one template and four possible answers (i.e., objects that when rotated match the base stimuli or objects that when rotated do not match the base stimuli). For every question, there are exactly two correct matching answers. To successfully answer the question, you must correctly identify both of the matching stimuli.

DESIGN AND PROCEDURE

Participants first signed a consent form and were then instructed on how to complete the mental rotation task (MRT-C). Each participant completed all four practice problems with the experimenter. Following practice, participants completed the first half of the test (i.e., problems 1–12). Participants were given five minutes to the complete the problems. Participants were then trained on the Financial Management task to ensure that they were familiar with the task and minimize potential learning effects. Participants were instructed to complete both tasks (primary and interruption) as quickly and accurately as possible. The trials took approximately 75 s each to complete with interruption time removed. During baseline, participants completed 9 total trials with 27 total interruptions. Interruptions occurred randomly after the successful completion of any one box. Researchers ensured the participants were actively completing the interruption task.

Following the baseline block, the tDCS unit was set up and stimulation was applied.

The DCS Sensation Questionnaire (Scheldrup et al., 2014) was administered at three time points throughout the stimulation block. This questionnaire is used to gauge the amount of itching, heat/burning, and tingling each participant felt as a result of the stimulation; participants responded by selecting their perceived sensations on a 11-point Likert scale where 0 represented no sensation at all and 10 represented the most intense sensation imaginable. This questionnaire is required by the George Mason University IRB to ensure participants safety during the experiment; thus, the data were not analyzed *post-hoc*. Once the current value reached 2.0 mA, the DCS Sensation Questionnaire was administered. Afterwards, participants completed the stimulation block of the Financial Management task, which was identical to the design of the baseline block (i.e., 9 trials with 27 random interruptions). The DCS Sensation Questionnaire was then administered a second time. Next, participants completed the second half (i.e, problems 13–24) of the mental rotation task (MRT-C). Once complete, the final DCS Sensation Questionnaire was administered. The tDCS unit was turned off and detached from the participant. They were thanked for their participation, given a short debrief about the experiment, and then left.

MEASURES

An error occurred when a participant attempted to place information in an incorrect box following an interruption; therefore, a maximum of 27 errors could be committed. Average trial completion time was computed in seconds for each participant. Performance on the interruption task was scored. Lastly, the mental rotation test (MRT-C) was scored for accuracy.

RESULTS

MANIPULATION VERIFICATION

We initially examined participants' engagement in the interruption task. Participants successfully answered 83% (SD = 5.1, range: 74–96%) of the multiple choice math problems, suggesting they were actively engaged in the interruption task and not rehearsing the primary task.

To determine if interruptions affected performance on the primary task, we compared the number of errors a participant made when completing the task without interruptions (M = 0.47, SD = 0.66) to the number of errors a participant made following an interruption (M = 12.71, SD = 2.81) in the baseline trials. A paired samples t-test confirmed that the interruptions negatively affected performance, $t_{(44)} = 27.51$, $p < 0.001$, $d = 4.10$.

Before determining if tDCS affected performance, we needed to ensure that no baseline differences existed between the three stimulation groups (anodal, cathodal, and sham). A one-way analysis of variance (ANOVA) revealed no differences existed in the number of errors made during the baseline trials between groups, $F_{(2,42)} = 0.076$, $p > 0.250$, $\eta^2_{\text{partial}} = 0.004$, see **Figure 2A**. A separate one-way ANOVA of the MRT-C revealed no differences existed in baseline scores (i.e., problems 1–12) between groups as well, $F_{(2,42)} = 0.056$, $p > 0.250$, $\eta^2_{\text{partial}} = 0.003$, see **Figure 2B**.

INTERRUPTION-RELATED ERRORS

A mixed-design ANOVA was performed to determine whether tDCS affected the number of errors participants made following an interruption. The within-subject factor was block (baseline and stimulation) and the between-subject factor was stimulation group (anodal, cathodal, and sham). Levene's test indicated equal error variances in both the baseline (F = 0.09, $p > 0.250$) and stimulation (F = 0.84, $p > 0.250$) data. There was a significant main effect of block, $F_{(1,42)} = 8.68$, $p = 0.005$, $\eta^2_{\text{partial}} = 0.17$ and a significant interaction between block and stimulation group, $F_{(2,42)} = 26.93$, $p < 0.001$, $\eta^2_{\text{partial}} = 0.56$, see **Figure 2A**.

Tests of simple main effects using a Bonferroni correction (α = 0.05) within the anodal stimulation group revealed that significant differences existed between the number of errors in baseline (M = 12.67, 95% CI [11.17, 14.16]) and stimulation (M = 9.8, 95% CI [8.35, 11.25]), $t_{(14)} = 7.56$, $p < 0.001$, d = 1.95. Tests of simple main effects using a Bonferroni correction (α = 0.05) within the cathodal stimulation group revealed that significant differences existed between the number of errors made at baseline (M = 12.93, 95% CI [11.44, 14.43]) and during stimulation (M = 13.8, 95% CI [12.35, 15.25]), $t_{(14)} = 2.29$, $p = 0.027$, $d = 0.59$. No differences existed within the sham group ($p > 0.250$). On average anodal stimulation resulted in three fewer spatial errors (i.e., 23% reduction), whereas cathodal stimulation increased spatial errors by one (i.e., 7% increase), and sham did not change performance.

Tests of simple main effects using a Bonferroni correction (α = 0.05) within the stimulation block revealed that significant differences existed between the number of errors committed in the anodal stimulation group (M = 9.8, 95% CI [8.35, 11.25]) compared to both the cathodal stimulation group (M = 13.8, 95% CI [12.35, 15.25]), $t_{(28)} = 3.94$, $p = 0.001$, d = 1.49) and sham stimulation group (M = 12.6, 95% CI [11.15, 14.05], $t_{(28)} = 2.76$, $p = 0.026$, $d = 1.04$), but not between the cathodal and sham stimulation groups ($p > 0.250$; see **Figure 2A**). On average individuals receiving anodal stimulation made three fewer errors (i.e., 22% reduction) in the stimulation block compared to individuals in the sham stimulation group and four fewer errors (i.e., 29% reduction) compared to individuals in the cathodal stimulation group.

We also correlated the number of errors each participant in the anodal stimulation group made at baseline to their change in errors (stimulation minus baseline), revealing a significant correlation, $r_{(14)} = -0.61$, $p = 0.016$, $R^2 = 0.37$. This suggests that individuals with worse initial performance (i.e., more errors in baseline) benefitted the most from anodal stimulation, see **Figure 3A**.

MENTAL ROTATION TASK (MRT-C)

A mixed-design ANOVA was performed to determine whether tDCS affected performance on the mental rotation task. The within-subject factor was block (baseline and stimulation) and the between-subject factor was stimulation group (anodal, cathodal, and sham). Levene's test indicated equal error variances in both the baseline (F = 0.218, $p > 0.250$) and stimulation (F = 0.074, $p > 0.250$) data. There was a significant main effect of block, $F_{(1,42)}$ 8.32, $p = 0.006$, $\eta^2_{\text{partial}} = 0.17$ and a significant interaction between block and stimulation group, $F_{(2,42)} = 21.96$, $p < 0.001$, $\eta^2_{\text{partial}} = 0.51$, see **Figure 2B**.

Tests of simple main effects using a Bonferroni correction (α = 0.05) within the anodal stimulation group revealed that significant differences existed between mental rotation accuracy during baseline (M = 5.133, 95% CI [4.00, 6.27]) and in stimulation (M = 7.07, 95% CI [5.96, 8.17]), $t_{(14)} = 6.90$, $p < 0.001$, d = 1.78. Tests of simple main using a Bonferroni correction (α = 0.05) effects within the cathodal stimulation group revealed that significant differences existed between mental rotation accuracy at baseline (M = 5.00, 95% CI [3.86, 6.14]) and during stimulation (M = 4.40, 95% CI [3.29, 5.51]), $t_{(14)} = 2.14$, p =.038, $d = 0.55$. No differences existed within the sham group ($p > 0.250$). On average participants in the anodal stimulation group improved mental rotation score by two (i.e., 27% improvement), cathodal stimulation decreased mental rotation score by half a point (i.e., 12% reduction), and sham did not change performance.

Tests of simple main effects using a Bonferroni correction (α = 0.05) within the stimulation block revealed that significant differences existed between MRT-C scores in the anodal stimulation group (M = 7.07, 95% CI [5.96, 8.17]) compared to both the cathodal stimulation group (M = 4.40, 95% CI [3.29, 5.51], $t_{(28)} = 3.44$, $p = 0.004$, $d = 0.89$) and sham stimulation group

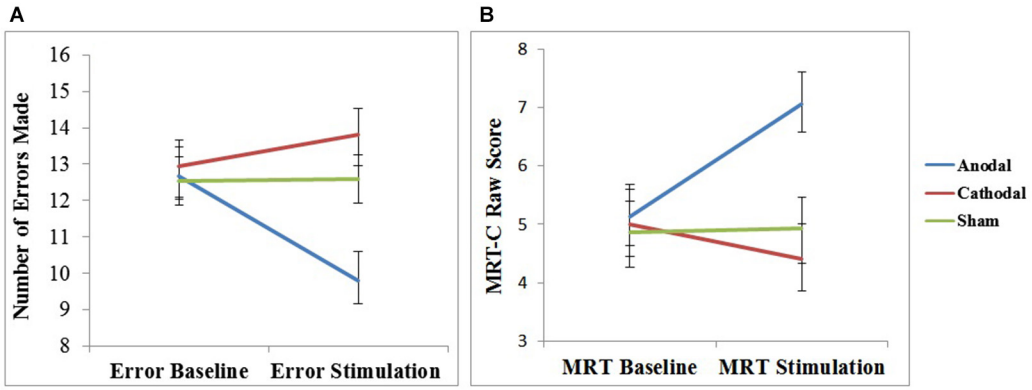

FIGURE 2 | The mean number of errors made (fewer errors represent improved performance) following an interruption (A) and the mean raw scores (higher scores represent improved performance) on the mental rotation task (B) during baseline and stimulation blocks for the three stimulation group (anodal, cathodal, and sham). Plotted with standard errors of the mean.

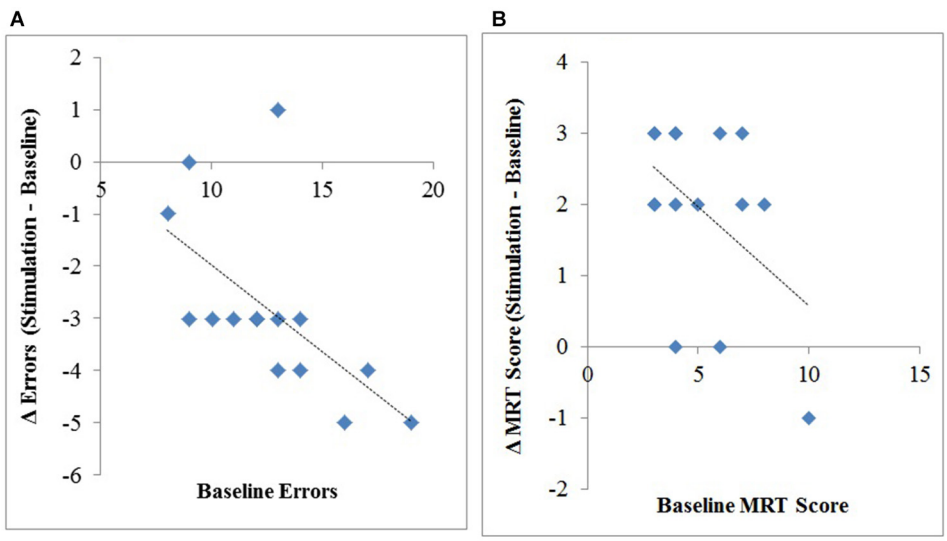

FIGURE 3 | Change (stimulation–baseline) in interruption-related errors (A) and MRT scores (B) plotted against the number of errors (A) and mean mental rotation scores (B) at baseline.

($M = 4.93$, 95% CI [3.83, 6.04], $t_{(28)} = 2.75$, $p = 0.026$, $d = 0.71$). Scores in the cathodal stimulation group were not significantly different from sham ($p > 0.250$; see **Figure 2B**). On average individuals receiving anodal stimulation scored two points higher (i.e., 30% improvement) on the mental rotation task compared to individuals in the sham stimulation group and two and a half points higher (i.e., 38% improvement) than individuals in the cathodal stimulation group.

Additionally, we correlated each participants MRT-C score in the anodal stimulation group at baseline to their change in MRT-C score (stimulation minus baseline), revealing a significant correlation, $r_{(14)} = -.47$, $p = 0.04$, $R^2 = 0.22$, however this effect is largely driven by one participant given the relatively low amount of variability ($s^2 = 4.5$) in MRT-C scores at baseline, see **Figure 3B**.

COMPLETION TIME

To determine whether tDCS affected average trial completion time, a mixed-design ANOVA was performed to determine whether tDCS affected average trial completion time across all three groups, with the within-subject factor being block (baseline and stimulation) and the between-subject factor being stimulation group (anodal, cathodal, and sham). Levene's test indicated equal error variances in both the baseline ($F = 0.859$, $p > 0.250$) and stimulation ($F = 0.331$, $p > 0.250$) data. There was a main effect of block, $F_{(1,42)} = 7.69$, $p = 0.008$, $\eta^2_{\text{partial}} = 0.16$ and a significant interaction between block and stimulation group, $F_{(2,42)} = 7.169$, $p = 0.002$, $\eta^2_{\text{partial}} = 0.25$.

Tests of simple main effects using a Bonferroni correction ($\alpha = 0.05$) within the anodal stimulation group revealed that a significant difference existed ($p < 0.001$) between average

trial completion time in baseline (M = 77.07s, 95% CI [72.69, 81.45]) and average trial completion time in stimulation (M = 72.13s, 95% CI [67.93, 76.34]). No differences existed between baseline and stimulation average completion time in the cathodal or sham stimulation groups ($p > 0.250$ for both). That is, individuals in the anodal stimulation group completed the task more quickly while stimulated compared to baseline. This may not be a surprise as these same individuals made fewer errors and making an error would result in more time spent on that trial.

MENTAL ROTATION AND ERRORS

Given that the processes that guide resumption after an interruption may recruit the same neural substrates as mental rotation, it is likely that changes in one (mental rotation) may be reflected in changes in the other (resumption process, i.e., errors). To examine the extent to which they are related, we correlated the difference scores (stimulation minus baseline) for both measures, including all three stimulation groups. The analysis revealed a significant correlation, $r_{(45)} = -.72$, $p < 0.001$, $R^2 = 0.52$, see **Figure 4**. The magnitudes of the changes in performance for each measure were significantly related.

DISCUSSION

The brain networks involved in the interruption process are not known and the neuromodulation of those networks has not been previously been examined. Given that spatial representations aid in the resumption process after an interruption (Ratwani and Trafton, 2008) and that the right PPC is activated during the maintenance of spatial representations (Cabeza and Nyberg, 2000; Cohen and Andersen, 2002), we hypothesized that anodal stimulation of this region would reduce the number of errors by enhancing memory for spatial information. Additionally, we hypothesized that cathodal stimulation applied to the PPC would increase the number of errors by diminishing spatial representation ability. The results supported these hypotheses: anodal stimulation of right PPC significantly reduced the number of interruption-related errors while increasing mental rotation accuracy, whereas cathodal stimulation of the same region had the opposite effects, and sham stimulation did not affect either performance measure.

To our knowledge, this is the first demonstration of bi-directional effects of activation and inhibition of PPC on spatial errors following interruptions and on mental rotation performance. The results provide evidence for a causal role for the PPC in the maintenance of spatial representations during interrupted task performance. We also found that the magnitude of the changes in interruption-related errors with tDCS was significantly related to changes in mental rotation performance, as measured by the MRT-C. Specifically, individuals who improved in mental rotation accuracy exhibited a reduction in the number of interruption errors to a similar degree. This finding supports the idea that spatial representation ability, as assessed using the MRT-C, guides resumption after an interruption. The findings are unlikely to reflect a placebo effect given that sham stimulation did not affect performance.

Additionally, we found that lower performing individuals at baseline testing, measured by both the number of interruption errors and MRT-C, showed the greatest improvements in performance following anodal stimulation of PPC. This result suggests that individual differences in baseline ability may modulate the behavioral effects of tDCS. Such "cognitive equalizing" due to tDCS was also previously reported in a study of change detection (Tseng et al., 2012). Our finding that lower-performing individuals showed greater benefits of tDCS than higher-performing ones diminishes concerns that tDCS and other non-invasive brain stimulation techniques may widen or exacerbate ability differences in the population, thereby leading to greater social inequality (Cohen Kadosh et al., 2012).

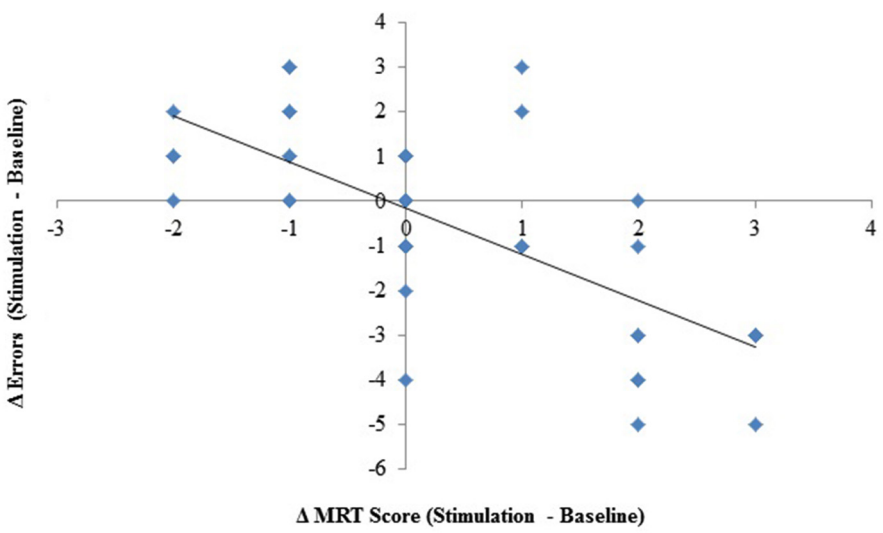

FIGURE 4 | Change (stimulation–baseline) in interruption-related errors plotted against change in mental rotation scores.

During stimulation, performance in both the interruption and mental rotation tasks was significantly greater in the anodal group than in the cathodal and sham groups. However, whereas cathodal stimulation significantly reduced performance on both tasks compared to baseline, the cathodal and sham groups did not differ significantly following stimulation. Some other previous tDCS studies have also found that effects of cathodal stimulation are often less pronounced than anodal effects (Fregni et al., 2005; Tseng et al., 2012; Coffman et al., 2014). Another limitation in the present study is that although we designed the tDCS montage to target the IPS based on current modeling (Datta et al., 2009) and previous literature (Harris and Miniussi, 2003), the relatively non-focal nature of tDCS means that other brain regions could also have been stimulated and could have played a role in the effects. In addition, each participant received only one type of stimulation; therefore it is possible that other individual differences that were not assessed in this study could have been responsible for the differential effects of anodal and cathodal stimulation on interruption errors and mental rotation performance. Additionally, math problems were included as the interruption task in the present study even though mental rotation has been shown to interfere with the resumption process to a greater extent (Ratwani and Trafton, 2008). Given that tDCS produced significant effects in resumption performance in the less interfering task (math problems), potentially greater effects may be found with mental rotation. Finally, many tasks that can be interrupted exist (e.g., giving verbal commands) that may not benefit from anodal stimulation of the PPC when interrupted because the task is not spatial in nature. Therefore, we cannot generalize our results to all tasks and forms of interruption.

This is the first study to show how noninvasive brain stimulation can reduce human error following interruptions. Interruptions are unavoidable, and while many only cause delays or reduce efficiency, they can also lead to serious errors (Westbrook et al., 2010; Prakash et al., 2014). Importantly, tDCS offers a safe, inexpensive, and easy to administer method to reduce errors during the resumption process. This study offers bi-directional causal support for the role of PPC in mental rotation ability and in the resumption process. Important issues that need to be addressed in future research include retention of tDCS-induced benefits on interruption performance and their transfer to other tasks (Parasuraman and McKinley, 2014).

ACKNOWLEDGMENTS

This research was supported by Air Force Office of Scientific Research Grant FA9550-10-1-0385 to Raja Parasuraman and the Center of Excellence in Neuroergonomics, Technology, and Cognition (CENTEC). We would like to thank Greg Trafton, Kevin Zish, and Malcolm McCurry for providing and assisting with the use of the Financial Management Task.

REFERENCES

Antal, A., Nitsche, M. A., and Paulus, W. (2001). External modulation of visual perception in humans. *Neuroreport* 12, 3553–3555. doi: 10.1097/00001756-200111160-00036

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J. Physiol.* 557, 175–190. doi: 10.1113/jphysiol.2003.055772

Blumberg, E. J., Foroughi, C. F., Scheldrup, M. R., Peterson, M. S., Boehm-Davis, D. A., and Parasuraman, R. (in press). Reducing the disruptive effects of interruptions with noninvasive brain stimulation. *Hum. Factors*

Brumby, D. P., Cox, A. L., Back, J., and Gould, S. J. (2013). Recovering from an interruption: investigating speed-accuracy trade-offs in task resumption behavior. *J. Exp. Psychol. Appl.* 19, 95–107. doi: 10.1037/a0032696

Cabeza, R., and Nyberg, L. (2000). Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J. Cogn. Neurosci.* 12, 1–47. doi: 10.1162/08989290051137585

Cades, D. M., Werner, N. E., Boehm-Davis, D. A., and Arshad, Z. (2010). Interruptions are disruptive in the real world: evidence from an office setting. *Proc. Hum. Factors U S A* 54, 448–452. doi: 10.1177/154193121005400437

Champod, A. S., and Petrides, M. (2007). Dissociable roles of the posterior parietal and the prefrontal cortex in manipulation and monitoring processes. *Proc. Natl. Acad. Sci. U S A* 104, 14837–14842. doi: 10.1073/pnas.0607101104

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85, 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Cohen, Y. E., and Andersen, R. A. (2002). A common reference frame for movement plans in the posterior parietal cortex. *Nat. Rev. Neurosci.* 3, 553–562. doi: 10.1038/nrn873

Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, 108–111. doi: 10.1016/j.cub.2012.01.013

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R., and Walsh, V. (2010). Modulating neuronal activity produces specific and long lasting changes in numerical competence. *Curr. Biol.* 20, 2016–2020. doi: 10.1016/j.cub.2010.10.007

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., and Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul.* 2, 201–207. doi: 10.1016/j.brs.2009.03.005

Dismukes, R. K., Loukopoulos, L. D., and Berman, B. A. (2012). *The Limits of Expertise: Rethinking Pilot Error and the Causes of Airline Accidents.* Hampshire, England: Ashgate Publishing, Ltd.

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp. Brain Res.* 166, 23–30. doi: 10.1007/s00221-005-2334-6

Harris, I. M., and Miniussi, C. (2003). Parietal lobe contribution to mental rotation demonstrated with rTMS. *J. Cogn. Neurosci.* 15, 315–323. doi: 10.1162/089892903321593054

Jonides, J., Lacey, S. C., and Nee, D. E. (2005). Processes of working memory in mind and brain. *Curr. Dir. Psychol. Sci.* 14, 2–5. doi: 10.1111/j.0963-7214.2005.00323.x

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Oostenveld, R., and Praamstra, P. (2001). The five percent electrode system for high-resolution EEG and ERP measurements. *Clin. Neurophysiol.* 112, 713–719. doi: 10.1016/s1388-2457(00)00527-7

Parasuraman, R., and McKinley, A. R. (2014). Using non-invasive brain stimulation to accelerate learning and enhance human performance. *Hum. Factors* 56, 816–824. doi: 10.1177/0018720814538815

Peters, M., Laeng, B., Latham, K., Jackson, M., Zaiyouna, R., and Richardson, C. (1995). A redrawn Vandenberg and Kuse mental rotations test-different versions and factors that affect performance. *Brain Cogn.* 28, 39–58. doi: 10.1006/brcg.1995.1032

Prakash, V., Koczmara, C., Savage, P., Trip, K., Stewart, J., McCurdie, T., et al. (2014). Mitigating errors caused by interruptions during medication verification and administration: interventions in a simulated ambulatory chemotherapy setting. *BMJ Qual. Saf.* 23, 884–892. doi: 10.1136/bmjqs-2013-002484

Ratwani, R. M., and Trafton, J. G. (2008). Spatial memory guides task resumption. *Vis. Cogn.* 16, 1001–1010. doi: 10.1080/13506280802025791

Scheldrup, M., Greenwood, P. M., McKendrick, R., Strohl, J., Bikson, M., Alam, M., et al. (2014). Transcranial direct current stimulation facilitates cognitive multitask performance differentially depending on anode location and subtask. *Front. Hum. Neurosci.* 8:665. doi: 10.3389/fnhum.2014.00665

Shen, Y. J., and Jiang, Y. V. (2006). Interrupted visual searches reveal volatile search memory. *J. Exp. Psychol. Hum. Percept. Perform.* 32, 1208–1220. doi: 10.1037/0096-1523.32.5.1208

Shepard, R. N., and Metzler, J. (1971). Mental rotation of three-dimensional objects. *Science* 171, 701–703. doi: 10.1126/science.171.3972.701

Sparing, R., Thimm, M., Hesse, M. D., Küst, J., Karbe, H., and Fink, G. R. (2009). Bidirectional alterations of interhemispheric parietal balance by non-invasive cortical stimulation. *Brain* 132, 3011–3020. doi: 10.1093/brain/awp154

Stone, D. B., and Tesche, C. D. (2009). Transcranial direct current stimulation modulates shifts in global/local attention. *Neuroreport* 20, 1115–1119. doi: 10.1097/wnr.0b013e32832e9aa2

Trafton, J. G., Altmann, E. M., Brock, D. P., and Mintz, F. E. (2003). Preparing to resume an interrupted task: effects of prospective goal encoding and retrospective rehearsal. *Int. J. Hum. Comput. Stud.* 58, 583–603. doi: 10.1016/s1071-5819(03)00023-5

Trafton, J. G., Altmann, E. M., and Ratwani, R. M. (2011). A memory for goals model of sequence errors. *Cogn. Syst. Res.* 12, 134–143. doi: 10.1016/j.cogsys.2010.07.010

Tseng, P., Hsu, T. Y., Chang, C. F., Tzeng, O. J., Hung, D. L., Muggleton, N. G., et al. (2012). Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. *J. Neurosci.* 32, 10554–10561. doi: 10.1523/jneurosci.0362-12.2012

Vandenberg, S. G., and Kuse, A. R. (1978). Mental rotations, a group test of three-dimensional spatial visualization. *Percept. Mot. Skills* 47, 599–604. doi: 10.2466/pms.1978.47.2.599

Westbrook, J. I., Woods, A., Rob, M. I., Dunsmuir, W. T., and Day, R. O. (2010). Association of interruptions with an increased risk and severity of medication administration errors. *Arch. Intern. Med.* 170, 683–690. doi: 10.1001/archinternmed.2010.65

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 November 2014; accepted: 15 December 2014; published online: 12 January 2015.

Citation: Foroughi CK, Blumberg EJ and Parasuraman R (2015) Activation and inhibition of posterior parietal cortex have bi-directional effects on spatial errors following interruptions. Front. Syst. Neurosci. ***8****:245. doi: 10.3389/fnsys.2014.00245*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 07 June 2016
doi: 10.3389/fnins.2016.00262

Weighing the Cost and Benefit of Transcranial Direct Current Stimulation on Different Reading Subskills

Jessica W. Younger[1,2]*, *Melissa Randazzo Wagner*[1,3] *and James R. Booth*[1,2]

[1] *Department of Communication Sciences and Disorders, Northwestern University, Evanston, IL, USA,* [2] *Department of Communication Sciences and Disorders, University of Texas at Austin, Austin, TX, USA,* [3] *Department of Communication Sciences and Disorders, Teachers College, Columbia University, New York, NY, USA*

Adults struggling with low reading skills are underserved by limited available treatments. While brain stimulation techniques such as transcranial direct current stimulation (tDCS) has the potential to improve a variety of cognitive functions, little work has been done examining its potential to treat reading disabilities. Research on the effects of tDCS on reading abilities has been somewhat inconsistent perhaps in part due to discrepancies between studies in the nature of the tasks. In the current study, we examined the effect of tDCS to the left inferior parietal lobe (L IPL) on two reading tasks in low-to-average readers. We compared performance on a sight word efficiency (SWE) task and a rhyme judgment task before and after either stimulation to the L IPL, right superior parietal lobe (R SPL), or sham stimulation. Readers who received stimulation to the L IPL showed greater improvements on the SWE task, but less improvement on the rhyme judgment task compared to the R SPL and sham groups. This study demonstrates for the first time both a positive and negative effect of stimulation under the same stimulation parameters within the same participants. The results highlight the need to consider multiple tasks when assessing the potential of using tDCS as a treatment.

Keywords: reading intervention, transcranial direct current stimulation, parietal lobes, sight word efficiency, rhyming

OPEN ACCESS

Edited by:
Manuel Fernando Casanova, University of Louisville, USA

Reviewed by:
Gabriele Miceli, University of Trento, Italy
Deniz Doruk, Harvard Medical School, USA

***Correspondence:**
Jessica W. Younger
jrwise@utexas.edu

Specialty section:
This article was submitted to Neural Technology, a section of the journal Frontiers in Neuroscience

Received: *11 February 2016*
Accepted: *24 May 2016*
Published: *07 June 2016*

Citation:
Younger JW, Randazzo Wagner M and Booth JR (2016) Weighing the Cost and Benefit of Transcranial Direct Current Stimulation on Different Reading Subskills. Front. Neurosci. 10:262. doi: 10.3389/fnins.2016.00262

INTRODUCTION

Over the last decade, interest in using brain stimulation techniques as a therapeutic tool to treat cognitive impairment in adults has received increasing attention (Dubljević et al., 2014). Brain stimulation is a non-invasive method using electrical currents to alter the firing potential of neurons in the affected area. While there are a variety of techniques that can be used to stimulate the brain, two primary techniques are transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). TMS delivers a larger current and is thought to cause neurons to fire (Ridding and Rothwell, 2007) while tDCS delivers a much smaller current and is believed to change the membrane potential of neurons (Nitsche et al., 2008; Priori et al., 2009) Both techniques have been used to enhance performance on tasks involved in a variety of cognitive processes in healthy and impaired adults (Miniussi et al., 2008; Nitsche et al., 2008; Williams et al., 2009; Nitsche and Paulus, 2011; Krause and Cohen Kadosh, 2013). While the evidence for brain stimulation

improving function in healthy adults has been somewhat controversial (see Horvath et al., 2015 but also Price et al., 2015), its use as a treatment in patient populations with brain injury has been promising (Fregni and Pascual-Leone, 2007; Miniussi et al., 2008; Wong and Tsang, 2013).

More recently, research using brain stimulation to treat learning disorders, such as dyslexia and dyscalculia, has been called for (Cohen Kadosh et al., 2013; Krause and Cohen Kadosh, 2013; Vicario and Nitsche, 2013). While those with learning disorders do not have frank insult to the brain, they are believed to have altered brain activation in key brain regions when compared to typical adults (Pugh et al., 2001; Price and Ansari, 2013; Norton et al., 2014; Kucian and von Aster, 2015). The case for using neuromodulation to treat learning disorders is thus conceptually straightforward; stimulation to modify the activity in a brain region shown to be integral to the cognitive process of interest and differentially activated in the disordered population should normalize activation in that region and therefore normalize performance. However, identifying the brain regions integral to the process is not trivial, particularly in the case of reading.

Neuroimaging research has identified several brain regions that show altered function in individuals with dyslexia that may serve as potential targets of brain stimulation. Three brain areas in particular have consistently shown altered functionality compared to typical readers—the inferior frontal gyrus (IFG), temporo-parietal areas, and occipito-temporal areas (Richlan et al., 2009, 2011). Meta-analysis of neuroimaging studies suggest the IFG may be hyperactive in adults with dyslexia as a compensatory region, while temporo-parietal and occipito-temporal areas may be hypoactive, reflecting impaired processing during reading. Brain stimulation could be used to either enhance potential compensatory regions in an attempt to strengthen these networks or enhance areas that are consistently underactive in people with dyslexia in an attempt to normalize their function.

So far, neuromodulation studies examining the tool's potential to improve reading ability have stimulated regions shown to be underactivated in poor readers, and all have had some success (Costanzo et al., 2012, 2013; Turkeltaub et al., 2012; Heth and Lavidor, 2015; Thomson et al., 2015). However, the exact nature of the reading improvements has been somewhat inconsistent across studies. Turkeltaub et al. (2012) first demonstrated the potential for tDCS to be used as a treatment for low-to-average readers by showing improved reading fluency after stimulation to the left superior temporal gyrus (STG) compared to sham stimulation. These findings were corroborated by Costanzo et al.'s TMS studies (Costanzo et al., 2012, 2013) that found TMS to the left STG increased real word reading speed and text reading accuracy in both dyslexic and average readers. However, a later tDCS study by Thomson et al. (2015) was inconsistent with the findings of Turkeltaub et al. (2012). Thomson et al. (2015) stimulated an overlapping, but slightly superior region to that stimulated in Turkeltaub et al. (2012) in average readers and found that right hemisphere stimulation led to improvements on real word reading ability, not left hemisphere.

The Costanzo et al.'s TMS studies (Costanzo et al., 2012, 2013) showed that the particular section of the temporo-parietal region that is stimulated leads to specific results. In contrast to the increases in real word reading following STG stimulation mentioned above, stimulation to the more superior temporo-parietal cortex, specifically, the left inferior parietal lobe (L IPL), led to increases in pseudoword reading. While the lack of improvements in real word reading, the ultimate goal of reading therapy, is discouraging, stimulation to the IPL and surrounding areas merits further investigation. In particular, tDCS to the more superior aspects of the temporo-parietal cortex may lead to greater gains in reading ability compared to the precisely targeted stimulation of TMS given the diffusivity of tDCS. The superior portion of the temporo-parietal cortex including not just IPL, but also the angular gyrus (AG) and supramarginal gyrus (SMG), have been specifically related to smaller-grained grapheme-to-phoneme mapping (Pugh et al., 2000; Simos et al., 2001; Booth et al., 2003; Jobard et al., 2003; Cao et al., 2006; Bitan et al., 2007b; He et al., 2013), which developmental and cross-linguistic studies of reading suggest is important for the initial development of the reading network (Pugh et al., 2000; Cao et al., 2006, 2015; Richlan et al., 2011; Martin et al., 2015). Therefore, we propose that facilitation of small-grain grapheme-to-phoneme processing via modulation of activation in superior portions of the temporo-parietal cortex may lead to gains in multiple aspects of reading ability including reading fluency and improved grapheme-phoneme mappings in low-to-average ability adults.

This hypothesis is supported by second language learning studies with adults (Hashimoto and Sakai, 2004; Mei et al., 2014) and reading remediation studies with children (Temple et al., 2003; Simos et al., 2007; Meyler et al., 2008; Rezaie et al., 2011b) and adults (Eden et al., 2004) that show greater ability related gains in superior vs. inferior temporo-parietal cortex. Further, functional connectivity between the IPL in particular and regions involved in orthographic processing such as the fusiform gyrus (FG) has been demonstrated to be related to word reading ability (Koyama et al., 2011; Simon et al., 2013). Developmental studies have suggested that the strength of the connection between grapheme-to-phoneme processing regions such as the IPL and the orthographic processing regions such as the FG may be critical for the specialization of these orthographic processing regions, in line with interactive specialization models of development that have been extended to reading (Johnson, 2001; Schlaggar and McCandliss, 2007; Price and Devlin, 2011). Indeed, dyslexic readers tend to have reduced connectivity between these two regions compared to controls. In contrast, there is little evidence suggesting the connectivity between the STG and FG is crucial for reading ability (Horwitz et al., 1998; Pugh et al., 2000; Booth et al., 2008; Cao et al., 2008; Quaglino et al., 2008; van der Mark et al., 2011; Finn et al., 2014).

In order to test the hypothesis that tDCS to superior portions of the temporo-parietal cortex will lead to reading improvement for low ability readers, we stimulated the left IPL in low-to-average readers and measured their improvement on two reading tasks; single word reading efficiency and a rhyme judgment task. Both require the use of phonological and orthographic information, but in different ways. Single word reading efficiency requires articulating the phonological output from orthographic input. This skill has been shown to be related to both overall

word reading ability and the ability to decode words based on grapheme-to-phoneme mappings (Adlof et al., 2006; Vellutino et al., 2007; Barth et al., 2009). Despite the orthographic processing component, neuroimaging studies have suggested that this skill is most related to parietal areas including the IPL and AG (He et al., 2013) typically implicated in grapheme-phoneme mappings, compared to areas involved in whole-word orthographic mappings. Further, training studies have shown that instruction in grapheme-to-phoneme mapping results in improvement in single word reading fluency (Ashmore et al., 2002; Simos et al., 2007) and these gains are related to activation in parietal areas (Rezaie et al., 2011a,b). The rhyme judgment task, in contrast, does not require articulation. Rather it requires the activation and memory of phonological representations, sometimes in the face of conflicting orthographic information. For this reason, this task is a measure of phonological working memory and the strength of the grapheme-to-phoneme maps needed to be activated to complete the task accurately. Behavioral studies have shown the rhyming task to be related to reading ability (Maclean et al., 1987; Ziegler and Goswami, 2005; Kovelman et al., 2012) and neuroimaging studies have shown activation in the left IPL to be related to ability on this task (Booth et al., 2003; Hoeft et al., 2006; Bitan et al., 2007a). Together, both tasks provide measures of orthographic and phonological processing that are related to activation in the left IPL. By using these two tasks, we can address how tDCS affects both the mental manipulation and articulation of phonological representation and therefore have a broader picture of what abilities can be impacted by tDCS.

METHODS

This study was carried out in accordance with the recommendations of the University of Texas at Austin Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Participants

In total, 100 right-handed 18–35 year-old native English speakers with normal or corrected-to-normal vision were screened for below average reading ability (<100 standard score) as determined by the Sight Word Efficiency (SWE) subtest of the Test of Word Reading Efficiency (TOWRE; Torgesen et al., 1999) in line with Turkeltaub et al. (2012). All participants reported no history of neurological disorder, psychiatric disorder, significant head trauma, hearing loss, substance abuse, seizure or migraine, metal implants, and current pregnancy. Of the initial 100, 54 participants scored below average, however, 14 did not complete both days of the experiment, and were therefore not included in the sample. An additional four participants were excluded for scoring <50% accuracy on behavioral measures. The remaining participants had at least average (>80 standard score) intelligence as measured by the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 2008). Scores on the SWE ranged from 74 to 99 pre-stimulation (within two standard deviations of 50th percentile performance of 100). Participants were randomly assigned to one of three groups, L IPL, right superior parietal lobe (R SPL), or Sham. Assignment to the R SPL group was done as part of an additional experiment not reported here. For the current experiment, the R SPL group served as a stimulation control condition in which participants received stimulation to a non-target region which complemented the no stimulation control condition fulfilled by the Sham group.

Of those who met all performance criteria, 11 (7 female) received real stimulation to the L IPL, 14 (6 female) received sham stimulation, and 11 (9 female) received real stimulation to the R SPL. Due to an imbalance in the run orders in the sham group, four participants were randomly eliminated for a final sample of 10 (4 female). One-way ANOVAs revealed no significant effects of group on all group characteristics and baseline measures as reported in **Table 1**.

Procedure

Participants took part in a single-blind, sham and stimulation controlled study comparing pre- and post-stimulation performance on two measures of reading ability: single word reading efficiency and rhyme judgment. Participants completed two sessions that took place 3–5 days apart. During the first session, participants completed standardized tests and baseline assessments of reading ability. During the second session, participants received either sham or real stimulation for 20 min, after which they completed an alternate form of the reading ability measures using different sets of stimuli. Alternate forms of the tasks were counterbalanced across participants.

Transcranial Direct Current Stimulation

Direct current was administered using a battery-driven DC stimulator device (NeuroConn) via two saline-soaked electrodes (5×5 cm; 25 cm^2). The anode electrode was placed over either the L IPL (P3) or R SPL (CP4) according to the international 10–20 system for electroencephalography (EEG) electrode placement (Herwig et al., 2003). The cathode (return) electrode was placed over the contralateral supraorbital frontal region. This montage allows the source of effects of stimulation to be more reliably attributed to the anodal stimulation of the target site instead of the cathodal stimulation of the reference site, as suggested by Turkeltaub et al. (2012). During real stimulation, 1.5 mA

TABLE 1 | Participant demographics and mean (SD) for performance for each participant group.

	L IPL	R SPL	Sham
Age (years)	26.8 (5.5)	25.2 (3.5)	26.2 (4.9)
Gender (f)	7	8	4
IQ	112.1 (10.9)	111.0 (11.70)	109.3 (9.9)
Pre-single word reading	88.5 (8.4)	88.1 (7.6)	89.2 (7.9)
Pre-rhyme judgment RT (ms)	854 (124)	972 (147)	998 (193)
Pre-rhyme judgment accuracy (%)	91.6 (7.0)	88.4 (9.5)	87.9 (12.0)

IQ measured by Wechsler Abbreviated Scale of Intelligence. Single Word Reading was measured by the Sight Word Efficiency subscale of the Test of Word Reading Efficiency. IQ and Single Word Reading have $\mu = 100$, $\sigma = 15$.

of current (current density 0.06 mA/cm2) was delivered for 20 min. During sham stimulation, the machine ramped up to 1.5 mA for 30 s, then extinguished over a 5 s fade-out. Using this procedure allows participants to feel the initial sensations (e.g., tingling or itching) associated with stimulation without any after-effects of stimulation being induced (Nitsche and Paulus, 2000). These stimulation parameters replicate the parameters used in Turkeltaub et al. (2012) and are within the safety limits established in prior studies on humans and animals (Iyer et al., 2005; Nitsche et al., 2008; Bikson et al., 2009).

Experimental Tasks

Single Word Reading Efficiency

Word reading efficiency was measured via the SWE subtest of the TOWRE. This test is a measure of ability to read real words accurately and quickly. Participants were given 45 s to read aloud as many of 104 words as possible. A standard score is determined by the number of words read correctly within 45 s, and this score was used as the metric of single word reading efficiency.

Rhyme Judgment Task

The ability to map orthography to phonology and phonological working memory were assessed with a rhyme judgment task in which participants were presented with a series of visual word pairs and asked to indicate whether the words rhymed or not. Word pairs were designed to manipulate orthographic and phonological similarity to ensure participants could not rely on orthography alone and phonological representations had to be used to accurately complete the task. There were two congruent conditions in which word pairs had either similar orthography and phonology (e.g., CAGE-RAGE) or not (e.g., TRIAL-FALL), and two incongruent conditions in which word pairs had either similar orthography but dissimilar phonology (e.g., PINT-MINT) or dissimilar orthography but similar phonology (e.g., GRADE-PAID) pairs. Each condition had 12 trials for a total of 48 trials in each session.

All words were monosyllabic, having neither homophones nor homographs and were matched across condition for written word frequency in children (Zeno, 1995) and the sum of written bigram frequency (Balota et al., 2007). Stimuli used in each version of the task were matched on average stimuli length, frequency, number of orthographic neighbors, and number of phonological neighbors (Balota et al., 2007).

Participants were asked to respond as quickly and as accurately as possible. The first word was presented for 800 ms followed by a 200 ms inter-stimulus interval and the presentation of the second word. Participants could respond as soon as the second word was presented up to 2500 ms after the onset of the word. After the participant responded, a red fixation cross appeared signaling the inter-trial interval. The task was self-paced and participants were able to control when the next trial began. Average reaction times (RT) to correct trials trimmed to include only responses within 2.5 standard deviations from an individual's average reaction time were used as the metric of rhyme judgment ability due to ceiling effects on accuracy.

Analysis

Performance on each experimental task was submitted to a 3 (Stimulation group; L IPL, Sham, R SPL) × 2 (Time; Session 1, Session 2) mixed-model ANOVA in order to determine whether a measure showed a Group × Time interaction. Planned follow-up tests were conducted using separate 2 (Stimulation group; L IPL, Sham or R SPL) × 2 (Time; Session 1, Session 2) mixed-model ANOVAs to examine potential Group × Time interactions for the L IPL group compared to the two control groups (Sham, R SPL) separately.

RESULTS

Single Word Reading

The 3 × 2 ANOVA revealed a significant main effect of Time [$F_{(1, 29)} = 24.68$, $p < 0.001$] and significant Group × Time interaction [$F_{(2, 29)} = 4.41$, $p = 0.021$], indicating that although all groups showed changes in their performance over time, the groups differed in the magnitude of these changes. Follow-up tests revealed the L IPL group showed significantly greater improvement compared to the Sham group [$F_{(1, 19)} = 7$, $p = 0.016$] and a trend toward greater improvement compared to the R SPL group [$F_{(1, 20)} = 4.22$, $p = 0.053$]. One sample *t*-tests indicated that the L IPL and R SPL groups' improvement was significantly greater than 0 [L IPL: $t_{(10)} = 4.17$, $p < 0.005$; R SPL: $t_{(10)} = 2.37$, $p < 0.05$], while the Sham group did not show improvement [$t_{(9)} = 1.72$, $p > 0.1$] see **Table 2** and **Figure 1**. The results of the current study result in an effect size (Cohen's d) of 1.57 for L IPL stimulation, greater than the 0.46 for left STG stimulation found by Turkeltaub et al. (2012).

Rhyme Judgment

All participants performed well on the rhyme judgment task as indicated by high accuracy at Time 1 and Time 2. A 3 × 3 ANOVA did not reveal a main effect of Time or any Group × Time interactions ($p > 0.1$). One sample *t*-tests for each group individually showed that no group's gain in accuracy was significantly greater than 0 ($p > 0.1$). These findings indicate that there was neither a practice effect nor an effect of stimulation on accuracy, possibly due to ceiling effects.

The 3 × 2 ANOVA again revealed a significant main effect of Time [$F_{(1, 29)} = 28.9$, $p < 0.001$] and significant Group × Time interaction [$F_{(2, 29)} = 4.13$, $p = 0.026$], indicating a change over time, but a group difference in the magnitude of the change. Follow-up tests showed the Sham group experienced significantly greater improvements in RT compared to the L IPL group [$F_{(1, 19)} = 7.27$, $p = 0.014$]. However, the R SPL group was not significantly different from either the Sham group [$F_{(1, 19)} = 2.62$, $p > 0.1$] or the L IPL group [$F_{(1, 20)} = 1.69$, $p > 0.1$]. *Post-hoc* one-sample *t*-tests, though, indicate that both the Sham and R SPL groups' improvement was significantly greater than 0 while the L IPL group did not improve [Sham: $t_{(9)} = 2.54$, $p = 0.006$; R SPL: $t_{(10)} = 2.31$, $p = 0.044$; L IPL: $t_{(10)} = 1.74$, $p > 0.1$] see **Table 2** and **Figure 2**.

TABLE 2 | Mean (SD) on reading measures pre- and post-stimulation.

	L IPL		R SPL		Sham	
	Pre	Post	Pre	Post	Pre	Post
Single word reading	88.5 (8.4)	98.8 (14.3)	88.1 (7.6)	92.2 (8.3)	89.2 (7.9)	91.7 (8.2)
Rhyme judgment RT (ms)	854 (124)	811 (142)	972 (147)	885 (141)	998 (193)	820 (99)
Rhyme judgment accuracy (%)	91.7 (7.0)	94.7 (7.5)	88.4 (9.5)	89.6 (10.6)	87.9 (12.1)	90.0 (9.0)

Single Word Reading has $\mu = 100$, $\sigma = 15$.

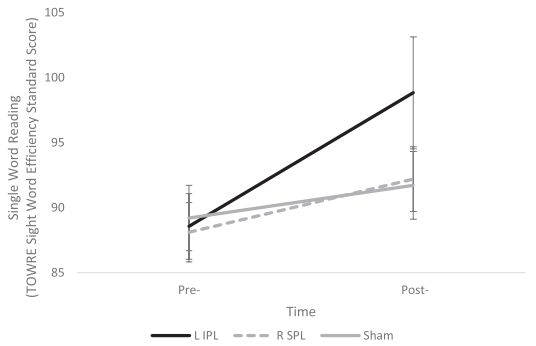

FIGURE 1 | Change in Single Word Reading Efficiency pre- and post-stimulation for each group. A significant Group × Time interaction indicates the L IPL group showed significantly greater improvement than the R SPL and Sham groups. Error bars indicate one standard deviation.

DISCUSSION

The goal of the current study was to assess whether stimulation of the L IPL improves multiple aspect of reading for low-to-average readers by measuring its impact on two tasks that tap into different subskills of reading. While L IPL stimulation did result in gains in single word reading efficiency, it resulted in relative impairment on the rhyme judgment task, demonstrating for the first time a significant positive and negative effect on two different tasks with the same stimulation parameters within the same group of participants. Although our results indicate stimulation to the L IPL may be a good site for improving reading fluency for low-to-average readers, the lack of improvement on the rhyme judgment task warrants caution in advocating the left IPL as a site to improve several aspects of reading.

The positive influence of L IPL stimulation on reading fluency measures for low-to-average readers was consistent with our hypothesis. Although there was a main effect of time, indicating there was a general practice effect for all groups, the L IPL stimulation resulted in greater improvement than the Sham or R SPL stimulation. Our finding that stimulation to the IPL led to greater improvements for low-to-average readers than previous reports of stimulation to the STG (Turkeltaub et al., 2012) are in line with studies indicating that single word reading fluency abilities depend on grapheme-to-phoneme mapping skills supported by the superior aspects of the temporo-parietal cortex (Ashmore et al., 2002; Simos et al., 2007; Rezaie et al., 2011a,b; He et al., 2013). This finding suggests that improvements in single word reading can result from increased grapheme-to-phoneme mapping abilities, even in adults. However, it should be noted that montage differences between the current study and that by Turkeltaub et al. (2012) could also account for the difference in effect size between the two studies. Turkeltaub et al. (2012) used a bilateral montage, meaning that the cathode or reference electrode was placed on the contralateral hemisphere (i.e., right STG). The results of that study cannot be attributed solely to facilitation of the left hemisphere, and the effects could have been due to alteration in the balance between the two hemispheres or even to inhibition of the right hemisphere. In contrast, in the current study, the cathode electrode was placed on the contralateral forehead. While we cannot rule out that the effects in the current study were due to inhibition of the frontal lobe, it is more reasonable to conclude that the effects are due to the modulation of activation in the stimulation site and surrounding areas. As Turkeltaub et al. (2012) themselves point out, it could be that unilateral stimulation may be more beneficial than bilateral stimulation. Further research should explore how montage affects the behavioral consequences of stimulation.

Our results that left hemisphere stimulation leads to improvement in SWE is in contrast to Thomson et al. (2015) who found that right, but not left, hemisphere stimulation led to behavioral improvements. These conflicting results are likely due to the difference in populations used in each study. In keeping with the Turkeltaub et al. (2012) findings that stimulation only led to improvements in low-to-average readers, the current study only used readers who had below average performance on the TOWRE. However, Thomson et al. (2015) used participants with a wide range of reading abilities. Because individual differences in skill have been shown to have an effect on the behavioral changes induced by tDCS, including individuals with a large range of reading ability may have diluted the effects for the left hemisphere stimulation in the Thomson et al. (2015) study. Future research should examine how individual differences in reading ability affect the impact of tDCS on behavioral performance.

In contrast to the expected results of tDCS on the SWE, the negative effect of L IPL stimulation on improvement on the rhyming judgment task in low-to-average readers was unexpected. Previous neuroimaging work with both children and adults has shown that increases in activation in the left IPL are associated with better performance on the rhyming task (Hoeft et al., 2006; Bitan et al., 2007a; Cao et al., 2015). If anodal stimulation to the L IPL did increase activation in that area as hypothesized, the stimulation group should have

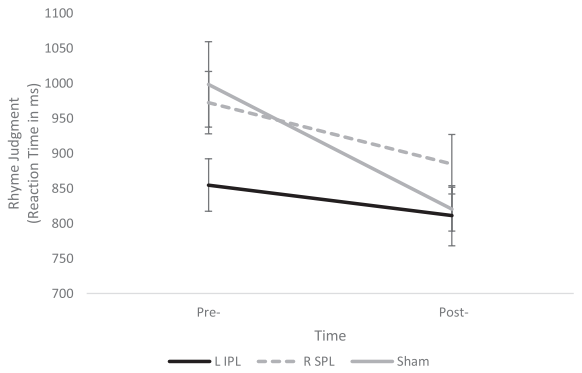

FIGURE 2 | Change in Rhyme Judgment performance pre- and post-stimulation for each group. A significant Group × Time interaction indicates the Sham group showed significantly greater improvement than the L IPL and R SPL groups. Error bars indicate one standard deviation.

shown increases in performance following stimulation. However, research with anodal stimulation to the IPL in the context of working memory has shown that stimulation actually impairs performance relative to sham, particularly in low performers (Jones and Berryhill, 2012; Sandrini et al., 2012). Specifically, Sandrini et al. (2012) showed that anodal stimulation abolished practice effects on a working memory task. These findings are in line with the results of the current study; while the L IPL stimulation group did not perform worse after stimulation, they did not demonstrate practice effects as seen in the sham group. The rhyming task involves phonological working memory when the phonological representation of the first word must be held in mind until the second word is presented and the two phonological representations can be compared to make a rhyme decision. Therefore, the effects of stimulation on working memory abilities may have prevented improvements on the task. Previous work examining the effect of stimulation to the parietal lobes has suggested that stimulation interferes with working memory by creating an imbalance of activation between the two hemispheres and interfering with the natural inter-hemispheric inhibition that occurs in the absence of stimulation (Sparing et al., 2009; Sandrini et al., 2012; Park and Friston, 2013; Krause and Cohen Kadosh, 2014). Inter-hemispheric inhibition may explain the seemingly contradictory results of the current study. As reading skills develop, activation during reading becomes more lateralized to the left hemisphere with the right hemisphere playing a decreasing role in reading (Turkeltaub et al., 2003; Eden et al., 2004; Shaywitz et al., 2004). Simulation causing disruptions to inter-hemispheric inhibition may thus be less likely to affect single word reading abilities, and therefore, we can expect improvements on single word reading tasks such as those seen in the current study. However, previous research has shown that low-to-average and dyslexic readers tend to have more bilateral activation during reading tasks with a phonological working memory component (Milne et al., 2002; Illingworth and Bishop, 2009; Xu et al., 2015). Given that the subjects in the current study were all low-to-average readers, it could be the case that the disruption to inter-hemispheric inhibition in the parietal lobes was particularly detrimental to their phonological working memory abilities, preventing the expected practice effects on the rhyming task. Our findings highlight the importance of considering the impact that individual differences may have on neural processing and subsequently the effects of tDCS.

From a clinical perspective, perhaps the most important finding from the current study is that tDCS can positively impact one skill while negatively impacting another. Our results underscore the importance of including multiple tasks that potentially tap into different underlying cognitive processes in order to assess whether the potential costs of stimulation outweigh the potential gains. Assessing multiple tasks becomes especially important when considering whether tDCS should be recommended as a treatment. In the current study, the cost in practice effect on speed during a rhyme judgment task is probably worth the gains seen in single word reading for low-to-average readers; accuracy was not affected and speed did not decrease after stimulation. However, this population was low-to-average in skill, not impaired. The cost to benefit ratio may increase as reading skill decreases. Further studies examining how individual differences impact the effects of tDCS on multiple reading tasks are needed before being able to advocate for tDCS as a treatment for reading disabilities.

Further, our findings that tDCS had a differential effect on two aspects of reading in low-to-average readers has implications for the design of future tDCS studies. In the current study, both tasks were reading-related, but the differences between tasks in the working memory component are in line with the literature that anodal tDCS does not have the expected positive effect on working memory abilities. These results support the idea that tDCS can affect cognitive processes differently, depending on how the target or surrounding brain area is involved in a given cognitive process. Future research is still needed to determine the circumstances in which the conventional idea that anodal stimulation leads to enhancement of activation and behavior while cathodal stimulation leads to inhibition of activation and behavior holds true (De Berker et al., 2013; Bestmann et al., 2015). When selecting target sites, the areas' involvement in multiple cognitive processes should be considered. The impact of stimulation on each of these processes should then be examined so that we might better understand whether tDCS can influence cognitive processes differently.

Limitations

The current study used a between subjects design, meaning that the different stimulation groups were composed of different individuals. While the groups were equated on task behavioral abilities, it is virtually impossible to equate them on all factors that could potentially impact the effects of stimulation. For example, other research groups have shown that individual differences in physiological measures such as skull thickness, and levels of certain hormones and neurotransmitters such as GABA, can affect the way stimulation affects an individual (Krause and Cohen Kadosh, 2014). Additionally, measures of non-reading related neurocognitive abilities were not collected. It is possible that differences in other cognitive processes between the two groups may have affected results. While the current study did

not control for such measures, the use of random assignment to group and ensuring the group was matched on task performance should minimize potential confounds from these factors.

Finally, as with all tDCS studies, without the use of neuroimaging techniques such as structural or functional magnetic resonance imaging (fMRI), we are unable to confirm that the stimulated area was in fact the targeted area. Individual differences in anatomy may have led to differences in how well the stimulation site aligned with the brain regions that are actually used to perform the tasks. Similarly, due to the distributed effects of tDCS we cannot make strong conclusions about whether the results are due to stimulation to the targeted region or surrounding and connected regions without neuroimaging measures. Future research with tDCS would benefit from using neuroimaging methods to have more precisely located targets and a better understanding of the locations that were actually affected in order to develop the most effective treatment methods.

CONCLUSIONS

Our study provides important cautionary evidence for the use of tDCS as a treatment for low reading ability. Although stimulation to the left IPL led to greater improvements in reading fluency than those previously demonstrated with a different stimulation site (2012), we also found a negative effect on another subcomponent of reading in low-to-average readers, i.e., rhyming two visually presented words. These positive and negative effects on two different subcomponents of reading were demonstrated using the same stimulation parameters within the same participants. These results stress the need for further research examining the effect of a set of stimulation parameters on complementary skills so that potential users of tDCS as a therapy can accurately weigh the costs and benefits of the treatment.

AUTHOR CONTRIBUTIONS

MR and JB conceived and designed the experiments. MR and JY performed the experiments. JY and JB analyzed and interpreted the data. JY drafted the manuscript. JY, MR, and JB performed a critical review of the manuscript. All the authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

This research was supported by the University of Texas at Austin. The authors would also like to thank the numerous research assistants who assisted in data collection throughout the study.

REFERENCES

Adlof, S. M., Catts, H. W., and Little, T. D. (2006). Should the simple view of reading include a fluency component? *Read. Writ.* 19, 933–958. doi: 10.1007/s11145-006-9024-z

Ashmore, R. A., Farrier, M. J., Paulson, L. H., and Chu, X. (2002). *The Effects of Phonemic Awareness Drills on Phonological Awareness and Word Reading Performance in a Later Learned Alphabetic Script*. Available online at: http://eric.ed.gov/?id=ED471632 (Accessed November 12, 2015).

Balota, D. A., Yap, M. J., Hutchison, K. A., Cortese, M. J., Kessler, B., Loftis, B., et al. (2007). The english lexicon project. *Behav. Res. Methods* 39, 445–459. doi: 10.3758/BF03193014

Barth, A. E., Catts, H. W., and Anthony, J. L. (2009). The component skills underlying reading fluency in adolescent readers: a latent variable analysis. *Read. Writ.* 22, 567–590. doi: 10.1007/s11145-008-9125-y

Bestmann, S., de Berker, A. O., and Bonaiuto, J. (2015). Understanding the behavioural consequences of noninvasive brain stimulation. *Trends Cogn. Sci.* 19, 13–20. doi: 10.1016/j.tics.2014.10.003

Bikson, M., Datta, A., and Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clin. Neurophysiol.* 120, 1033–1034. doi: 10.1016/j.clinph.2009.03.018

Bitan, T., Burman, D. D., Chou, T.-L., Lu, D., Cone, N. E., Cao, F., et al. (2007a). The interaction between orthographic and phonological information in children: an fMRI study. *Hum. Brain Mapp.* 28, 880–891. doi: 10.1002/hbm.20313

Bitan, T., Cheon, J., Lu, D., Burman, D. D., Gitelman, D. R., Mesulam, M.-M., et al. (2007b). Developmental changes in activation and effective connectivity in phonological processing. *Neuroimage* 38, 564–575. doi: 10.1016/j.neuroimage.2007.07.048

Booth, J. R., Burman, D. D., Meyer, J. R., Gitelman, D. R., Parrish, T. B., and Mesulam, M. M. (2003). Relation between brain activation and lexical performance. *Hum. Brain Mapp.* 19, 155–169. doi: 10.1002/hbm.10111

Booth, J. R., Mehdiratta, N., Burman, D. D., and Bitan, T. (2008). Developmental increases in effective connectivity to brain regions involved in phonological processing during tasks with orthographic demands. *Brain Res.* 1189, 78–89. doi: 10.1016/j.brainres.2007.10.080

Cao, F., Bitan, T., and Booth, J. R. (2008). Effective brain connectivity in children with reading difficulties during phonological processing. *Brain Lang.* 107, 91–101. doi: 10.1016/j.bandl.2007.12.009

Cao, F., Bitan, T., Chou, T.-L., Burman, D. D., and Booth, J. R. (2006). Deficient orthographic and phonological representations in children with dyslexia revealed by brain activation patterns. *J. Child Psychol. Psychiatry* 47, 1041–1050. doi: 10.1111/j.1469-7610.2006.01684.x

Cao, F., Brennan, C., and Booth, J. R. (2015). The brain adapts to orthography with experience: evidence from English and Chinese. *Dev. Sci.* 18, 785–798. doi: 10.1111/desc.12245

Cohen Kadosh, R., Dowker, A., Heine, A., Kaufmann, L., and Kucian, K. (2013). Interventions for improving numerical abilities: present and future. *Trends Neurosci. Educ.* 2, 85–93. doi: 10.1016/j.tine.2013.04.001

Costanzo, F., Menghini, D., Caltagirone, C., Oliveri, M., and Vicari, S. (2012). High frequency rTMS over the left parietal lobule increases non-word reading accuracy. *Neuropsychologia* 50, 2645–2651. doi: 10.1016/j.neuropsychologia.2012.07.017

Costanzo, F., Menghini, D., Caltagirone, C., Oliveri, M., and Vicari, S. (2013). How to improve reading skills in dyslexics: the effect of high frequency rTMS. *Neuropsychologia* 51, 2953–2959. doi: 10.1016/j.neuropsychologia.2013.10.018

De Berker, A. O., Bikson, M., and Bestmann, S. (2013). Predicting the behavioral impact of transcranial direct current stimulation: issues and limitations. *Front. Hum. Neurosci.* 7:613. doi: 10.3389/fnhum.2013.00613

Dubljević, V., Saigle, V., and Racine, E. (2014). The rising tide of tDCS in the media and academic literature. *Neuron* 82, 731–736. doi: 10.1016/j.neuron.2014.05.003

Eden, G. F., Jones, K. M., Cappell, K., Gareau, L., Wood, F. B., Zeffiro, T. A., et al. (2004). Neural changes following remediation in adult developmental dyslexia. *Neuron* 44, 411–422. doi: 10.1016/j.neuron.2004.10.019

Finn, E. S., Shen, X., Holahan, J. M., Scheinost, D., Lacadie, C., Papademetris, X., et al. (2014). Disruption of functional networks in dyslexia: a whole-brain, data-driven analysis of connectivity. *Biol. Psychiatry* 76, 397–404. doi: 10.1016/j.biopsych.2013.08.031

Fregni, F., and Pascual-Leone, A. (2007). Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. *Nat. Clin. Pract. Neurol.* 3, 383–393. doi: 10.1038/ncpneuro0530

Hashimoto, R., and Sakai, K. L. (2004). Learning letters in adulthood. *Neuron* 42, 311–322. doi: 10.1016/S0896-6273(04)00196-5

He, Q., Xue, G., Chen, C., Chen, C., Lu, Z.-L., and Dong, Q. (2013). Decoding the neuroanatomical basis of reading ability: a multivoxel morphometric study. *J. Neurosci.* 33, 12835–12843. doi: 10.1523/JNEUROSCI.0449-13.2013

Herwig, U., Satrapi, P., and Schönfeldt-Lecuona, C. (2003). Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. *Brain Topogr.* 16, 95–99. doi: 10.1023/B:BRAT.0000006333.93597.9d

Heth, I., and Lavidor, M. (2015). Improved reading measures in adults with dyslexia following transcranial direct current stimulation treatment. *Neuropsychologia* 70, 107–113. doi: 10.1016/j.neuropsychologia.2015.02.022

Hoeft, F., Hernandez, A., McMillon, G., Taylor-Hill, H., Martindale, J. L., Meyler, A., et al. (2006). Neural basis of dyslexia: a comparison between dyslexic and nondyslexic children equated for reading ability. *J. Neurosci.* 26, 10700–10708. doi: 10.1523/JNEUROSCI.4931-05.2006

Horvath, J. C., Forte, J. D., and Carter, O. (2015). Quantitative review finds no evidence of cognitive effects in healthy populations from single-session Transcranial Direct Current Stimulation (tDCS). *Brain Stimul.* 8, 535–550. doi: 10.1016/j.brs.2015.01.400

Horwitz, B., Rumsey, J. M., and Donohue, B. C. (1998). Functional connectivity of the angular gyrus in normal reading and dyslexia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 8939–8944.

Illingworth, S., and Bishop, D. V. M. (2009). Atypical cerebral lateralisation in adults with compensated developmental dyslexia demonstrated using functional transcranial Doppler ultrasound. *Brain Lang.* 111, 61–65. doi: 10.1016/j.bandl.2009.05.002

Iyer, M. B., Mattu, U., Grafman, J., Lomarev, M., Sato, S., and Wassermann, E. M. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology* 64, 872–875. doi: 10.1212/01.WNL.0000152986.07469.E9

Jobard, G., Crivello, F., and Tzourio-Mazoyer, N. (2003). Evaluation of the dual route theory of reading: a metanalysis of 35 neuroimaging studies. *Neuroimage* 20, 693–712. doi: 10.1016/S1053-8119(03)00343-4

Johnson, M. H. (2001). Functional brain development in humans. *Nat. Rev. Neurosci.* 2, 475–483. doi: 10.1038/35081509

Jones, K. T., and Berryhill, M. E. (2012). Parietal contributions to visual working memory depend on task difficulty. *Front. psychiatry* 3:81. doi: 10.3389/fpsyt.2012.00081

Kovelman, I., Norton, E. S., Christodoulou, J. A., Gaab, N., Lieberman, D. A., Triantafyllou, C., et al. (2012). Brain basis of phonological awareness for spoken language in children and its disruption in dyslexia. *Cereb. Cortex* 22, 754–764. doi: 10.1093/cercor/bhr094

Koyama, M. S., Di Martino, A., Zuo, X.-N., Kelly, C., Mennes, M., Jutagir, D. R., et al. (2011). Resting-state functional connectivity indexes reading competence in children and adults. *J. Neurosci.* 31, 8617–8624. doi: 10.1523/JNEUROSCI.4865-10.2011

Krause, B., and Cohen Kadosh, R. (2013). Can transcranial electrical stimulation improve learning difficulties in atypical brain development? A future possibility for cognitive training. *Dev. Cogn. Neurosci.* 6, 176–194. doi: 10.1016/j.dcn.2013.04.001

Krause, B., and Cohen Kadosh, R. (2014). Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation. *Front. Syst. Neurosci.* 8:25. doi: 10.3389/fnsys.2014.00025

Kucian, K., and von Aster, M. (2015). Developmental dyscalculia. *Eur. J. Pediatr.*. 174, 1–13. doi: 10.1007/s00431-014-2455-7

Maclean, M., Bryant, P., and Bradley, L. (1987). Rhymes, nursery rhymes, and reading in early childhood. *Merril-Palmer Q.* 33, 255–281.

Martin, A., Schurz, M., Kronbichler, M., and Richlan, F. (2015). Reading in the brain of children and adults: a meta-analysis of 40 functional magnetic resonance imaging studies. *Hum. Brain Mapp.* 36, 1963–1981. doi: 10.1002/hbm.22749

Mei, L., Xue, G., Lu, Z.-L., He, Q., Zhang, M., Wei, M., et al. (2014). Artificial language training reveals the neural substrates underlying addressed and assembled phonologies. *PLoS ONE* 9:e93548. doi: 10.1371/journal.pone.0093548

Meyler, A., Keller, T. A., Cherkassky, V. L., Gabrieli, J. D. E., and Just, M. A. (2008). Modifying the brain activation of poor readers during sentence comprehension with extended remedial instruction: a longitudinal study of neuroplasticity. *Neuropsychologia* 46, 2580–2592. doi: 10.1016/j.neuropsychologia.2008.03.012

Milne, R. D., Syngeniotis, A., Jackson, G., and Corballis, M. C. (2002). Mixed lateralization of phonological assembly in developmental dyslexia. *Neurocase* 8, 205–209. doi: 10.1093/neucas/8.3.205

Miniussi, C., Cappa, S. F., Cohen, L. G., Floel, A., Fregni, F., Nitsche, M. A., et al. (2008). Efficacy of repetitive transcranial magnetic stimulation/transcranial direct current stimulation in cognitive neurorehabilitation. *Brain Stimul.* 1, 326–336. doi: 10.1016/j.brs.2008.07.002

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2011). Transcranial direct current stimulation–update 2011. *Restor. Neurol. Neurosci.* 29, 463–492. doi: 10.3233/RNN-2011-0618

Norton, E. S., Beach, S. D., and de Gabrieli, J. (2014). Neurobiology of dyslexia. *Curr. Opin. Neurobiol.* 30C, 73–78. doi: 10.1016/j.conb.2014.09.007

Park, H.-J., and Friston, K. (2013). Structural and functional brain networks: from connections to cognition. *Science* 342:1238411. doi: 10.1126/science.1238411

Price, A. R., McAdams, H., Grossman, M., and Hamilton, R. H. (2015). A meta-analysis of transcranial direct current stimulation studies examining the reliability of effects on language measures. *Brain Stimul.* 8, 1093–1100. doi: 10.1016/j.brs.2015.06.013

Price, C. J., and Devlin, J. T. (2011). The interactive account of ventral occipitotemporal contributions to reading. *Trends Cogn. Sci.* 15, 246–253. doi: 10.1016/j.tics.2011.04.001

Price, G. R., and Ansari, D. (2013). Dyscalculia : characteristics, causes, and treatments. *Numeracy* 6, 1–18. doi: 10.5038/1936-4660.6.1.2

Priori, A., Hallett, M., and Rothwell, J. C. (2009). Repetitive transcranial magnetic stimulation or transcranial direct current stimulation? *Brain Stimul.* 2, 241–245. doi: 10.1016/j.brs.2009.02.004

Pugh, K. R., Mencl, W. E., Jenner, A. R., Katz, L., Frost, S. J., Lee, J. R., et al. (2001). Neurobiological studies of reading and reading disability. *J. Commun. Disord.* 34, 479–492. doi: 10.1016/S0021-9924(01)00060-0

Pugh, K. R., Mencl, W. E., Shaywitz, B. A., Shaywitz, S. E., Fulbright, R. K., Constable, R., et al. (2000). The angular gyrus in developmental dyslexia: task-specific differences in functional connectivity within posterior cortex. *Psychol. Sci.* 11, 51–56. doi: 10.1111/1467-9280.00214

Quaglino, V., Bourdin, B., Czternasty, G., Vrignaud, P., Fall, S., Meyer, M. E., et al. (2008). Differences in effective connectivity between dyslexic children and normal readers during a pseudoword reading task: an fMRI study. *Neurophysiol. Clin.* 38, 73–82. doi: 10.1016/j.neucli.2007.12.007

Rezaie, R., Simos, P. G., Fletcher, J. M., Cirino, P. T., Vaughn, S., and Papanicolaou, A. C. (2011a). Engagement of temporal lobe regions predicts response to educational interventions in adolescent struggling readers. *Dev. Neuropsychol.* 36, 869–888. doi: 10.1080/87565641.2011.606404

Rezaie, R., Simos, P. G., Fletcher, J. M., Cirino, P. T., Vaughn, S., and Papanicolaou, A. C. (2011b). Temporo-parietal brain activity as a longitudinal predictor of response to educational interventions among middle school struggling readers. *J. Int. Neuropsychol. Soc.* 17, 875–885. doi: 10.1017/S1355617711000890

Richlan, F., Kronbichler, M., and Wimmer, H. (2009). Functional abnormalities in the dyslexic brain: a quantitative meta-analysis of neuroimaging studies. *Hum. Brain Mapp.* 30, 3299–3308. doi: 10.1002/hbm.20752

Richlan, F., Kronbichler, M., and Wimmer, H. (2011). Meta-analyzing brain dysfunctions in dyslexic children and adults. *Neuroimage* 56, 1735–1742. doi: 10.1016/j.neuroimage.2011.02.040

Ridding, M. C., and Rothwell, J. C. (2007). Is there a future for therapeutic use of transcranial magnetic stimulation? *Nat. Rev. Neurosci.* 8, 559–567. doi: 10.1038/nrn2169

Sandrini, M., Fertonani, A., Cohen, L. G., and Miniussi, C. (2012). Double dissociation of working memory load effects induced by bilateral parietal modulation. *Neuropsychologia* 50, 396–402. doi: 10.1016/j.neuropsychologia.2011.12.011

Schlaggar, B. L., and McCandliss, B. D. (2007). Development of neural systems for reading. *Annu. Rev. Neurosci.* 30, 475–503. doi: 10.1146/annurev.neuro.28.061604.135645

Shaywitz, B. A., Shaywitz, S. E., Blachman, B. A., Pugh, K. R., Fulbright, R. K., Skudlarski, P., et al. (2004). Development of left occipitotemporal systems for skilled reading in children after a phonologically- based intervention. *Biol. Psychiatry* 55, 926–933. doi: 10.1016/j.biopsych.2003.12.019

Simon, G., Lanoë, C., Poirel, N., Rossi, S., Lubin, A., Pineau, A., et al. (2013). Dynamics of the anatomical changes that occur in the brains of schoolchildren as they learn to read. *PLoS ONE* 8:e81789. doi: 10.1371/journal.pone.0081789

Simos, P. G., Breier, J. I., and Jack, M. (2001). Age-related changes in regional brain activation during phonological decoding and printed word recognition. *Dev. Neuropsychol.* 19, 37–41. doi: 10.1207/S15326942DN1902

Simos, P. G., Fletcher, J. M., Sarkari, S., Billingsley, R. L., Denton, C., and Papanicolaou, A. C. (2007). Altering the brain circuits for reading through intervention: a magnetic source imaging study. *Neuropsychology* 21, 485–496. doi: 10.1037/0894-4105.21.4.485

Sparing, R., Thimm, M., Hesse, M. D., Küst, J., Karbe, H., and Fink, G. R. (2009). Bidirectional alterations of interhemispheric parietal balance by non-invasive cortical stimulation. *Brain* 132, 3011–3020. doi: 10.1093/brain/awp154

Temple, E., Deutsch, G. K., Poldrack, R. A., Miller, S. L., Tallal, P., Merzenich, M. M., et al. (2003). Neural deficits in children with dyslexia ameliorated by behavioral remediation: evidence from functional MRI. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2860–2865. doi: 10.1073/pnas.0030098100

Thomson, J. M., Doruk, D., Mascio, B., Fregni, F., and Cerruti, C. (2015). Transcranial direct current stimulation modulates efficiency of reading processes. *Front. Hum. Neurosci.* 9:114. doi: 10.3389/fnhum.2015.00114

Torgesen, J., Wagner, R., and Rashotte, C. (1999). *TOWRE–2 Test of Word Reading Efficiency*. Austin, TX: Pro-Ed. Available online at: http://www.mindresources.com/pdfs/SR2011/pages/PAGE_202.pdf (Accessed January 15, 2016).

Turkeltaub, P. E., Benson, J., Hamilton, R. H., Datta, A., Bikson, M., and Coslett, H. B. (2012). Left lateralizing transcranial direct current stimulation improves reading efficiency. *Brain Stimul.* 5, 201–207. doi: 10.1016/j.brs.2011.04.002

Turkeltaub, P. E., Gareau, L., Flowers, D. L., Zeffiro, T. A., and Eden, G. F. (2003). Development of neural mechanisms for reading. *Nat. Neurosci.* 6, 767–773. doi: 10.1038/nn1065

van der Mark, S., Klaver, P., Bucher, K., Maurer, U., Schulz, E., Brem, S., et al. (2011). The left occipitotemporal system in reading: disruption of focal fMRI connectivity to left inferior frontal and inferior parietal language areas in children with dyslexia. *Neuroimage* 54, 2426–2436. doi: 10.1016/j.neuroimage.2010.10.002

Vellutino, F. R., Tunmer, W. E., Jaccard, J. J., and Chen, R. (2007). Components of reading ability: multivariate evidence for a convergent skills model of reading development. *Sci. Stud. Read.* 11, 3–32. doi: 10.1080/10888430709336632

Vicario, C. M., and Nitsche, M. A. (2013). Transcranial direct current stimulation: a remediation tool for the treatment of childhood congenital dyslexia? *Front. Hum. Neurosci.* 7:139. doi: 10.3389/fnhum.2013.00139

Wechsler, D. (2008). *Wechsler Adult Intelligence Scale–Fourth Edition (WAIS–IV)*. San Antonio, TX: NCS Pearson. Available online.at: http://www.statisticssolutions.com/academic-solutions/resources/directory-of-survey-instruments/wechsler-adult-intelligence-scale-fourth-edition-wais-iv/ (Accessed January 15, 2016).

Williams, J. A., Imamura, M., and Fregni, F. (2009). Updates on the use of non-invasive brain stimulation in physical and rehabilitation medicine. *J. Rehabil. Med.* 41, 305–311. doi: 10.2340/16501977-0356

Wong, I. S. Y., and Tsang, H. W. H. (2013). A review on the effectiveness of repetitive transcranial magnetic stimulation (rTMS) on post-stroke aphasia. *Rev. Neurosci.* 24, 105–114. doi: 10.1515/revneuro-2012-0072

Xu, M., Yang, J., Siok, W. T., and Tan, L. H. (2015). Atypical lateralization of phonological working memory in developmental dyslexia. *J. Neurolinguist.* 33, 67–77. doi: 10.1016/j.jneuroling.2014.07.004

Zeno, S. (1995). *The Educator's Word Frequency Guide*. Touchstone Applied Science Associates. Available online at: https://scholar.google.com/scholar?q=zeno$+$1995&btnG=&hl=en&as_sdt=0%2C44#0 (Accessed January 15, 2016).

Ziegler, J. C., and Goswami, U. (2005). Reading acquisition, developmental dyslexia, and skilled reading across languages: a psycholinguistic grain size theory. *Psychol. Bull.* 131, 3–29. doi: 10.1037/0033-2909.131.1.3

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 30 July 2014
doi: 10.3389/fnsys.2014.00132

Best of both worlds: promise of combining brain stimulation and brain connectome

Caroline Di Bernardi Luft[1], Ernesto Pereda[2], Michael J. Banissy[1] and Joydeep Bhattacharya[1]*

[1] *Department of Psychology, Goldsmiths, University of London, London, UK*
[2] *Lab. of Electrical Engineering and Bioengineering, Department of Industrial Engineering, Institute of Biomedical Technology, University of La Laguna, Tenerife, Spain*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Kohitij Kar, Rutgers University, USA
Diego Kaski, Imperial College London, UK

***Correspondence:**
Joydeep Bhattacharya, Department of Psychology, Goldsmiths, University of London, New Cross, London SE14 6NW, UK
e-mail: j.bhattacharya@gold.ac.uk

Transcranial current brain stimulation (tCS) is becoming increasingly popular as a non-pharmacological non-invasive *neuromodulatory* method that alters cortical excitability by applying weak electrical currents to the scalp via a pair of electrodes. Most applications of this technique have focused on enhancing motor and learning skills, as well as a therapeutic agent in neurological and psychiatric disorders. In these applications, similarly to lesion studies, tCS was used to provide a causal link between a function or behavior and a specific brain region (e.g., primary motor cortex). Nonetheless, complex cognitive functions are known to rely on functionally connected multitude of brain regions with dynamically changing patterns of information flow rather than on isolated areas, which are most commonly targeted in typical tCS experiments. In this review article, we argue in favor of combining tCS method with other neuroimaging techniques (e.g., fMRI, EEG) and by employing state-of-the-art connectivity data analysis techniques (e.g., graph theory) to obtain a deeper understanding of the underlying spatiotemporal dynamics of functional connectivity patterns and cognitive performance. Finally, we discuss the possibilities of using these combined techniques to investigate the neural correlates of human creativity and to enhance creativity.

Keywords: tCS, connectome, graph theory, functional connectivity, structural connectivity, tDCS, tACS, tRNS

INTRODUCTION

The possibility of non-invasively modulating the activity of the brain using transcranial current brain stimulation (tCS) has been intriguing the researchers in a variety of fields as it allows to improve cognition in various domains (Fregni et al., 2005; Santiesteban et al., 2012; Schaal et al., 2013; Snowball et al., 2013) or treat many human psychiatric conditions (Boggio et al., 2007, 2008; Rigonatti et al., 2008; Nitsche et al., 2009; Terhune and Cohen Kadosh, 2013). There are a number of tCS techniques available, including, but not limited to, transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), and transcranial random noise stimulation (tRNS) (for a review on the tCS methods, see: Nitsche et al., 2008; Ruffini et al., 2013). In tDCS, a small direct current (DC) is passed from anodal (positive) to cathodal (negative) electrodes positioned in the head surface in order to target specific brain areas underneath the electrodes (Nitsche and Paulus, 2000; Faria et al., 2011). Early studies with animals demonstrated an increase in excitation through membrane depolarization in the neurons underneath anodal electrode but an inhibition under the cathodal one (Bindman et al., 1962, 1964; Purpura and McMurtry, 1965). In humans, there is evidence for an increase in excitability in areas underneath the anodal electrode and a decrease underneath the cathodal following tDCS on the motor (Nitsche and Paulus, 2000) and visual cortex (Antal et al., 2004). Although this rationale of higher excitability under anodal and inhibition under cathodal has been used for determining the stimulation protocol in many studies, it remains unclear if this is so in all cases, as other variables such as the position of the cathodal in relation to anodal (Nitsche and Paulus, 2000; Antal et al., 2004; Moliadze et al., 2010) and the intensity of the stimulation (Batsikadze et al., 2013) seem to interfere with the excitability effects observed under anodal and cathodal stimulation sites. In tRNS the areas underneath both electrodes are stimulated with a current whose amplitude varies randomly in time within the frequency range of 100–640 Hz (Terney et al., 2008; Ruffini et al., 2013). In tACS, an alternating current (AC) with a pre-determined frequency passes from anodal to cathodal and the frequency is usually set within the EEG frequency spectrum (1–100 Hz) (Antal et al., 2008; Kanai et al., 2010).

The protocol for tCS stimulation, especially the anodal and cathodal electrodes location, is usually determined based on neuroimaging findings (e.g., EEG, fMRI) evidencing that a certain region is involved in the target brain function which the researcher wants to modulate. Therefore, most tCS studies hitherto are grounded on the modular paradigm, in which complex cognitive functions are thought to be mediated by independent brain areas (e.g., Kanwisher et al., 1997). Despite the great advance in the knowledge made through the modular paradigm in the last decades, the understanding that each cognitive function is mediated by independent brain areas is challenged by an increasing number of studies supporting that most cognitive

functions are mediated by widely distributed areas functioning in parallel (Fuster, 2000; Sporns, 2014). For example, dyslexia was for a long time thought to be caused by a problem in the phonetic representations located in the primary and secondary auditory cortices (Goswami, 2000). However, recent work (Boets et al., 2013) has shown that dyslexic individuals have intact phonetic representations, but presented a problem in connectivity, both structural and functional, between inferior frontal gyrus (IFG) and the bilateral auditory cortex, which is associated with retrieving these representations. Other disorders such as schizophrenia (van den Heuvel et al., 2013), epilepsy (Bettus et al., 2008), and autism (Barttfeld et al., 2011) are also associated with abnormal (increased or decreased) brain connectivity rather than abnormal activity of isolated brain regions. In such cases, in seems logical that brain stimulation should not target one or the other isolated area, but the connection between them, which is certainly a challenging aim because most of the brain stimulation effects are assumed to be caused by the excitation/inhibition of the specific areas underneath anodal/cathodal electrodes.

Thus, in order to target specific connections rather than specific areas, it is necessary to understand how (or even whether) brain networks respond under or after tCS. In fact, the notion that tCS effects are brought about by increases/decreases in activation of the stimulated area has been challenged by studies showing that the effects of tDCS are not restricted to the stimulated sites (Lang et al., 2005; Kwon et al., 2008; Keeser et al., 2011). Moreover, there are some recent studies showing that tDCS affects brain connectivity patterns during both task and rest (Keeser et al., 2011; Polania et al., 2011a,b, 2012a; Meinzer et al., 2012, 2013), suggesting that the tCS has an impact not only on the target areas, but also on the brain networks. In this review paper, we discuss the possibility of tracking tCS-induced changes in the brain network by combining neuroimaging with advanced connectivity analysis techniques (e.g., graph theory). We briefly review the mechanisms of tCS and the basics of brain network analysis through graph theory as a framework to develop new brain stimulation protocols able to produce relevant changes in brain connectivity and, ultimately, in the features of a given brain network. In particular, we discuss the rationale for determining the stimulation protocol for improving creativity as an example of a complex cognitive construct which requires complex associations between multiple brain areas.

POSSIBLE MECHANISMS OF tCS

Before discussing the macro effects of tCS on connectivity, a brief discussion of its potential mechanisms is needed. Dissertating in detail on how the distinct tCS techniques can affect brain and behavior is out of the scope of this paper, but some of the key issues are elucidated here as they may be important for understanding how tCS can shape brain functional connectivity. Previous research with animals (Bindman et al., 1962, 1964; Purpura and McMurtry, 1965) showed that a small electrical DC passing through the anodal electrode can depolarize the cell membrane at subthreshold intensity, making the neurons more susceptible to excitatory activity as they become less negative, whereas the current passing the cathodal electrode polarizes the cell membrane making it more negative, inhibiting neural firing. Gartside (1968) found that a weak electrical current induced an increase in cortical firing under the stimulated area (anodal) in rats. Importantly, the same study observed that turning off the electrical current after 5 min of stimulation did not cease this increased neural discharge, termed the "aftereffect." Although in humans the currents reaching the brain through tCS are much weaker than the ones in animal studies (even if the external stimulating current is the same as humans have thicker skull), there is evidence that the brain areas underneath the anodal electrode are more prone to excitatory activity (Nitsche and Paulus, 2000, 2001; Antal et al., 2004). Repeated firing, or high-frequency stimulation, may result in long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Lomo, 1973), which are thought to be the main mechanisms by which tCS modulates brain activity, as it does during learning (Rioult-Pedotti et al., 2000). In the LTP process, sustained activation of the cell through the binding of glutamate to α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the post-synaptic membrane causes the magnesium (Mg^{2+}) to leave the N-methyl-D-aspartate receptor (NMDA) ion channel, allowing large quantities of calcium (Ca^{2+}) to enter the cell through this channel (Mg^{2+} blocks the NMDA channel). These large quantities of Ca^{2+} in the post-synaptic dendrites can improve the synaptic efficiency for an extended time period by activating second messengers (calcium-dependent kinases such as the Ca^{2+}/calmodulin-dependent ones: CaMKs), which create more AMPA receptors and protein expressions (growth factor), thereby facilitating neural plasticity (Malenka and Bear, 2004). The role of LTP on tCS effects is supported by pharmacological studies showing that the administration of an antagonist of the NMDA receptor blocks the effects of anodal and cathodal stimulation on the motor evoked potential (MEP), as triggered by a transcranial magnetic stimulation (TMS) pulse, an indirect measure of motor cortex excitability (Liebetanz et al., 2002; Nitsche et al., 2003; Monte-Silva et al., 2013). Saturation of the LTP can induce LTD (Rioult-Pedotti et al., 2000), which might be one of the reasons why the tCS effects were found to be dosage dependant (Batsikadze et al., 2013). It is important to notice that the electrical currents delivered by tDCS/tACS and tRNS are not strong enough to fire an action potential (Radman et al., 2009), but they can cause a bimodal polarization effect, namely soma depolarization and apical dendrite hyperpolarization (Bikson et al., 2004). Therefore tCS, as opposed to TMS (Terao and Ugawa, 2002), affects the post-synaptic potential by promoting a change in the cell gain (Rahman et al., 2013), and not by increasing the firing itself. Furthermore, single neuron response to weak current stimulation seems to be rather dependant on the network dynamics. It was found that low frequency AC currents can induce changes in gamma oscillations (25–35 Hz) in a zero-sum fashion as increases in excitability were balanced by complimentary inhibitory activity according to the network dynamics (Reato et al., 2010). Other studies (Parra and Bikson, 2004; Deans et al., 2007) also revealed that the network plays a role even at the cellular level (*in vitro* studies), as the neuronal firing behavior was largely determined by the network it belongs to.

The ongoing neuronal oscillatory activity during stimulation seems to shape the effects of tDCS and tACS on the resulting

network activity (Frohlich and McCormick, 2010; Ali et al., 2013; Frohlich and Schmidt, 2013). This appears to happen through a feedback loop between the neural activity and the endogenous electric field (Frohlich and McCormick, 2010). Thus, tACS at the endogenous oscillation frequency (*in vitro*) produced a higher enhancement of this oscillation as compared to tDCS (Ali et al., 2013). In addition, these authors found that the network oscillatory effects were more pronounced if the stimulated frequency matches the endogenous oscillation frequency, suggesting a resonance-like effect. The importance of network activity was not only evidenced at the cellular level, but also at the cortical level in awake human beings. In relation to tDCS, it was found that stimulating over the premotor cortex (PMC) resulted in increased excitability (as measured by the MEP) over the primary motor cortex (M1) (Boros et al., 2008), which suggests that stimulating one area can affect others structurally connected to it. This might explain why many studies found that the stimulation with weak currents affects a number of areas other than the region underneath the anodal electrode (Lang et al., 2005; Kwon et al., 2008; Keeser et al., 2011). The possibility that network dynamics also plays a role in the effects of tCS at the macro level is supported by studies showing that tDCS brings about changes in functional connectivity, especially when those changes are assessed during task performance (Polania et al., 2012a; Weber et al., 2014). The default mode network (DMN) (Keeser et al., 2011; Amadi et al., 2014) and the attention network (AN) (Pena-Gomez et al., 2012) also seem to be affected by brain stimulation, even when the stimulated area is not within the same network. However, studies investigating the effects of tCS on brain connectivity during different tasks are still scarce (Polania et al., 2011b, 2012a,b; Meinzer et al., 2012, 2013; Weber et al., 2014), but they may shed new light into how tCS affects the brain dynamics and behavior. One of the challenges on this enterprise is to develop a suitable framework that can guide not only the analysis and interpretation of complex connectivity results derived from various neuroimaging techniques, but also to develop more efficient protocols to tackle connectivity.

NETWORK ANALYSIS AND BRAIN STIMULATION

There are many ways of measuring how different brain areas interact or communicate with each other. Connectivity is usually investigated from three perspectives: structural, functional and effective (Friston, 2011). Structural connectivity refers to the anatomical connections within the brain, such as axons and synapses, that can be measured non-invasively through diffusion tensor imaging (DTI) (van den Heuvel and Sporns, 2013). The brain structure is thought to shape or determine the paths for the communication between brain areas while the brain is engaged in various tasks or even at rest (Friston, 2011). While structural connectivity traces the paths between regions based on the physical connections between them, functional and effective connectivity estimate these connections based on the relationship between the time series from each of these brain regions (voxels) or the corresponding electrode / sensor (van den Heuvel and Sporns, 2013). Functional connectivity refers to how interdependent the activity between two areas (or more) is, with no information on the direction of their communication, whereas effective connectivity refers to the directed (source and sink) connection between two areas (Horwitz, 2003; Friston, 2011). Both functional and effective connectivity are dynamic and can be measured from data collected using various neuroimaging techniques, such as EEG, MEG, and fMRI. A variety of algorithms has been developed to estimate functional (e.g., coherence, phase synchronization, correlation, synchronization likelihood), and effective (e.g., dynamic causal modeling, Granger causality, phase slope index) connectivity, and they are available in many neuroimaging analysis toolboxes (e.g., Delorme and Makeig, 2004; Oostenveld et al., 2011; Niso et al., 2013). These measures allow us to estimate the strength of the communication between regions/sensors and they all have different advantages and limitations (Pereda et al., 2005; Friston, 2011).

In order to understand the organizing principles of the brain networks (estimated using the techniques mentioned in the previous paragraph), we can use graph theory, which has emerged as an important model for understanding and quantifying the global properties of brain networks (Bassett and Bullmore, 2006; Bressler and Menon, 2010; Sporns, 2011). In graph theory, networks are mathematically represented as a set of *nodes*, which in this framework are the brain regions or the electrodes/sensors for EEG/MEG, connected through *edges*, which are the paths or lines representing the direct relation between the nodes (**Figure 1A**).

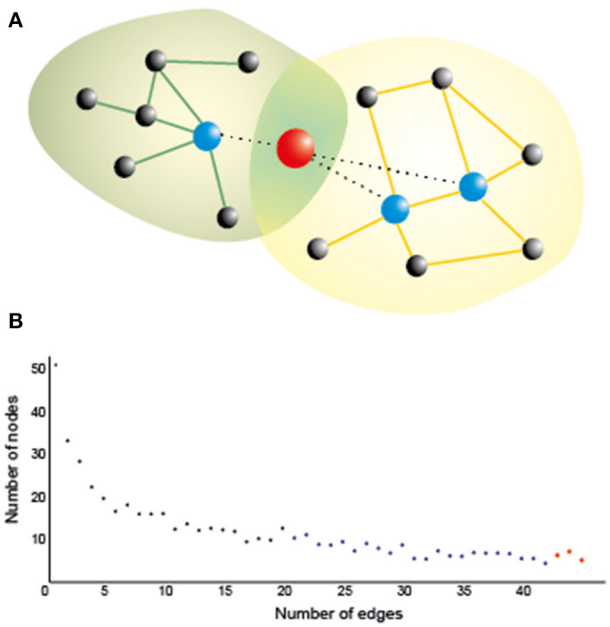

FIGURE 1 | (A) A hub naturally serves as a bridge between two or more networks, as it is connected to many nodes in all of them, thereby playing an important role in brain functioning. Stimulating a hub is likely to affect many nodes even at a long distance, which could maximize the effect of stimulation. Yet networks are also vulnerable against a directed attack on the hubs, so that their inhibition, however partial and/or transient, may crucially affect the corresponding cognitive functions if it is not properly controlled (e.g., by multisite stimulation of nearby nodes). *Green and orange lines*: intra-network edges. *Dotted lines*: edges connecting the hub with nodes in both networks; **(B)** A typical degree distribution for brain nodes as assessed by a functional connectivity index. Most of the nodes (black) have low degree (i.e., are connected only to a few nodes); some of them (blue) have moderate degree, and a few of them (in red) are hubs, which are connected to many nodes.

Out of the many measures that can be used to characterize these networks (Rubinov and Sporns, 2010; Sporns, 2014), two of them are allegedly the most commonly used in neuroscience: (1) the average clustering coefficient, and (2) the average shortest path length. The clustering coefficient is the probability that neighboring nodes will be connected. The average shortest path length is the average minimum number of edges or connections that need to be traversed between two nodes. The arrangement of the edges in the network can take three main forms, which in turn determines the character of the network itself: regular, random, and small-world (Watts and Strogatz, 1998). In a regular network, each node is connected to its neighbors, resulting in high clustering and large average path length. If we randomly rewire most of the edges in a regular network, we would reduce the path length and the clustering coefficient, which characterizes a random network. However, if only a small number of edges are rewired to connect distant nodes, we have a "small-world" network, characterized by a small shortest path length and a high clustering coefficient (Watts and Strogatz, 1998). In small-world networks, the nodes containing the long-range connections have a similar number of connections in comparison to the other nodes, which makes the network resistant to random attack to the nodes. In the brain, however, there are some regions (termed network hubs) that are more heavily connected than others, and also more connected among themselves (van den Heuvel and Sporns, 2013). This type of network organization, with only a few nodes more connected than others and also more connected among themselves, is known as "scale-free," because such networks have a power law degree distribution (Barabasi and Albert, 1999; Sporns et al., 2004), which means that most nodes have only few connections or edges, whereas a few hubs have a large number of connections, as represented in **Figure 1B**. The presence of hubs (blue and red nodes in **Figure 1**), which are heavily connected and usually also centrally located nodes, and associated with locally connected specialized nodes, allows the network to have both local and global information processing (van den Heuvel and Sporns, 2011, 2013). The hubs are essential for brain communication, albeit energetically expensive as they are highly connected, and occupy a privileged position in the network, connecting distant communities of nodes to other hubs. In addition these hubs are organized as a "rich-club," in which densely connected nodes tend to be more connected to each other (van den Heuvel and Sporns, 2013). There are two types of rich-club hubs: connector hubs (red node **Figure 1A**), which interconnect different modules, and provincial hubs (blue nodes **Figure 1A**), which connect nodes within the same module. A study with DTI (van den Heuvel and Sporns, 2011) identified three main cortical areas (both hemispheres, near the midline) which are connector hubs: superior parietal, precuneus, and superior frontal cortex; and three subcortical regions (both hemispheres near medial regions) which are provincial hubs: putamen, hippocampus, and thalamus. Importantly, they found that attacking the rich-club connections (links between members of the rich-club) in comparison to random attacks or attacks to other hub connections caused a larger decrease in the global efficiency of the network. These hubs were defined based on structural connectivity, but there is accumulating evidence on the large overlap between brain network structure and functional connectivity especially during resting state (Cabral et al., 2014; Goni et al., 2014). Regions within the DMN, medially, contain most of the functional hubs, including most of the medial regions, including anterior cingulate cortex (ACC), precuneus/posterior cingulate gyrus. Using a measure of global functional connectivity, a study (Cole et al., 2010) found that not only the DMN regions have high functional connectivity with all other regions, but the cognitive control network (CCN) (Cole and Schneider, 2007), which comprises the dorsolateral prefrontal cortex (DLPFC), rostrolateral prefrontal cortex (RLPFC), dorsal-caudal ACC, inferior frontal junction (IFJ), posterior parietal cortex (PPC), PMC, and anterior insular cortex (AIC), has also high global connectivity. The regions in the DMN and in the CCN are among the top 5% most connected regions in the brain (Cole et al., 2010). Recently, the concept of flexible hubs (Cole et al., 2013a,b), which are brain regions (e.g., DLPFC) that quickly shift their functional connectivity patterns (become highly connected) to implement cognitive control, has been discussed as an important part of network's ability for flexible behavior. Besides, a recent theoretical work (Aguirre et al., 2014) shows that communication through heavily connected nodes facilitates synchronization between different networks. Therefore, the structural and functional hubs seem to be an essential part of the scale-free brain network organization.

Making use of its structural and functional hubs, which can flexibly adapt to different environmental demands, the scale-free configuration allows dynamical exchange of information that facilitates parallel processing and rapid changes on its own configuration (Bassett and Bullmore, 2006). There is evidence that the scale-free network characteristics, as measured by graph theory, are optimized during awake compared to sleep periods (Uehara et al., 2014), suggesting that the functional organization of the network is relevant for cognitive processing. Therefore, graph-theory can be used to investigate structural, functional and effective connectivity. Functional connectivity gives undirected edges to the network, whereas effective connectivity provides information on the direction of the interactions investigated. Moreover, the edges can be weighted according to the degree of coupling between two nodes, as estimated by the techniques mentioned previously. Knowing and estimating the changes in network properties as a result of brain stimulation, during task and resting state alike, has important implications on the understanding of its effects over behavior. In addition, the knowledge of the networks representing the target cognitive process might provide insight into optimizing stimulation protocols.

BRAIN STIMULATION AND CONNECTIVITY

There is a great deal of research analysing the impact of TMS on brain connectivity patterns during task and rest (for a review, see Shafi et al., 2012). As TMS is not covered in this paper, we only summarize four main findings on TMS-induced alterations in connectivity, which can shed light onto how connectivity changes in response to brain stimulation: (1) *state dependency:* the brain state during stimulation affects how it modifies connectivity (Massimini et al., 2005; Davare et al., 2008; Morishima et al., 2009); (2) *rich-club spreading:* stimulating areas that have more connections (rich-club nodes) will affect a larger network

(Bestmann et al., 2003, 2005; Chouinard et al., 2003); (3) *structural connectivity spreading:* stimulating one area affects other regions that are structurally connected to the main stimulated region (Pascual-Leone and Walsh, 2001; Mochizuki et al., 2004); (4) *compensatory connectivity:* inhibiting certain brain areas may trigger compensatory activity in the task related network (O'Shea et al., 2007). Although there are not many studies on the effects of tCS on brain connectivity, we will discuss some of these premises in the context of the few available ones published hitherto, which are listed in **Table 1**.

Most of the papers investigating the effect of tCS on brain connectivity analyzed the changes in the functional network during resting state (Alon et al., 2011; Keeser et al., 2011; Polania et al., 2011b, 2012a; Meinzer et al., 2012, 2013; Pena-Gomez et al., 2012). Studies vary in how they define the regions/nodes of the network. Using fMRI, some studies looked into the connectivity using M1 as a seed (Alon et al., 2011; Polania et al., 2012a; Sehm et al., 2013), while others looked into specific regions of interest (Pena-Gomez et al., 2012; Polania et al., 2012b; Chib et al., 2013; Weber et al., 2014), or into the resting state networks (Keeser et al., 2011; Pena-Gomez et al., 2012). Furthermore, graph-theory was also used for tracking connectivity changes after brain stimulation (Polania et al., 2011a).

The results on stimulating motor cortex (M1) are somehow mixed: some found increased functional connectivity within M1 (Polania et al., 2012a), whereas others found a decrease (Alon et al., 2011) or both a decrease during the stimulation but an increase afterwards (Sehm et al., 2013). One of the issues with these studies is that functional connectivity before and after stimulation was assessed during rest, yet M1 is not typically a region which is highly active during rest (Boros et al., 2008), so the impact of stimulation on brain connectivity may not be pronounced or strong as the motor network is relatively idle during rest. This possibility is supported by an EEG study on the effects of tDCS over M1 on functional connectivity during rest and during a finger tapping task (Polania et al., 2011a). These authors found that stimulation of the left motor cortex (anodal over C3/C5) with the cathodal electrode over right frontopolar electrodes (Fp2) was associated with higher connectivity of the motor areas in the gamma frequency band (60–90 Hz) during finger tapping. In addition, they found an increase in frontal connectivity in theta (4–7 Hz) and alpha (8–12 Hz) frequency bands during rest after tDCS stimulation, but this result was weaker than during task. They also found reduced coupling between frontal and occipital and areas after tDCS as compared to sham stimulation, which indicates that brain stimulation can shape connectivity not only by increasing communication between areas directly associated with the performing task, but also by reducing communication between other areas. Altogether, these results showed that the findings 1 (state-dependency) and 4 (compensatory connectivity) mentioned above can also explain some of the changes in functional connectivity following tDCS, while the aftereffects of the stimulation are still in place. The state-dependency in the cited studies is not related to the exact activity during the stimulation itself as referred in the TMS studies (Massimini et al., 2005; Davare et al., 2008; Morishima et al., 2009), but with the task conducted during the aftereffects of the stimulation. This means that the increase in coupling resulting from the stimulation is dependent on the task performed during the aftereffect period and on whether it recruits the stimulated network. In relation to finding 4 (compensatory connectivity), it seems that connectivity also changes in a zero-sum fashion, as it was suggested to be the case for most neuroenhancement interventions (Brem et al., 2014).

The state dependency seems to be important not only for tDCS, but also for tACS. By investigating how it can boost motion discrimination and lower adaptation, a study found that the method was only effective when the 10 Hz stimulation over the motion area (left hMT+) was administered during visual stimulation, but not before or after it (Kar and Krekelberg, 2014). This suggests that, differently from the tDCS, where the task conducted during the aftereffects can shape the effects of the stimulation, tACS effects on brain synchronization are dependent of the ongoing activity/task or brain state during which the stimulation is administered. In the latter study (Kar and Krekelberg, 2014), administering tACS during rest is unlikely to boost motion discrimination since the effects seem to be very dependent on the precise moment of visual perception in each trial.

The structural connections between brain areas, as in findings 2 (rich-club spreading) and 3 (structural connectivity spreading), also seem to be of importance for understanding how tCS affects the brain networks. It is possible to use the knowledge about the connectivity between areas to target deeper brain structures (Takano et al., 2011; Chib et al., 2013) which up until recently could only be targeted pharmacologically. Research with animals (Takano et al., 2011) observed an increased activation in the nucleus accumbens in rats after 10 min of stimulation over the frontal cortex. A recent study in humans (Chib et al., 2013) used tDCS to target midbrain areas (subcortical), including substantia nigra (SN) and the ventral tegmental area (VTA) by stimulating the ventral medial prefrontal cortex (VMPFC), during a face attractiveness judgment task, which is associated with the dopaminergic system for reward processing. The anodal and cathodal electrode locations were defined based on the knowledge that excitation of the VMPFC combined with inhibition of the DLPFC can bring about an increase in activity (together with dopamine release) of the midbrain (Takano et al., 2011). They found that the main stimulation groups (anodal over the VMPFC and cathodal over the DLPFC) increased their ratings of face attractiveness after the stimulation, but not the active sham group, which was stimulated for the same time, but with the opposite locations (anode over DLPFC and cathode over VMPFC). Importantly, the main stimulation elicited an increase in the BOLD signal in the midbrain areas and an increase in functional connectivity between VMPFC and the midbrain area. This increase in connectivity was correlated to the participants' ratings of attractiveness. In addition, the behavioral effect was only evident when the cathodal electrode was placed over the DLPFC, as the same effects were not found when the cathode was positioned on the vertex (Cz). This result is consistent with the idea that coordinated activity between different brain areas, in this case VMPFC and DLPFC, can influence the outcomes of the stimulation in subcortical areas. Relevantly, it also demonstrates that it is possible to exploit the brain networks structure to target

Table 1 | Studies on the effects of tCS on brain connectivity.

Study	Technique	Parameters/location	Duration	Study design	Results
Polania et al., 2011a	tDCS	1 mA 4 × 4 cm^2 Anode: left M1 Cathode: right frontopolar	10 min during rest	Within participants (two sessions) Conditions: (1) tDCS; (2) Sham	After tDCS, not sham, there was an increase in synchronization between the stimulated area with premotor and sensorimotor more pronounced in the gamma band and during the motor task
Polania et al., 2012a	tDCS	1 mA 7 × 5cm^2 Anode: left M1 Cathode: right frontopolar	10 min during rest	Within participants Conditions: (1) Anode left M1/cathode right frontopolar; (2) Anode right M1/cathode left frontopolar; (3) Sham	Anode over left M1 was associated with increased connectivity between left thalamus and the ipsilateral M1, and between left caudate and parietal association cortex. Connectivity between the caudate and regions of the default mode network (DMN) was reduced, especially with the PCC. Cathode over the left M1 resulted reduced connectivity: between right putamen and left precentral gyrus and between right thalamus and left superior frontal gyrus
Meinzer et al., 2012	tDCS	1 mA Anode: 5 × 7 cm^2 Cathode: 10 × 10 cm^2 Anode: left IFG (BA44/45) Cathode: right supraorbital	20 min of which: ∼6 min during rest ∼11 min during task	Within participants Conditions: (1) tDCS; (2) Sham	Anodal tDCS over the left IFG was associated with better performance in a semantic word generation task. The BOLD response reduced at stimulated areas (left ventral IFG) compared to sham during the task. An increase in connectivity in the language network areas (left IFG and anterior insula) was also observed during the tDCS compared to sham. During rest, anodal stimulation resulted in increased connectivity between the left ventral IFG and other major language network hubs which partially overlapped with the task related changes in connectivity
Meinzer et al., 2013	tDCS	1 mA Anode: 5 × 7 cm^2 Cathode: 10 × 10 cm^2 Anode: left IFG (BA44/45) Cathode: right supraorbital	20 min of which: ∼6 min during rest ∼11 min during task	Within participants Conditions: (1) tDCS; (2) Sham	Older participants under sham stimulation performed a semantic word generation task worse than the younger group. However, during the tDCS, there was no difference in the performance between old and young participants. During task, the stimulation was associated with lower BOLD response at the stimulated site (left IFG—anodal), but with increased connectivity between this and other areas of the language network. The differences in BOLD and connectivity between young and older were reduced during tDCS stimulation, evidencing a "youth-like" effect in the older participants' brains under stimulation
Keeser et al., 2011	tDCS	2 mA 7 × 5 cm^2 Anode: left DLPFC Cathode: right supraorbital	20 min during rest	Within participants Conditions: (1) tDCS; (2) Sham	Among four resting state networks: DMN, left and right frontal-parietal networks (FPNs) and the self-referential network, it was found that tDCS induced a change in connectivity within the DMN and the FPNs

(Continued)

Table 1 | Continued

Study	Technique	Parameters/location	Duration	Study design	Results
Pena-Gomez et al., 2012	tDCS	2 mA 7 × 5 cm^2 Anode: DLPFC Cathode: right supraorbital	20 min during rest	Within participants Conditions: (1) Anodal left DLPFC; (2) Anodal right DLPFC; (3) Sham	Anodal tDCS to the DLPFC resulted in an increase in functional connectivity between prefrontal and parietal regions. There was also a decrease in the spatial configuration of the DMN following both right and left DLPFC anodal stimulation
Sehm et al., 2013	tDCS	1 mA Unilateral: Anode: right M1 Cathode: contralateral orbit; Bilateral: Anode: right M1 Cathode: left M1	20 min during rest	Within participants Conditions: (1) Unilateral; (2) Bilateral; (3) Sham	Bilateral tDCS was associated with reduced interhemispheric connectivity during stimulation and with an increase in intracortical connectivity within right M1 after the stimulation. Unilateral tDCS was associated with reduced interhemispheric connectivity, but not with increased connectivity after the stimulation as did the bilateral
Alon et al., 2011	tDCS, tPCS	tDCS: 2 mA 7 × 4.5 cm^2 Anode: right M1 Cathode: left supra-orbital tPCS: Monophasic waveform with pulse duration of 33 us and interval of 33.3 us Stimulator's carrier frequency 15 kHz	12 min 48 s (split in two—STIM 1 and STIM 2 with 6 min 24 s each)	Within participants Conditions: (1) tDCS; (2) tPCS	A reduced resting functional connectivity between right and left M1 was found after stimulation in both tDCS and tPCS
Weber et al., 2014	tDCS	1.5mA 5 × 5 cm^2 Anode: right DLPFC (F4) Cathode: left DLPFC (F3)	15 min rest outside scanner	Between subjects: Groups: (1) tDCS; (2) Sham	The tDCS group showed reduced connectivity between the right ACC and the rest of the brain after the stimulation during rest
Chib et al., 2013	tDCS	2 mA Anode: 3.5 × 3.5 cm^2 VMPFC (Fpz) Cathode: 5 × 5 cm^2 Right DLPFC (F4)	15 min during rest	Between subjects Groups*: (1) Main Stimulation; (2) Active sham group: Anode 5 × 5 cm over right DLPFC; and Cathode 3.5 × 3.5 cm over VMPFC Within-subjects: pre- vs. post-stimulation	Functional connectivity changes elicited by tDCS were evaluated during a face attractiveness judgment task, before and after stimulation. The main stimulation was associated with an increase in connectivity between the VMPFC and the midbrain area (substantia nigra and ventral tegmental area). The higher the functional connectivity between these two areas during stimulation, the better the performance in the face judgment task
Neuling et al., 2013	tACS	Individualized current around 1500 mA 5 × 7 cm^2 Anode: Oz Cathode: Cz Stimulation frequency: individual alpha peak frequency (IAF)	20 min during an auditory detection task	Between subjects Experiment eyes-closed groups: (1) tACS; (2) Sham Experiment eyes-open groups: (1)tACS; (2) Sham	They found that the aftereffects of tACS were higher for the group whose stimulation was done with eyes-open, whereas it did not differ between stimulation and sham for the eyes-closed experiment. However, the coherence between right and left parietal (P4-P3) increased only for the tACS group of the eyes-closed experiment

**Other four conditions were tested behaviorally, but only the main stimulation was effective in improving attractiveness judgments, leading them to scan only this and the active sham group for comparison.*

subcortical areas and their connections. The ability to affect connections between cortical and subcortical areas by stimulating the cerebral cortex has been also demonstrated during rest (Polania et al., 2012a).

Studies looking at resting state networks (Keeser et al., 2011; Pena-Gomez et al., 2012) found changes in functional connectivity after stimulation of the DLPFC. One of these studies (Pena-Gomez et al., 2012) found that stimulating either the right or the left DLPFC resulted in robust changes in the AN and in the DMN. It was observed that anodal tDCS over DLPFC was associated with a disruption in the DMN topography, as if the anterior (medial prefrontal) and posterior components (medial posterior) of the network become temporally independent after the stimulation. On the other hand, there was an increase in functional connectivity between frontal and parietal areas, which are part of the AN. An increase in fronto-parietal connectivity related to the AN was also found by others (Keeser et al., 2011), along with an increase in connectivity between an area near the anodal electrode on the left DLPFC and the DMN regions. It may be that tDCS over the DLPFC increases the alertness for action as indicated by the networks affected by stimulation, which it was suggested by the authors of both studies (Keeser et al., 2011; Pena-Gomez et al., 2012). As we mentioned in the previous section, the DLPFC can be considered a functional "flexible hub," which plays an important role in switching from one state to the other in order to attend to the necessary task demands (Cole et al., 2013b). This might be one of the reasons why stimulating DLPFC seems to affect functional connectivity in the DMN, as it reflects the change from one to the other network. These studies support that tCS can alter the connectivity in the brain during rest. In order to improve the interpretation of the results, new studies should target specific networks and bring new hypothesis of possible behavioral correlates. For example, what does it mean to increase or decrease connectivity in certain brain networks? Would these changes, for instance, improve mood, reduce depression, increase alertness, and others? The need for behavioral correlates in these studies is crucial for understanding their functional meaning.

Hitherto, two recent studies (Meinzer et al., 2012, 2013) used fMRI to monitor the brain activity during rest as well as during a semantic word generation task while the participants received anodal tDCS over the left IFG (targeting language areas—BA 44/45—see **Table 1** for details). Both studies observed a reduction in the BOLD signal at the stimulated sites during the language task. Importantly, the connectivity between the stimulated area (left IFG) and other language-related area was increased during stimulation for both task and rest (Meinzer et al., 2012, 2013). It has been argued that these changes are related to increased neural efficiency at the stimulated site and its networks (Kar and Wright, 2014) similar to the changes observed as a result of learning (Buchel et al., 1999). These changes were found to be behaviorally relevant as the performance in the semantic word generation task improved when the participants were receiving active stimulation compared to sham. One of these studies (Meinzer et al., 2013) observed an interesting effect whereby older adults showed a pattern similar to that of their younger counterparts in terms of performance in the semantic task when receiving anodal stimulation over the left IFG, despite performing worse without stimulation. In a similar vein, the differences in BOLD activation and functional connectivity between young and older adults were reduced during anodal tDCS. In relation to the connectivity, older participants showed higher anterior (fronto-temporal, and medial frontal regions) and lower posterior (temporo-occipital, precentral, and postcentral cortices) functional connectivity than younger, which was related to worse task performance. Anodal tDCS stimulation over the left IFG seems to reverse these effects, as it reduced functional connectivity among anterior cortical areas and increased it among the posterior cortices (note that not all the age related connectivity differences were reversed though).

Considering that all but one of these studies (except Polania et al., 2011a), were conducted using tDCS in combination with fMRI, more research is needed using other tCS techniques, especially tACS and tRNS, combined with higher temporal resolution neuroimaging techniques such as EEG/MEG, as we cannot expect that all tCS techniques would impact brain connectivity in the same way. Nonetheless, there is an EEG study (Neuling et al., 2013) in which the participants' occipital cortex (anode: Oz) was stimulated in the individual alpha frequency (IAF) to compare the aftereffects of stimulating in two different conditions: eyes-closed and eyes-open. These authors found that alpha power only differs between tACS stimulation and sham in the eyes-open condition. The coherence between two parietal electrodes (P3-P4), however, was only increased after tACS stimulation with eyes-closed (not with eyes-open). Therefore, there was a difference in the aftereffects of stimulating the areas with eyes-open and eyes-closed, which support the idea that the effects of tCS are dependent on the brain state, and for tACS in particular, on the ongoing brain oscillations. Considering the time varying nature of the oscillatory brain activity, new real time protocols are being developed to adjust the stimulation frequency according to the brain oscillations in real time (Boyle and Frohlich, 2013).

CREATIVITY, BRAIN STIMULATION, AND NETWORK ANALYSIS

Creativity is a multidimensional construct that can be investigated from a number of different perspectives, from its associated processes such as convergent and divergent thinking (Sawyer, 2012), passing through the creative person, product and press or environment (Rhodes, 1961). In this paper, we borrowed the following definition of creativity from Plucker et al., 2004: *"Creativity is the interaction among aptitude, process, and environment by which an individual or group produces a perceptible product that is both novel and useful as defined within a social context" (p. 90).* In the field of Cognitive Neuroscience, the main focus of creativity research is on the processes involved in the creative thinking, including divergent and convergent thinking (Luft and Bhattacharya, 2014). Divergent thinking refers to the capacity of generating novel and original ideas to open-ended problems (e.g., think of as many unusual uses of a brick). Convergent thinking, on the other hand, refers to the process of finding a correct solution to a closed-ended problem, such as a puzzle. In the real world, however, the creative process involves both divergent and convergent thinking (Sawyer, 2012).

There are only a few studies on how we can boost creativity using tCS (Cerruti and Schlaug, 2009; Chi and Snyder, 2011, 2012; Metuki et al., 2012; Chrysikou et al., 2013), which are described in **Table 2**. On the divergent thinking study (Chrysikou et al., 2013), the authors positioned the cathodal electrode over the left or the right prefrontal cortex and the anode over the mastoid, in an attempt to inhibit the left or right DLPFC. They found that cathodal stimulation over the left DLPFC, but not over the right DLPFC, was associated with quicker responses on the uncommon uses task (the participants were asked to generate common or uncommon uses for presented objects). The authors suggested that this result is coherent with the idea that divergent thinking depends on transient hypo-frontality, but in this case, it was found to be specific to the left hemisphere.

Most of the studies cited in **Table 2** investigated creative insight, which is a convergent thinking process. In one of the insight studies (Cerruti and Schlaug, 2009) it was found that anodal stimulation over the left DLPFC increases the solution rate in a convergent thinking task, the "Remote Associate Task—RAT." In this task, the participants have to find a fourth word which makes a compound word with three words presented on the screen (e.g., food/forward/break; solution: fast). However, another study (Metuki et al., 2012) observed that anodal stimulation over the left DLPFC did not improve the solution rate when subjects were given less time to solve the problem (7 s compared to 30 s), but it did improve subjects' ability to recognize correct solutions to hard RAT problems. The authors suggested that the left DLPFC is involved in recognizing the correct solution rather than generating it, a role that has been attributed to the right hemisphere (Bowden and Jung-Beeman, 2003), especially the right anterior area (Jung-Beeman et al., 2004). A slightly different account can be suggested based on two experiments (Anderson et al., 2009) monitoring the subjects brain responses (fMRI) while they solved a compound-word RAT task (Experiment 1) and another similar paradigm which allowed faster responses (Experiment 2). They observed that while the LIPFC was associated with the word search in memory or memory retrieval, the ACC was associated with the processing of solutions. In both experiments, the LIPFC activity increased when the participants were trying to find a solution, but from the moment they reached it, the ACC activity increased and the LIPFC returned to baseline levels.

The other two insight studies (Chi and Snyder, 2011, 2012) focused on other insight problems that are less dependent on verbal processes (matchstick and 9-dot problems). In both studies (Chi and Snyder, 2011, 2012), the stimulation protocol positioned the cathodal electrode over the left anterior temporal lobe (ATL), and the anodal over the right ATL. The authors found that this protocol increased the solution rates to these problems in relation to sham stimulation (between groups).

Therefore, for insight studies, it seems that the left DLPFC, and the right ATL play a role in finding solutions for convergent thinking. Nonetheless, little is still known about how these areas communicate to generate a solution as none of these studies combined tCS with any neuroimaging techniques, making it difficult to know whether these changes in performance are indeed caused by the excited/inhibited areas (Chi and Snyder, 2011, 2012; Chrysikou et al., 2013), by a connection between the stimulated/inhibited area, or by a compensatory network mechanisms that may be triggered by inhibiting those (as in finding 4 described in the previous section). In addition, in the human motor system evidence that bilateral tDCS evokes a suppression of one hemisphere but a facilitation of the other is somewhat mixed (see Nitsche and Paulus, 2000; Mordillo-Mateos et al., 2012; Hasan et al., 2013). Thus, the biological impact of bilateral tDCS (and by virtue the mechanisms that modulate previously reported enhancements in creativity following bilateral tDCS—e.g., Chi and Snyder, 2011, 2012) remains unclear.

Previous studies on the brain activity underlying creativity found, for example, that divergent thinking is associated with higher functional connectivity between medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC), both of which are key nodes of the DMN (Takeuchi et al., 2012). It could be that inhibiting the lateral prefrontal cortex may result in higher activation of the medial prefrontal, but this can only be tested by combining tCS with neuroimaging. There are a number of studies suggesting that creativity is associated with higher connectivity, both structural (Jung et al., 2010a,b; Takeuchi et al., 2010) and functional during rest (Jaušovec and Jaušovec, 2000a; Kounios et al., 2008; Takeuchi et al., 2012) and task alike (Jaušovec and Jaušovec, 2000b; Bhattacharya and Petsche, 2002, 2005; Razumnikova and Larina, 2005). Further, there are recent suggestions that creative ideas may reside in dynamic activation patterns of spontaneous brain networks (Wiggins and Bhattacharya, 2014). On account of these works, we believe that an important step towards improving creativity through tCS would be to combine brain stimulation with neuroimaging and advanced analysis techniques, in order to find how the brain networks mediate the improvements in creative processes observed in the cited studies. Importantly, techniques such as tACS allow targeting both long range and local synchronization (Ali et al., 2013).

Based on the previous discussions, we propose a hypothetical approach for combining neuroimaging, connectivity and brain stimulation for improving creativity (**Figure 2**). In this approach, the research starts with the graph theoretical analysis of the connectivity between the regions involved in a certain process (e.g., convergent thinking—insight) as in **Figure 2A**. Using the discovered cognitive connections (**Figure 2B**), a protocol for stimulation is determined (**Figure 2C**). In **Figure 2A**, we showed a couple of regions involved in creativity. Immediately after the stimulation, the connectivity patterns (in identical conditions of the analyzed patterns in **Figures 2A,B**) are analyzed against control stimulation protocol, e.g., sham stimulation. The results are presented as a map, with the edges linking the nodes representing here the strength of the difference in connectivity between active and sham stimulation (or any other control or contrast of the experiment), as in **Figure 2D**. In **Figure 2** hypothetical example, the connectivity changed after stimulation of the left DLPFC during a RAT paradigm, especially among the temporal and frontal areas (**Figure 2D**). In the hypothetical example, there was an increase in communication between ATL and DLPFC and between the DLPFC and the ACC, which could represent coordinated (DLPFC) search of the solution in memory (ATL) and

Table 2 | Studies on tCS and creativity.

Study	Creative process	Technique	Parameters/location	Duration	Design	Paradigm	Results
Chi and Snyder, 2012	Insight	tDCS	1.6 mA (30 s raising), electrode 35 cm^2 Anode: right ATL Cathode: left ATL	10 min	Within-participants: pre-during-post Between: active vs. sham	9-dot problem	40% of the active group were able to solve the problem; none of the sham group solved it
Chi and Snyder, 2011	Insight	tDCS	1.6 mA (30 s raising) electrode 35 cm^2 Anode: right ATL Cathode: left ATL	10 min	Between participants: (1) L− R+; (2) L+ R−; (3) Sham stimulation	Matchstick problems	60% of the participants in the L-R+ group were able to solve the difficult problems whereas lower than 20% in the other groups solved it
Metuki et al., 2012	Insight	tDCS	1 mA (30 s raising) electrode 35 cm^2 Unilateral active Anode: left DLPFC (F3); Cathode: right OFC (Fp2)	11 min (5 min pre + 6 online)	2 × 2Within-participants: (1) Active vs. sham (sessions separated by a week); (2) Easy vs. Hard	RAT (CRA) with limited time (to investigate solution identification rather than generation)	They found that stimulation did not affect the rate of solution for either hard or easy problems. However, they found an interaction between stimulation and difficulty for solution recognition, as the participants in the active stimulation group were more able to recognize correct solutions for hard problems
Cerruti and Schlaug, 2009	Insight	tDCS	1 mA Anode: 16.3 cm^2 Cathode: 30 cm^2 Experiment 1 Anode: left DLPFC Cathode: right OFC Experiment 2 Anode: left DLPFC Cathode: right DLPFC	20 min 16 min stimulation + 4 min stimulation during the verbal fluency task	Within-participants design with (3 h session): (1) Anodal electrode location: F3 vs. right supraorbital region (Experiment 1); F3 vs. F4 (Experiment 2); (2) Condition: active anodal, active cathodal, sham	Verbal Fluency (VF) + RAT (CRA) with 30 s to solve	They found that the stimulation did not improve VF, but was associated with higher solution rates when the stimulated area was above the left DLPFC. The two experiments showed the same result, with higher solution rates for anodal on the left DLPFC
Chrysikou et al., 2013	Divergent thinking (flexible tool use)	tDCS	1.5 mA 25 cm^2 electrodes Cathode: F7 or F8 Anode: on the contralateral mastoid (the main purpose was to cause inhibition of PFC)	20 min (including 10 s ramp-up + 10 s ramp down). Stimulation began for 180 s prior to the tasks	Between-subjects design with two factors: Stimulation protocol (groups): (1) Cathodal Left (F7) and anodal on mastoid; (2) Cathodal Right (F8) and anodal on mastoid; (3) Sham Task (groups): (1) Common uses; (2) Uncommon uses	Div. Thinking: participants were asked to generate either (1) common vs. (2) uncommon uses for the objects presented on the screen (60 grayscale pictures). Each participant was assigned to only one of these two conditions. The performance was measure to response onset time	There was a significant interaction between stimulation protocol and task condition, since cathodal over the left PFC was associated with in an decrease in the response times for the uncommon uses task. There was no difference in performance between stimulation conditions in the common uses task. In addition, cathodal stimulation over the left PFC was associated with lower number of response omissions in the uncommon uses task only (no difference in the common uses task)

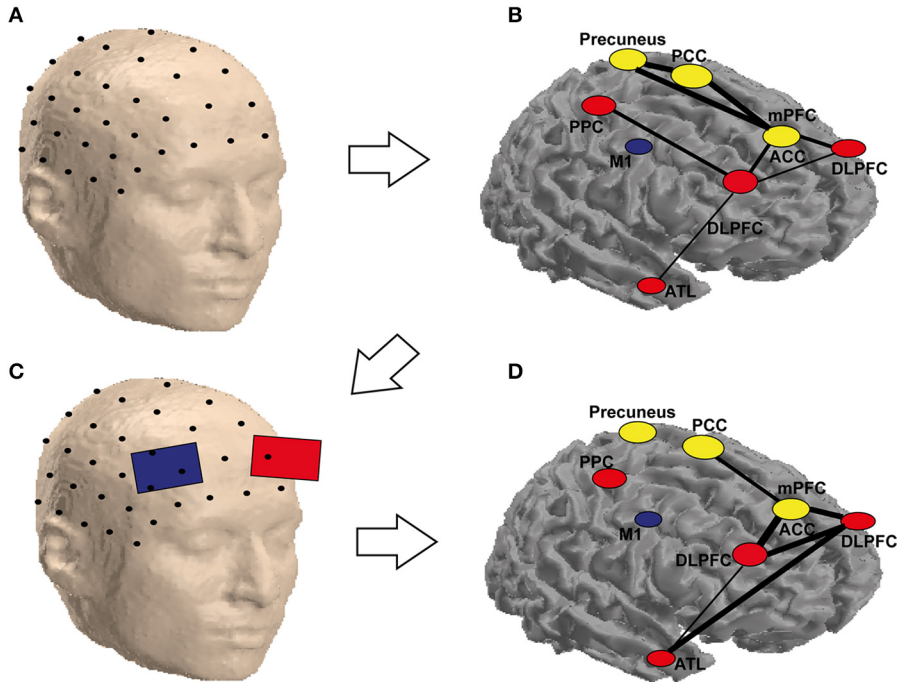

FIGURE 2 | Schematic representation of the possible combination of tCS and functional (EEG) brain connectivity to enhance creativity. An array of scalp electrodes, a subset of which is depicted in **(A)**, is recorded. In **(B)** is possible to identify ROIs which are highly connected (yellow) and the ones that are flexible hubs (red) or less connected areas (blue) during a solution vs. non-solution RAT task. This helps identifying the target electrodes to stimulate as in **(C)**. Stimulating these electrodes may not only eliminate the differences for the corresponding nodes, but also reduce them for areas in the same network, which are not stimulated but functionally connected to the targeted ones as depicted in **(D)**. It is worth noting that the labels and the networks drawn in the figure are only for demonstration, as they are not precisely equivalent to their anatomical locations (they are only approximate locations on a surface, the areas in yellow are located in the medial area of the brain, which cannot be seen in a cortical mesh). The areas are abbreviated as follows: PCC, posterior cingulate cortex; PPC, posterior parietal cortex; M1, primary motor cortex; mPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; ATL, anterior temporal lobe.

the recognition of the correct solution (ACC), from DLPFC to ACC. Note that this approach does not account for the direction of the interactions, which could be also tested using effective connectivity analysis techniques such as dynamic causal modeling or Granger causality. In divergent thinking, the approach would probably result in a higher change over the posterior rather than anterior regions.

LIMITATIONS OF GRAPH-THEORY AND BRAIN STIMULATION

Notwithstanding the advances emerging from the combination of tCS and neuroimaging for the understanding of the connectivity changes in response to brain stimulation, there are many limitations of both methods which can be thought as new challenges for this enterprise. First, despite its usefulness in characterizing brain network during cognitive functions (Sporns, 2014), the application of graph theory is not problem-free (Fornito et al., 2013). The approach, after all, is based on sophisticated mathematical techniques that require judicious choices at various steps of the analysis. Perhaps the most obvious one is the need to choose among a number of possible strategies to reconstruct the networks, which do not always lead to a convergent or consistent outcome. For instance, one has to decide whether the links will be weighted or unweighted, directed or not, whether to use a fixed value or a fixed link density across participants as a threshold or to use statistical controls such as surrogate data analysis in order to determine the significance in a per-link basis. Further, especially in the case of EEG/MEG, many different bivariate indices of functional/effective connectivity are available (e.g., Niso et al., 2013). Besides, spurious characterization of a network could result from an inappropriate temporal and/or spatial sampling of the underlying systems (Bialonski et al., 2010, 2011). Last, but not least, the relationship between connectivity at the sensor/electrode level and connectivity at the neural source level is more complicated than traditionally assumed (Ewald et al., 2012). Nevertheless, we argue that graph theory is a very useful tool to study the synchronized activity between different brain areas underlying most cognitive functions, as it can be used to characterize these patterns of brain connectivity. Moreover, it can be also used to understand how local changes (whether internally or externally generated) are able to affect other distant brain areas. For instance, in focal epilepsy research a paradigm shift is currently taking place whereby attention is increasingly not toward the epileptic focus itself, the classical and obvious target area of the neuroscientific research on epilepsy, but the epileptic network and its characteristics (Lehnertz et al., 2014), offering a tremendous potential in minimizing the extent of surgical intervention.

The tCS methods also face limitations that have to be considered when attempting to develop protocols to boost specific cognitive functions. Currently, there are many issues related to our lack of understanding of how tCS, in its different modalities,

shapes neural activity. First, as tCS methods in humans are non-invasive, the stimulation is applied to the skull rather than directly to the brain, meaning that the spatial resolution of tCS is diffuse due to skull dispersion. The spatial resolution can be improved with small stimulation electrodes (Datta et al., 2009), but it still does not overcome the problem that the stimulation is indirect and the electrodes often too large for such focal stimulation of a small cortical area. The second limitation, which is closely related to the first, is the lack of control of the stimulation current reaching the brain as the current is altered as it passes through the skull to be then conducted by the cerebrospinal fluid to the brain. Individual differences in skull thickness and shape may interact with how much current is actually reaching the brain (Datta et al., 2009). Therefore, even with advanced modeling of the current (Wagner et al., 2007; Shahid et al., 2014), it is difficult to predict how much current is actually reaching the brain. Third, the stimulation effects, as stated earlier in the Introduction, are not straightforward. For example, anodal stimulation does not always cause increase excitability, and vice versa for cathodal (Nitsche and Paulus, 2000; Antal et al., 2004; Moliadze et al., 2010). Further, other factors such as increasing the intensity (Batsikadze et al., 2013) or including a task during stimulation (Antal et al., 2007) can also change the effects in a complicated fashion. Therefore, it is important for these factors to be controlled carefully in tCS studies. Moreover, when one considers these limitations in conjunction with the fact that stimulating one area of the brain can affect a network of regions, it hampers the possibility of claiming "cause-effects" relations between a single stimulated brain region and specific cognitive functions as the stimulation effects are not entirely known.

CONCLUSION

In this paper, we discussed the possibility of combining tCS with neuroimaging and graph theory for analysing the impact of brain stimulation on brain connectivity. In doing so, we highlight how graph theoretical analysis can help in understanding how the brain networks are affected by tCS in specific locations. In addition, we suggest that the knowledge of structural connectivity pathways can be used to target a network of brain areas rather than a single area and that ongoing brain connectivity during and just after (during the aftereffects period) tCS is an important factor in determining the connectivity changes in response to stimulation. In the future, we suggest using graph theory not only to understand the network effects of stimulating different brain areas, but also to develop tCS protocols that can target connectivity rather than individual brain areas.

ACKNOWLEDGMENTS

The authors are supported by the CREAM project that has been funded by the European Commission under Grant Agreement no 612022. This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use which may be made of the information contained therein. Michael J. Banissy is also supported by the Economic and Social Research Council (ES/K00882X/1).

REFERENCES

Aguirre, J., Sevilla-Escoboza, R., Gutiérrez, R., Papo, D., and Buldú, J. M. (2014). Synchronization of interconnected networks: the role of connector nodes. *Phys. Rev. Lett.* 112:248701. doi: 10.1103/PhysRevLett.112.248701

Ali, M. M., Sellers, K. K., and Frohlich, F. (2013). Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. *J. Neurosci.* 33, 11262–11275. doi: 10.1523/JNEUROSCI.5867-12.2013

Alon, G., Roys, S. R., Gullapalli, R. P., and Greenspan, J. D. (2011). Non-invasive electrical stimulation of the brain (ESB) modifies the resting-state network connectivity of the primary motor cortex: a proof of concept fMRI study. *Brain Res.* 1403, 37–44. doi: 10.1016/j.brainres.2011.06.013

Amadi, U., Ilie, A., Johansen-Berg, H., and Stagg, C. J. (2014). Polarity-specific effects of motor transcranial direct current stimulation on fMRI resting state networks. *Neuroimage* 88, 155–161. doi: 10.1016/j.neuroimage.2013.11.037

Anderson, J. R., Anderson, J. F., Ferris, J. L., Fincham, J. M., and Jung, K. J. (2009). Lateral inferior prefrontal cortex and anterior cingulate cortex are engaged at different stages in the solution of insight problems. *Proc. Natl. Acad. Sci. U.S.A.* 106, 10799–10804. doi: 10.1073/pnas.0903953106

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., and Paulus, W. (2008). Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. *Brain Stimul.* 1, 97–105. doi: 10.1016/j.brs.2007.10.001

Antal, A., Kincses, T. Z., Nitsche, M. A., Bartfai, O., and Paulus, W. (2004). Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Invest. Ophthalmol. Vis. Sci.* 45, 702–707. doi: 10.1167/iovs.03-0688

Antal, A., Terney, D., Poreisz, C., and Paulus, W. (2007). Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. *Eur. J. Neurosci.* 26, 2687–2691. doi: 10.1111/j.1460-9568.2007.05896.x

Barabasi, A. L., and Albert, R. (1999). Emergence of scaling in random networks. *Science* 286, 509–512. doi: 10.1126/science.286.5439.509

Barttfeld, P., Wicker, B., Cukier, S., Navarta, S., Lew, S., and Sigman, M. (2011). A big-world network in ASD: dynamical connectivity analysis reflects a deficit in long-range connections and an excess of short-range connections. *Neuropsychologia* 49, 254–263. doi: 10.1016/j.neuropsychologia.2010.11.024

Bassett, D. S., and Bullmore, E. (2006). Small-world brain networks. *Neuroscientist* 12, 512–523. doi: 10.1177/1073858406293182

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., and Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., and Frahm, J. (2003). Subthreshold high-frequency TMS of human primary motor cortex modulates interconnected frontal motor areas as detected by interleaved fMRI-TMS. *Neuroimage* 20, 1685–1696. doi: 10.1016/j.neuroimage.2003.07.028

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., and Frahm, J. (2005). BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. *Neuroimage* 28, 22–29. doi: 10.1016/j.neuroimage.2005.05.027

Bettus, G., Wendling, F., Guye, M., Valton, L., Regis, J., Chauvel, P., et al. (2008). Enhanced EEG functional connectivity in mesial temporal lobe epilepsy. *Epilepsy Res.* 81, 58–68. doi: 10.1016/j.eplepsyres.2008.04.020

Bhattacharya, J., and Petsche, H. (2002). Shadows of artistry: cortical synchrony during perception and imagery of visual art. *Brain Res. Cogn. Brain Res.* 13, 179–186. doi: 10.1016/S0926-6410(01)00110-0

Bhattacharya, J., and Petsche, H. (2005). Drawing on mind's canvas: differences in cortical integration patterns between artists and non-artists. *Hum. Brain Mapp.* 26, 1–14. doi: 10.1002/hbm.20104

Bialonski, S., Horstmann, M. T., and Lehnertz, K. (2010). From brain to earth and climate systems: small-world interaction networks or not? *Chaos* 20, 013134. doi: 10.1063/1.3360561

Bialonski, S., Wendler, M., and Lehnertz, K. (2011). Unraveling spurious properties of interaction networks with tailored random networks. *PLoS ONE* 6:e22826. doi: 10.1371/journal.pone.0022826

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices *in vitro*. *J. Physiol.* 557, 175–190. doi: 10.1113/jphysiol.2003.055772

Bindman, L. J., Lippold, O. C., and Redfearn, J. W. (1962). Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents. *Nature* 196, 584–585. doi: 10.1038/196584a0

Bindman, L. J., Lippold, O. C., and Redfearn, J. W. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382.

Bliss, T. V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232, 331–356.

Boets, B., Op De Beeck, H. P., Vandermosten, M., Scott, S. K., Gillebert, C. R., Mantini, D., et al. (2013). Intact but less accessible phonetic representations in adults with dyslexia. *Science* 342, 1251–1254. doi: 10.1126/science.1244333

Boggio, P. S., Nunes, A., Rigonatti, S. P., Nitsche, M. A., Pascual-Leone, A., and Fregni, F. (2007). Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restor. Neurol. Neurosci.* 25, 123–129.

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., et al. (2008). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *Int. J. Neuropsychopharmacol.* 11, 249–254. doi: 10.1017/S1461145707007833

Boros, K., Poreisz, C., Munchau, A., Paulus, W., and Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *Eur. J. Neurosci.* 27, 1292–1300. doi: 10.1111/j.1460-9568.2008.06090.x

Bowden, E. M., and Jung-Beeman, M. (2003). Aha! Insight experience correlates with solution activation in the right hemisphere. *Psychon. Bull. Rev.* 10, 730–737. doi: 10.3758/BF03196539

Boyle, M. R., and Frohlich, F. (2013). "EEG feedback-controlled transcranial alternating current stimulation," in *2013 6th International IEEE/EMBS Conference on Neural Engineering* (San Diego, CA: NER), 140–143. doi: 10.1109/NER.2013.6695891

Brem, A. K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *Neuroimage* 85(pt 3), 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Bressler, S. L., and Menon, V. (2010). Large-scale brain networks in cognition: emerging methods and principles. *Trends Cogn. Sci.* 14, 277–290. doi: 10.1016/j.tics.2010.04.004

Buchel, C., Coull, J. T., and Friston, K. J. (1999). The predictive value of changes in effective connectivity for human learning. *Science* 283, 1538–1541. doi: 10.1126/science.283.5407.1538

Cabral, J., Luckhoo, H., Woolrich, M., Joensson, M., Mohseni, H., Baker, A., et al. (2014). Exploring mechanisms of spontaneous functional connectivity in MEG: how delayed network interactions lead to structured amplitude envelopes of band-pass filtered oscillations. *Neuroimage* 90, 423–435. doi: 10.1016/j.neuroimage.2013.11.047

Cerruti, C., and Schlaug, G. (2009). Anodal transcranial direct current stimulation of the prefrontal cortex enhances complex verbal associative thought. *J. Cogn. Neurosci.* 21, 1980–1987. doi: 10.1162/jocn.2008.21143

Chi, R. P., and Snyder, A. W. (2011). Facilitate insight by non-invasive brain stimulation. *PLoS ONE* 6:e16655. doi: 10.1371/journal.pone.0016655

Chi, R. P., and Snyder, A. W. (2012). Brain stimulation enables the solution of an inherently difficult problem. *Neurosci. Lett.* 515, 121–124. doi: 10.1016/j.neulet.2012.03.012

Chib, V. S., Yun, K., Takahashi, H., and Shimojo, S. (2013). Noninvasive remote activation of the ventral midbrain by transcranial direct current stimulation of prefrontal cortex. *Transl. Psychiatry* 3, e268. doi: 10.1038/tp.2013.44

Chouinard, P. A., Van Der Werf, Y. D., Leonard, G., and Paus, T. (2003). Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. *J. Neurophysiol.* 90, 1071–1083. doi: 10.1152/jn.01105.2002

Chrysikou, E. G., Hamilton, R. H., Coslett, H. B., Datta, A., Bikson, M., and Thompson-Schill, S. L. (2013). Noninvasive transcranial direct current stimulation over the left prefrontal cortex facilitates cognitive flexibility in tool use. *Cogn. Neurosci.* 4, 81–89. doi: 10.1080/17588928.2013.768221

Cole, M. W., Laurent, P., and Stocco, A. (2013a). Rapid instructed task learning: a new window into the human brain's unique capacity for flexible cognitive control. *Cogn. Affect. Behav. Neurosci.* 13, 1–22. doi: 10.3758/s13415-012-0125-7

Cole, M. W., Pathak, S., and Schneider, W. (2010). Identifying the brain's most globally connected regions. *Neuroimage* 49, 3132–3148. doi: 10.1016/j.neuroimage.2009.11.001

Cole, M. W., Reynolds, J. R., Power, J. D., Repovs, G., Anticevic, A., and Braver, T. S. (2013b). Multi-task connectivity reveals flexible hubs for adaptive task control. *Nat. Neurosci.* 16, 1348–1355. doi: 10.1038/nn.3470

Cole, M. W., and Schneider, W. (2007). The cognitive control network: integrated cortical regions with dissociable functions. *Neuroimage* 37, 343–360. doi: 10.1016/j.neuroimage.2007.03.071

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., and Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul.* 2, 201–207, 207.e1. doi: 10.1016/j.brs.2009.03.005

Davare, M., Lemon, R., and Olivier, E. (2008). Selective modulation of interactions between ventral premotor cortex and primary motor cortex during precision grasping in humans. *J. Physiol.* 586, 2735–2742. doi: 10.1113/jphysiol.2008.152603

Deans, J. K., Powell, A. D., and Jefferys, J. G. (2007). Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *J. Physiol.* 583, 555–565. doi: 10.1113/jphysiol.2007.137711

Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009

Ewald, A., Marzetti, L., Zappasodi, F., Meinecke, F. C., and Nolte, G. (2012). Estimating true brain connectivity from EEG/MEG data invariant to linear and static transformations in sensor space. *Neuroimage* 60, 476–488. doi: 10.1016/j.neuroimage.2011.11.084

Faria, P., Hallett, M., and Miranda, P. C. (2011). A finite element analysis of the effect of electrode area and inter-electrode distance on the spatial distribution of the current density in tDCS. *J. Neural Eng.* 8:066017. doi: 10.1088/1741-2560/8/6/066017

Fornito, A., Zalesky, A., and Breakspear, M. (2013). Graph analysis of the human connectome: promise, progress, and pitfalls. *Neuroimage* 80, 426–444. doi: 10.1016/j.neuroimage.2013.04.087

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp. Brain Res.* 166, 23–30. doi: 10.1007/s00221-005-2334-6

Friston, K. J. (2011). Functional and effective connectivity: a review. *Brain Connect.* 1, 13–36. doi: 10.1089/brain.2011.0008

Frohlich, F., and McCormick, D. A. (2010). Endogenous electric fields may guide neocortical network activity. *Neuron* 67, 129–143. doi: 10.1016/j.neuron.2010.06.005

Frohlich, F., and Schmidt, S. L. (2013). Rational design of transcranial current stimulation (TCS) through mechanistic insights into cortical network dynamics. *Front. Hum. Neurosci.* 7:804. doi: 10.3389/fnhum.2013.00804

Fuster, J. M. (2000). The module: crisis of a paradigm. *Neuron* 26, 51–53. doi: 10.1016/S0896-6273(00)81137-X

Gartside, I. B. (1968). Mechanisms of sustained increases of firing rate of neurons in the rat cerebral cortex after polarization: reverberating circuits or modification of synaptic conductance? *Nature* 220, 382–383. doi: 10.1038/220382a0

Goni, J., van den Heuvel, M. P., Avena-Koenigsberger, A., Velez De Mendizabal, N., Betzel, R. F., Griffa, A., et al. (2014). Resting-brain functional connectivity predicted by analytic measures of network communication. *Proc. Natl. Acad. Sci. U.S.A.* 111, 833–838. doi: 10.1073/pnas.1315529111

Goswami, U. (2000). Phonological representations, reading development and dyslexia: towards a cross-linguistic theoretical framework. *Dyslexia* 6, 133–151. doi: 10.1002/(sici)1099-0909(200004/06)6:2<133::aid-dys160>3.0.co;2-a

Hasan, A., Bergener, T., Nitsche, M. A., Strube, W., Bunse, T., Falkai, P., et al. (2013). Impairments of motor-cortex responses to unilateral and bilateral direct current stimulation in schizophrenia. *Front. Psychiatry* 4:121. doi: 10.3389/fpsyt.2013.00121

Horwitz, B. (2003). The elusive concept of brain connectivity. *Neuroimage* 19, 466–470. doi: 10.1016/S1053-8119(03)00112-5

Jaušovec, N., and Jaušovec, K. (2000a). Differences in resting EEG related to ability. *Brain Topogr.* 12, 229–240. doi: 10.1023/A:1023446024923

Jaušovec, N., and Jaušovec, K. (2000b). EEG activity during the performance of complex mental problems. *Int. J. Psychophysiol.* 36, 73–88. doi: 10.1016/S0167-8760(99)00113-0

Jung, R. E., Grazioplene, R., Caprihan, A., Chavez, R. S., and Haier, R. J. (2010a). White matter integrity, creativity, and psychopathology: disentangling constructs with diffusion tensor imaging. *PLoS ONE* 5:e9818. doi: 10.1371/journal.pone.0009818

Jung, R. E., Segall, J. M., Jeremy Bockholt, H., Flores, R. A., Smith, S. M., Chavez, R. S., et al. (2010b). Neuroanatomy of creativity. *Hum. Brain Mapp.* 31, 398–409. doi: 10.1002/hbm.20874

Jung-Beeman, M., Bowden, E. M., Haberman, J., Frymiare, J. L., Arambel-Liu, S., Greenblatt, R., et al. (2004). Neural activity when people solve verbal problems with insight. *PLoS Biol.* 2:E97. doi: 10.1371/journal.pbio.0020097

Kanai, R., Paulus, W., and Walsh, V. (2010). Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clin. Neurophysiol.* 121, 1551–1554. doi: 10.1016/j.clinph.2010.03.022

Kanwisher, N., McDermott, J., and Chun, M. M. (1997). The fusiform face area: a module in human extrastriate cortex specialized for face perception. *J. Neurosci.* 17, 4302–4311.

Kar, K., and Krekelberg, B. (2014). Transcranial alternating current stimulation attenuates visual motion adaptation. *J. Neurosci.* 34, 7334–7340. doi: 10.1523/JNEUROSCI.5248-13.2014

Kar, K., and Wright, J. (2014). Probing the mechanisms underlying the mitigation of cognitive aging with anodal transcranial direct current stimulation. *J. Neurophysiol.* 111, 1397–1399. doi: 10.1152/jn.00736.2013

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., et al. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J. Neurosci.* 31, 15284–15293. doi: 10.1523/JNEUROSCI.0542-11.2011

Kounios, J., Fleck, J., Green, D., Payne, L., Stevenson, J. L., Bowden, E. M., et al. (2008). The origins of insight in resting-state brain activity. *Neuropsychologia* 46, 281–291. doi: 10.1016/j.neuropsychologia.2007.07.013

Kwon, Y. H., Ko, M. H., Ahn, S. H., Kim, Y. H., Song, J. C., Lee, C. H., et al. (2008). Primary motor cortex activation by transcranial direct current stimulation in the human brain. *Neurosci. Lett.* 435, 56–59. doi: 10.1016/j.neulet.2008.02.012

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., et al. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *Eur. J. Neurosci.* 22, 495–504. doi: 10.1111/j.1460-9568.2005.04233.x

Lehnertz, K., Ansmann, G., Bialonski, S., Dickten, H., Geier, C., and Porz, S. (2014). Evolving networks in the human epileptic brain. *Physica D Nonlin. Phenom.* 267, 7–15. doi: 10.1016/j.physd.2013.06.009

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

Luft, C. D. B., and Bhattacharya, J. (2014). "Correlatos neurales de la creatividad," in *Conectividad Funcional y Anatómica en el Crebro Humano: Análisis De Señales y Aplicaciones en Ciencias de la Salud*, eds F. Maestú, E. Pereda, and F. Del Pozo (Madrid: Elsevier), (in press).

Malenka, R. C., and Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron* 44, 5–21. doi: 10.1016/j.neuron.2004.09.012

Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., and Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science* 309, 2228–2232. doi: 10.1126/science.1117256

Meinzer, M., Antonenko, D., Lindenberg, R., Hetzer, S., Ulm, L., Avirame, K., et al. (2012). Electrical brain stimulation improves cognitive performance by modulating functional connectivity and task-specific activation. *J. Neurosci.* 32, 1859–1866. doi: 10.1523/JNEUROSCI.4812-11.2012

Meinzer, M., Lindenberg, R., Antonenko, D., Flaisch, T., and Floel, A. (2013). Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. *J. Neurosci.* 33, 12470–12478. doi: 10.1523/JNEUROSCI.5743-12.2013

Metuki, N., Sela, T., and Lavidor, M. (2012). Enhancing cognitive control components of insight problems solving by anodal tDCS of the left dorsolateral prefrontal cortex. *Brain Stimul.* 5, 110–115. doi: 10.1016/j.brs.2012.03.002

Mochizuki, H., Huang, Y. Z., and Rothwell, J. C. (2004). Interhemispheric interaction between human dorsal premotor and contralateral primary motor cortex. *J. Physiol.* 561, 331–338. doi: 10.1113/jphysiol.2004.072843

Moliadze, V., Antal, A., and Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clin. Neurophysiol.* 121, 2165–2171. doi: 10.1016/j.clinph.2010.04.033

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., et al. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul.* 6, 424–432. doi: 10.1016/j.brs.2012.04.011

Mordillo-Mateos, L., Turpin-Fenoll, L., Millan-Pascual, J., Nunez-Perez, N., Panyavin, I., Gomez-Arguelles, J. M., et al. (2012). Effects of simultaneous bilateral tDCS of the human motor cortex. *Brain Stimul.* 5, 214–222. doi: 10.1016/j.brs.2011.05.001

Morishima, Y., Akaishi, R., Yamada, Y., Okuda, J., Toma, K., and Sakai, K. (2009). Task-specific signal transmission from prefrontal cortex in visual selective attention. *Nat. Neurosci.* 12, 85–91. doi: 10.1038/nn.2237

Neuling, T., Rach, S., and Herrmann, C. S. (2013). Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Front. Hum. Neurosci.* 7:161. doi: 10.3389/fnhum.2013.00161

Niso, G., Bruna, R., Pereda, E., Gutierrez, R., Bajo, R., Maestu, F., et al. (2013). HERMES: towards an integrated toolbox to characterize functional and effective brain connectivity. *Neuroinformatics* 11, 405–434. doi: 10.1007/s12021-013-9186-1

Nitsche, M. A., Boggio, P. S., Fregni, F., and Pascual-Leone, A. (2009). Treatment of depression with transcranial direct current stimulation (tDCS): a review. *Exp. Neurol.* 219, 14–19. doi: 10.1016/j.expneurol.2009.03.038

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J. Physiol.* 553, 293–301. doi: 10.1113/jphysiol.2003.049916

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527(pt 3), 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 57, 1899–1901. doi: 10.1212/WNL.57.10.1899

Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J. M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput. Intell. Neurosci.* 2011:156869. doi: 10.1155/2011/156869

O'Shea, J., Johansen-Berg, H., Trief, D., Gobel, S., and Rushworth, M. F. (2007). Functionally specific reorganization in human premotor cortex. *Neuron* 54, 479–490. doi: 10.1016/j.neuron.2007.04.021

Parra, L. C., and Bikson, M. (2004). Model of the effect of extracellular fields on spike time coherence. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 6, 4584–4587. doi: 10.1109/IEMBS.2004.1404271

Pascual-Leone, A., and Walsh, V. (2001). Fast backprojections from the motion to the primary visual area necessary for visual awareness. *Science* 292, 510–512. doi: 10.1126/science.1057099

Pena-Gomez, C., Sala-Lonch, R., Junque, C., Clemente, I. C., Vidal, D., Bargallo, N., et al. (2012). Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. *Brain Stimul.* 5, 252–263. doi: 10.1016/j.brs.2011.08.006

Pereda, E., Quiroga, R. Q., and Bhattacharya, J. (2005). Nonlinear multivariate analysis of neurophysiological signals. *Prog. Neurobiol.* 77, 1–37. doi: 10.1016/j.pneurobio.2005.10.003

Plucker, J. A., Beghetto, R. A., and Dow, G. T. (2004). Why isn't creativity more important to educational psychologists? Potentials, pitfalls, and future directions in creativity research. *Educ. Psychol.* 39, 83–96. doi: 10.1207/s15326985ep3902_1

Polania, R., Nitsche, M. A., and Paulus, W. (2011a). Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. *Hum. Brain Mapp.* 32, 1236–1249. doi: 10.1002/hbm.21104

Polania, R., Paulus, W., Antal, A., and Nitsche, M. A. (2011b). Introducing graph theory to track for neuroplastic alterations in the resting human brain: a transcranial direct current stimulation study. *Neuroimage* 54, 2287–2296. doi: 10.1016/j.neuroimage.2010.09.085

Polania, R., Paulus, W., and Nitsche, M. A. (2012a). Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Hum. Brain Mapp.* 33, 2499–2508. doi: 10.1002/hbm.21380

Polania, R., Paulus, W., and Nitsche, M. A. (2012b). Reorganizing the intrinsic functional architecture of the human primary motor cortex during rest with non-invasive cortical stimulation. *PLoS ONE* 7:e30971. doi: 10.1371/journal.pone.0030971

Purpura, D. P., and McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *J. Neurophysiol.* 28, 166–185.

Radman, T., Ramos, R. L., Brumberg, J. C., and Bikson, M. (2009). Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation *in vitro*. *Brain Stimul.* 2, 215–228, 228. e1–e3. doi: 10.1016/j.brs.2009.03.007

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *J. Physiol.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Razumnikova, O. M., and Larina, E. N. (2005). Hemispheric interactions during a search of original verbal associations: EEG coherence in creative men and women. *Zh. Vyssh. Nerv. Deiat. Im. I P Pavlova* 55, 777–787.

Reato, D., Rahman, A., Bikson, M., and Parra, L. C. (2010). Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *J. Neurosci.* 30, 15067–15079. doi: 10.1523/JNEUROSCI.2059-10.2010

Rhodes, M. (1961). An analysis of creativity. *Phi Delta Kappan* 42, 305–310.

Rigonatti, S. P., Boggio, P. S., Myczkowski, M. L., Otta, E., Fiquer, J. T., Ribeiro, R. B., et al. (2008). Transcranial direct stimulation and fluoxetine for the treatment of depression. *Eur. Psychiatry* 23, 74–76. doi: 10.1016/j.eurpsy.2007.09.006

Rioult-Pedotti, M. S., Friedman, D., and Donoghue, J. P. (2000). Learning-induced LTP in neocortex. *Science* 290, 533–536. doi: 10.1126/science.290.5491.533

Rubinov, M., and Sporns, O. (2010). Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52, 1059–1069. doi: 10.1016/j.neuroimage.2009.10.003

Ruffini, G., Wendling, F., Merlet, I., Molaee-Ardekani, B., Mekonnen, A., Salvador, R., et al. (2013). Transcranial current brain stimulation (tCS): models and technologies. *IEEE Trans. Neural Syst. Rehabil. Eng.* 21, 333–345. doi: 10.1109/TNSRE.2012.2200046

Santiesteban, I., Banissy, M. J., Catmur, C., and Bird, G. (2012). Enhancing social ability by stimulating right temporoparietal junction. *Curr. Biol.* 22, 2274–2277. doi: 10.1016/j.cub.2012.10.018

Sawyer, K. (2012). *Explaining Creativity: The Science of Human Innovation.* New York, NY: Oxford University Press, Inc.

Schaal, N. K., Williamson, V. J., and Banissy, M. J. (2013). Anodal transcranial direct current stimulation over the supramarginal gyrus facilitates pitch memory. *Eur. J. Neurosci.* 38, 3513–3518. doi: 10.1111/ejn.12344

Sehm, B., Kipping, J., Schafer, A., Villringer, A., and Ragert, P. (2013). A comparison between uni- and bilateral tDCS effects on functional connectivity of the human motor cortex. *Front. Hum. Neurosci.* 7:183. doi: 10.3389/fnhum.2013.00183

Shafi, M. M., Westover, M. B., Fox, M. D., and Pascual-Leone, A. (2012). Exploration and modulation of brain network interactions with noninvasive brain stimulation in combination with neuroimaging. *Eur. J. Neurosci.* 35, 805–825. doi: 10.1111/j.1460-9568.2012.08035.x

Shahid, S. S., Bikson, M., Salman, H., Wen, P., and Ahfock, T. (2014). The value and cost of complexity in predictive modelling: role of tissue anisotropic conductivity and fibre tracts in neuromodulation. *J. Neural Eng.* 11:036002. doi: 10.1088/1741-2560/11/3/036002

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Sporns, O. (2011). *Networks of the Brain.* Cambridge, MA: MIT Press.

Sporns, O. (2014). Contributions and challenges for network models in cognitive neuroscience. *Nat. Neurosci.* 17, 652–660. doi: 10.1038/nn.3690

Sporns, O., Chialvo, D. R., Kaiser, M., and Hilgetag, C. C. (2004). Organization, development and function of complex brain networks. *Trends Cogn. Sci.* 8, 418–425. doi: 10.1016/j.tics.2004.07.008

Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., and Hironaka, N. (2011). A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neurosci. Lett.* 491, 40–43. doi: 10.1016/j.neulet.2011.01.004

Takeuchi, H., Taki, Y., Hashizume, H., Sassa, Y., Nagase, T., Nouchi, R., et al. (2012). The association between resting functional connectivity and creativity. *Cereb. Cortex* 22, 2921–2929. doi: 10.1093/cercor/bhr371

Takeuchi, H., Taki, Y., Sassa, Y., Hashizume, H., Sekiguchi, A., Fukushima, A., et al. (2010). White matter structures associated with creativity: evidence from diffusion tensor imaging. *Neuroimage* 51, 11–18. doi: 10.1016/j.neuroimage.2010.02.035

Terao, Y., and Ugawa, Y. (2002). Basic mechanisms of TMS. *J. Clin. Neurophysiol.* 19, 322–343. doi: 10.1097/00004691-200208000-00006

Terhune, D. B., and Cohen Kadosh, R. (2013). Modulating cognitive control in major depression with transcranial electrical stimulation. *Biol. Psychiatry* 73, 595–596. doi: 10.1016/j.biopsych.2013.01.006

Terney, D., Chaieb, L., Moliadze, V., Antal, A., and Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *J. Neurosci.* 28, 14147–14155. doi: 10.1523/JNEUROSCI.4248-08.2008

Uehara, T., Yamasaki, T., Okamoto, T., Koike, T., Kan, S., Miyauchi, S., et al. (2014). Efficiency of a "small-world" brain network depends on consciousness level: a resting-state FMRI study. *Cereb. Cortex* 24, 1529–1539. doi: 10.1093/cercor/bht004

van den Heuvel, M. P., and Sporns, O. (2011). Rich-club organization of the human connectome. *J. Neurosci.* 31, 15775–15786. doi: 10.1523/JNEUROSCI.3539-11.2011

van den Heuvel, M. P., and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci.* 17, 683–696. doi: 10.1016/j.tics.2013.09.012

van den Heuvel, M. P., Sporns, O., Collin, G., Scheewe, T., Mandl, R. C., Cahn, W., et al. (2013). Abnormal rich club organization and functional brain dynamics in schizophrenia. *JAMA Psychiatry* 70, 783–792. doi: 10.1001/jamapsychiatry.2013.1328

Wagner, T., Fregni, F., Fecteau, S., Grodzinsky, A., Zahn, M., and Pascual-Leone, A. (2007). Transcranial direct current stimulation: a computer-based human model study. *Neuroimage* 35, 1113–1124. doi: 10.1016/j.neuroimage.2007.01.027

Watts, D. J., and Strogatz, S. H. (1998). Collective dynamics of 'small-world' networks. *Nature* 393, 440–442. doi: 10.1038/30918

Weber, M. J., Messing, S. B., Rao, H., Detre, J. A., and Thompson-Schill, S. L. (2014). Prefrontal transcranial direct current stimulation alters activation and connectivity in cortical and subcortical reward systems: a tDCS-fMRI study. *Hum. Brain Mapp.* 35, 3673–3686. doi: 10.1002/hbm.22429

Wiggins, G., and Bhattacharya, J. (2014). Mind the gap: an attempt to bridge computational and neuroscientific approaches to study creativity. *Front. Hum. Neurosci.* 8:540. doi: 10.3389/fnhum.2014.00540

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 June 2014; accepted: 07 July 2014; published online: 30 July 2014.
Citation: Luft CDB, Pereda E, Banissy MJ and Bhattacharya J (2014) Best of both worlds: promise of combining brain stimulation and brain connectome. Front. Syst. Neurosci. ***8****:132. doi: 10.3389/fnsys.2014.00132*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 26 June 2014
doi: 10.3389/fnsys.2014.00119

Hypothesis-driven methods to augment human cognition by optimizing cortical oscillations

Jörn M. Horschig[1]*, Johanna M. Zumer[1,2] and Ali Bahramisharif[1]

[1] *Radboud University Nijmegen, Donders Institute for Brain, Behaviour and Cognition, Nijmegen, Netherlands*
[2] *School of Psychology, University of Birmingham, Birmingham, UK*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Markus Werkle-Bergner, Max Planck Institute for Human Development, Germany
Sepideh Sadaghiani, University of California Berkeley, USA
Ali Mazaheri, University of Amsterdam, Netherlands

****Correspondence:***
Jörn M. Horschig, Radboud University Nijmegen, Donders Institute for Brain, Behaviour and Cognition, Kapittelweg 29, 6525 EN Nijmegen, Netherlands
e-mail: jm.horschig@donders.ru.nl

Cortical oscillations have been shown to represent fundamental functions of a working brain, e.g., communication, stimulus binding, error monitoring, and inhibition, and are directly linked to behavior. Recent studies intervening with these oscillations have demonstrated effective modulation of both the oscillations and behavior. In this review, we collect evidence in favor of how hypothesis-driven methods can be used to augment cognition by optimizing cortical oscillations. We elaborate their potential usefulness for three target groups: healthy elderly, patients with attention deficit/hyperactivity disorder, and healthy young adults. We discuss the relevance of neuronal oscillations in each group and show how each of them can benefit from the manipulation of functionally-related oscillations. Further, we describe methods for manipulation of neuronal oscillations including direct brain stimulation as well as indirect task alterations. We also discuss practical considerations about the proposed techniques. In conclusion, we propose that insights from neuroscience should guide techniques to augment human cognition, which in turn can provide a better understanding of how the human brain works.

Keywords: neuronal oscillations, working memory, attention, elderly, ADHD, brain stimulation, brain-computer interfacing, brain state dependent tasks

INTRODUCTION

Recent advances in cognitive neuroscience have provided insight into the functional mechanisms of the human brain. Neuroscientists have identified specific brain patterns, for example neuronal oscillations, that co-fluctuate with the task and behavioral performance (Buzsáki, 2006). These fluctuations are not random but depend on the specific task and cognitive settings; these findings have allowed functional hypotheses to be formed, directly tested, and confirmed. Throughout the previous decades, huge progress has been made in understanding how the human brain works, and in understanding differences across age groups, pathologies, and individuals. Applying this in-depth knowledge in practice might therefore be a key to creating brain tools for different target groups to improve different aspects of human cognition.

Cognitive functioning declines with age (Deary et al., 2009) and does not necessarily occur in the presence of a neurological disorder. Healthy elderly suffer from problems with memory and attention more than healthy, young individuals. With an increasing aging population, it has become a societal priority to look into approaches that can delay or prevent functional degeneracy or even augment cognitive abilities in the elderly. Brain tools might have the potential to rejuvenate the functionality of an aging brain.

Many people suffer from cognitive deficiencies in daily activities, but there are population groups in which these problems are more severe. Attention deficit/hyperactivity disorder (ADHD) is a well-studied disorder with problems of attention, hyperactivity, and impulsivity. Although the cause of ADHD is unknown, there have been many attempts to treat it using medication (e.g., Chang et al., 2012). Next to many unknown side-effects of medication, about 30% of the ADHD population do not respond to any medication, which calls for alternative treatments (Kidd, 2000). Brain tools might serve this population by normalizing their brain activities.

Most of our knowledge from cognitive neuroscience about the human brain stems from studies on healthy, young individuals, which have helped to form functional hypotheses about traits of human brain activity. These hypotheses can serve as a benchmark for other populations groups. In addition, also healthy young adults show large task variability in cognitive tasks. Next to individual differences, individuals' performance varies momentarily in cognitive tasks (Kane and Engle, 2002; Paulus et al., 2009). Thus, while constituting a proper control group, we will also discuss our current knowledge on whether young, healthy adults can benefit from cognitive improvements.

The brain is a highly flexible organ which can adapt to different manipulations very quickly (Pascual-Leone et al., 2011). Entrainment of neuronal oscillations to augment human behavior has already been proposed in the past (see e.g., Thut et al., 2011a; Herrmann et al., 2013; Calderone et al., 2014; Enriquez-Geppert et al., 2014). We complement these reviews by proposing different techniques in different target groups to relate the to-be-augmented aspect of cognition to associated neuronal signatures,

specifically neuronal oscillations. Identifying the neural signatures of different tasks will allow for proposing protocols for manipulating the brain and thereby the individual's cognitive abilities. Recent studies suggest a causal role of neuronal oscillations in cognitive tasks (Thut and Miniussi, 2009; Romei et al., 2010). Based on this hypothesis and the possibility of manipulating neuronal oscillations in several ways, we propose that by using "hypothesis-driven" approaches, one can augment human cognition by optimizing cortical oscillations. In this paper, we begin with discussing the functional role of neuronal oscillations and their cognitive relevance. We then continue with more details about three target population groups, healthy elderly, patients with ADHD, and healthy young adults, and elaborate on how cognitive improvement can be gained. Next, we go into different ways of manipulating functional oscillations in order to improve cognitive performance in the three target groups. The paper ends with practical considerations and conclusions.

FUNCTIONAL ROLE OF NEURONAL OSCILLATIONS

Spontaneous and goal-related fluctuations of the brain state are reflected in electrophysiological activity that can be measured non-invasively using various techniques like electroencephalography (EEG) and magnetoencephalography (MEG). EEG and MEG measure the strength of the voltage potentials and magnetic fields at the scalp associated with postsynaptic potentials along the dendrites of pyramidal neurons, i.e., the synaptic input to these cells (Nunez, 2000; Niedermeyer and Lopes da Silva, 2005; Wang, 2010; Lopes da Silva, 2013). Non-invasive measurements require strongly synchronized activity across nearby neurons to result in a measurable signal at scalp level. Neuronal oscillations at the scalp level are rhythmic patterns that represent the degree of synchronized neuronal input to the underlying neuronal ensemble (Lopes da Silva, 1991; Buzsáki and Draguhn, 2004), which are reflected as power increases (commonly known as event-related synchronization, ERS) or power decreases (event-related desynchronization, ERD; see Pfurtscheller and Lopes da Silva, 1999).

Neuronal oscillations are commonly divided into different frequency bands. While lower frequencies are often associated with long-range connectivity between cortical regions (von Stein and Sarnthein, 2000), higher frequencies reflect the local firing pattern of neurons (Xing et al., 2012). Furthermore, different neuronal oscillations have been associated with specific neuronal processes (e.g., Engel et al., 2001; Kopell et al., 2010), which have been related to behavioral performance, e.g., in attention and working memory tasks (reviewed in more detail below). In this paper, we will focus on cortical oscillations in three frequency bands: alpha oscillations (8–13 Hz), theta oscillations (5–8 Hz), and gamma oscillations (>30 Hz). In the following, we will introduce these three oscillations, describe the current dominant views on their function, and elucidate their role on qualitative aspects of cognition.[1]

ALPHA OSCILLATIONS (8–13 HZ): FUNCTIONAL INHIBITION OF NEURONAL REGIONS

Human alpha oscillations are the dominant rhythms in EEG and MEG. They were observed almost a century ago (Berger, 1929; Adrian and Matthews, 1934) and, while originally having been associated with the idle state of the visual cortex, the current dominant view has changed towards a functional, inhibitory role, elaborated upon below. Alpha is generated via thalamocortical and cortico-cortical loops (Lopes Da Silva and Storm Van Leeuwen, 1977; Lopes da Silva et al., 1980; Suffczynski et al., 2001; Bollimunta et al., 2011). Cortical alpha has been shown to be modulated by the pulvinar nucleus of the thalamus (Saalmann et al., 2012) as well as by frontal regions (Capotosto et al., 2009). The precise mediation of cortical alpha via the interplay between frontocortical and subcortical mechanisms still requires further investigation. Individual variations of alpha power appear as stable traits. The peak frequency of alpha oscillations has been related to the latent factors of general cognitive abilities, and the frequency and power of alpha oscillations have been shown to change with age (Klimesch, 1999; Grandy et al., 2013).

Alpha oscillations are strongly involved in attention processes. While low alpha activity can be observed in regions that are processing information, sensory regions that are not involved in the current task show high alpha activity. For example, in covert spatial attention studies, selective attention and successful inhibition of the task-irrelevant hemifield are often indexed using the extent of alpha power lateralization. Highly lateralized alpha activity thereby suggests a high inhibition of the task-irrelevant hemifield and has been shown to lead to better task performance (Worden et al., 2000; Thut et al., 2006; Kelly et al., 2009), whereas a weak or reverse alpha lateralization leads to failures in motor inhibition (Bengson et al., 2012). During the retention interval of working memory tasks, it has been shown that the degree of alpha power increase in early sensory regions scales with memory load (Jensen et al., 2002). This effect also holds true for paradigms in non-visual domains, e.g., occipital alpha increases during a somatosensory delayed-match-to-sample task (Haegens et al., 2010; Spitzer and Blankenburg, 2012). Additionally, strong alpha during the immediate rehearsal of an item for long-term memory encoding has been shown to predict the long-term memory encoding success (Meeuwissen et al., 2011). Alpha activity has therefore been suggested to reflect the amount of top-down controlled cortical inhibition (reviewed in Klimesch, 1999, 2012; Klimesch et al., 2007; Jensen and Mazaheri, 2010; Foxe and Snyder, 2011; Jensen et al., 2012).

Alpha oscillations are also strongly involved in the anticipation of upcoming stimuli, which has been convincingly shown by Rohenkohl and Nobre (2011). They showed that when stimulation was temporally anticipated but absent, alpha power decreased to a similar degree compared to when stimulation actually occurred when anticipated, see **Figure 1A**. Moreover, the degree of anticipatory alpha power correlates with subsequent

[1] Note that we focus on power of the oscillation and do not cover its phasic role. Other frequency bands, such as delta (1–3 Hz) or beta (13–30 Hz) oscillations, or slow cortical potentials lie outside the scope of this article. Some alternative approaches are shortly discussed in Section Alternative Aspects of Neuronal Oscillations to Utilize.

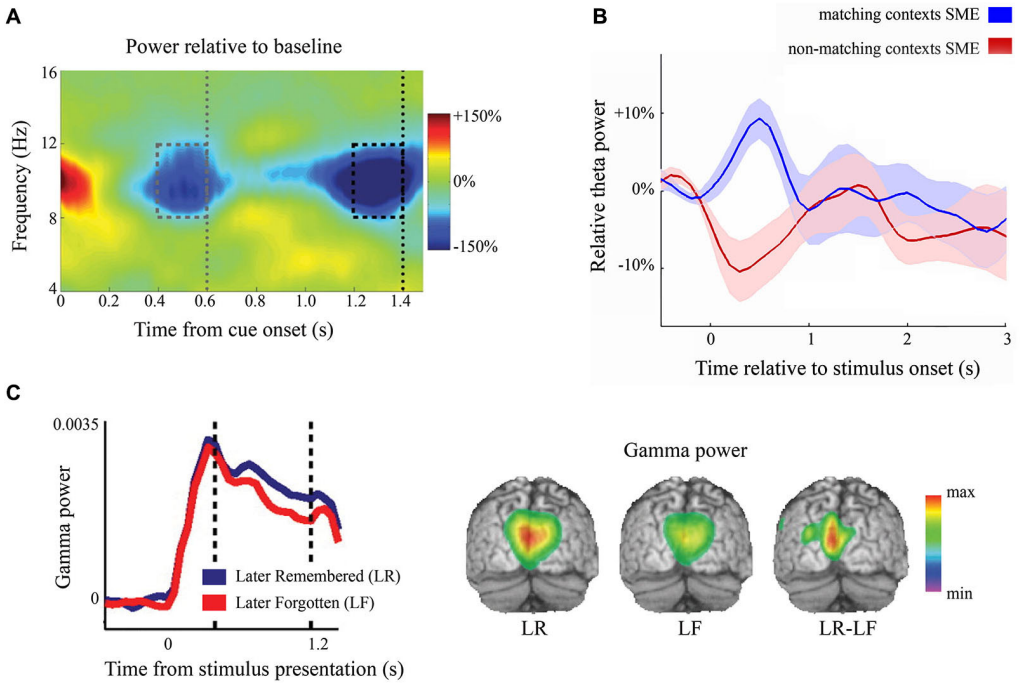

FIGURE 1 | Example of the functional involvement of neuronal oscillations. (A) The time-frequency representation of an anticipation task. The gray dotted line shows the time that the stimulus could have been presented, but was not. The black dotted line indicates the onset of the target. Alpha power decreases in anticipation of a stimulus even without subsequent stimulation. Reproduced with permission from Rohenkohl and Nobre (2011). **(B)** The dynamics of frontal theta power. Depending on the encoding task, both increases and decreases of frontal theta power were found for successful versus unsuccessful remembering. SME = subsequent memory effect. Reproduced with permission from Hanslmayr and Staudigl (2014). **(C)** During visual stimulus encoding, posterior gamma power (60–90 Hz) is predictive of subsequent memory performance in young, healthy adults. The vertical dashed bars indicate the window for significance testing and beamformer application. The left panel shows gamma power from one significant posterior sensor and the right panel is the beamformer projection (Osipova et al., 2006).

behavioral performance in attention tasks (Worden et al., 2000; Thut et al., 2006; Kelly et al., 2009) and correlates with subsequent long-term memory performance (Park et al., 2014). Anticipatory alpha power also scales with stimulation likelihood (Gould et al., 2011; Haegens et al., 2012; Horschig et al., 2014). Upon stimulation, however, alpha oscillations robustly decrease in early sensory regions. Recently, Hanslmayr et al. (2012) suggested an entropy-based explanation for this: the more complex the information is that needs to be encoded, the less structured (i.e., the more de-synchronized) the activity in a network has to be. An alternative explanation, which is not mutually exclusive, is that early sensory regions become disinhibited, i.e., functionally engaged, and therefore show low alpha power.

THETA OSCILLATIONS (5–8 HZ): WORKING MEMORY AND NEURONAL ORGANIZATION

Depending on where in the brain theta oscillations are observed, they can be divided into two groups of hippocampal and cortical theta rhythms (reviewed in Mitchell et al., 2008). In this paper we only focus on the cortical theta rhythms that can be measured non-invasively and are probably generated in hippocampal-cortical feedback loops (Klimesch, 1999). Cortical theta power has been related to encoding, retaining, and retrieving items in working memory (Kahana et al., 2001; Klimesch et al., 2010; Sauseng et al., 2010). The frequency of theta rhythms shows a large inter-individual variability similar to alpha oscillations, and the individual theta peak frequency has been shown to be significantly correlated to the individual alpha peak frequency (Klimesch et al., 1996).

During working memory tasks, theta power increases over temporal sites during encoding, maintenance, and retrieval (Raghavachari et al., 2001; Fell et al., 2011). Over frontal regions, theta power increases proportionally with task demands (Gevins et al., 1997). In working memory tasks, for example, a higher memory load produces stronger frontal theta activity (Jensen and Tesche, 2002). However, both frontal theta increases and decreases have been found to be beneficial for successful memory retrieval (Staudigl and Hanslmayr, 2013; see **Figure 1B**). Hanslmayr and Staudigl (2014) recently suggested that the context of the memory and the probe items is the crucial factor for whether theta increases or decreases upon successful retrieval. Raghavachari et al. (2006), however, argued for local theta generators where frontal theta exerts executive control and parietal theta serves to maintain items in working memory.

Apart from their role in working memory, frontal theta oscillations link prediction errors to behavioral adaptations (Cavanagh et al., 2010). Theta oscillations are also involved in

long-range neuronal communication between cortical and subcortical regions including the hippocampus (Mitchell et al., 2008) and nucleus accumbens (Cohen et al., 2009, 2012) as well as for corticocortical communication (reviewed in von Stein and Sarnthein, 2000). In conclusion, contemporary theory posits that theta oscillations are crucially involved in the organization and coordination of information and for memory storage and retrieval (Jensen and Lisman, 1996; Buzsáki and Draguhn, 2004; Lisman, 2005, 2010; Sauseng et al., 2010; Lisman and Jensen, 2013).

GAMMA (>30 HZ): PERCEPTUAL AND MULTISENSORY BINDING AND MEMORY MAINTENANCE

Gamma oscillations are defined as frequencies above 30 Hz, subdivided into the lower gamma range (30–80 Hz) and the higher gamma range (>80 Hz; Buzsáki and Draguhn, 2004; Ray and Maunsell, 2011). Gamma oscillations have been implicated with active processing of information and thus increase with stimulation intensity and amount of attention to stimulation (Engel and Singer, 2001; Engel et al., 2001; Fries et al., 2001; Jensen et al., 2007). A large inter-individual variability in the gamma peak frequency has been demonstrated (van Pelt et al., 2012) as well as a correspondence between the gamma peak frequency and the characteristics of the visual stimuli (van Pelt and Fries, 2013).

Gamma oscillations are also observed during working memory maintenance (Tallon-Baudry et al., 1998; Miltner et al., 1999; Jokisch and Jensen, 2007), possibly reflecting active processing and binding of the to-be-maintained information in frontal and parietal cortices (Polanía et al., 2012b). The degree of gamma power during encoding of visual items has been found to correlate with working memory load (Howard et al., 2003) and predicts successful memory encoding, see **Figure 1C** (Osipova et al., 2006). Also, during successful multisensory integration, an increase of gamma band power has been observed (Schneider et al., 2008; Kanayama et al., 2012), strengthening the idea that gamma band oscillations serve to form a coherent object representation in working memory. Gamma band activity has been suggested to reflect the process of mentally forming and binding objects (Tallon-Baudry et al., 1996; Tallon-Baudry, 1999).

Many studies report a simultaneous decrease of alpha power and increase in gamma power in task relevant brain regions, whereas a number of studies have shown that alpha and gamma power are not always inversely coupled (Haegens et al., 2010; Scheeringa et al., 2011). For example, while alpha power has been shown to decrease in anticipation of a stimulus (Rohenkohl and Nobre, 2011; see **Figure 1A**), gamma activity is induced by stimulation and active maintenance but is not observable in anticipation to stimulation (Hoogenboom et al., 2006). Thus, while alpha decreases may serve to disinhibit a brain region, gamma oscillations reflect the active, ongoing processing and binding of information.

POPULATION TARGET GROUPS AND THEIR NEURONAL SIGNATURES

Many common individual differences in cognition can be traced back to differences in memory or attention processes. In the previous section we outlined the functional role of neuronal oscillations in cognition and presented strong evidence for a relationship between oscillatory power and behavioral performance in specific tasks. In this section, we will outline how optimizing neuronal oscillations might improve cognition in three target groups: the elderly, who commonly have problems with attention and working memory, patients suffering from ADHD, and also healthy, young adults who show remarkable inter-individual differences in cognitive tasks. We will propose that optimizing neuronal oscillations might help to alleviate symptoms and improve cognition.

THE ELDERLY AND THEIR PROBLEMS WITH ATTENTION AND WORKING MEMORY

In the western world, there is a continuous demographic change with an increased percentage of elderly people in the population (Cohen, 2005; Peters et al., 2010). Several studies have statistically assessed the areas of compromised cognition in the elderly. Elderly people have more trouble in task switching paradigms compared to younger adults (Kray and Lindenberger, 2000). The elderly show lower performance in working memory tasks (Salthouse et al., 1991) and have reduced working memory capacity (at least in part) due to problems in binding multiple low-level features (Brockmole and Logie, 2013). Additionally, it has been shown that elderly have trouble inhibiting distracting information in unimodal tasks (Folk and Lincourt, 1996; Groth and Allen, 2000; Gaeta et al., 2001; Tales et al., 2002; Andrés et al., 2006; Fabiani et al., 2006; Rowe et al., 2006; Yang and Hasher, 2007), in cross-modal tasks (Alain and Woods, 1999; Poliakoff et al., 2006; Hugenschmidt et al., 2009b), and multi-modal tasks (Hugenschmidt et al., 2009a). The studies by Hugenschmidt et al. and others suggest that while elderly have trouble ignoring task-irrelevant items, they do show intact attention abilities. For example, elderly showed behavioral cueing effects, even in more complex environments (Hugenschmidt et al., 2009a,b).

In addition, a number of structural and functional changes have been reported in the brain of the elderly. For example, with increasing age, alpha peak frequency and power decrease while theta power increases (Dustman et al., 1993; Grandy et al., 2013) and the amount of evoked gamma band activity is reduced in the elderly compared to young adults (Werkle-Bergner et al., 2009). Recently, Sander et al. (2012a) proposed to disassemble working memory into two components: a global top-down control mechanism and a local perceptual binding mechanism. They further hypothesized that both components are differentially impaired in the elderly. By incorporating the reviewed evidence from the previous section, global top-down control is reflected by the power of frontal theta and posterior alpha oscillations (reviewed in Klimesch, 1999; Klimesch et al., 2007; Jensen and Mazaheri, 2010; Foxe and Snyder, 2011; Jensen et al., 2012; Klimesch, 2012), whereas local binding is reflected by gamma band oscillations (Tallon-Baudry et al., 1996; Tallon-Baudry, 1999). Under this framework, reduced alpha or theta power would suggest a problem with top down control, whereas a reduction in evoked gamma-power would predict a problem with perceptual binding.

A number of studies showed the absence of correlations between behavior and oscillatory activity in the elderly, while present in healthy young adults. For example, a strong increase in frontal theta power with working memory load has been reported in young adults (Jensen and Tesche, 2002), but there was no such relation in the elderly (McEvoy et al., 2001). Also, McEvoy et al. (2001) found that with increasing task difficulty, alpha power in both parietal and frontal cortices decreased in the elderly, whereas in young adults alpha power only decreased over parietal regions. In a similar vein, Gazzaley et al. (2008) showed that frontal theta power scaled with the relevance of the to-be-processed item in young healthy adults, but not in the elderly, see **Figure 2A**. Sander et al. (2012b) showed that in a visual covert attention working memory paradigm, the degree of lateralized alpha power was maximal under high memory load in healthy, young adults, whereas it peaked under medium memory load in the elderly and was nearly absent in the high memory load condition. Despite the absence of this correlation, the elderly were still able to successfully perform well in this task, although worse than the young adults.

All studies in elderly are fraught with the problem that compensatory mechanisms seem to be active (Reuter-Lorenz et al., 2000; Cabeza et al., 2002; Logan et al., 2002; Nielson et al., 2002; Riis et al., 2008). While a reduced amount of activity might highlight the locus of the problem, an increase in activity elsewhere might indicate the compensation. Hence, in the above finding by Sander et al. (2012b) it is likely that a compensatory mechanism took over the functional role of posterior alpha lateralization under high memory load. Identifying such mechanisms and studying whether they are compensatory or competing with normal functioning could help in determining whether this mechanism is causing or resulting in the degraded bottom up sensory processing and binding.

ATTENTION DEFICIT/HYPERACTIVITY DISORDER

ADHD is the most common psychiatric disorder in the western world (Cantwell, 1996; Barry et al., 2003) with an estimated prevalence of 3–6% (Pelham et al., 1992; Polanczyk et al., 2007). Diagnosis of ADHD is characterized by two components: an attention and a hyperactivity component. While some patients show traits of both components (the "combined" subtype), a large proportion of patients show only one component strongly with the other component weaker or absent (Barkley et al., 1990; Lockwood et al., 2001). Here, we will focus on the combined and the inattentive subgroups, i.e., those with attention deficits. However, the combined patient subgroup is characterized by different attention deficits in cognitive tasks than the inattentive subgroup (Weiss et al., 2003; Booth et al., 2007; Adams et al., 2008), leading to the proposition of categorizing patients showing inattentive without hyperactivity symptoms as a patient group distinct from ADHD (Barkley, 2001; Milich et al., 2001; Derefinko et al., 2008). Nonetheless, we will discuss characteristics and possible treatments for attention deficits of both the combined and the inattentive subgroups.

The inattentive subtype is characterized, as the name implies, by problems in engaging and sustaining attention. In behavioral paradigms it has been found that the inattentive subgroup showed either reduced attention resources (Carr et al., 2010) or reduced visual processing power (Weiler et al., 2002). The inattentive subtype also showed a lack of response cueing effect (Lockwood et al., 2001; Derefinko et al., 2008). Behavioral deficits and oscillatory power differences between ADHD patients and

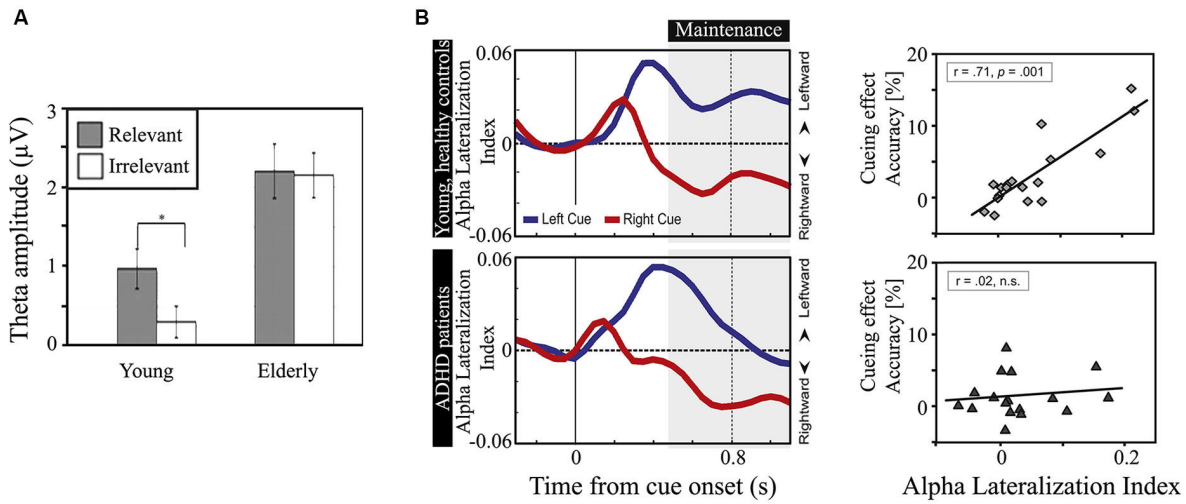

FIGURE 2 | Oscillatory power differences in different task settings in the three discussed population groups. (A) Frontal theta power during stimulus encoding is significantly higher for elderly versus young adults, and scales with relevance of the stimuli in young, healthy adults, but not in the elderly (* $p < 0.001$). Reproduced with permission from Gazzaley et al. (2008). **(B)** During visual spatial covert attention, the degree of alpha lateralization indexes the relative disengagement of the task-irrelevant ipsilateral hemisphere versus the task-relevant contralateral hemisphere. While young, healthy adults are able to modulate their alpha lateralization symmetrically around zero, inattentive ADHD patients show a lack of maintaining a high degree of alpha lateralization to the left side. In addition, there is an absence of correlation between alpha lateralization index and behavioral cueing effect in ADHD patients, whereas there is a strong relationship in healthy, young adults. Reproduced with permission from ter Huurne et al. (2013).

control subjects have been investigated recently. In adolescents, Mazaheri et al. (2013) found that ADHD patients of the inattentive subgroup showed a reduced behavioral cueing effect and weaker suppression of posterior alpha in response to the cue that indicates with which hand to respond. In young adults with the inattentive subtype, ter Huurne et al. (2013) showed an absence of maintaining posterior anticipatory alpha lateralization in response to a left attention cue, but not to a right attention cue. Interestingly, they found the same initial level of alpha lateralization in control and ADHD groups. In both studies, the degree of alpha power modulation with task showed a strong correlation with behavior for the control group but not for the ADHD group; see **Figure 2B**. These studies suggest that inattentive ADHD patients do not only suffer from reduced but also from inefficient integration of posterior alpha power as it does not seem to be beneficial for behavior, whereas it is for healthy people.

Studies on the combined ADHD subgroup and neuronal oscillations in task settings are sparse. Recently Karch et al. (2012) found that young adults suffering from ADHD showed increased frontocentral gamma band activity shortly after auditory stimulation followed by a voluntary motor response. Yordanova et al. (2001) found that ADHD children between 9 and 12 years of age showed increased induced fronto-central gamma band responses for right auditory stimulation compared to normally developing children, but not for left auditory stimulation. Lenz et al. (2008) found that children of the combined ADHD group showed enhanced visual stimulus-induced gamma power. While gamma power correlated with long-term memory performance for typically developing children, it did not for the ADHD children. In addition, Mazaheri et al. (2013) tested children of the inattentive subgroup and the combined subgroup and compared these two groups with typically developing children. They found significantly more alpha power to response cues for the inattentive subtypes compared to typically developing children (discussed above), but there was no such effect for the combined group. The combined group, however, showed no significant difference in alpha power from the inattentive subgroup. Also, the combined subgroup showed no correlation between behavior and anticipatory alpha, whereas the typically developing children did. In an earlier study, Mazaheri et al. (2010) investigated the response preparation ability in a perceptual switching task in typically developing children compared to children classified in the combined ADHD group in age range from 8 to 12 years. Typically developing children showed a strong alpha power increase in parieto-occipital cortex in preparation for auditory versus visual stimulation, in line with the idea of shutting down the visual stream when preparing for auditory input. ADHD children, however, showed no such difference, but a frontal theta increase instead. Additionally, although in typically developing children parieto-occipital alpha power was inversely correlated with the behavioral cueing effect in the visual condition, there was no such correlation for the ADHD children. Recently, Lenartowicz et al. (2014) investigated the neuronal patterns in a group of children between 7 and 14 years of age comprised of both the inattentive and combined subgroups. In a working memory paradigm, they found reduced vigilance attributed to a less pronounced alpha depression during encoding (i.e., higher occipital alpha activity) in ADHD children than in typically developing children, but in return a stronger alpha power synchronization during stimulus maintenance in ADHD children. Frontal theta during the maintenance period was also elevated in ADHD children, which they interpreted as a compensation for the lack of vigilance during encoding.

The above studies indicate a versatile interplay of hyper- and hypo-activity in specific phases during a task. In line with Lenartowicz et al. (2014) we propose that this might be anchored to improper preparation for the task, indicated by a lack of modulating anticipatory alpha activity to stimulation. Higher gamma during stimulation and higher alpha and theta during stimulus maintenance might thus be the product of compensation for this improper preparation.

COGNITION IN HEALTHY, YOUNG ADULTS

Within young healthy adults there is a large spread of inter- and intra-individual differences in both neuronal oscillations and behavior. For example, healthy individuals differ in visual working memory capacity (Luck and Vogel, 2013). Individuals with better working memory capacity have greater frontal theta power during stimulus encoding (Gevins and Smith, 2000). Similarly, individuals with greater pre-stimulus frontal theta power better remembered source context (Addante et al., 2011). Also within subjects, high frontal pre-stimulus theta power was predictive of whether an item was remembered (Addante et al., 2011), as was increased gamma power during stimulus encoding; see **Figure 1C** (Osipova et al., 2006).

The ability to sustain attention varies among individuals. Individual differences in the degree of alpha lateralization have been linked to differential abilities to ignore the task-irrelevant hemifield (Fu et al., 2001; Haegens et al., 2011a; Horschig et al., 2014) as well as to working memory performance (Sauseng et al., 2009b). Gamma power has been linked to improved attention as well. Apart from the fact that gamma power is commonly increased for attended versus unattended stimuli (Tallon-Baudry et al., 2005; Bauer et al., 2006), individuals with stronger gamma activity have been associated with improved perceptual processing (Jokeit and Makeig, 1994; Fründ et al., 2007). Moreover, the ratio between theta and gamma peak frequency has been successfully linked to short-term memory capacity (Jensen and Lisman, 1996, 1998), which both consistently vary from individual to individual (Kamiński et al., 2011). In future studies, explicit perturbation of the specific neuronal oscillations is required to identify whether they are causally involved in cognition and, if so, the findings can serve to form strong hypotheses on how to augment human cognition.

TECHNIQUES TO MANIPULATE NEURONAL OSCILLATIONS

In this section we will discuss how to apply our framework in practice. We will combine the fundamental insights of neuronal oscillations from the second section with the studies on different population groups discussed in the third section to answer which oscillatory components might be suitable for optimization and whether and how this optimization could increase cognitive

performance. Since there are oscillations-behavior correlations in healthy adults and oscillations-pathology correlations, optimization of neuronal oscillations could lead to optimized behavior. We will introduce different, non-invasive approaches to manipulate neuronal oscillations, outline how these techniques work, and present studies on what has already been achieved. In addition we will suggest steps to fill the gap in current literature on successful augmentation of human cognition.

TRANSCRANIAL STIMULATION OF THE HUMAN BRAIN

The most direct way to manipulate neuronal firing is by electrical stimulation to manipulate the neuron's membrane potential causing it to de- or hyperpolarize. The two common non-physically-invasive techniques to do so are transcranial magnetic stimulation (TMS) and transcranial current stimulation (tCS). TMS utilizes the fact that a changing current in a wire induces a changing external magnetic field that, if in the presence of a conducting material such as neural tissue, induces a secondary electric current in the opposite direction (Pascual-Leone et al., 1999, 2000). This secondary current then affects local membrane potentials. Although the exact mechanism is still not completely understood, it is assumed that TMS pulses primarily influence the axons of both excitatory and inhibitory neurons and might actively elicit action potentials (Dayan et al., 2013). Two basic TMS approaches are commonly used. The "online" approach applies single, double, or brief bursts of pulses, each lasting a few 100 ms, during the task. The "offline" approach applies repetitive stimulation (rTMS) before a task or other measurement (Huang et al., 2005). Depending on the exact pattern and frequency of rTMS, cortical excitability can be facilitated or inhibited for a period outlasting the stimulation itself from 15 up to 90 min (Thut and Pascual-Leone, 2010). However, it has also been suggested that the frequency of stimulation entrains the neuronal oscillations at the stimulation frequency, which outlasts stimulation for a short period of time (hundreds of milliseconds; Thut and Miniussi, 2009). There is some first direct evidence for rhythmically entraining alpha oscillations in the visual cortex by means of brief bursts of rTMS around 10 Hz (Thut et al., 2011b and **Figure 3A**), yet stimulation not necessarily in the alpha frequency range can induce an alpha power increase (Thut and Pascual-Leone, 2010). Several studies have provided convincing indirect evidence for rTMS entrainment by its impact on behavior (Romei et al., 2010 and **Figure 3B**; Klimesch et al., 2003; Sauseng et al., 2009a).

The basic principle of tCS is that a weak electrical current is established between an anode and cathode, thereby altering neural membrane potentials. Importantly, because of the weak electrical current, tCS cannot trigger action potentials but rather slightly facilitates or inhibits spontaneous neuronal firing, depending on its polarity (Dayan et al., 2013; Reato et al., 2013). Transcranial current stimulation can be applied in two different ways: transcranial alternating current stimulation (tACS) and

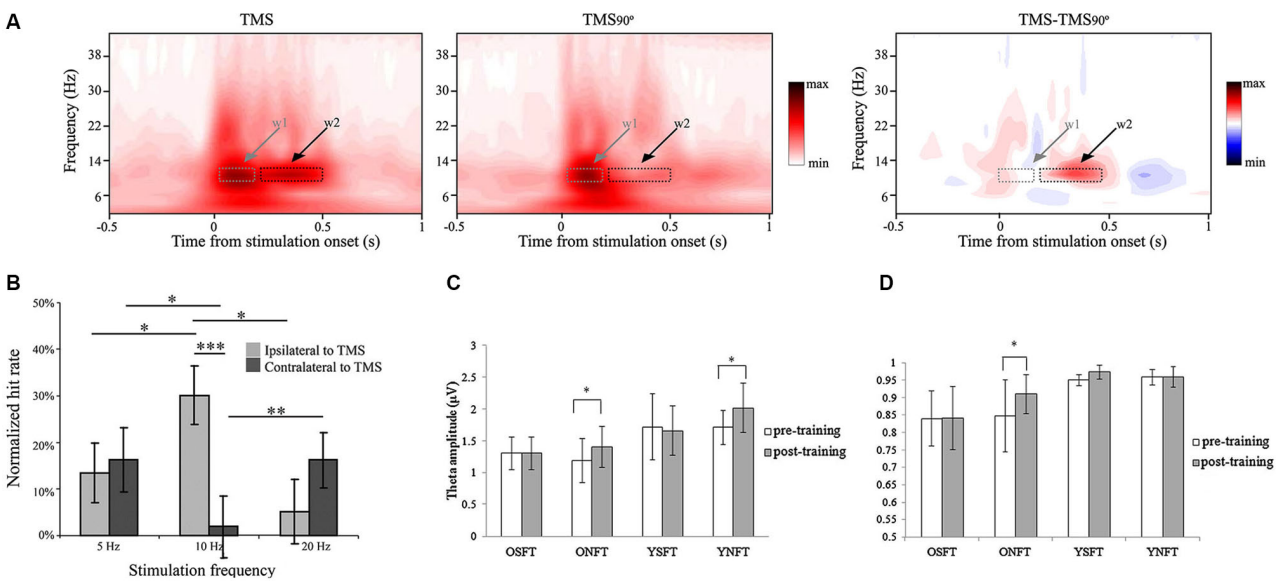

FIGURE 3 | Examples of manipulating neuronal oscillations and their impact on cognition. (A) rTMS stimulation at 10 Hz to right parietal cortex results in alpha oscillations outlasting the stimulation period (t = 0 s), compared to the control condition of rotating the TMS coil by 90° (TMS90). The condition contrast with other control conditions confirmed the exclusive effect of rTMS at 10 Hz. w1 = time window of the first two pulses and w2 = time window of the last three pulses. Reproduced with permission from Thut et al. (2011b). **(B)** 10 Hz rTMS stimulation, but not 5 or 20 Hz, of parietal cortex ipsilateral to stimulation results in behavioral improvement, whereas contralateral stimulation results in decreased performance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Reproduced with permission from Romei et al. (2010). **(C)** Frontal theta neurofeedback training results in increased frontal theta in both young and old adults. OSFT = old subjects, sham feedback; ONFT = old subjects, neurofeedback group; YSFT = young subjects, sham feedback; YNFT = young subjects, neurofeedback (* $p < 0.01$). Taken with permission from Wang and Hsieh (2013). **(D)** Only old adults receiving neurofeedback on frontal theta increased working memory accuracy in a Sternberg task (depicted on the y-axis). Young adults were already performing at ceiling level. (for acronym, see **panel C**; * $p < 0.01$). Taken with permission from Wang and Hsieh (2013).

transcranial direct current stimulation (tDCS). While with tDCS the polarity between anode and cathode stays constant (see Priori, 2003, for a review), with tACS the polarity constantly changes, thereby producing an alternating flow of current similar to an oscillation (Antal and Paulus, 2013; Herrmann et al., 2013; Reato et al., 2013). Sometimes tACS is used in conjunction with a DC offset. In that case, the polarity between anode and cathode stays fixed, as the current strength oscillates around the DC offset instead of zero. Consequently current only flows in one direction, from the cathode to the anode, which is in contrast to the classical tACS definition, where not only current strength but also direction changes throughout each oscillatory cycle. Here, we will explicitly clarify if we refer to tACS studies with a DC offset.

In the past a number of studies has shown improved cognition or performance in tasks using tCS or TMS (Meinzer et al., 2012, 2013; Snowball et al., 2013; Vollmann et al., 2013; Coffman et al., 2014; Luber and Lisanby, 2014; Schutter, 2014). However, most of these studies did not use tCS or tACS to stimulate neuronal oscillations or concurrently recorded them by EEG or MEG. In the following we will summarize a number of studies that used tACS or TMS to stimulate in the alpha, theta, or gamma range.

Although this is a relatively recent field, a number of studies has demonstrated that stimulating in the alpha range results in sustained alpha power increases in the cortex. Zaehle et al. (2010) showed that applying tACS offline for 10 min at the subject's individual peak alpha frequency over left and right posterior cortex resulted in an increase in alpha power for at least 3 min after the stimulation. Neuling et al. (2012) used tACS with a DC offset to entrain a 10 Hz rhythm during the task. After 3 blocks of 7 min stimulation, alpha power increased post-stimulation during a measurement lasting 3 min. In a more recent study, Neuling et al. (2013) found that the increase in alpha power using tACS without a DC offset lasts for up to 30 min. However, neither of these two studies found an influence of the power increase on behavior.

In contrast, several studies have found effects of entrainment in the alpha band on behavior using TMS, albeit they do not *per se* show that they were successful in changing cortical alpha power. Hamidi et al. (2009) applied rTMS at 10 Hz in parieto-central regions during the 3 s maintenance period in a delayed-match-to-sample task and found beneficial effects for a spatial working memory task, but not for a non-spatial working memory task. This is in line with the idea that high alpha power is needed during working memory maintenance to block additional incoming information and that the dorsal (parietal) regions encode spatial information but inferior (temporal) regions encode identity information (Goodale and Milner, 1992). Interestingly however, while Hamidi et al. (2009) did not find a change in alpha power due to TMS at the group level, they did find an across-subjects correlation of alpha power with behavior. Romei et al. (2010) found that entraining the alpha rhythm ipsilateral to the attended hemifield increased perceptual accuracy, whereas entraining alpha contralaterally decreased accuracy, strongly in line with the idea that strong alpha inhibits processing (**Figure 3B**). However, the effect of TMS stimulations on neuronal oscillations was not concurrently assessed by EEG, so it remains to be investigated whether the stimulation also resulted in changes in cortical oscillations.

Brignani et al. (2013) also tried to increase the ipsilateral alpha rhythm but with tACS to parieto-occipital cortex. Surprisingly, they found no spatially specific effect but a general task impairment during a covert spatial attention task. This might have been caused because the stimulated area using tCS is widespread and not easily inferable from the placement of the anode and cathode (Manoli et al., 2012; Bai et al., 2014). Also in this study, no concurrent EEG recordings were done, so the effect of stimulation on cortical oscillations could not be directly assessed. These studies show that different brain regions are affected differently by different techniques on different target areas and that precise *a priori* hypotheses and knowledge about the brain region to be stimulated are necessary for successful augmentation of human cognition. *A priori* computational modeling of the stimulation protocol using advanced physical models has recently been demonstrated (tCS: Manoli et al., 2012; Bai et al., 2014; TMS: Bijsterbosch et al., 2012; Janssen et al., 2013; Wagner et al., 2014). This could help in applying protocols and stimulation electrodes and thereby aid in studying whether modulation of cortical alpha power in specific regions has behavioral consequences.

Fewer studies are available which attempt to induce theta oscillations. Meiron and Lavidor (2014) applied tACS in the theta range to bilateral frontal cortex during a working memory task and found an improvement in online working memory capacity. Jaušovec and Jaušovec (2014) used tACS in the theta range but around left parietal cortex and showed that this leads to an improved memory working memory span. Jaušovec et al. (2014) replicated their finding on stimulating the theta rhythm, but this time in bilateral parietal cortices and replicated the effect on improved working memory capacity. They also report a null-effect for stimulating frontal cortex. While these findings suggest that entrainment of the theta rhythm is spatially specific and results in increasing working memory, they did not report oscillatory responses to assess whether the theta power was successfully increased over the stimulated area. In addition, none of these studies used control frequencies to show a frequency specific effect. Further studies are required to measure spatial and spectral specificity, disentangle which part of working memory is affected, and show convincingly that theta oscillations are entrained or at least modulated during stimulation.

In the gamma range, Chanes et al. (2013) showed a differential effect on performance in a perceptual detection task for 30 Hz stimulation versus 50 Hz stimulation using TMS to right frontal eye fields, where the former enhanced perceptual sensitivity and the latter shifted the response criterion. Using tACS, Santarnecchi et al. (2013) showed that entraining gamma around 40 Hz at the left mid-frontal gyrus improved performance in conditioning and reasoning tasks. Laczó et al. (2012) showed that gamma stimulation to early visual cortex around 60 Hz but not at 40 or 80 Hz resulted in a lower contrast detection threshold. Importantly, none of the stimulation frequencies resulted in behavioral changes in spatial detection tasks. While showing promising results, studies on rhythmically entraining the gamma range are sparse and differ in exact frequency range and spatial

location. Reproductions of these findings are required in order to draw definite conclusions on where and how to stimulate to improve what aspect of perceptual performance by gamma entrainment.

All of the above reviewed literature was on healthy, young adults and not on other target groups. Given the partly incomplete literature and understanding of TMS and tACS, it remains to be tested whether such hypothesis-driven brain stimulation shows success in the elderly and ADHD patients, and whether this results in a reduction of cognitive problems.

HYPOTHESIS-DRIVEN BRAIN COMPUTER INTERFACING AND NEUROFEEDBACK

Brain-computer interfacing (BCI) commonly refers to the technique to use a signal measured from the brain to control a computer or a machine without the use of the peripheral muscle system (Wolpaw et al., 2002). This requires the user's awareness of the ongoing brain activity, which is made perceivable by means of visual, tactile, or auditory feedback (van Gerven et al., 2009a). The original goal of BCI was to provide the user with an additional output channel for the purpose of communication.

Neurofeedback serves a different purpose by similar means. The goal of neurofeedback is to make the users aware of their brain activity to learn to enhance or decrease the fed back aspects, e.g., theta power, and thereby alleviate pathological symptoms, such as in ADHD (Lubar et al., 1995; Fuchs et al., 2003). An unfortunate major criticism in the field of neurofeedback, however, is often the absence of control conditions, significant effects, a scientifically grounded hypothesis, or reproducibility (see Vollebregt et al., 2014). Recently it has been proposed to start using hypothesis-driven BCI to improve subsequent behavior (Jensen et al., 2011), on which we will now elaborate.

Hypothesis-driven BCI (hdBCI) refers to the idea that insights from fundamental and cognitive neuroscience are applied to infer a robust and reliable control signal to improve cognition. Such a control signal must show strong single trial correlation with behavior and must be trainable. As described above, neuronal oscillations have been well studied and show strong correlation with cognition in a multitude of task settings ranging from attention or working memory studies to studies on long-term memory encoding and retrieval. This robustness and reliability allows making a concrete hypothesis to be tested when applying neurofeedback techniques. We suggest using a control signal that shows strong correlations with behavior, for example frontal theta during working memory or alpha lateralization during covert visual spatial attention tasks, and train the subjects to gain awareness and control of that signal. Before and after hdBCI training, a number of behavioral tasks orthogonal to the hdBCI task should be used to assess whether the neurofeedback training translates to subsequent improvements in cognition. The neuronal oscillation and the hdBCI paradigm need to reflect the underlying cognitive mechanism to be trained. For example, we described problems with general attention and reduced ability to suppress distracting stimuli in the elderly or in ADHD patients - a functional role that alpha oscillations are supposed to fulfill. The aim of hdBCI training is to study whether the training effect on oscillatory power brings along changes in behavior, in particular ideally, that has been shown to be correlated to that oscillation. If subsequent behavioral improvements in untrained, but related tasks are found, this would serve as a strong case that neuronal oscillations are causally involved in cognition. In the following we will review studies that can serve as templates for how hdBCI training can be used to study the functional role of neuronal oscillations.

While alpha-based neurofeedback has been shown to modulate not only the alpha power but also performance on a mental rotation task (Hanslmayr et al., 2005b; Zoefel et al., 2011), we link the findings of alpha power's role in inhibition of distracters (see Section Functional Role of Neuronal Oscillations) to hdBCI training with the goal of improving resilience to distraction. No such study has been conducted so far. If successful, one might ask whether the same rationale can be used for treating inattentive symptoms of the elderly or ADHD patients. As a concrete example, one could study whether the alpha lateralization pattern during covert spatial attention can be trained and strengthened. Alpha lateralization has been studied intensively in the past and has been shown to be a reliable control signal for BCI (Kelly et al., 2005; van Gerven and Jensen, 2009; van Gerven et al., 2009b; Bahramisharif et al., 2010; Tonin et al., 2012). In healthy young adults, high memory load coincided with strong alpha lateralization during a covert attention working memory task—an aspect that was missing in the elderly (Sander et al., 2012b). An hdBCI can consist of training alpha lateralization in the elderly asking whether strong alpha lateralization will be of beneficial nature as observed in young adults and whether a correlation between alpha lateralization and high memory load will reemerge. In a similar line a lack of maintaining alpha lateralization has been found in inattentive ADHD patients (ter Huurne et al., 2013). hdBCI training on maintaining a high degree of alpha lateralization could help to restore this ability. Again, a correlation between behavioral performance and degree of alpha lateralization was observed in healthy adults, but lacking in ADHD patients. The most critical question is, if the ability to maintain a high degree of alpha lateralization is restored in inattentive ADHD patients, will the underlying mechanisms leading to the alpha power shifts be beneficial for their performance again? No studies in this direction have been pursued so far, but they would elucidate the functional role of alpha oscillations.

Complementarily, during working memory paradigms it has been shown that frontal theta reflects the memory load in healthy individuals (Jensen and Tesche, 2002), but no such relation was found in the elderly (McEvoy et al., 2001). One might ask whether training the elderly to increase their frontal theta proportional to memory load during a working memory task results in improved working memory performance. Recently, Enriquez-Geppert et al. (2014) and Wang and Hsieh (2013) provide preliminary evidence that frontal theta neurofeedback training does work. The latter shows that both young and old participants learned to increase frontal theta by neurofeedback in contrast to control groups (see **Figures 3C,D**). In addition elderly receiving frontal theta neurofeedback training showed improved performance in a subsequent working memory task in contrast to the control group. However, they do not show whether the neurofeedback training restored the correlation between memory load and frontal theta in the

elderly. Also their study was confounded by several other issues, for example young, healthy, adults were performing at ceiling level already before the training, which resulted in a null-effect training for them. Thus, this study is inconclusive on whether frontal theta training increases working memory capacity in young, healthy adults. For ADHD patients it has been found that frontal theta is already relatively high compared to young healthy adults, e.g., during working memory encoding, which might be caused by a lack of proper preparation to the task, quantified by weaker anticipatory alpha oscillations in posterior regions (Lenartowicz et al., 2014; see also Section Population Target Groups and Their Neuronal Signatures). Given all reviewed literature, it seems plausible that an additional increase in frontal theta during encoding or increase in posterior alpha in anticipation of the stimulus would further boost working memory performance.

Neurofeedback on gamma oscillations has been studied by Keizer et al. (2010), who found that successful increases in gamma band power in young, healthy adults correlated with an increase in fluid intelligence and reduced cost of feature binding reflected in the lower reaction times. In line with the current view on feature-binding problems in the elderly (cf. Sander et al., 2012a), Staufenbiel et al. (2014) used neurofeedback training of gamma oscillations in the elderly. Although the neurofeedback training resulted in increased gamma, they failed to show a beneficial nature of this training for fluid intelligence, working memory, and quality of life. However, these studies did not feedback gamma power during stimulation, but during resting state. It might have been beneficial to give feedback of gamma power during stimulation, as the neural sources processing stimuli are likely to differ from resting state sources, when no stimulus is being processed. No studies on training gamma band in ADHD patients have been conducted.

For a more complete overview on neurofeedback studies, the interested reader is referred to Gruzelier (2013). In general, we advise that neurofeedback studies should follow hypotheses and paradigms that are more focused and grounded in insights gained from fundamental and cognitive research conducted in the last decades, in particular using information on the functional role of neuronal oscillations.

BRAIN STATE DEPENDENT TASKS (BSDT): ADAPTING THE ENVIRONMENT TO THE USER'S MENTAL STATE

The former approach aimed at shaping the user's brain activity for optimal stimulus processing or task performance. Recent insight in the field of cognitive neuroscience (see Section Functional Role of Neuronal Oscillations) suggests that we can predict cognitive behavior by neuronal oscillations. This knowledge can be used to adapt the task environment based on the user's current brain activity to allow for optimal performance. Ultimately this could aid the user to develop an optimal brain state more quickly or efficiently. Specifically, the timing and properties of the task would be determined by an online read-out of the current brain state, as quantified by ongoing neuronal oscillatory activity (Hartmann et al., 2011; Jensen et al., 2011). It is even possible to combine this with active brain stimulation (Silvanto and Pascual-Leone, 2008), which was recently demonstrated by Gharabaghi et al. (2014), who applied TMS and provided haptic feedback according to relevant neural oscillatory activity.

Brain state dependent tasks (BSDT) serve two purposes. First, by adapting the environment to the ongoing brain activity, individual cognition could be improved as described below. Second, BSDT informs the user about his ongoing brain state and rewards the user for a "good" brain state in a similar manner as in BCI. BSDT therefore could help the subject's ability to modulate his brain activity to reach a certain mind setting, or brain state. BSDT can be used in two complementary manners. First, stimulus presentation can be triggered to ongoing oscillatory activity. For example, it has been found that strong prestimulus alpha power in task-relevant regions negatively affects subsequent stimulus processing (e.g., Ergenoglu et al., 2004; Hanslmayr et al., 2005a, 2007; van Dijk et al., 2008; Mazaheri et al., 2009). In a BSDT paradigm, stimulus presentation could thus be triggered only when alpha power is relatively low, thereby increasing efficiency of stimulus processing. A reverse rationale applies, where the task is to inhibit some aspect of the environment: stimulate during high alpha power in task-irrelevant brain regions. For example, background speech might be distracting when visually learning vocabularies. Thus, in a BSDT environment one could first reward high temporal alpha by removing some artificially-added, auditory distraction while visually presenting vocabularies and, in a second step, additionally only present vocabularies when posterior alpha power is low. This could lead to increased processing of the visually presented vocabularies and also increased inhibition of the distracting auditory noise. To generalize this idea, training could be provided in a variety of tasks where the direction and location of alpha modulation varies; this way, it is the control of alpha that is important and learned, not just a focal or unidirectional lesson which might interfere with other tasks. The subject thereby learns the skill to consciously modulate brain oscillations in similar manner to neurofeedback.

A second manner for BSDT is the fact that based on the activity during stimulus processing or maintenance, subsequent behavior can be predicted, as already proposed e.g., by Mazaheri et al. (2009). For example, increased alpha activity during visual processing (Park et al., 2014) and during the retention interval (Meeuwissen et al., 2011) has been shown to strongly correlate with long-term memory encoding performance. Based on these findings, one could predict which items are most likely to be forgotten and present these items again to the subject in order to facilitate long-term memory performance. In addition, measures correlating with memory load, e.g., frontal theta or occipital gamma for visual items, can be read out to predict the current load. This could also be used to prevent memory overload (discussed in Huggins et al., 2014). This second manner represents an online adaptation of the environment and cannot necessarily be utilized offline or result in an offline skill.

For young, healthy adults, strong correlations between oscillatory power and cognition have been found as reviewed in Sections Functional Role of Neuronal Oscillations and Techniques to Manipulate Neuronal Oscillations. However, in Section Population Target Groups and Their Neuronal Signatures we also review evidence that this correlation seems to be absent in the elderly and ADHD patients. In these population groups, compensatory

mechanisms might have taken over the function that some oscillations usually represent. While brain stimulation techniques aim to restore the beneficial nature of these oscillations, BSDT aims to predict cognition. It remains to be tested whether BSDT is beneficial for the elderly and ADHD patients when utilizing hypotheses based on another population group. Both manners of applying BSDT—waiting with stimulation until a good distribution of oscillatory power and predicting subsequent behavior by the distribution of oscillatory power—might prove useless, without a correlation between oscillation and behavior. We would therefore advise to identify neural signatures of the compensatory mechanisms, and find appropriate hypotheses for these population groups, or to manipulate the neural oscillations as proposed in the previous two subsections before applying BSDT in a patient group.

PRACTICAL CONSIDERATIONS

In the previous section we made some concrete suggestions on how insights from cognitive neuroscience can be applied to augment human behavior. In this section, we will discuss how to define successful interventions, outline practical considerations about our hypothesis that should be regarded when following above suggestions, suggest alternative approaches, and point to possible pitfalls when applying these techniques in the lab, at home, or when valorizing these ideas for commercial use. For ethical considerations, we encourage the reader to read the Nuffield report on neurotechnology (Nuffield Councils on Bioethics, 2013).

DEFINING AND ASSESSING SUCCESS

We have collected supporting evidence for hypothesis-driven approaches to augment human behavior. Although literature on this topic is relatively sparse, studies often differ in crucial aspects such as motivation for the study (i.e., the original hypothesis), methodology (e.g., control conditions, number or duration of training sessions, or the number of hours or days after training to test for long-term effects), and conclusions on how to generalize the findings. The most important question to ask when drawing conclusions about these studies is how best to quantify if the applied technique resulted in augmentation of cognition. This question can be disentangled into two parts.

First, we need to quantify that the augmentation effect is caused by the applied technique and not by confounding other reasons. For example, proper control conditions in neurofeedback settings yield results similar to neurofeedback protocols often used for ADHD treatment (van Dongen-Boomsma et al., 2013; Vollebregt et al., 2014). Thus, special care needs to be taken when attributing the beneficial effect to the applied technique, and that the effect is specific to the modulated frequency-band. As another example, it has been shown that auditory perception of the clicking sound of TMS stimulation alone is sufficient to induce an effect in visual cortex (Romei et al., 2012). Other control conditions aside from sham stimulation, such as cognitive behavioral training (Safren et al., 2010; Strenziok et al., 2014) or physical exercise (Halperin and Healey, 2011; Verret et al., 2012), are often more easily applied than brain stimulation techniques. Furthermore, it is very important to be aware of misleading causes of experimental observations, such as temporal ordering of the tasks; a randomized design order is a crucial part of any paradigm.

Second, we need to define what we mean by augmentation of cognition. We first need to define a baseline level of performance before starting the intervention and we need to show a strong, significant increase from this baseline, above the appropriate control discussed above. Additionally, an improvement in one skill might come at a cost in another skill (Brem et al., 2014; Reinhart and Woodman, 2014). Therefore, one core assertion of successful augmentation of human cognition needs to be measured by transfer learning (Klingberg et al., 2002; Dahlin et al., 2008; Klingberg, 2010), i.e., quantifying how the intervention translates to other domains that were not explicitly trained and tempered by any deficits gained in other domains. In other words, one need to clearly establish the scope of the intervention and what does and does not work.

THE DANGER OF UNDESIRED SIDE EFFECTS

One important aspect to consider when proposing any study with stimulation is to *a priori* think about which brain region to stimulate and for what purpose. In the vast majority of this article, we talked about how to manipulate neuronal oscillations, but for successful augmentation of human cognition one needs to understand that the human brain is organized into different cortical and subcortical structures and each serves multiple, partly overlapping functionalities. When considering augmenting human cognition, one needs to be precise on which part of human cognition. Let us take the example of increased alpha power in task-irrelevant regions. Firstly, one needs to define "task-irrelevant"; for example, in a covert visual spatial attention task, the posterior ipsilateral hemisphere is "task-irrelevant", but not the contralateral hemisphere (Worden et al., 2000; Thut et al., 2006; Kelly et al., 2009). Secondly, the natural region(s) exerting modulation of the task-irrelevant region(s) should be noted; for example, it has been found that the intraparietal sulcus and the frontal eye fields exert top-down control on posterior areas and that stimulating them has consequences for posterior alpha and subsequent behavior (Capotosto et al., 2009, 2012; Sauseng et al., 2011). Thirdly, stimulation of the "wrong" brain region can lead to unexpected, reversed effects, i.e., where stimulation of one brain region is beneficial for one task and impedimental for another task (Romei et al., 2010; Iuculano and Cohen Kadosh, 2013); for example, when conducting a purely auditory task, the whole visual cortex becomes "task-irrelevant". Note that the anatomical precision of non-invasive EEG recordings is not high enough to verify the spatial specificity of the measured oscillations. While source reconstruction techniques can increase spatial certainty beyond sensor level information, invasive recordings are necessary for precise spatial localization.

Additional side effects can arise from improper task settings. For example, one might think to save time by concurrently testing for items in memory while memorizing new items. This, however, has been found to be inefficient and led to deteriorated memory performance (Huijbers et al., 2009). In addition, the functional hypothesis has to be correct and grounded in previous findings. For example, while many studies have convincingly related alpha power with inhibition of task-irrelevant regions, this might not

hold true for all brain regions (Mo et al., 2011). Thus the exact experimental paradigm has to be vigorously thought through, which requires intensive knowledge from an expert in the field of cognitive neuroscience and/or brain stimulation or hdBCI techniques. Therefore, one needs to be sure which region requires which treatment (e.g., excitation is distinct from release from inhibition) in order to augment cognition successfully without burdensome side effects.

Non-optimal stimulation protocols and task settings might not be the only cause for side effects. As the mechanism behind electromagnetic brain stimulation is not fully understood, a number of unforeseen side effects can occur. Manufacturers restrict the maximum amplitude in their amplifier to a rather low value to reduce the possible risks (e.g., infrequent reports of inducing seizures, kindling, mood changes and scalp burnings) which can be further minimized by following general guidelines (Wassermann, 1998; Rossi et al., 2009). In addition, effects of long-term electromagnetic brain stimulation are rarely studied and not well understood. As Antal and Paulus (2013) wrote about the motor evoked potential (MEP): "Increasing the duration of tDCS results in a prolongation of the induced aftereffects (Nitsche and Paulus, 2000) up to about 13 min whereas doubling the 13 stimulation to 26 min inverses MEP aftereffects into inhibition (Batsikadze et al., 2013). It is unclear if this can be translated to tACS, too". While an aftereffect reversal is an obvious crucial side-effect and would be undesired, e.g., in the case of attention boosting, even stronger side effects might occur, especially due to the unknown long-term effects on plasticity and anatomical and functional connectivity. Plastic white matter changes in humans have been found following behavioral training (Zatorre et al., 2012; Sampaio-Baptista et al., 2013), and there is some preliminary evidence for white matter changes following electric stimulation (Allendorfer et al., 2012) and neurofeedback (Ghaziri et al., 2013).

INDIVIDUAL DIFFERENCES

Cognitive neuroscience aims to infer general mechanisms of the brain by studying a subgroup of some homogenous population. Significant statistical tests using random effects analysis then allow making inference from the subgroup to the population. However, finding a group level effect does not automatically mean that all individuals show the same effect, or even a significant effect. An example is found in the strength and adaptation of alpha lateralization (e.g., Händel et al., 2011; Horschig et al., 2014). Inter-individual variability has extensively been described as an issue in the field of BCI, where it has been found that about 1/5 of all individuals are unable to gain control over the control signal (Dickhaus et al., 2009; Vidaurre and Blankertz, 2010). A similar kind of inter-subject variability is reported in tCS techniques, which might arise due to a multitude of factors (Horvath et al., 2014; Krause and Cohen Kadosh, 2014). Thus, while we are proposing that these techniques can be used to decrease interindividual variability, it might be that interindividual variability requires different solutions for different subjects, e.g., different frequency bands or a different location of stimulation sites.

ALTERNATIVE ASPECTS OF NEURONAL OSCILLATIONS TO UTILIZE

In this review, we focused on the region-specific power of neuronal oscillations because, as discussed in Section Functional Role of Neuronal Oscillations, they are a robust read-out of the brain state and show strong across-trials correlations with behavior. However, investigating the power of neuronal oscillations is not the only means of quantifying electrophysiological data. The alternatives we discuss below, as well as others not mentioned, may also provide a handle with which to augment cognition via manipulation as discussed in Section Techniques to Manipulate Neuronal Oscillations, if a suitable hypothesis can be formed linking neural activity to behavior. We believe however that our main point has been sufficiently illustrated by the examples (i.e., oscillatory power and patient groups) used.

One natural alternative to power is the phase. In particular, the phase of the alpha and theta oscillations has been studied intensively in the past (Buzsáki and Draguhn, 2004; Montemurro et al., 2008; VanRullen et al., 2014). Recent evidence suggests that specific phases are more optimal for specific tasks (Gho and Varela, 1988; Kruglikov and Schiff, 2003; Mathewson et al., 2009). For example, ignoring a distracter is more successful in a specific phase of alpha oscillations than in its opposed phase (Bonnefond and Jensen, 2012). Interestingly, it has also been shown that tACS can be used to gain control over the phase of an oscillation (Helfrich et al., 2014; Jaegle and Ro, 2014; Strüber et al., 2014; Zanto et al., 2014), which has corresponding, orthogonal behavioral effects. Recent studies also indicate that a visual stimulus regularly flickering at 10 Hz can entrain alpha oscillations in visual cortex outlasting the stimulation period and also that subsequent behavior was modulated in a phasic manner according to the phase of the stimulation flickering (Mathewson et al., 2012; de Graaf et al., 2013; Spaak et al., 2014). In conclusion, studying the phasic nature of oscillations will provide further insight into human perception and provide important, additional information for brain stimulation techniques, hypothesis-driven brain-computer interfaces, and BSDT.

Furthermore another quantification of oscillations includes cross-frequency phase-amplitude coupling, whereby the phase of a slower oscillation is linked to the amplitude of a faster oscillation. For example it has been shown that gamma power waxes and wanes with the phase of alpha or theta oscillations (Canolty et al., 2006; Jensen et al., 2012; Bonnefond and Jensen, 2013; Lisman and Jensen, 2013; Roux and Uhlhaas, 2014). This also has important implications for behavior as, already discussed briefly in Section Functional Role of Neuronal Oscillations, the ratio between a theta cycle and a gamma cycle has been suggested to determine working memory capacity (Jensen and Lisman, 1996, 1998; Kamiński et al., 2011). This knowledge could be used to optimize cross-frequency coupling in order to study its direct effect on behavior.

In addition, we also chose not to focus on connectivity between brain regions, which can be a phase adjustment across regions (Varela et al., 2001; Gross et al., 2004; Fries, 2005; Sauseng and Klimesch, 2008) or a simultaneous power adjustment (e.g., Mazaheri et al., 2009). For example, several studies found a different functional connectivity pattern in the elderly compared to healthy, young adults (Hogan et al., 2011; Onoda et al., 2012;

Geerligs et al., 2013, 2014; Oh and Jagust, 2013; Waring et al., 2013). In children with ADHD compared to typically developing children the pattern of functional connectivity is also different (Murias et al., 2007; Mazaheri et al., 2010). Further evidence for the importance of functional connectivity comes from TMS/tCS studies showing that not only is the activity in the stimulated region modified, but also connectivity of the stimulated region to other regions (Strens et al., 2002; Polanía et al., 2012a; Veniero et al., 2013; Shafi et al., 2014) and in some cases this altered connectivity correlated with task modulations (Vidal-Piñeiro et al., 2014) or improved/altered behavior (Lee and D'Esposito, 2012). As the human brain is a huge network of neurons, it seems logical that an optimization of functional connectivity within and across brain-regions is a crucial aspect to optimize human behavior. However, we have just begun to understand the mechanisms behind inter- and intra-regional coupling (Felleman and Essen, 1991; Varela et al., 2001; Callaway, 2004; Fox et al., 2005; Canolty et al., 2006; Lakatos et al., 2008; Schroeder and Lakatos, 2009; Maier et al., 2010; Haegens et al., 2011b; Spaak et al., 2012), so we still have a long way to go until we fully grasp the effect that artificially manipulating functional connectivity has on the human brain and human cognition.

PROBLEMS OF USAGE AT HOME

Applying some of these techniques at home can be a challenge on its own. The most obvious question to ask is whether the proposed techniques can be used alone at home or whether an expert, e.g., a neuroscientist or a physician with proper training, should be visited. Currently, there is a trend in crowd funding and open source projects, which allow individuals to propose and share ideas. This has led to projects such as OpenrTMS[2] or OpenBCI,[3] which in theory allow everyone to create their own TMS or BCI protocol. As the human brain is overly complex, however, special care has to be taken on this path of neurohacking.[4] Obviously side-effects, as discussed above, are likely to occur during improper application. Apart from that, additional problems can be expected when applying the discussed brain augmentation techniques at home, e.g., motivational reasons can cause irregular, inefficient use and eventually lead to stopping the treatment. In addition, environmental noise may influence the measurements at home differently than in a well-controlled laboratory. Studies in the lab under special, controlled circumstances might not easily transfer to use at home or other situations of daily life (see e.g., Vaughan et al., 2006). Therefore and especially due to medical reasons we would propose that, first, more research is required to investigate side-effects and long-term effects. Second, application should only be administered by trained experts. Only in the far future does usage at home seem realistic.

CONSIDERATIONS FOR SUCCESSFUL VALORIZATION AND COMMERCIALIZATION

The above suggestions and hypotheses might seem like a great promise for augmentation of human behavior, so it is natural that commercial companies will pick up on these ideas in the near future. However, when considering augmentation of human behavior from a commercial perspective, there are additional considerations apart from those already discussed, foremost safety issues. Modern advertisements focus on short, catchy messages to attract potential customers (Dahlén and Rosengren, 2005; Kohli et al., 2007). The human brain, however, is a complicated machine and we are just beginning to understand its fundamental mechanisms and functions. Companies have to take responsibility for the products they sell and be wary of linking false promises to their commercial products. Also, companies need to be explicit to the customer on the consequences of not using devices properly as intended. Yet, there is no consensus on proper use of brain stimulation technique. Thus, extreme caution needs to be exercised before bringing a product onto the market. We therefore advise any company with serious perspectives on developing and selling these devices to collaborate strongly with established neuroscientists in the field and ethical committees and to conduct extensive studies on their products.

CONCLUSIONS

In this review, we started from the hypothesis that neuronal oscillations serve as strong neuronal correlates of behavior and are involved in human cognition. We reviewed this hypothesis in light of three target groups: the healthy elderly, ADHD patients, and healthy young adults. Most of the evidence supporting our hypothesis stemmed from studies on healthy young adults showing reliable correlations between the power of the oscillations and cognitive aspects of human behavior, e.g., working memory capacity or detection accuracy. Our hypothesis was corroborated by brain stimulation studies using TMS and tACS showing a strong link between the strength of induced neuronal oscillations and behavioral performance (see Section Techniques to Manipulate Neuronal Oscillations).

This hypothesis has important implications for the realm of cognitive neuroscience: if an oscillation is causally involved in cognition, a manipulation of that oscillation has to lead to a subsequent change in behavior. Such a strong, functional hypothesis helps to drive the field of cognitive neuroscience forward in understanding how the human brain functions.

Further, we hypothesized in Section Population Target Groups and Their Neuronal Signatures that the elderly and ADHD patients suffer from a lack of integrating neuronal oscillations properly, due to an absence of correlation with behavior, and that compensatory mechanisms could have taken over the functional shaping of cognition. We further hypothesized that manipulating neuronal oscillations in these target groups might restore their beneficial nature. This is a far-fetched claim without any empirical evidence and should be further studied. First of all, we need to ask whether the absence of the correlation between neuronal oscillations and behavior still allows the control and modulation of oscillations. Further, if these target groups could increase some neuronal oscillation during tasks, would that have behavioral consequences? Although we hypothesized that there would be, even if there would not be a behavioral benefit, we would gain important, fundamental insight in different neuronal mechanisms to shape human cognition.

[2]http://open-rtms.sourceforge.net/
[3]http://www.openbci.com/
[4]http://en.wikipedia.org/wiki/Neurohacking

In sum, we conclude that many more studies need to be conducted and reproduced, in both young, healthy and other target groups, to elucidate the role of and effects of manipulation of neuronal oscillations on behavior. Further insight from fundamental research into neuronal mechanisms is required to develop robust products for augmenting cognition.

ACKNOWLEDGMENTS

The authors would like to thank Madelon Vollebregt for useful feedback and discussion on Section Attention Deficit/Hyperactivity Disorder, and Til Ole Bergmann for useful feedback and discussion on Section Transcranial Stimulation of the Human Brain. The authors are or have been supported by a VICI grant of the Organization for Scientific Research (NWO) to Prof. Ole Jensen under grant number 453-09-002, and the research program "The healthy brain" funded by the Netherlands Initiative Brain and Cognition (NIHC), a part of NWO, to Prof. Ole Jensen under grant number 056-14-011. All authors declare no conflict of interest or other financial support.

REFERENCES

Adams, Z. W., Derefinko, K. J., Milich, R., and Fillmore, M. T. (2008). Inhibitory functioning across ADHD subtypes: recent findings, clinical implications and future directions. *Dev. Disabil. Res. Rev.* 14, 268–275. doi: 10.1002/ddrr.37

Addante, R. J., Watrous, A. J., Yonelinas, A. P., Ekstrom, A. D., and Ranganath, C. (2011). Prestimulus theta activity predicts correct source memory retrieval. *Proc. Natl. Acad. Sci. U S A* 108, 10702–10707. doi: 10.1073/pnas.1014528108

Adrian, E. D., and Matthews, B. H. C. (1934). The berger rhythm: potential changes from the occipital lobes in man. *Brain* 57, 355–385. doi: 10.1093/brain/57.4.355

Alain, C., and Woods, D. L. (1999). Age-related changes in processing auditory stimuli during visual attention: evidence for deficits in inhibitory control and sensory memory. *Psychol. Aging* 14, 507–519. doi: 10.1037//0882-7974.14.3.507

Allendorfer, J. B., Storrs, J. M., and Szaflarski, J. P. (2012). Changes in white matter integrity follow excitatory rTMS treatment of post-stroke aphasia. *Restor. Neurol. Neurosci.* 30, 103–113. doi: 10.3233/RNN-2011-0627

Andrés, P., Parmentier, F. B. R., and Escera, C. (2006). The effect of age on involuntary capture of attention by irrelevant sounds: a test of the frontal hypothesis of aging. *Neuropsychologia* 44, 2564–2568. doi: 10.1016/j.neuropsychologia.2006.05.005

Antal, A., and Paulus, W. (2013). Transcranial alternating current stimulation (tACS). *Front. Hum. Neurosci.* 7:317. doi: 10.3389/fnhum.2013.00317

Bahramisharif, A., van Gerven, M., Heskes, T., and Jensen, O. (2010). Covert attention allows for continuous control of brain-computer interfaces. *Eur. J. Neurosci.* 31, 1501–1508. doi: 10.1111/j.1460-9568.2010.07174.x

Bai, S., Dokos, S., Ho, K.-A., and Loo, C. (2014). A computational modelling study of transcranial direct current stimulation montages used in depression. *Neuroimage* 87, 332–344. doi: 10.1016/j.neuroimage.2013.11.015

Barkley, R. A. (2001). The Inattentive type of ADHD As a distinct disorder: what remains to be done. *Clin. Psychol. Sci. Pract.* 8, 489–493. doi: 10.1093/clipsy.8.4.489

Barkley, R. A., DuPaul, G. J., and McMurray, M. B. (1990). Comprehensive evaluation of attention deficit disorder with and without hyperactivity as defined by research criteria. *J. Consult. Clin. Psychol.* 58, 775–789. doi: 10.1037//0022-006x.58.6.775

Barry, R. J., Clarke, A. R., and Johnstone, S. J. (2003). A review of electrophysiology in attention-deficit/hyperactivity disorder: I. Qualitative and quantitative electroencephalography. *Clin. Neurophysiol.* 114, 171–183. doi: 10.1016/s1388-2457(02)00362-0

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M.-F., and Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bauer, M., Oostenveld, R., Peeters, M., and Fries, P. (2006). Tactile spatial attention enhances gamma-band activity in somatosensory cortex and reduces low-frequency activity in parieto-occipital areas. *J. Neurosci.* 26, 490–501. doi: 10.1523/JNEUROSCI.5228-04.2006

Bengson, J. J., Mangun, G. R., and Mazaheri, A. (2012). The neural markers of an imminent failure of response inhibition. *Neuroimage* 59, 1534–1539. doi: 10.1016/j.neuroimage.2011.08.034

Berger, P. D. H. (1929). Über das Elektrenkephalogramm des Menschen. *Arch. Für Psychiatr. Nervenkrankh.* 87, 527–570.

Bijsterbosch, J. D., Barker, A. T., Lee, K.-H., and Woodruff, P. W. R. (2012). Where does transcranial magnetic stimulation (TMS) stimulate? Modelling of induced field maps for some common cortical and cerebellar targets. *Med. Biol. Eng. Comput.* 50, 671–681. doi: 10.1007/s11517-012-0922-8

Bollimunta, A., Mo, J., Schroeder, C. E., and Ding, M. (2011). Neuronal mechanisms and attentional modulation of corticothalamic α oscillations. *J. Neurosci.* 31, 4935–4943. doi: 10.1523/jneurosci.5580-10.2011

Bonnefond, M., and Jensen, O. (2012). Alpha oscillations serve to protect working memory maintenance against anticipated distracters. *Curr. Biol.* 22, 1969–1974. doi: 10.1016/j.cub.2012.08.029

Bonnefond, M., and Jensen, O. (2013). The role of gamma and alpha oscillations for blocking out distraction. *Commun. Integr. Biol.* 6:e22702. doi: 10.4161/cib.22702

Booth, J. E., Carlson, C. L., and Tucker, D. M. (2007). Performance on a neurocognitive measure of alerting differentiates ADHD combined and inattentive subtypes: a preliminary report. *Arch. Clin. Neuropsychol.* 22, 423–432. doi: 10.1016/j.acn.2007.01.017

Brem, A.-K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *Neuroimage* 85(Pt. 3), 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Brignani, D., Ruzzoli, M., Mauri, P., and Miniussi, C. (2013). Is transcranial alternating current stimulation effective in modulating brain oscillations? *PLoS One* 8:e56589. doi: 10.1371/journal.pone.0056589

Brockmole, J. R., and Logie, R. H. (2013). Age-related change in visual working memory: a study of 55,753 participants aged 8–75. *Front. Psychol.* 4:12. doi: 10.3389/fpsyg.2013.00012

Buzsáki, G. (2006). *Rhythms of the Brain.* New York, NY: Oxford University Press.

Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science* 304, 1926–1929. doi: 10.1126/science.1099745

Cabeza, R., Anderson, N. D., Locantore, J. K., and McIntosh, A. R. (2002). Aging gracefully: compensatory brain activity in high-performing older adults. *Neuroimage* 17, 1394–1402. doi: 10.1006/nimg.2002.1280

Calderone, D. J., Lakatos, P., Butler, P. D., and Castellanos, F. X. (2014). Entrainment of neural oscillations as a modifiable substrate of attention. *Trends Cogn. Sci.* 18, 300–309. doi: 10.1016/j.tics.2014.02.005

Callaway, E. M. (2004). Feedforward, feedback and inhibitory connections in primate visual cortex. *Neural Netw.* 17, 625–632. doi: 10.1016/j.neunet.2004.04.004

Canolty, R. T., Edwards, E., Dalal, S. S., Soltani, M., Nagarajan, S. S., Kirsch, H. E., et al. (2006). High gamma power is phase-locked to theta oscillations in human neocortex. *Science* 313, 1626–1628. doi: 10.1126/science.1128115

Cantwell, D. P. (1996). Attention deficit disorder: a review of the past 10 years. *J. Am. Acad. Child Adolesc. Psychiatry* 35, 978–987. doi: 10.1097/00004583-199608000-00008

Capotosto, P., Babiloni, C., Romani, G. L., and Corbetta, M. (2009). Frontoparietal cortex controls spatial attention through modulation of anticipatory alpha rhythms. *J. Neurosci.* 29, 5863–5872. doi: 10.1523/jneurosci.0539-09.2009

Capotosto, P., Babiloni, C., Romani, G. L., and Corbetta, M. (2012). Differential contribution of right and left parietal cortex to the control of spatial attention: a simultaneous EEG-rTMS study. *Cereb. Cortex* 22, 446–454. doi: 10.1093/cercor/bhr127

Carr, L., Henderson, J., and Nigg, J. T. (2010). Cognitive control and attentional selection in adolescents with ADHD versus ADD. *J. Clin. Child Adolesc. Psychol.* 39, 726–740. doi: 10.1080/15374416.2010.517168

Cavanagh, J. F., Frank, M. J., Klein, T. J., and Allen, J. J. B. (2010). Frontal theta links prediction errors to behavioral adaptation in reinforcement learning. *Neuroimage* 49, 3198–3209. doi: 10.1016/j.neuroimage.2009.11.080

Chanes, L., Quentin, R., Tallon-Baudry, C., and Valero-Cabré, A. (2013). Causal Frequency-specific contributions of frontal spatiotemporal patterns induced by non-invasive neurostimulation to human visual performance. *J. Neurosci.* 33, 5000–5005. doi: 10.1523/JNEUROSCI.4401-12.2013

Chang, Z., Lichtenstein, P., and Larsson, H. (2012). The effects of childhood ADHD symptoms on early-onset substance use: a Swedish twin study. *J. Abnorm. Child Psychol.* 40, 425–435. doi: 10.1007/s10802-011-9575-6

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85(Pt. 3), 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Cohen, J. E. (2005). Human population grows up. *Sci. Am.* 293, 48–55. doi: 10.1038/scientificamerican0905-48

Cohen, M. X., Axmacher, N., Lenartz, D., Elger, C. E., Sturm, V., and Schlaepfer, T. E. (2009). Nuclei accumbens phase synchrony predicts decision-making reversals following negative feedback. *J. Neurosci.* 29, 7591–7598. doi: 10.1523/jneurosci.5335-08.2009

Cohen, M. X., Bour, L., Mantione, M., Figee, M., Vink, M., Tijssen, M. A. J., et al. (2012). Top-down-directed synchrony from medial frontal cortex to nucleus accumbens during reward anticipation. *Hum. Brain Mapp.* 33, 246–252. doi: 10.1002/hbm.21195

Dahlén, M., and Rosengren, S. (2005). Brands affect slogans affect brands? Competitive interference, brand equity and the brand-slogan link. *J. Brand Manag.* 12, 151–164. doi: 10.1057/palgrave.bm.2540212

Dahlin, E., Neely, A. S., Larsson, A., Bäckman, L., and Nyberg, L. (2008). Transfer of learning after updating training mediated by the striatum. *Science* 320, 1510–1512. doi: 10.1126/science.1155466

Dayan, E., Censor, N., Buch, E. R., Sandrini, M., and Cohen, L. G. (2013). Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat. Neurosci.* 16, 838–844. doi: 10.1038/nn.3422

de Graaf, T. A., Gross, J., Paterson, G., Rusch, T., Sack, A. T., and Thut, G. (2013). Alpha-Band rhythms in visual task performance: phase-locking by rhythmic sensory stimulation. *PLoS One* 8:e60035. doi: 10.1371/journal.pone.0060035

Deary, I. J., Corley, J., Gow, A. J., Harris, S. E., Houlihan, L. M., Marioni, R. E., et al. (2009). Age-associated cognitive decline. *Br. Med. Bull.* 92, 135–152. doi: 10.1093/bmb/ldp033

Derefinko, K. J., Adams, Z. W., Milich, R., Fillmore, M. T., Lorch, E. P., and Lynam, D. R. (2008). Response style differences in the inattentive and combined subtypes of attention-deficit/hyperactivity disorder. *J. Abnorm. Child Psychol.* 36, 745–758. doi: 10.1007/s10802-007-9207-3

Dickhaus, T., Sannelli, C., Müller, K.-R., Curio, G., and Blankertz, B. (2009). Predicting BCI performance to study BCI illiteracy. *BMC Neurosci.* 10:84. doi: 10.1186/1471-2202-10-s1-p84

Dustman, R. E., Shearer, D. E., and Emmerson, R. Y. (1993). EEG and event-related potentials in normal aging. *Prog. Neurobiol.* 41, 369–401. doi: 10.1016/0301-0082(93)90005-d

Engel, A. K., Fries, P., and Singer, W. (2001). Dynamic predictions: oscillations and synchrony in top-down processing. *Nat. Rev. Neurosci.* 2, 704–716. doi: 10.1038/35094565

Engel, A. K., and Singer, W. (2001). Temporal binding and the neural correlates of sensory awareness. *Trends Cogn. Sci.* 5, 16–25. doi: 10.1016/s1364-6613(00)01568-0

Enriquez-Geppert, S., Huster, R. J., Scharfenort, R., Mokom, Z. N., Zimmermann, J., and Herrmann, C. S. (2014). Modulation of frontal-midline theta by neurofeedback. *Biol. Psychol.* 95, 59–69. doi: 10.1016/j.biopsycho.2013.02.019

Ergenoglu, T., Demiralp, T., Bayraktaroglu, Z., Ergen, M., Beydagi, H., and Uresin, Y. (2004). Alpha rhythm of the EEG modulates visual detection performance in humans. *Brain Res. Cogn. Brain Res.* 20, 376–383. doi: 10.1016/j.cogbrainres.2004.03.009

Fabiani, M., Low, K. A., Wee, E., Sable, J. J., and Gratton, G. (2006). Reduced suppression or labile memory? Mechanisms of inefficient filtering of irrelevant information in older adults. *J. Cogn. Neurosci.* 18, 637–650. doi: 10.1162/jocn.2006.18.4.637

Fell, J., Ludowig, E., Staresina, B. P., Wagner, T., Kranz, T., Elger, C. E., et al. (2011). Medial temporal theta/alpha power enhancement precedes successful memory encoding: evidence based on intracranial EEG. *J. Neurosci.* 31, 5392–5397. doi: 10.1523/jneurosci.3668-10.2011

Felleman, D. J., and Essen, D. C. V. (1991). Distributed hierarchical processing in the primate. *Cereb. Cortex* 1, 1–47. doi: 10.1093/cercor/1.1.1

Folk, C. L., and Lincourt, A. E. (1996). The effects of age on guided conjunction search. *Exp. Aging Res.* 22, 99–118. doi: 10.1080/03610739608254000

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Essen, D. C. V., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U S A* 102, 9673–9678. doi: 10.1073/pnas.0504136102

Foxe, J. J., and Snyder, A. C. (2011). The role of alpha-band brain oscillations as a sensory suppression mechanism during selective attention. *Front. Psychol.* 2:154. doi: 10.3389/fpsyg.2011.00154

Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *Trends Cogn. Sci.* 9, 474–480. doi: 10.1016/j.tics.2005.08.011

Fries, P., Reynolds, J. H., Rorie, A. E., and Desimone, R. (2001). Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291, 1560–1563. doi: 10.1126/science.1055465

Fründ, I., Busch, N. A., Schadow, J., Körner, U., and Herrmann, C. S. (2007). From perception to action: phase-locked gamma oscillations correlate with reaction times in a speeded response task. *BMC Neurosci.* 8:27. doi: 10.1186/1471-2202-8-27

Fu, K. M., Foxe, J. J., Murray, M. M., Higgins, B. A., Javitt, D. C., and Schroeder, C. E. (2001). Attention-dependent suppression of distracter visual input can be cross-modally cued as indexed by anticipatory parieto-occipital alpha-band oscillations. *Brain Res. Cogn. Brain Res.* 12, 145–152. doi: 10.1016/s0926-6410(01)00034-9

Fuchs, T., Birbaumer, N., Lutzenberger, W., Gruzelier, J. H., and Kaiser, J. (2003). Neurofeedback treatment for attention-deficit/hyperactivity disorder in children: a comparison with methylphenidate. *Appl. Psychophysiol. Biofeedback* 28, 1–12. doi: 10.1023/A:1022353731579

Gaeta, H., Friedman, D., Ritter, W., and Cheng, J. (2001). An event-related potential evaluation of involuntary attentional shifts in young and older adults. *Psychol. Aging* 16, 55–68. doi: 10.1037//0882-7974.16.1.55

Gazzaley, A., Clapp, W., Kelley, J., McEvoy, K., Knight, R. T., and D'Esposito, M. (2008). Age-related top-down suppression deficit in the early stages of cortical visual memory processing. *Proc. Natl. Acad. Sci.* 105, 13122–13126. doi: 10.1073/pnas.0806074105

Geerligs, L., Maurits, N. M., Renken, R. J., and Lorist, M. M. (2014). Reduced specificity of functional connectivity in the aging brain during task performance. *Hum. Brain Mapp.* 35, 319–330. doi: 10.1002/hbm.22175

Geerligs, L., Saliasi, E., Renken, R. J., Maurits, N. M., and Lorist, M. M. (2013). Flexible connectivity in the aging brain revealed by task modulations. *Hum. Brain Mapp.* doi: 10.1002/hbm.22437. [Epub ahead of print].

Gevins, A., and Smith, M. E. (2000). Neurophysiological measures of working memory and individual differences in cognitive ability and cognitive style. *Cereb. Cortex* 10, 829–839. doi: 10.1093/cercor/10.9.829

Gevins, A., Smith, M. E., McEvoy, L., and Yu, D. (1997). High-resolution EEG mapping of cortical activation related to working memory: effects of task difficulty, type of processing and practice. *Cereb. Cortex* 7, 374–385. doi: 10.1093/cercor/7.4.374

Gharabaghi, A., Kraus, D., Leao, M. T., Spüler, M., Walter, A., Bogdan, M., et al. (2014). Coupling brain-machine interfaces with cortical stimulation for brain-state dependent stimulation: enhancing motor cortex excitability for neurorehabilitation. *Front. Hum. Neurosci.* 8:122. doi: 10.3389/fnhum.2014.00122

Ghaziri, J., Tucholka, A., Larue, V., Blanchette-Sylvestre, M., Reyburn, G., Gilbert, G., et al. (2013). Neurofeedback training induces changes in white and gray matter. *Clin. EEG Neurosci.* 44, 265–272. doi: 10.1177/1550059413476031

Gho, M., and Varela, F. J. (1988). A quantitative assessment of the dependency of the visual temporal frame upon the cortical rhythm. *J. Physiol. (Paris)* 83, 95–101.

Goodale, M. A., and Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends Neurosci.* 15, 20–25. doi: 10.1016/0166-2236(92)90344-8

Gould, I. C., Rushworth, M. F., and Nobre, A. C. (2011). Indexing the graded allocation of visuospatial attention using anticipatory alpha oscillations. *J. Neurophysiol.* 105, 1318–1326. doi: 10.1152/jn.00653.2010

Grandy, T. H., Werkle-Bergner, M., Chicherio, C., Lövdén, M., Schmiedek, F., and Lindenberger, U. (2013). Individual alpha peak frequency is related to latent factors of general cognitive abilities. *Neuroimage* 79, 10–18. doi: 10.1016/j.neuroimage.2013.04.059

Gross, J., Schmitz, F., Schnitzler, I., Kessler, K., Shapiro, K., Hommel, B., et al. (2004). Modulation of long-range neural synchrony reflects temporal limitations of visual attention in humans. *Proc. Natl. Acad. Sci. U S A* 101, 13050–13055. doi: 10.1073/pnas.0404944101

Groth, K. E., and Allen, P. A. (2000). Visual attention and aging. *Front. Biosci.* 5, D284–D297. doi: 10.2741/Groth

Gruzelier, J. H. (2013). EEG-neurofeedback for optimising performance. I: a review of cognitive and affective outcome in healthy participants. *Neurosci. Biobehav. Rev.* doi: 10.1016/j.neubiorev.2013.09.015

Haegens, S., Händel, B. F., and Jensen, O. (2011a). Top-down controlled alpha band activity in somatosensory areas determines behavioral performance in a discrimination task. *J. Neurosci.* 31, 5197–5204. doi: 10.1523/jneurosci.5199-10.2011

Haegens, S., Luther, L., and Jensen, O. (2012). Somatosensory anticipatory alpha activity increases to suppress distracting input. *J. Cogn. Neurosci.* 24, 677–685. doi: 10.1162/jocn_a_00164

Haegens, S., Nácher, V., Luna, R., Romo, R., and Jensen, O. (2011b). α-Oscillations in the monkey sensorimotor network influence discrimination performance by rhythmical inhibition of neuronal spiking. *Proc. Natl. Acad. Sci. U S A* 108, 19377–19382. doi: 10.1073/pnas.1117190108

Haegens, S., Osipova, D., Oostenveld, R., and Jensen, O. (2010). Somatosensory working memory performance in humans depends on both engagement and disengagement of regions in a distributed network. *Hum. Brain Mapp.* 31, 26–35. doi: 10.1002/hbm.20842

Halperin, J. M., and Healey, D. M. (2011). The influences of environmental enrichment, cognitive enhancement and physical exercise on brain development: can we alter the developmental trajectory of ADHD? *Neurosci. Biobehav. Rev.* 35, 621–634. doi: 10.1016/j.neubiorev.2010.07.006

Hamidi, M., Slagter, H. A., Tononi, G., Postle, B. R., Hamidi, M., Slagter, H. A., et al. (2009). Repetitive transcranial magnetic stimulation affects behavior by biasing endogenous cortical oscillations. *Front. Integr. Neurosci.* 3:14. doi: 10.3389/neuro.07.014.2009

Händel, B. F., Haarmeier, T., and Jensen, O. (2011). Alpha oscillations correlate with the successful inhibition of unattended stimuli. *J. Cogn. Neurosci.* 23, 2494–2502. doi: 10.1162/jocn.2010.21557

Hanslmayr, S., Aslan, A., Staudigl, T., Klimesch, W., Herrmann, C. S., and Bäuml, K.-H. (2007). Prestimulus oscillations predict visual perception performance between and within subjects. *Neuroimage* 37, 1465–1473. doi: 10.1016/j.neuroimage.2007.07.011

Hanslmayr, S., Klimesch, W., Sauseng, P., Gruber, W., Doppelmayr, M., Freunberger, R., et al. (2005a). Visual discrimination performance is related to decreased alpha amplitude but increased phase locking. *Neurosci. Lett.* 375, 64–68. doi: 10.1016/j.neulet.2004.10.092

Hanslmayr, S., Sauseng, P., Doppelmayr, M., Schabus, M., and Klimesch, W. (2005b). Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects. *Appl. Psychophysiol. Biofeedback* 30, 1–10. doi: 10.1007/s10484-005-2169-8

Hanslmayr, S., and Staudigl, T. (2014). How brain oscillations form memories–a processing based perspective on oscillatory subsequent memory effects. *Neuroimage* 85(Pt. 2), 648–655. doi: 10.1016/j.neuroimage.2013.05.121

Hanslmayr, S., Staudigl, T., and Fellner, M.-C. (2012). Oscillatory power decreases and long-term memory: the information via desynchronization hypothesis. *Front. Hum. Neurosci.* 6:74. doi: 10.3389/fnhum.2012.00074

Hartmann, T., Schulz, H., and Weisz, N. (2011). Probing of brain states in real-time: introducing the console environment. *Front. Psychol.* 2:36. doi: 10.3389/fpsyg.2011.00036

Helfrich, R. F., Schneider, T. R., Rach, S., Trautmann-Lengsfeld, S. A., Engel, A. K., and Herrmann, C. S. (2014). Entrainment of brain oscillations by transcranial alternating current stimulation. *Curr. Biol.* 24, 333–339. doi: 10.1016/j.cub.2013.12.041

Herrmann, C. S., Rach, S., Neuling, T., and Strüber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Front. Hum. Neurosci.* 7:279. doi: 10.3389/fnhum.2013.00279

Hogan, M., Collins, P., Keane, M., Kilmartin, L., Kaiser, J., Kenney, J., et al. (2011). Electroencephalographic coherence, aging and memory: distinct responses to background context and stimulus repetition in younger, older and older declined groups. *Exp. Brain Res.* 212, 241–255. doi: 10.1007/s00221-011-2726-8

Hoogenboom, N., Schoffelen, J.-M., Oostenveld, R., Parkes, L. M., and Fries, P. (2006). Localizing human visual gamma-band activity in frequency, time and space. *Neuroimage* 29, 764–773. doi: 10.1016/j.neuroimage.2005.08.043

Horschig, J. M., Jensen, O., van Schouwenburg, M. R., Cools, R., and Bonnefond, M. (2014). Alpha activity reflects individual abilities to adapt to the environment. *Neuroimage* 89, 235–243. doi: 10.1016/j.neuroimage.2013.12.018

Horvath, J. C., Carter, O., and Forte, J. D. (2014). Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Front. Syst. Neurosci.* 8:2. doi: 10.3389/fnsys.2014.00002

Howard, M. W., Rizzuto, D. S., Caplan, J. B., Madsen, J. R., Lisman, J., Aschenbrenner-Scheibe, R., et al. (2003). Gamma oscillations correlate with working memory load in humans. *Cereb. Cortex* 13, 1369–1374. doi: 10.1093/cercor/bhg084

Huang, Y.-Z., Edwards, M. J., Rounis, E., Bhatia, K. P., and Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron* 45, 201–206. doi: 10.1016/j.neuron.2004.12.033

Hugenschmidt, C. E., Mozolic, J. L., and Laurienti, P. J. (2009a). Suppression of multisensory integration by modality-specific attention in aging. *Neuroreport* 20, 349–353. doi: 10.1097/wnr.0b013e328323ab07

Hugenschmidt, C. E., Peiffer, A. M., McCoy, T. P., Hayasaka, S., and Laurienti, P. J. (2009b). Preservation of crossmodal selective attention in healthy aging. *Exp. Brain Res.* 198, 273–285. doi: 10.1007/s00221-009-1816-3

Huggins, J. E., Guger, C., Allison, B., Anderson, C. W., Batista, A., Brouwer, A.-M., et al. (2014). Workshops of the fifth international brain-computer interface meeting: defining the future. *Brain-Comput. Interfaces* 1, 27–49. doi: 10.1080/2326263x.2013.876724

Huijbers, W., Pennartz, C. M., Cabeza, R., and Daselaar, S. M. (2009). When learning and remembering compete: a functional MRI study. *PLoS Biol.* 7:e1000011. doi: 10.1371/journal.pbio.1000011

Iuculano, T., and Cohen Kadosh, R. (2013). The mental cost of cognitive enhancement. *J. Neurosci.* 33, 4482–4486. doi: 10.1523/JNEUROSCI.4927-12.2013

Jaegle, A., and Ro, T. (2014). Direct control of visual perception with phase-specific modulation of posterior parietal cortex. *J. Cogn. Neurosci.* 26, 422–432. doi: 10.1162/jocn_a_00494

Janssen, A. M., Rampersad, S. M., Lucka, F., Lanfer, B., Lew, S., Aydin, U., et al. (2013). The influence of sulcus width on simulated electric fields induced by transcranial magnetic stimulation. *Phys. Med. Biol.* 58, 4881–4896. doi: 10.1088/0031-9155/58/14/4881

Jaušovec, N., and Jaušovec, K. (2014). Increasing working memory capacity with theta transcranial alternating current stimulation (tACS). *Biol. Psychol.* 96, 42–47. doi: 10.1016/j.biopsycho.2013.11.006

Jaušovec, N., Jaušovec, K., and Pahor, A. (2014). The influence of theta transcranial alternating current stimulation (tACS) on working memory storage and processing functions. *Acta Psychol. (Amst)* 146, 1–6. doi: 10.1016/j.actpsy.2013.11.011

Jensen, O., Bahramisharif, A., Oostenveld, R., Klanke, S., Hadjipapas, A., Okazaki, Y. O., et al. (2011). Using brain-computer interfaces and brain-state dependent stimulation as tools in cognitive neuroscience. *Front. Psychol.* 2:100. doi: 10.3389/fpsyg.2011.00100

Jensen, O., Bonnefond, M., and VanRullen, R. (2012). An oscillatory mechanism for prioritizing salient unattended stimuli. *Trends Cogn. Sci.* 16, 200–206. doi: 10.1016/j.tics.2012.03.002

Jensen, O., Gelfand, J., Kounios, J., and Lisman, J. E. (2002). Oscillations in the alpha band (9–12 Hz) increase with memory load during retention in a short-term memory task. *Cereb. Cortex* 12, 877–882. doi: 10.1093/cercor/12.8.877

Jensen, O., Kaiser, J., and Lachaux, J.-P. (2007). Human gamma-frequency oscillations associated with attention and memory. *Trends Neurosci.* 30, 317–324. doi: 10.1016/j.tins.2007.05.001

Jensen, O., and Lisman, J. E. (1996). Novel lists of 7 +/− 2 known items can be reliably stored in an oscillatory short-term memory network: interaction with long-term memory. *Learn. Mem.* 3, 257–263. doi: 10.1101/lm.3.2-3.257

Jensen, O., and Lisman, J. E. (1998). An oscillatory short-term memory buffer model can account for data on the Sternberg task. *J. Neurosci.* 18, 10688–10699.

Jensen, O., and Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Front. Hum. Neurosci.* 4:186. doi: 10.3389/fnhum.2010.00186

Jensen, O., and Tesche, C. D. (2002). Frontal theta activity in humans increases with memory load in a working memory task. *Eur. J. Neurosci.* 15, 1395–1399. doi: 10.1046/j.1460-9568.2002.01975.x

Jokeit, H., and Makeig, S. (1994). Different event-related patterns of gamma-band power in brain waves of fast- and slow-reacting subjects. *Proc. Natl. Acad. Sci. U S A* 91, 6339–6343. doi: 10.1073/pnas.91.14.6339

Jokisch, D., and Jensen, O. (2007). Modulation of gamma and alpha activity during a working memory task engaging the dorsal or ventral stream. *J. Neurosci.* 27, 3244–3251. doi: 10.1523/jneurosci.5399-06.2007

Kahana, M. J., Seelig, D., and Madsen, J. R. (2001). Theta returns. *Curr. Opin. Neurobiol.* 11, 739–744. doi: 10.1016/s0959-4388(01)00278-1

Kamiński, J., Brzezicka, A., and Wróbel, A. (2011). Short-term memory capacity (7 ± 2) predicted by theta to gamma cycle length ratio. *Neurobiol. Learn. Mem.* 95, 19–23. doi: 10.1016/j.nlm.2010.10.001

Kanayama, N., Tamè, L., Ohira, H., and Pavani, F. (2012). Top down influence on visuo-tactile interaction modulates neural oscillatory responses. *Neuroimage* 59, 3406–3417. doi: 10.1016/j.neuroimage.2011.11.076

Kane, M. J., and Engle, R. W. (2002). The role of prefrontal cortex in working-memory capacity, executive attention and general fluid intelligence: an individual-differences perspective. *Psychon. Bull. Rev.* 9, 637–671. doi: 10.3758/bf03196323

Karch, S., Segmiller, F., Hantschk, I., Cerovecki, A., Opgen-Rhein, M., Hock, B., et al. (2012). Increased gamma oscillations during voluntary selection processes in adult patients with attention deficit/hyperactivity disorder. *J. Psychiatr. Res.* 46, 1515–1523. doi: 10.1016/j.jpsychires.2012.07.017

Keizer, A. W., Verschoor, M., Verment, R. S., and Hommel, B. (2010). The effect of gamma enhancing neurofeedback on the control of feature bindings and intelligence measures. *Int. J. Psychophysiol.* 75, 25–32. doi: 10.1016/j.ijpsycho.2009.10.011

Kelly, S. P., Gomez-Ramirez, M., and Foxe, J. J. (2009). The strength of anticipatory spatial biasing predicts target discrimination at attended locations: a high-density EEG study. *Eur. J. Neurosci.* 30, 2224–2234. doi: 10.1111/j.1460-9568.2009.06980.x

Kelly, S. P., Lalor, E. C., Reilly, R. B., and Foxe, J. J. (2005). Visual spatial attention tracking using high-density SSVEP data for independent brain-computer communication. *IEEE Trans. Neural. Syst. Rehabil. Eng.* 13, 172–178. doi: 10.1109/tnsre.2005.847369

Kidd, P. M. (2000). Attention deficit/hyperactivity disorder (ADHD) in children: rationale for its integrative management. *Altern. Med. Rev.* 5, 402–428.

Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res. Brain Res. Rev.* 29, 169–195. doi: 10.1016/s0165-0173(98)00056-3

Klimesch, W. (2012). α-band oscillations, attention and controlled access to stored information. *Trends Cogn. Sci.* 16, 606–617. doi: 10.1016/j.tics.2012.10.007

Klimesch, W., Doppelmayr, M., Russegger, H., and Pachinger, T. (1996). Theta band power in the human scalp EEG and the encoding of new information. *Neuroreport* 7, 1235–1240. doi: 10.1097/00001756-199605170-00002

Klimesch, W., Freunberger, R., and Sauseng, P. (2010). Oscillatory mechanisms of process binding in memory. *Neurosci. Biobehav. Rev.* 34, 1002–1014. doi: 10.1016/j.neubiorev.2009.10.004

Klimesch, W., Sauseng, P., and Gerloff, C. (2003). Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. *Eur. J. Neurosci.* 17, 1129–1133. doi: 10.1046/j.1460-9568.2003.02517.x

Klimesch, W., Sauseng, P., and Hanslmayr, S. (2007). EEG alpha oscillations: the inhibition-timing hypothesis. *Brain Res. Rev.* 53, 63–88. doi: 10.1016/j.brainresrev.2006.06.003

Klingberg, T. (2010). Training and plasticity of working memory. *Trends Cogn. Sci.* 14, 317–324. doi: 10.1016/j.tics.2010.05.002

Klingberg, T., Forssberg, H., and Westerberg, H. (2002). Training of working memory in children with ADHD. *J. Clin. Exp. Neuropsychol.* 24, 781–791. doi: 10.1076/jcen.24.6.781.8395

Kohli, C., Leuthesser, L., and Suri, R. (2007). Got slogan? Guidelines for creating effective slogans. *Bus. Horiz.* 50, 415–422. doi: 10.1016/j.bushor.2007.05.002

Kopell, N., Kramer, M. A., Malerba, P., and Whittington, M. A. (2010). Are different rhythms good for different functions? *Front. Hum. Neurosci.* 4:187. doi: 10.3389/fnhum.2010.00187

Krause, B., and Cohen Kadosh, R. (2014). Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation. *Front. Syst. Neurosci.* 8:25. doi: 10.3389/fnsys.2014.00025

Kray, J., and Lindenberger, U. (2000). Adult age differences in task switching. *Psychol. Aging* 15, 126–147. doi: 10.1037//0882-7974.15.1.126

Kruglikov, S. Y., and Schiff, S. J. (2003). Interplay of electroencephalogram phase and auditory-evoked neural activity. *J. Neurosci.* 23, 10122–10127.

Laczó, B., Antal, A., Niebergall, R., Treue, S., and Paulus, W. (2012). Transcranial alternating stimulation in a high gamma frequency range applied over V1 improves contrast perception but does not modulate spatial attention. *Brain Stimulat.* 5, 484–491. doi: 10.1016/j.brs.2011.08.008

Lakatos, P., Karmos, G., Mehta, A. D., Ulbert, I., and Schroeder, C. E. (2008). Entrainment of neuronal oscillations as a mechanism of attentional selection. *Science* 320, 110–113. doi: 10.1126/science.1154735

Lee, T. G., and D'Esposito, M. (2012). The dynamic nature of top-down signals originating from prefrontal cortex: a combined fMRI-TMS study. *J. Neurosci.* 32, 15458–15466. doi: 10.1523/JNEUROSCI.0627-12.2012

Lenartowicz, A., Delorme, A., Walshaw, P. D., Cho, A. L., Bilder, R. M., McGough, J. J., et al. (2014). Electroencephalography correlates of spatial working memory deficits in attention-deficit/hyperactivity disorder: vigilance, encoding and maintenance. *J. Neurosci.* 34, 1171–1182. doi: 10.1523/JNEUROSCI.1765-13.2014

Lenz, D., Krauel, K., Schadow, J., Baving, L., Duzel, E., and Herrmann, C. S. (2008). Enhanced gamma-band activity in ADHD patients lacks correlation with memory performance found in healthy children. *Brain Res.* 1235, 117–132. doi: 10.1016/j.brainres.2008.06.023

Lisman, J. (2005). The theta/gamma discrete phase code occuring during the hippocampal phase precession may be a more general brain coding scheme. *Hippocampus* 15, 913–922. doi: 10.1002/hipo.20121

Lisman, J. (2010). Working memory: the importance of theta and gamma oscillations. *Curr. Biol.* 20, R490–R492. doi: 10.1016/j.cub.2010.04.011

Lisman, J. E., and Jensen, O. (2013). The θ-γ neural code. *Neuron* 77, 1002–1016. doi: 10.1016/j.neuron.2013.03.007

Lockwood, K. A., Marcotte, A. C., and Stern, C. (2001). Differentiation of attention-deficit/hyperactivity disorder subtypes: application of a neuropsychological model of attention. *J. Clin. Exp. Neuropsychol.* 23, 317–330. doi: 10.1076/jcen.23.3.317.1179

Logan, J. M., Sanders, A. L., Snyder, A. Z., Morris, J. C., and Buckner, R. L. (2002). Under-recruitment and nonselective recruitment: dissociable neural mechanisms associated with aging. *Neuron* 33, 827–840. doi: 10.1016/s0896-6273(02)00612-8

Lopes da Silva, F. (1991). Neural mechanisms underlying brain waves: from neural membranes to networks. *Electroencephalogr. Clin. Neurophysiol.* 79, 81–93. doi: 10.1016/0013-4694(91)90044-5

Lopes da Silva, F. (2013). EEG and MEG: relevance to neuroscience. *Neuron* 80, 1112–1128. doi: 10.1016/j.neuron.2013.10.017

Lopes Da Silva, F. H., and Storm Van Leeuwen, W. (1977). The cortical source of the alpha rhythm. *Neurosci. Lett.* 6, 237–241. doi: 10.1016/0304-3940(77)90024-6

Lopes da Silva, F. H., Vos, J. E., Mooibroek, J., and Van Rotterdam, A. (1980). Relative contributions of intracortical and thalamo-cortical processes in the generation of alpha rhythms, revealed by partial coherence analysis. *Electroencephalogr. Clin. Neurophysiol.* 50, 449–456. doi: 10.1016/0013-4694(80)90011-5

Lubar, J. F., Swartwood, M. O., Swartwood, J. N., and O'Donnell, P. H. (1995). Evaluation of the effectiveness of EEG neurofeedback training for ADHD in a clinical setting as measured by changes in T.O.V.A. scores, behavioral ratings and WISC-R performance. *Biofeedback Self Regul.* 20, 83–99. doi: 10.1007/bf01712768

Luber, B., and Lisanby, S. H. (2014). Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* 85(Pt. 3), 961–970. doi: 10.1016/j.neuroimage.2013.06.007

Luck, S. J., and Vogel, E. K. (2013). Visual working memory capacity: from psychophysics and neurobiology to individual differences. *Trends Cogn. Sci.* 17, 391–400. doi: 10.1016/j.tics.2013.06.006

Maier, A., Adams, G. K., Aura, C., and Leopold, D. A. (2010). Distinct superficial and deep laminar domains of activity in the visual cortex during rest and stimulation. *Front. Syst. Neurosci.* 4:31. doi: 10.3389/fnsys.2010.00031

Manoli, Z., Grossman, N., and Samaras, T. (2012). Theoretical investigation of transcranial alternating current stimulation using realistic head model. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2012, 4156–4159. doi: 10.1109/EMBC.2012.6346882

Mathewson, K. E., Gratton, G., Fabiani, M., Beck, D. M., and Ro, T. (2009). To see or not to see: prestimulus alpha phase predicts visual awareness. *J. Neurosci.* 29, 2725–2732. doi: 10.1523/JNEUROSCI.3963-08.2009

Mathewson, K. E., Prudhomme, C., Fabiani, M., Beck, D. M., Lleras, A., and Gratton, G. (2012). Making waves in the stream of consciousness: entraining oscillations in EEG alpha and fluctuations in visual awareness with rhythmic visual stimulation. *J. Cogn. Neurosci.* 24, 2321–2333. doi: 10.1162/jocn_a_00288

Mazaheri, A., Coffey-Corina, S., Mangun, G. R., Bekker, E. M., Berry, A. S., and Corbett, B. A. (2010). Functional disconnection of frontal cortex and visual cortex in attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 67, 617–623. doi: 10.1016/j.biopsych.2009.11.022

Mazaheri, A., Fassbender, C., Coffey-Corina, S., Hartanto, T. A., Schweitzer, J. B., and Mangun, G. R. (2013). Differential oscillatory electroencephalogram between attention-deficit/hyperactivity disorder subtypes and typically developing adolescents. *Biol. Psychiatry* doi: 10.1016/j.biopsych.2013.08.023. [Epub ahead of print].

Mazaheri, A., Nieuwenhuis, I. L. C., van Dijk, H., and Jensen, O. (2009). Prestimulus alpha and mu activity predicts failure to inhibit motor responses. *Hum. Brain Mapp.* 30, 1791–1800. doi: 10.1002/hbm.20763

McEvoy, L. K., Pellouchoud, E., Smith, M. E., and Gevins, A. (2001). Neurophysiological signals of working memory in normal aging. *Brain Res. Cogn. Brain Res.* 11, 363–376. doi: 10.1016/s0926-6410(01)00009-x

Meeuwissen, E. B., Takashima, A., Fernández, G., and Jensen, O. (2011). Increase in posterior alpha activity during rehearsal predicts successful long-term memory formation of word sequences. *Hum. Brain Mapp.* 32, 2045–2053. doi: 10.1002/hbm.21167

Meinzer, M., Antonenko, D., Lindenberg, R., Hetzer, S., Ulm, L., Avirame, K., et al. (2012). Electrical brain stimulation improves cognitive performance by modulating functional connectivity and task-specific activation. *J. Neurosci.* 32, 1859–1866. doi: 10.1523/JNEUROSCI.4812-11.2012

Meinzer, M., Lindenberg, R., Antonenko, D., Flaisch, T., and Flöel, A. (2013). Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. *J. Neurosci.* 33, 12470–12478. doi: 10.1523/JNEUROSCI.5743-12.2013

Meiron, O., and Lavidor, M. (2014). Prefrontal oscillatory stimulation modulates access to cognitive control references in retrospective metacognitive commentary. *Clin. Neurophysiol.* 125, 77–82. doi: 10.1016/j.clinph.2013.06.013

Milich, R., Balentine, A. C., and Lynam, D. R. (2001). ADHD combined type and adhd predominantly inattentive type are distinct and unrelated disorders. *Clin. Psychol. Sci. Pract.* 8, 463–488. doi: 10.1093/clipsy.8.4.463

Miltner, W. H., Braun, C., Arnold, M., Witte, H., and Taub, E. (1999). Coherence of gamma-band EEG activity as a basis for associative learning. *Nature* 397, 434–436. doi: 10.1038/17126

Mitchell, D. J., McNaughton, N., Flanagan, D., and Kirk, I. J. (2008). Frontal-midline theta from the perspective of hippocampal "theta". *Prog. Neurobiol.* 86, 156–185. doi: 10.1016/j.pneurobio.2008.09.005

Mo, J., Schroeder, C. E., and Ding, M. (2011). Attentional modulation of alpha oscillations in macaque inferotemporal cortex. *J. Neurosci.* 31, 878–882. doi: 10.1523/JNEUROSCI.5295-10.2011

Montemurro, M. A., Rasch, M. J., Murayama, Y., Logothetis, N. K., and Panzeri, S. (2008). Phase-of-firing coding of natural visual stimuli in primary visual cortex. *Curr. Biol.* 18, 375–380. doi: 10.1016/j.cub.2008.02.023

Murias, M., Swanson, J. M., and Srinivasan, R. (2007). Functional connectivity of frontal cortex in healthy and ADHD children reflected in EEG coherence. *Cereb. Cortex* 17, 1788–1799. doi: 10.1093/cercor/bhl089

Neuling, T., Rach, S., and Herrmann, C. S. (2013). Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Front. Hum. Neurosci.* 7:161. doi: 10.3389/fnhum.2013.00161

Neuling, T., Rach, S., Wagner, S., Wolters, C. H., and Herrmann, C. S. (2012). Good vibrations: oscillatory phase shapes perception. *Neuroimage* 63, 771–778. doi: 10.1016/j.neuroimage.2012.07.024

Niedermeyer, E., and Lopes da Silva, F. H. (2005). *Electroencephalography: Basic Principles, Clinical Applications and Related Fields.* Philadelphia, PA: Lippincott Williams and Wilkins.

Nielson, K. A., Langenecker, S. A., and Garavan, H. (2002). Differences in the functional neuroanatomy of inhibitory control across the adult life span. *Psychol. Aging* 17, 56–71. doi: 10.1037//0882-7974.17.1.56

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527(Pt. 3), 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nuffield Councils on Bioethics. (2013). Novel neurotechnologies: intervening in the brain. Available at: http://www.nuffieldbioethics.org/sites/default/files/Novel_neurotechnologies_report_PDF_web_0.pdf

Nunez, P. L. (2000). Toward a quantitative description of large-scale neocortical dynamic function and EEG. *Behav. Brain Sci.* 23, 371–398; discussion 399–437. doi: 10.1017/s0140525x00003253

Oh, H., and Jagust, W. J. (2013). Frontotemporal network connectivity during memory encoding is increased with aging and disrupted by beta-amyloid. *J. Neurosci.* 33, 18425–18437. doi: 10.1523/JNEUROSCI.2775-13.2013

Onoda, K., Ishihara, M., and Yamaguchi, S. (2012). Decreased functional connectivity by aging is associated with cognitive decline. *J. Cogn. Neurosci.* 24, 2186–2198. doi: 10.1162/jocn_a_00269

Osipova, D., Takashima, A., Oostenveld, R., Fernández, G., Maris, E., and Jensen, O. (2006). Theta and gamma oscillations predict encoding and retrieval of declarative memory. *J. Neurosci.* 26, 7523–7531. doi: 10.1523/jneurosci.1948-06.2006

Park, H., Lee, D. S., Kang, E., Kang, H., Hahm, J., Kim, J. S., et al. (2014). Blocking of irrelevant memories by posterior alpha activity boosts memory encoding. *Hum. Brain Mapp.* doi: 10.1002/hbm.22452. [Epub ahead of print].

Pascual-Leone, A., Bartres-Faz, D., and Keenan, J. P. (1999). Transcranial magnetic stimulation: studying the brain-behaviour relationship by induction of "virtual lesions". *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 354, 1229–1238. doi: 10.1098/rstb.1999.0476

Pascual-Leone, A., Freitas, C., Oberman, L., Horvath, J. C., Halko, M., Eldaief, M., et al. (2011). Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI. *Brain Topogr.* 24, 302–315. doi: 10.1007/s10548-011-0196-8

Pascual-Leone, A., Walsh, V., and Rothwell, J. (2000). Transcranial magnetic stimulation in cognitive neuroscience—virtual lesion, chronometry and functional connectivity. *Curr. Opin. Neurobiol.* 10, 232–237. doi: 10.1016/s0959-4388(00)00081-7

Paulus, M. P., Potterat, E. G., Taylor, M. K., Van Orden, K. F., Bauman, J., Momen, N., et al. (2009). A neuroscience approach to optimizing brain resources for human performance in extreme environments. *Neurosci. Biobehav. Rev.* 33, 1080–1088. doi: 10.1016/j.neubiorev.2009.05.003

Pelham, W. E. Jr., Gnagy, E. M., Greenslade, K. E., and Milich, R. (1992). Teacher ratings of DSM-III-R Symptoms for the disruptive behavior disorders. *J. Am. Acad. Child Adolesc. Psychiatry* 31, 210–218. doi: 10.1097/00004583-199203000-00006

Peters, E., Pritzkuleit, R., Beske, F., and Katalinic, A. (2010). [Demographic change and disease rates: a projection until 2050]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 53, 417–426. doi: 10.1007/s00103-010-1050-y

Pfurtscheller, G., and Lopes da Silva, F. H. (1999). Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* 110, 1842–1857. doi: 10.1016/s1388-2457(99)00141-8

Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., and Rohde, L. A. (2007). The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am. J. Psychiatry* 164, 942–948. doi: 10.1176/appi.ajp.164.6.942

Polanía, R., Nitsche, M. A., Korman, C., Batsikadze, G., and Paulus, W. (2012a). The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr. Biol.* 22, 1314–1318. doi: 10.1016/j.cub.2012.05.021

Polanía, R., Paulus, W., and Nitsche, M. A. (2012b). Noninvasively decoding the contents of visual working memory in the human prefrontal cortex within high-gamma oscillatory patterns. *J. Cogn. Neurosci.* 24, 304–314. doi: 10.1162/jocn_a_00151

Poliakoff, E., Ashworth, S., Lowe, C., and Spence, C. (2006). Vision and touch in ageing: crossmodal selective attention and visuotactile spatial interactions. *Neuropsychologia* 44, 507–517. doi: 10.1016/j.neuropsychologia.2005.07.004

Priori, A. (2003). Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clin. Neurophysiol.* 114, 589–595. doi: 10.1016/s1388-2457(02)00437-6

Raghavachari, S., Kahana, M. J., Rizzuto, D. S., Caplan, J. B., Kirschen, M. P., Bourgeois, B., et al. (2001). Gating of human theta oscillations by a working memory task. *J. Neurosci.* 21, 3175–3183.

Raghavachari, S., Lisman, J. E., Tully, M., Madsen, J. R., Bromfield, E. B., and Kahana, M. J. (2006). Theta oscillations in human cortex during a working-memory task: evidence for local generators. *J. Neurophysiol.* 95, 1630–1638. doi: 10.1152/jn.00409.2005

Ray, S., and Maunsell, J. H. R. (2011). Different origins of gamma rhythm and high-gamma activity in macaque visual cortex. *PLoS Biol.* 9:e1000610. doi: 10.1371/journal.pbio.1000610

Reato, D., Rahman, A., Bikson, M., and Parra, L. C. (2013). Effects of weak transcranial alternating current stimulation on brain activity—a review of known mechanisms from animal studies. *Front. Hum. Neurosci.* 7:687. doi: 10.3389/fnhum.2013.00687

Reinhart, R. M. G., and Woodman, G. F. (2014). Causal control of medial–frontal cortex governs electrophysiological and behavioral indices of performance monitoring and learning. *J. Neurosci.* 34, 4214–4227. doi: 10.1523/JNEUROSCI.5421-13.2014

Reuter-Lorenz, P. A., Jonides, J., Smith, E. E., Hartley, A., Miller, A., Marshuetz, C., et al. (2000). Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. *J. Cogn. Neurosci.* 12, 174–187. doi: 10.1162/089892900561814

Riis, J. L., Chong, H., Ryan, K. K., Wolk, D. A., Rentz, D. M., Holcomb, P. J., et al. (2008). Compensatory neural activity distinguishes different patterns of normal cognitive aging. *Neuroimage* 39, 441–454. doi: 10.1016/j.neuroimage.2007.08.034

Rohenkohl, G., and Nobre, A. C. (2011). α oscillations related to anticipatory attention follow temporal expectations. *J. Neurosci.* 31, 14076–14084. doi: 10.1523/JNEUROSCI.3387-11.2011

Romei, V., Gross, J., and Thut, G. (2010). On the role of prestimulus alpha rhythms over occipito-parietal areas in visual input regulation: correlation or causation? *J. Neurosci.* 30, 8692–8697. doi: 10.1523/JNEUROSCI.0160-10.2010

Romei, V., Gross, J., and Thut, G. (2012). Sounds reset rhythms of visual cortex and corresponding human visual perception. *Curr. Biol.* 22, 807–813. doi: 10.1016/j.cub.2012.03.025

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2009). Safety, ethical considerations and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Nerophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Roux, F., and Uhlhaas, P. J. (2014). Working memory and neural oscillations: alpha-gamma versus theta-gamma codes for distinct WM information? *Trends Cogn. Sci.* 18, 16–25. doi: 10.1016/j.tics.2013.10.010

Rowe, G., Valderrama, S., Hasher, L., and Lenartowicz, A. (2006). Attentional disregulation: a benefit for implicit memory. *Psychol. Aging* 21, 826–830. doi: 10.1037/0882-7974.21.4.826

Saalmann, Y. B., Pinsk, M. A., Wang, L., Li, X., and Kastner, S. (2012). The pulvinar regulates information transmission between cortical areas based on attention demands. *Science* 337, 753–756. doi: 10.1126/science.1223082

Safren, S. A., Sprich, S., Mimiaga, M. J., Surman, C., Knouse, L., Groves, M., et al. (2010). Cognitive behavioral therapy vs relaxation with educational support for medication-treated adults with adhd and persistent symptoms: a randomized controlled trial. *JAMA* 304, 875–880. doi: 10.1001/jama.2010.1192

Salthouse, T. A., Babcock, R. L., and Shaw, R. J. (1991). Effects of adult age on structural and operational capacities in working memory. *Psychol. Aging* 6, 118–127. doi: 10.1037/0882-7974.6.1.118

Sampaio-Baptista, C., Khrapitchev, A. A., Foxley, S., Schlagheck, T., Scholz, J., Jbabdi, S., et al. (2013). Motor skill learning induces changes in white matter microstructure and myelination. *J. Neurosci.* 33, 19499–19503. doi: 10.1523/JNEUROSCI.3048-13.2013

Sander, M. C., Lindenberger, U., and Werkle-Bergner, M. (2012a). Lifespan age differences in working memory: a two-component framework. *Neurosci. Biobehav. Rev.* 36, 2007–2033. doi: 10.1016/j.neubiorev.2012.06.004

Sander, M. C., Werkle-Bergner, M., and Lindenberger, U. (2012b). Amplitude modulations and inter-trial phase stability of alpha-oscillations differentially reflect working memory constraints across the lifespan. *Neuroimage* 59, 646–654. doi: 10.1016/j.neuroimage.2011.06.092

Santarnecchi, E., Polizzotto, N. R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., et al. (2013). Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr. Biol.* 23, 1449–1453. doi: 10.1016/j.cub.2013.06.022

Sauseng, P., Feldheim, J. F., Freunberger, R., and Hummel, F. C. (2011). Right prefrontal TMS disrupts interregional anticipatory EEG alpha activity during shifting of visuospatial attention. *Front. Psychol.* 2:241. doi: 10.3389/fpsyg.2011.00241

Sauseng, P., Griesmayr, B., Freunberger, R., and Klimesch, W. (2010). Control mechanisms in working memory: a possible function of EEG theta oscillations. *Neurosci. Biobehav. Rev.* 34, 1015–1022. doi: 10.1016/j.neubiorev.2009.12.006

Sauseng, P., and Klimesch, W. (2008). What does phase information of oscillatory brain activity tell us about cognitive processes? *Neurosci. Biobehav. Rev.* 32, 1001–1013. doi: 10.1016/j.neubiorev.2008.03.014

Sauseng, P., Klimesch, W., Gerloff, C., and Hummel, F. C. (2009a). Spontaneous locally restricted EEG alpha activity determines cortical excitability in the motor cortex. *Neuropsychologia* 47, 284–288. doi: 10.1016/j.neuropsychologia.2008.07.021

Sauseng, P., Klimesch, W., Heise, K. F., Gruber, W. R., Holz, E., Karim, A. A., et al. (2009b). Brain oscillatory substrates of visual short-term memory capacity. *Curr. Biol.* 19, 1846–1852. doi: 10.1016/j.cub.2009.08.062

Scheeringa, R., Fries, P., Petersson, K.-M., Oostenveld, R., Grothe, I., Norris, D. G., et al. (2011). Neuronal dynamics underlying high- and low-frequency EEG oscillations contribute independently to the human BOLD signal. *Neuron* 69, 572–583. doi: 10.1016/j.neuron.2010.11.044

Schneider, T. R., Debener, S., Oostenveld, R., and Engel, A. K. (2008). Enhanced EEG gamma-band activity reflects multisensory semantic matching in visual-to-auditory object priming. *Neuroimage* 42, 1244–1254. doi: 10.1016/j.neuroimage.2008.05.033

Schroeder, C. E., and Lakatos, P. (2009). Low-frequency neuronal oscillations as instruments of sensory selection. *Trends Neurosci.* 32, 9–18. doi: 10.1016/j.tins.2008.09.012

Schutter, D. J. (2014). Syncing your brain: electric currents to enhance cognition. *Trends Cogn. Sci.* doi: 10.1016/j.tics.2014.02.011. [Epub ahead of print].

Shafi, M. M., Brandon Westover, M., Oberman, L., Cash, S. S., and Pascual-Leone, A. (2014). Modulation of EEG functional connectivity networks in subjects undergoing repetitive transcranial magnetic stimulation. *Brain Topogr.* 27, 172–191. doi: 10.1007/s10548-013-0277-y

Silvanto, J., and Pascual-Leone, A. (2008). State-dependency of transcranial magnetic stimulation. *Brain Topogr.* 21, 1–10. doi: 10.1007/s10548-008-0067-0

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Spaak, E., Bonnefond, M., Maier, A., Leopold, D. A., and Jensen, O. (2012). Layer-specific entrainment of gamma-band neural activity by the alpha rhythm in monkey visual cortex. *Curr. Biol.* 22, 2313–2318. doi: 10.1016/j.cub.2012.10.020

Spaak, E., de Lange, F. P., and Jensen, O. (2014). Local entrainment of Alpha oscillations by visual stimuli causes cyclic modulation of perception. *J. Neurosci.* 34, 3536–3544. doi: 10.1523/jneurosci.4385-13.2014

Spitzer, B., and Blankenburg, F. (2012). Supramodal parametric working memory processing in humans. *J. Neurosci.* 32, 3287–3295. doi: 10.1523/jneurosci.5280-11.2012

Staudigl, T., and Hanslmayr, S. (2013). Theta oscillations at encoding mediate the context-dependent nature of human episodic memory. *Curr. Biol.* 23, 1101–1106. doi: 10.1016/j.cub.2013.04.074

Staufenbiel, S. M., Brouwer, A.-M., Keizer, A. W., and van Wouwe, N. C. (2014). Effect of beta and gamma neurofeedback on memory and intelligence in the elderly. *Biol. Psychol.* 95, 74–85. doi: 10.1016/j.biopsycho.2013.05.020

Strens, L. H. A., Oliviero, A., Bloem, B. R., Gerschlager, W., Rothwell, J. C., and Brown, P. (2002). The effects of subthreshold 1 Hz repetitive TMS on cortico-cortical and interhemispheric coherence. *Clin. Neurophysiol.* 113, 1279–1285. doi: 10.1016/s1388-2457(02)00151-7

Strenziok, M., Parasuraman, R., Clarke, E., Cisler, D. S., Thompson, J. C., and Greenwood, P. M. (2014). Neurocognitive enhancement in older adults: comparison of three cognitive training tasks to test a hypothesis of training transfer in brain connectivity. *Neuroimage* 85(Pt. 3), 1027–1039. doi: 10.1016/j.neuroimage.2013.07.069

Strüber, D., Rach, S., Trautmann-Lengsfeld, S. A., Engel, A. K., and Herrmann, C. S. (2014). Antiphasic 40 Hz oscillatory current stimulation affects bistable motion perception. *Brain Topogr.* 27, 158–171. doi: 10.1007/s10548-013-0294-x

Suffczynski, P., Kalitzin, S., Pfurtscheller, G., and Lopes da Silva, F. H. (2001). Computational model of thalamo-cortical networks: dynamical control of alpha rhythms in relation to focal attention. *Int. J. Psychophysiol.* 43, 25–40. doi: 10.1016/s0167-8760(01)00177-5

Tales, A., Troscianko, T., Wilcock, G. K., Newton, P., and Butler, S. R. (2002). Age-related changes in the preattentional detection of visual change. *Neuroreport* 13, 969–972. doi: 10.1097/00001756-200205240-00014

Tallon-Baudry, C. (1999). Oscillatory gamma activity in humans and its role in object representation. *Trends Cogn. Sci.* 3, 151–162. doi: 10.1016/s1364-6613(99)01299-1

Tallon-Baudry, C., Bertrand, O., Delpuech, C., and Pernier, J. (1996). Stimulus specificity of phase-locked and non-phase-locked 40 Hz visual responses in human. *J. Neurosci.* 16, 4240–4249.

Tallon-Baudry, C., Bertrand, O., Hénaff, M.-A., Isnard, J., and Fischer, C. (2005). Attention modulates gamma-band oscillations differently in the human lateral occipital cortex and fusiform gyrus. *Cereb. Cortex* 15, 654–662. doi: 10.1093/cercor/bhh167

Tallon-Baudry, C., Bertrand, O., Peronnet, F., and Pernier, J. (1998). Induced gamma-band activity during the delay of a visual short-term memory task in humans. *J. Neurosci.* 18, 4244–4254.

ter Huurne, N., Onnink, M., Kan, C., Franke, B., Buitelaar, J., and Jensen, O. (2013). Behavioral consequences of aberrant alpha lateralization in attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 74, 227–233. doi: 10.1016/j.biopsych.2013.02.001

Thut, G., and Miniussi, C. (2009). New insights into rhythmic brain activity from TMS–EEG studies. *Trends Cogn. Sci.* 13, 182–189. doi: 10.1016/j.tics.2009.01.004

Thut, G., and Pascual-Leone, A. (2010). A review of combined TMS-EEG studies to characterize lasting effects of repetitive TMS and assess their usefulness in cognitive and clinical neuroscience. *Brain Topogr.* 22, 219–232. doi: 10.1007/s10548-009-0115-4

Thut, G., Nietzel, A., Brandt, S. A., and Pascual-Leone, A. (2006). Alpha-band electroencephalographic activity over occipital cortex indexes visuospatial attention bias and predicts visual target detection. *J. Neurosci.* 26, 9494–9502. doi: 10.1523/jneurosci.0875-06.2006

Thut, G., Schyns, P. G., and Gross, J. (2011a). Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Front. Psychol.* 2:170. doi: 10.3389/fpsyg.2011.00170

Thut, G., Veniero, D., Romei, V., Miniussi, C., Schyns, P., and Gross, J. (2011b). Rhythmic TMS causes local entrainment of natural oscillatory signatures. *Curr. Biol.* 21, 1176–1185. doi: 10.1016/j.cub.2011.05.049

Tonin, L., Leeb, R., and Del R Millán, J. (2012). Time-dependent approach for single trial classification of covert visuospatial attention. *J. Neural Eng.* 9:045011. doi: 10.1088/1741-2560/9/4/045011

van Dijk, H., Schoffelen, J.-M., Oostenveld, R., and Jensen, O. (2008). Prestimulus oscillatory activity in the alpha band predicts visual discrimination ability. *J. Neurosci.* 28, 1816–1823. doi: 10.1523/jneurosci.1853-07.2008

van Dongen-Boomsma, M., Vollebregt, M. A., Slaats-Willemse, D., and Buitelaar, J. K. (2013). A randomized placebo-controlled trial of electroencephalographic (EEG) neurofeedback in children with attention-deficit/hyperactivity disorder. *J. Clin. Psychiatry* 74, 821–827. doi: 10.4088/jcp.12m08321

van Gerven, M. A. J., Farquhar, J., Schaefer, R., Vlek, R., Geuze, J., Nijholt, A., et al. (2009a). The brain–computer interface cycle. *J. Neural Eng.* 6:041001. doi: 10.1088/1741-2560/6/4/041001

van Gerven, M., and Jensen, O. (2009). Attention modulations of posterior alpha as a control signal for two-dimensional brain-computer interfaces. *J. Neurosci. Methods* 179, 78–84. doi: 10.1016/j.jneumeth.2009.01.016

van Gerven, M., Bahramisharif, A., Heskes, T., and Jensen, O. (2009b). Selecting features for BCI control based on a covert spatial attention paradigm. *Neural Netw.* 22, 1271–1277. doi: 10.1016/j.neunet.2009.06.004

van Pelt, S., Boomsma, D. I., and Fries, P. (2012). Magnetoencephalography in twins reveals a strong genetic determination of the peak frequency of visually induced γ-band synchronization. *J. Neurosci.* 32, 3388–3392. doi: 10.1523/jneurosci.5592-11.2012

van Pelt, S., and Fries, P. (2013). Visual stimulus eccentricity affects human gamma peak frequency. *Neuroimage* 78, 439–447. doi: 10.1016/j.neuroimage.2013.04.040

VanRullen, R., Zoefel, B., and Ilhan, B. (2014). On the cyclic nature of perception in vision versus audition. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:20130214. doi: 10.1098/rstb.2013.0214

Varela, F., Lachaux, J. P., Rodriguez, E., and Martinerie, J. (2001). The brainweb: phase synchronization and large-scale integration. *Nat. Rev. Neurosci.* 2, 229–239. doi: 10.1038/35067550

Vaughan, T. M., McFarland, D. J., Schalk, G., Sarnacki, W. A., Krusienski, D. J., Sellers, E. W., et al. (2006). The wadsworth BCI research and development program: at home with BCI. *IEEE Trans. Neural Syst. Rehabil. Eng.* 14, 229–233. doi: 10.1109/tnsre.2006.875577

Veniero, D., Ponzo, V., and Koch, G. (2013). Paired associative stimulation enforces the communication between interconnected areas. *J. Neurosci.* 33, 13773–13783. doi: 10.1523/jneurosci.1777-13.2013

Verret, C., Guay, M.-C., Berthiaume, C., Gardiner, P., and Béliveau, L. (2012). A physical activity program improves behavior and cognitive functions in children with ADHD an exploratory study. *J. Atten. Disord.* 16, 71–80. doi: 10.1177/1087054710379735

Vidal-Piñeiro, D., Martin-Trias, P., Arenaza-Urquijo, E. M., Sala-Llonch, R., Clemente, I. C., Mena-Sánchez, I., et al. (2014). Task-dependent activity and connectivity predict episodic memory network-based responses to brain stimulation in healthy aging. *Brain Stimul.* 7, 287–296. doi: 10.1016/j.brs.2013.12.016

Vidaurre, C., and Blankertz, B. (2010). Towards a cure for BCI illiteracy. *Brain Topogr.* 23, 194–198. doi: 10.1007/s10548-009-0121-6

Vollebregt, M. A., van Dongen-Boomsma, M., Buitelaar, J. K., and Slaats-Willemse, D. (2014). Does EEG-neurofeedback improve neurocognitive functioning in children with attention-deficit/hyperactivity disorder? A systematic review and a double-blind placebo-controlled study. *J. Child Psychol. Psychiatry* 55, 460–472. doi: 10.1111/jcpp.12143

Vollmann, H., Conde, V., Sewerin, S., Taubert, M., Sehm, B., Witte, O. W., et al. (2013). Anodal transcranial direct current stimulation (tDCS) over supplementary motor area (SMA) but not pre-SMA promotes short-term visuomotor learning. *Brain Stimul.* 6, 101–107. doi: 10.1016/j.brs.2012.03.018

von Stein, A., and Sarnthein, J. (2000). Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. *Int. J. Psychophysiol.* 38, 301–313. doi: 10.1016/s0167-8760(00)00172-0

Wagner, T., Eden, U., Rushmore, J., Russo, C. J., Dipietro, L., Fregni, F., et al. (2014). Impact of brain tissue filtering on neurostimulation fields: A modeling study. *Neuroimage* 85(Pt. 3), 1048–1057. doi: 10.1016/j.neuroimage.2013.06.079

Wang, J.-R., and Hsieh, S. (2013). Neurofeedback training improves attention and working memory performance. *Clin. Neurophysiol.* 124, 2406–2420. doi: 10.1016/j.clinph.2013.05.020

Wang, X.-J. (2010). Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol. Rev.* 90, 1195–1268. doi: 10.1152/physrev.00035.2008

Waring, J. D., Addis, D. R., and Kensinger, E. A. (2013). Effects of aging on neural connectivity underlying selective memory for emotional scenes. *Neurobiol. Aging* 34, 451–467. doi: 10.1016/j.neurobiolaging.2012.03.011

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the international workshop on the safety of repetitive transcranial magnetic stimulation, June 5–7, 1996. *Electroencephalogr. Clin. Neurophysiol.* 108, 1–16. doi: 10.1016/s0168-5597(97)00096-8

Weiler, M. D., Bernstein, J. H., Bellinger, D., and Waber, D. P. (2002). Information processing deficits in children with attention-deficit/hyperactivity disorder, inattentive type and children with reading disability. *J. Learn. Disabil.* 35, 448–461. doi: 10.1177/00222194020350050501

Weiss, M., Worling, D., and Wasdell, M. (2003). A chart review study of the inattentive and combined types of ADHD. *J. Atten. Disord.* 7, 1–9. doi: 10.1177/108705470300700101

Werkle-Bergner, M., Shing, Y. L., Müller, V., Li, S.-C., and Lindenberger, U. (2009). EEG gamma-band synchronization in visual coding from childhood to old age: evidence from evoked power and inter-trial phase locking. *Clin. Neurophysiol.* 120, 1291–1302. doi: 10.1016/j.clinph.2009.04.012

Wolpaw, J. R., Birbaumer, N., McFarland, D. J., Pfurtscheller, G., and Vaughan, T. M. (2002). Brain-computer interfaces for communication and control. *Clin. Neurophysiol.* 113, 767–791. doi: 10.1016/S1388-2457(02)00057-3

Worden, M. S., Foxe, J. J., Wang, N., and Simpson, G. V. (2000). Anticipatory biasing of visuospatial attention indexed by retinotopically specific alpha-band electroencephalography increases over occipital cortex. *J. Neurosci.* 20:RC63.

Xing, D., Yeh, C.-I., Burns, S., and Shapley, R. M. (2012). Laminar analysis of visually evoked activity in the primary visual cortex. *Proc. Natl. Acad. Sci. U S A* 109, 13871–13876. doi: 10.1073/pnas.1201478109

Yang, L., and Hasher, L. (2007). The enhanced effects of pictorial distraction in older adults. *J. Gerontol. B. Psychol. Sci. Soc. Sci.* 62, P230–P233. doi: 10.1093/geronb/62.4.p230

Yordanova, J., Banaschewski, T., Kolev, V., Woerner, W., and Rothenberger, A. (2001). Abnormal early stages of task stimulus processing in children with attention-deficit hyperactivity disorder—evidence from event-related gamma oscillations. *Clin. Neurophysiol.* 112, 1096–1108. doi: 10.1016/s1388-2457(01)00524-7

Zaehle, T., Rach, S., and Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One* 5:e13766. doi: 10.1371/journal.pone.0013766

Zanto, T. P., Chadick, J. Z., and Gazzaley, A. (2014). Anticipatory alpha phase influences visual working memory performance. *Neuroimage* 85(Pt. 2), 794–802. doi: 10.1016/j.neuroimage.2013.07.048

Zatorre, R. J., Fields, R. D., and Johansen-Berg, H. (2012). Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat. Neurosci.* 15, 528–536. doi: 10.1038/nn.3045

Zoefel, B., Huster, R. J., and Herrmann, C. S. (2011). Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance. *Neuroimage* 54, 1427–1431. doi: 10.1016/j.neuroimage.2010.08.078

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 08 April 2014; accepted: 03 June 2014; published online: 26 June 2014.
Citation: Horschig JM, Zumer JM and Bahramisharif A (2014) Hypothesis-driven methods to augment human cognition by optimizing cortical oscillations. Front. Syst. Neurosci. ***8****:119. doi: 10.3389/fnsys.2014.00119*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

PERSPECTIVE
published: 10 November 2015
doi: 10.3389/fnsys.2015.00154

Two is More Than One: How to Combine Brain Stimulation Rehabilitative Training for Functional Recovery?

Satoko Koganemaru[1,2]*, *Hidenao Fukuyama*[2] *and Tatsuya Mima*[2]

[1] *Brain Integrative Science, Kyoto University School of Medicine, Sakyo-ku, Kyoto, Japan,* [2] *Human Brain Research Center, Kyoto University School of Medicine, Sakyo-ku, Kyoto, Japan*

A number of studies have shown that non-invasive brain stimulation has an additional effect in combination with rehabilitative therapy to enhance functional recovery than either therapy alone. The combination enhances use-dependent plasticity induced by repetitive training. The neurophysiological mechanism of the effects of this combination is based on associative plasticity. However, these effects were not reported in all cases. We propose a list of possible strategies to achieve an effective association between rehabilitative training with brain stimulation for plasticity: (1) control of temporal aspect between stimulation and task execution; (2) the use of a shaped task for the combination; (3) the appropriate stimulation of neuronal circuits where use-dependent plastic changes occur; and (4) phase synchronization between rhythmically patterned brain stimulation and task-related patterned activities of neurons. To better utilize brain stimulation in neuro-rehabilitation, it is important to develop more effective techniques to combine them.

Keywords: use-dependent plasticity, associative plasticity, neuro-rehabilitation, transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS)

OPEN ACCESS

Edited by:
Mikhail Lebedev,
Duke University, USA

Reviewed by:
Po-Yi Tsai,
Yang-Ming National University, Taiwan
Anna-Katharine Brem,
University of Oxford, UK
Marco Sandrini,
University of Roehampton, UK

***Correspondence:**
Satoko Koganemaru
kogane@kuhp.kyoto-u.ac.jp

Received: *13 November 2014*
Accepted: *26 October 2015*
Published: *10 November 2015*

Citation:
Koganemaru S, Fukuyama H and Mima T (2015) Two is More Than One: How to Combine Brain Stimulation and Rehabilitative Training for Functional Recovery? Front. Syst. Neurosci. 9:154. doi: 10.3389/fnsys.2015.00154

USE-DEPENDENT PLASTICITY ENHANCED BY THE COMBINATION OF REHABILITATIVE TRAINING AND BRAIN STIMULATION

Repetitive training is one of the fundamental strategies in neuro-rehabilitation regardless of what type of damage has occurred in the central or peripheral nervous system. Task-specific training induces task-specific neuronal changes lasting for a long period i.e., use-dependent plasticity that can lead to functional recovery (Butefisch et al., 1995; Nudo and Milliken, 1996; Nudo et al., 1996a,b; Hummelsheim, 1999; Masiero and Carraro, 2008; Richards et al., 2008; Dimyan and Cohen, 2011). Use-dependent plasticity induced by motor training has been demonstrated within the human primary motor cortex (M1). The long-term potentiation (LTP)-like changes in specific cortcospinal motoneurons were induced for the trained task after repetitive simple finger movements (Classen et al., 1998; Butefisch et al., 2000; Rossini and Pauri, 2000).

In recent decades, a number of studies have shown that non-invasive brain stimulation such as repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS) has an add-on effect in combination with rehabilitative therapy

(Platz and Rothwell, 2010; Edwardson et al., 2013; Sandrini and Cohen, 2013; Floel, 2014). Furthermore, this combination may better enhance functional recovery in post-stroke patients, as compared with rehabilitation training alone, which may not sufficiently induce functional recovery (Khedr et al., 2005, 2010; Kim et al., 2006, 2010; Takeuchi et al., 2008; Chang et al., 2010, 2012; Emara et al., 2010; Koganemaru et al., 2010; Conforto et al., 2012; Meehan et al., 2011; Nair et al., 2011; Stagg and Nitsche, 2011; Wang et al., 2012; Hsu et al., 2013), especially in the chronic phase when it is difficult to produce plastic changes (Nakayama et al., 1994; Verheyden et al., 2008). A single intervention of brain stimulation alone without rehabilitative therapy seems to have limited effects on patients with mild motor symptoms (Hummel and Cohen, 2005; Koganemaru et al., 2010) and insufficient sustainability of effects (Takeuchi et al., 2005; Kim et al., 2009). Whereas the combination may enhance use-dependent plasticity induced by repetitive training.

ASSOCIATIVE PLASTICITY TO PRODUCE THE COMBINATION EFFECTS

Although the exact neurophysiological mechanism of this combination effect is not known yet, it may be based on Hebbian associative plasticity (Hebb, 1949). For example, a post-synaptic neuron (A) receives low-frequency weak inputs from one pre-synaptic neuron (B) (the inputs themselves cannot induce LTP in a synaptic connection). Simultaneously, neuron (A) receives high-frequency weak inputs (the inputs themselves can induce LTP) from another pre-synaptic neuron (C). According to the Hebbian rule, LTP is also induced in the weak synaptic connection between neurons (A) and (B) as well as between neurons (A) and (C). A similar mechanism would work in the case of the combination of training with brain stimulation. Training alone may only produce a weak activation of neuronal circuits, which do not lead to long term changes. On the other hand, brain stimulation can induce LTP-like changes for synaptic strength in stimulated areas (Pascual-Leone et al., 1994; Hallett, 2000; Fritsch et al., 2010; Dayan et al., 2013; Karabanov et al., 2015). Therefore, simultaneous training with brain stimulation would enable weak synaptic connections to induce associative LTP-like effects through the Hebbian rule.

However, a recent study reported no additional effects of theta-burst stimulation (TBS) in combination with standardized rehabilitative therapy in chronic stroke patients. That might be possibly due to a failure to induce associative plasticity. Neuronal activities enhanced by TBS may not have been associated with task-specific neuronal activities produced by the rehabilitative therapy (Talelli et al., 2012).

We can speculate and make a list of possible factors that may have weakened the therapeutic effects of combined rehabilitation and brain stimulation:

1. Diversities in diseases, particularly the locations of lesions
   – The effects of facilitatory rTMS over M1 depended on lesion location in post-stroke hemiparetic patients. The deterioration of finger function was seen in the patients with cortical lesions, whereas improvement in finger function was seen in patients with subcortical lesions (Ameli et al., 2009).
2. Small sample size
   – The responses to brain stimulation show a large variability even if patients are similar in lesion location, severity of paresis and time after stroke onset in patients. Genetic factors are responsible for individual susceptibility to rTMS-induced plasticity (Cheeran et al., 2008).
3. Insufficient intensity and/or too short duration of the intervention
   – Patients with severe paresis show reduced or no motor evoked potentials (MEP) with TMS (Pennisi et al., 1999; Hendricks et al., 2002). If the intensity of brain stimulation is determined by the excitability of the healthy hemisphere, it may be too weak to induce plasticity in the affected hemisphere. Unless brain stimulation is repeated daily for days to weeks, its effects might not be sustainable (Khedr et al., 2009; Emara et al., 2010; Bolognini et al., 2011; Conforto et al., 2012; Edwardson et al., 2013).
4. Inappropriate affinity between rehabilitation task and brain stimulation modality
   – This will be discussed in detail in the following section.

Future clinical studies should give careful consideration to these factors. We have considered how effectively we can induce associative plasticity through the combination of training and brain stimulation.

EFFECTIVE METHODS OF COMBINING REHABILITATIVE TRAINING AND BRAIN STIMULATION

Control of the Temporal Aspect Between Stimulation and Task Execution

First, we should control the temporal aspect between stimulation and task execution. Spike timing-dependent associative plasticity has been proven in both animals (Hess and Donoghue, 1996; Hess et al., 1996; Egger et al., 1999) and humans (Stefan et al., 2000, 2002; Ueki et al., 2006; Koganemaru et al., 2009). Associative LTP occurs when a post-synaptic neuron fires less than 10–20 ms after a pre-synaptic neuron. Recently, we have demonstrated that associative plasticity is induced within human M1. The repetitive pairing of TMS and paired bihemispheric stimulation (PBS) applied at a time interval of 15 ms, produced an associative LTP-like effect within the targeted M1 and facilitated fine finger movements (Koganemaru et al., 2009). Furthermore, associative use-dependent plasticity has been demonstrated within human M1. Thabit et al. (2010) showed that associative LTP-like changes were induced by the repetitive paring of a unidirectional finger movement and a single TMS pulse over the contralateral M1 with a specific interval in healthy subjects. It resulted in a faster reaction in the trained direction. By decrease or increase of the interval, LTP-like effects can disappear or be reversed. Buetefisch et al. (2011) showed that the extensor-specific M1 reorganization was induced by robot-assisted training of paretic wrist extension combined with TMS over the ipsilesional M1 in a strict temporal relationship

in chronic post-stroke patients. Particularly, a decrease of motor threshold and a shift of motor mapping for the extensor carpi ulnaris muscles, not the biceps muscles, were demonstrated in the combination therapy with TMS over the ipsilesional M1. The training alone and the simulation protocol with TMS over the contralesional M1 did not show those changes. The results suggest that temporal associative plasticity is induced specifically for the extensor-related activity in the ipsilesional M1. If we can associate brain stimulation and task execution with proper timing, a task-specific associative plasticity may be induced. There is a large variability in movement onset of the paretic limbs after neurological insults. The central motor conduction time is prolonged in stroke and other neurodegenerative diseases due to lesions in the corticospinal tract (Kaviraja and Robert, 2013). It is influenced by time after disease onset or disease progression. On the other hand, electromyogram (EMG) onsets are often variable since it is difficult to increase firing rate in central nervous diseases (Barnes, 1980). Therefore, when we do simultaneous TMS with training, we may as well be careful for both the timing for TMS and task execution.

Use of a Shaped Task for the Combination

An effective rehabilitative approach should consist of various types of training pertaining to body movements in enriched environments that encourage patterns and combinations of movement for improving recovery. Different types of task-specific training help their effects transfer into actual activities of daily life in patients (Teasell et al., 2005). However, a specific task or tasks for specific movements are better combined with brain stimulation. If a task consists of various gross and precise movements, it may evoke conflicting neuronal activities such as inhibition and activation. Then, it may reduce the effect of brain stimulation. In recent animal studies, it was shown that training general gross movements inhibited recovery of skilled movements (Garcia-Alias et al., 2009). Neural competition for newly available neural resources may occur when multiple tasks are trained (Reinkensmeyer and Boninger, 2012). In clinical studies, task-specificity of training is more important than the intensity of training (Page, 2003; Bayona et al., 2005). The repetition of task-specific training produced long-lasting cortical reorganization and use-dependent plasticity specific to the areas that were activated during the trained movements in healthy subjects (Classen et al., 1998; Butefisch et al., 2000). In post-stroke patients, shaped task-specific training resulted in a better recovery of their paretic upper-limb function as compared with general training (Butefisch et al., 1995; Woldag et al., 2010).

Recently, we have investigated the effect of repetitive motor tasks in the paretic upper-limb combined with brain stimulation in post-stroke patients (Koganemaru et al., 2010). Patients with chronic stroke with moderate-to-severe hemiparesis often suffer from motor deficits associated with flexor hypertonia. A possible therapeutic strategy is to selectively induce use-dependent plasticity in the extensors to counteract the flexor hypertonia. However, the beneficial effects of training in chronic-phase patients are relatively limited due to resistance to induction of use-dependent plasticity in the chronic phase (Nakayama et al., 1994; Verheyden et al., 2008). When 5 Hz rTMS over the ipsilesional M1 was combined with extensor training assisted by electrical neuromuscular stimulation, the combined intervention resulted in an improvement of extensor movement with a reduction of flexor hypertonia, whereas neither of the single interventions alone demonstrated any improvements. The extensor-specific change in M1 was likely attributable to a functional recovery of the paretic upper limb (Koganemaru et al., 2010). Our study is an exemplary case showing the relevance of task selection combined with brain stimulation to enhance use-dependent plasticity for functional recovery.

The Stimulation of Neuronal Circuits Where Use-Dependent Plastic Changes Occur

Stimulation should be given to neuronal circuits and brain areas where use-dependent plastic changes occur. Because use-dependent plasticity is task-specific, changed circuits and areas depend on what type of task was trained. For repetitive simple motor tasks, use-dependent plastic changes have been reported within M1 (Classen et al., 1998; Butefisch et al., 2000; Rossini and Pauri, 2000). However, it is unknown whether it occurs within M1 alone or in combination with the multi-regional functional reorganization of the motor-related brain network. If it occurs in a multi-regional brain network, other non-M1 regions would be the possible targets of stimulation.

Recently, we used neuroimaging to investigate whether use-dependent changes occurred in a multiregional brain network in chronic post-stroke patients (Koganemaru et al., 2015). In process of post-stroke recovery with rehabilitative

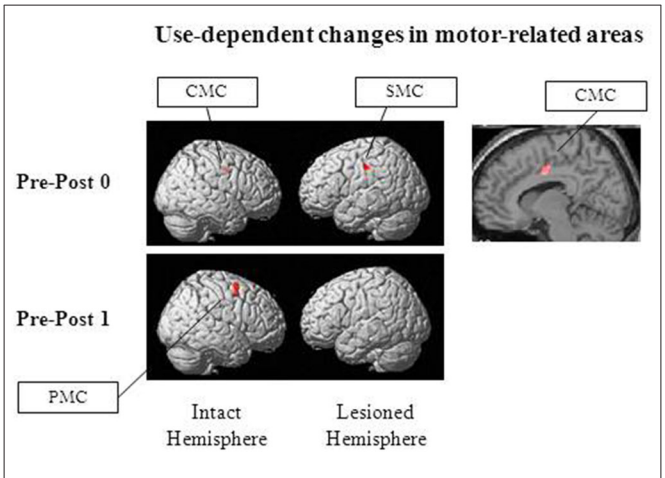

FIGURE 1 | Brain regions with use-dependent task-specific changes. Reduced activation was observed in the ipsilesional SMC, the contralesional cingulate motor cortex (CMC) and the contralesional premotor cortices (PMC), specifically for extensor movements of the paretic upper-limb after our combination therapy consisting of 5 Hz repetitive transcranial magnetic stimulation (rTMS) and the paretic extensor training in post-stroke patients with flexor hypertonia ("Post 0" and "Post 1", immediately after and 2 weeks after the combination therapy, respectively). Adapted from Koganemaru et al. (2015).

training, neuroimaging studies demonstrated that multi-regional brain reorganization occurred in several motor-related regions, including bilateral M1, premotor cortices (PMC), cingulate motor cortex (CMC), basal ganglia, and cerebellum (Nelles et al., 2001; Carey et al., 2002; Johansen-Berg et al., 2002a; Jang et al., 2003, 2005; Ward et al., 2003a, 2004; Luft et al., 2004; Ward and Cohen, 2004). The over-activity of non-M1 regions such as bilateral PMC in the acute stage was progressively decreased with an improvement in motor performance of the hemiparetic limbs (Calautti et al., 2001; Small et al., 2002; Ward et al., 2003a). In the chronic stage, the magnitude of brain activity in the non-M1 regions was negatively correlated with clinical outcome (Ward et al., 2003a,b) and positively correlated with the extent of damage in the corticospinal system (Ward et al., 2006). The findings suggest that the compensatory mechanism of these regions may be due to insufficient motor recovery (Feydy et al., 2002; Ward et al., 2003b). Those patients with larger brain damage and poorer clinical recovery may rely on activity in secondary motor areas to drive residual hand function (Johansen-Berg et al., 2002b). If a combination therapy of a task-specific training and brain stimulation could restore the ipsilesional M1 function by use-dependent plasticity, compensatory drive from secondary motor areas would be changed in post-stroke patients.

As previously described, we have developed a new combination therapy consisting of 5 Hz rTMS and an electrical neuromuscular stimulation assisted extensor training of the paretic upper-limb for stroke patients with flexor hypertonia. The extensor-specific plastic change in M1 was associated with beneficial functional effects (Koganemaru et al., 2010). We investigated whether extensor-specific multiregional brain reorganization occurred after our combination therapy by using functional magnetic resonance imaging (fMRI). The patients were scanned while performing upper-limb extensor movements. Untrained flexor movements were used as a control condition. Assessments were performed before, immediately after, and 2 weeks after the hybrid rehabilitation protocol. Analysis of the imaging data showed a significant reduction of brain activity in the ipsilesional SMC and the contralesional CMC immediately after (Post 0) and in the contralesional PMC 2 weeks after the intervention (Post 1; **Figure 1**). It suggests that the effects of the hybrid-rehabilitation appeared to differ temporally in each brain area. The process of motor learning consists of a fast learning stage and a slow learning stage. Specific neural representations are known in each stage (Karni et al., 1998; Kantak et al., 2012). The changes in activity in the ipsilesional SMC and the contralesional CMC may have shown combined effects of the fast learning stage, whereas the activity change in the contralesional PMC may have been involved in a consolidative process of the slow learning stage. Furthermore, the changes were associated with functional improvements of the paretic hands. They were not shown for the control condition (Koganemaru et al., 2015). Use-dependent plasticity induced by repetitive training may be related to the task-specific multi-regional brain reorganization. Thus, we expect that possible future targets for brain stimulation could include secondary motor areas. Artificial control of compensatory drive from secondary motor areas in accordance with the recovery process may be the next target for a combination therapy.

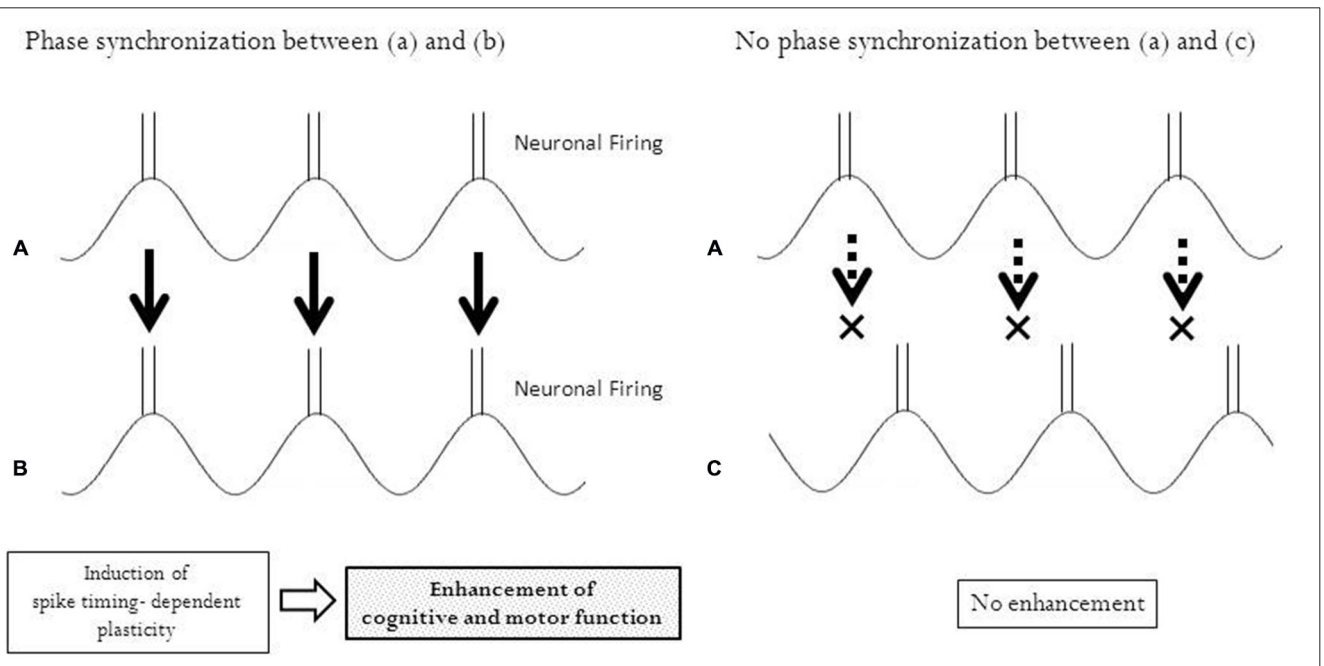

FIGURE 2 | Phase synchronization and temporal associative plasticity. Phase synchronization enables neurons in two different regions **(A)** and **(B)** to fire at the same timing, leading to an induction of spike-timing dependent plasticity between these regions. No synchronization of phases between the two regions **(A)** and **(C)** with different timing of neuronal firing does not induce any plasticity.

Phase Synchronization Between Rhythmically Patterned Brain Stimulation and Task-Related Patterned Activities of Neurons

In line with the theory of associative plasticity, we may be able to utilize the synchronization of phases between rhythmically patterned brain stimulation and task-related rhythmical activities of neurons. The phase synchronization of pre- and post-synaptic oscillations (wave-like neuronal signals) enabled researchers to correlate the timing of pre- and post-synaptic action potentials, resulting in the induction of temporal associative plasticity (Fell and Axmacher, 2011). Oscillatory non-invasive brain stimulation such as transcranial alternative current stimulation (tACS) and oscillatory tDCS (otDCS) has been reported to modulate oscillatory brain activity (Herrmann et al., 2013). Both tACS and otDCS use a sinusoidal form of electrical currents; however, tACS has no DC offset (net current = 0) and otDCS has a DC offset (net current = DC offset). In otDCS, the alternating current is superimposed onto a direct current. These protocols of stimulation may enhance neuronal circuits associated with intrinsic rhythmicity, leading to the enhancement of cognitive function (Marshall et al., 2006; Castro-Alamancos et al., 2007; Kanai et al., 2008, 2010; Kirov et al., 2009; Zaehle et al., 2010). In a recent study, phase-synchronized tACS suppressed Parkinson tremor by adjusting the phase to an abnormal cycle of the movements (Brittain et al., 2013). Rhythmical movements are produced with neuronal rhythmicity, a periodical repeat of excitation and inhibition. If oscillatory brain stimulation is synchronized with them at an appropriate phase, temporal associative plasticity may be induced (**Figure 2**).

One of the most familiar rhythmical movements in our daily life is locomotion, which requires the repeated patterned activation of specific neurons and muscles. Recently, in a preliminary experiment, we found that otDCS simulating gait rhythm induced gait-specific plasticity in healthy subjects (Koganemaru et al., 2014). Oscillatory patterned brain stimulation could be a new and powerful approach for the association of neuronal activities involved with training.

CONCLUSION

We have proposed the possible strategies for combination therapy of stimulation and rehabilitative trainings: (1) the control of temporal aspect between stimulation and task execution; (2) the use of a shaped task for the combination; (3) the appropriate stimulation of neuronal circuits where use-dependent plastic changes occur; and (4) phase synchronization between rhythmically patterned brain stimulation and task-related patterned activities of neurons. Associative brain plasticity induced by the combination therapy can bring functional improvements in patients.

There are still many diseases that are resistant to neuro-rehabilitative approaches. To better utilize brain stimulation in neuro-rehabilitation, we must explore more effective techniques for combining brain stimulation and rehabilitative training. An efficient association between brain stimulation and rehabilitative training could improve brain plasticity and promote functional recovery of patients.

ACKNOWLEDGMENTS

This study was partly supported by Grant-in-Aid for JSPS Fellows (25-5251), by Grant-in-Aid for Young Scientists (B) (26870321) from the Japan Society for the Promotion of Science, by the research grant (2015) from the Nakatomi Foundation (to SK), by Grant-in-Aid for Scientific Research (B) 15H03044, Grant-in-Aid for Scientific Research on Innovative Areas (15H05880, 15H05871) from the Japan Society for the Promotion of Science, STEP2 (15ek0109078h0001) from Japan Agency for Medical Research and development, AMED and the research grant (2015) from the Brain Science Foundation (to TM).

REFERENCES

Ameli, M., Grefkes, C., Kemper, F., Riegg, F. P., Rehme, A. K., Karbe, H., et al. (2009). Differential effects of high-frequency repetitive transcranial magnetic stimulation over ipsilesional primary motor cortex in cortical and subcortical middle cerebral artery stroke. *Ann. Neurol.* 66, 298–309. doi: 10.1002/ana.21725

Barnes, W. S. (1980). The relationship of motor-unit activation to isokinetic muscular contraction at different contractile velocities. *Phys. Ther.* 60, 1152–1158.

Bayona, N. A., Bitensky, J., Salter, K., and Teasell, R. (2005). The role of task-specific training in rehabilitation therapies. *Top Stroke Rehabil.* 12, 58–65. doi: 10.1310/bqm5-6ygb-mvj5-wvcr

Bolognini, N., Vallar, G., Casati, C., Latif, L. A., El-Nazer, R., Williams, J., et al. (2011). Neurophysiological and behavioral effects of tDCS combined with constraint-induced movement therapy in poststroke patients. *Neurorehabil. Neural Repair* 25, 819–829. doi: 10.1177/1545968311411056

Brittain, J. S., Probert-Smith, P., Aziz, T. Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. *Curr. Biol.* 23, 436–440. doi: 10.1016/j.cub.2013.01.068

Buetefisch, C., Heger, R., Schicks, W., Seitz, R., and Netz, J. (2011). Hebbian-type stimulation during robot-assisted training in patients with stroke. *Neurorehabil. Neural Repair* 25, 645–655. doi: 10.1177/1545968311402507

Butefisch, C., Hummelsheim, H., Denzler, P., and Mauritz, K. H. (1995). Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. *J. Neurol. Sci.* 130, 59–68. doi: 10.1016/0022-510x(95)00003-k

Butefisch, C. M., Davis, B. C., Wise, S. P., Sawaki, L., Kopylev, L., Classen, J., et al. (2000). Mechanisms of use-dependent plasticity in the human motor cortex. *Proc. Natl. Acad. Sci. U S A* 97, 3661–3665. doi: 10.1073/pnas.97.7.3661

Calautti, C., Leroy, F., Guincestre, J. Y., and Baron, J. C. (2001). Dynamics of motor network overactivation after striatocapsular stroke: a longitudinal PET study using a fixed-performance paradigm. *Stroke* 32, 2534–2542. doi: 10.1161/hs1101.097401

Carey, J. R., Kimberley, T. J., Lewis, S. M., Auerbach, E. J., Dorsey, L., Rundquist, P., et al. (2002). Analysis of fMRI and finger tracking training in subjects with chronic stroke. *Brain* 125, 773–788. doi: 10.1093/brain/awf091

Castro-Alamancos, M. A., Rigas, P., and Tawara-Hirata, Y. (2007). Resonance (approximately 10 Hz) of excitatory networks in motor cortex: effects of voltage-dependent ion channel blockers. *J. Physiol.* 578, 173–191. doi: 10.1113/jphysiol.2006.119016

Chang, W. H., Kim, Y. H., Bang, O. Y., Kim, S. T., Park, Y. H., and Lee, P. K. (2010). Long-term effects of rTMS on motor recovery in patients after subacute stroke. *J. Rehabil. Med.* 42, 758–764. doi: 10.2340/16501977-0590

Chang, W. H., Kim, Y. H., Yoo, W. K., Goo, K. H., Park, C. H., Kim, S. T., et al. (2012). rTMS with motor training modulates cortico-basal ganglia-thalamocortical circuits in stroke patients. *Restor. Neurol. Neurosci.* 30, 179–189. doi: 10.3233/RNN-2012-110162

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., et al. (2008). A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *J. Physiol. Lond.* 586, 5717–5725. doi: 10.1113/jphysiol.2008

Classen, J., Liepert, J., Wise, S. P., Hallett, M., and Cohen, L. G. (1998). Rapid plasticity of human cortical movement representation induced by practice. *J. Neurophysiol.* 79, 1117–1123.

Conforto, A. B., Anjos, S. M., Saposnik, G., Mello, E. A., Nagaya, E. M., Santos, W., et al. (2012). Transcranial magnetic stimulation in mild to severe hemiparesis early after stroke: a proof of principle and novel approach to improve motor function. *J. Neurol.* 259, 1399–1405. doi: 10.1007/s00415-011-6364-7

Dayan, E., Censor, N., Buch, E. R., Sandrini, M., and Cohen, L. G. (2013). Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat. Neurosci.* 16, 838–844. doi: 10.1038/nn.3422

Dimyan, M. A., and Cohen, L. G. (2011). Neuroplasticity in the context of motor rehabilitation after stroke. *Nat. Rev. Neurol.* 7, 76–85. doi: 10.1038/nrneurol.2010.200

Edwardson, M. A., Lucas, T. H., Carey, J. R., and Fetz, E. E. (2013). New modalities of brain stimulation for stroke rehabilitation. *Exp. Brain Res.* 224, 335–358. doi: 10.1007/s00221-012-3315-1

Egger, V., Feldmeyer, D., and Sakmann, B. (1999). Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nat. Neurosci.* 2, 1098–1105.

Emara, T. H., Moustafa, R. R., Elnahas, N. M., Elganzoury, A. M., Abdo, T. A., Mohamed, S. A., et al. (2010). Repetitive transcranial magnetic stimulation at 1Hz and 5Hz produces sustained improvement in motor function and disability after ischaemic stroke. *Eur. J. Neurol.* 17, 1203–1209. doi: 10.1111/j.1468-1331.2010.03000.x

Fell, J., and Axmacher, N. (2011). The role of phase synchronization in memory processes. *Nat. Rev. Neurosci.* 12, 105–118. doi: 10.1038/nrn2979

Feydy, A., Carlier, R., Roby-Brami, A., Bussel, B., Cazalis, F., Pierot, L., et al. (2002). Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. *Stroke* 33, 1610–1617. doi: 10.1161/01.str.0000017100.68294.52

Floel, A. (2014). tDCS-enhanced motor and cognitive function in neurological diseases. *Neuroimage* 85, 934–947. doi: 10.1016/j.neuroimage.2013.05.098

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Garcia-Alias, G., Barkhuysen, S., Buckle, M., and Fawcett, J. W. (2009). Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. *Nat. Neurosci.* 12, 1145–1151. doi: 10.1038/nn.2377

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature* 406, 147–150. doi: 10.1038/35018000

Hebb, D. O. (1949). *The Organization of Behavior: A Neuropsychological Theory*. New York, NY: Wiley.

Hendricks, H. T., Van Limbeek, J., Geurts, A. C., and Zwarts, M. J. (2002). Motor recovery after stroke: a systematic review of the literature. *Arch. Phys. Med. Rehabil.* 83, 1629–1637. doi: 10.1053/apmr.2002.35473

Herrmann, C. S., Rach, S., Neuling, T., and Struber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Front. Hum. Neurosci.* 7:279. doi: 10.3389/fnhum.2013.00279

Hess, G., Aizenman, C. D., and Donoghue, J. P. (1996). Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. *J. Neurophysiol.* 75, 1765–1778.

Hess, G., and Donoghue, J. P. (1996). Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. *Acta. Neurobiol. Exp. (Wars)* 56, 397–405.

Hsu, Y. F., Huang, Y. Z., Lin, Y. Y., Tang, C. W., Liao, K. K., Lee, P. L., et al. (2013). Intermittent theta burst stimulation over ipsilesional primary motor cortex of subacute ischemic stroke patients: a pilot study. *Brain Stimul.* 6, 166–174. doi: 10.1016/j.brs.2012.04.007

Hummel, F. C., and Cohen, L. G. (2005). Drivers of brain plasticity. *Curr. Opin. Neurol.* 18, 667–674. doi: 10.1097/01.wco.0000189876.37475.42

Hummelsheim, H. (1999). Rationales for improving motor function. *Curr. Opin. Neurol.* 12, 697–701. doi: 10.1097/00019052-199912000-00007

Jang, S. H., Kim, Y. H., Cho, S. H., Chang, Y., Lee, Z. I., and Ha, J. S. (2003). Cortical reorganization associated with motor recovery in hemiparetic stroke patients. *Neuroreport* 14, 1305–1310. doi: 10.1097/01.wnr.0000078700.79393.09

Jang, S. H., You, S. H., Hallett, M., Cho, Y. W., Park, C. M., Cho, S. H., et al. (2005). Cortical reorganization and associated functional motor recovery after virtual reality in patients with chronic stroke: an experimenter-blind preliminary study. *Arch. Phys. Med. Rehabil.* 86, 2218–2223. doi: 10.1016/j.apmr.2005.04.015

Johansen-Berg, H., Dawes, H., Guy, C., Smith, S. M., Wade, D. T., and Matthews, P. M. (2002a). Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. *Brain* 125, 2731–2742. doi: 10.1093/brain/awf282

Johansen-Berg, H., Rushworth, M. F., Bogdanovic, M. D., Kischka, U., Wimalaratna, S., and Matthews, P. M. (2002b). The role of ipsilateral premotor cortex in hand movement after stroke. *Proc. Natl. Acad. Sci. U S A* 99, 14518–14523. doi: 10.1073/pnas.222536799

Kanai, R., Chaieb, L., Antal, A., Walsh, V., and Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Curr. Biol.* 18, 1839–1843. doi: 10.1016/j.cub.2008.10.027

Kanai, R., Paulus, W., and Walsh, V. (2010). Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clin. Neurophysiol.* 121, 1551–1554. doi: 10.1016/j.clinph.2010.03.022

Kantak, S. S., Stinear, J. W., Buch, E. R., and Cohen, L. G. (2012). Rewiring the brain: potential role of the premotor cortex in motor control, learning and recovery of function following brain injury. *Neurorehabil. Neural Repair* 26, 282–292. doi: 10.1177/1545968311420845

Karabanov, A., Ziemann, U., Hamada, M., George, M. S., Quartarone, A., Classen, J., et al. (2015). Consensus paper: probing homeostatic plasticity of human cortex with non-invasive transcranial brain stimulation. *Brain Stimul.* 8, 442–454. doi: 10.1016/j.brs.2015.01.404

Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M. M., Turner, R., et al. (1998). The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. *Proc. Natl. Acad. Sci. U S A* 95, 861–868. doi: 10.1073/pnas.95.3.861

Kaviraja, U., and Robert, C. (2013). "Central motor conduction time," in *Handbook of Clinical Neurology, Brain Stimulation*, eds A. M. Lozano and M. Hallett (Amsterdam: Elsevier B.V.), 375–386. doi: 10.1007/978-3-540-29678-2_891

Khedr, E. M., Abdel-Fadeil, M. R., Farghali, A., and Qaid, M. (2009). Role of 1 and 3 Hz repetitive transcranial magnetic stimulation on motor function recovery after acute ischaemic stroke. *Eur. J. Neurol.* 16, 1323–1330. doi: 10.1111/j.1468-1331.2009.02746.x

Khedr, E. M., Ahmed, M. A., Fathy, N., and Rothwell, J. C. (2005). Therapeutic trial of repetitive transcranial magnetic stimulation after acute ischemic stroke. *Neurology* 65, 466–468. doi: 10.1212/01.wnl.0000173067.84247.36

Khedr, E. M., Etraby, A. E., Hemeda, M., Nasef, A. M., and Razek, A. A. (2010). Long-term effect of repetitive transcranial magnetic stimulation on motor function recovery after acute ischemic stroke. *Acta. Neurol. Scand.* 121, 30–37. doi: 10.1111/j.1600-0404.2009.01195.x

Kim, D. Y., Lim, J. Y., Kang, E. K., You, D. S., Oh, M. K., Oh, B. M., et al. (2010). Effect of transcranial direct current stimulation on motor recovery in patients with subacute stroke. *Am. J. Phys. Med. Rehabil.* 89, 879–886. doi: 10.1097/phm.0b013e3181f70aa7

Kim, D. Y., Ohn, S. H., Yang, E. J., Park, C. I., and Jung, K. J. (2009). Enhancing motor performance by anodal transcranial direct current stimulation in subacute stroke patients. *Am. J. Phys. Med. Rehabil.* 88, 829–836. doi: 10.1097/phm.0b013e3181b811e3

Kim, Y. H., You, S. H., Ko, M. H., Park, J. W., Lee, K. H., Jang, S. H., et al. (2006). Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. *Stroke* 37, 1471–1476. doi: 10.1161/01.str.000221233.55497.51

Kirov, R., Weiss, C., Siebner, H. R., Born, J., and Marshall, L. (2009). Slow oscillation electrical brain stimulation during waking promotes EEG theta

activity and memory encoding. *Proc. Natl. Acad. Sci. U S A* 106, 15460–15465. doi: 10.1073/pnas.0904438106

Koganemaru, S., Mikami, Y., Fukuyama, H., and Mima, T. (2014). Slow oscillatory stimulation simulating gait rhythm induces LTP-like effects on corticospinal excitability in foot. *Clin. Neurophysiol.* 125, Suppl 1, Abstracts of the 30th International Congress of Clinical Neurophysiology (ICCN) of the IFCN, March 20–23 2014, Berlin, Germany, S223.

Koganemaru, S., Mima, T., Nakatsuka, M., Ueki, Y., Fukuyama, H., and Domen, K. (2009). Human motor associative plasticity induced by paired bihemispheric stimulation. *J. Physiol.* 587, 4629–4644. doi: 10.1113/jphysiol.2009.174342

Koganemaru, S., Mima, T., Thabit, M. N., Ikkaku, T., Shimada, K., Kanematsu, M., et al. (2010). Recovery of upper-limb function due to enhanced use-dependent plasticity in chronic stroke patients. *Brain* 133, 3373–3384. doi: 10.1093/brain/awq193

Koganemaru, S., Sawamoto, N., Aso, T., Sagara, A., Ikkaku, T., Shimada, K., et al. (2015). Task-specific brain reorganization in motor recovery induced by a hybrid-rehabilitation combining training with brain stimulation after stroke. *Neurosci. Res.* 92, 29–38. doi: 10.1016/j.neures.2014.10.004

Luft, A. R., Mccombe-Waller, S., Whitall, J., Macko, R., Sorkin, J. D., Schulz, J. B., et al. (2004). Repetitive bilateral arm training and motor cortex activation in chronic stroke: A randomized controlled trial. *JAMA* 292, 1853–1861. doi: 10.1001/jama.292.20.2470-b

Marshall, L., Helgadottir, H., Molle, M., and Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature* 444, 610–613. doi: 10.1038/nature05278

Masiero, S., and Carraro, E. (2008). Upper limb movements and cerebral plasticity in post-stroke rehabilitation. *Aging Clin. Exp. Res.* 20, 103–108. doi: 10.1007/bf03324755

Meehan, S. K., Dao, E., Linsdell, M. A., and Boyd, L. A. (2011). Continuous theta burst stimulation over the contralesional sensory and motor cortex enhances motor learning post-stroke. *Neurosci. Lett.* 500, 26–30. doi: 10.1016/j.neulet.2011.05.237

Nair, D. G., Renga, V., Lindenberg, R., Zhu, L., and Schlaug, G. (2011). Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS. *Restor. Neurol. Neurosci.* 29, 411–420. doi: 10.3233/RNN-2011-0612

Nakayama, H., Jorgensen, H. S., Raaschou, H. O., and Olsen, T. S. (1994). Recovery of upper extremity function in stroke patients: the Copenhagen Stroke Study. *Arch. Phys. Med. Rehabil.* 75, 394–398.

Nelles, G., Jentzen, W., Jueptner, M., Müller, S., and Diener, H. C. (2001). Arm training induced brain plasticity in stroke studied with serial positron emission tomography. *NeuroImage* 13, 1146–1154. doi: 10.1006/nimg.2001.0757

Nudo, R. J., and Milliken, G. W. (1996). Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J. Neurophysiol.* 75, 2144–2149.

Nudo, R. J., Milliken, G. W., Jenkins, W. M., and Merzenich, M. M. (1996a). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J. Neurosci.* 16, 785–807.

Nudo, R. J., Wise, B. M., Sifuentes, F., and Milliken, G. W. (1996b). Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science* 272, 1791–1794. doi: 10.1126/science.272.5269.1791

Page, S. J. (2003). Intensity versus task-specificity after stroke: how important is intensity? *Am. J. Phys. Med. Rehabil.* 82, 730–732. doi: 10.1097/01.phm.0000078226.36000.a5

Pascual-Leone, A., Valls-Solé, J., Wassermann, E. M., and Hallett, M. (1994). Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain* 117, 847–858. doi: 10.1093/brain/117.4.847

Pennisi, G., Rapisarda, G., Bella, R., Calabrese, V., Maertens De Noordhout, A., and Delwaide, P. J. (1999). Absence of response to early transcranial magnetic stimulation in ischemic stroke patients: prognostic value for hand motor recovery. *Stroke* 30, 2666–2670. doi: 10.1161/01.str.30.12.2666

Platz, T., and Rothwell, J. C. (2010). Brain stimulation and brain repair–rTMS: from animal experiment to clinical trials–what do we know? *Restor. Neurol. Neurosci.* 28, 387–398. doi: 10.3233/RNN-2010-0570

Reinkensmeyer, D. J., and Boninger, M. L. (2012). Technologies and combination therapies for enhancing movement training for people with a disability. *J. Neuroeng. Rehabil.* 9, 17. doi: 10.1186/1743-0003-9-17

Richards, L. G., Stewart, K. C., Woodbury, M. L., Senesac, C., and Cauraugh, J. H. (2008). Movement-dependent stroke recovery: A systematic review and meta-analysis of TMS and fMRI evidence. *Neuropsychologia* 46, 3–11. doi: 10.1016/j.neuropsychologia.2007.08.013

Rossini, P. M., and Pauri, F. (2000). Neuromagnetic integrated methods tracking human brain mechanisms of sensorimotor areas 'plastic' reorganisation. *Brain Res. Rev.* 33, 131–154. doi: 10.1016/s0169-328x(00)00090-5

Sandrini, M., and Cohen, L. G. (2013). "Noninvasive brain stimulation in neurorehabilitation," in *Handbook of Clinical Neurology, Brain Stimulation*, eds A. M. Lozano and M. Hallett. (Amsterdam: Elsevier B.V.), 499–524. doi: 10.1016/b978-0-444-53497-2.00040-1

Small, S. L., Hlustik, P., Noll, D. C., Genovese, C., and Solodkin, A. (2002). Cerebellar hemispheric activation ipsilateral to the paretic hand correlates with functional recovery after stroke. *Brain* 125, 1544–1557. doi: 10.1093/brain/awf148

Stagg, C. J., and Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *Neuroscientist* 17, 37–53. doi: 10.1177/1073858410386614

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., and Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J. Physiol. Lond.* 543, 699–708. doi: 10.1113/jphysiol.2002.023317

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., and Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 123, 572–584. doi: 10.1093/brain/123.3.572

Takeuchi, N., Chuma, T., Matsuo, Y., Watanabe, I., and Ikoma, K. (2005). Repetitive transcranial magnetic stimulation of contralesional primary motor cortex improves hand function after stroke. *Stroke* 36, 2681–2686. doi: 10.1161/01.str.0000189658.51972.34

Takeuchi, N., Tada, T., Toshima, M., Chuma, T., Matsuo, Y., and Ikoma, K. (2008). Inhibition of the unaffected motor cortex by 1 Hz repetitive transcranical magnetic stimulation enhances motor performance and training effect of the paretic hand in patients with chronic stroke. *J. Rehabil. Med.* 40, 298–303. doi: 10.2340/16501977-0181

Talelli, P., Wallace, A., Dileone, M., Hoad, D., Cheeran, B., Oliver, R., et al. (2012). Theta burst stimulation in the rehabilitation of the upper limb: a semirandomized, placebo-controlled trial in chronic stroke patients. *Neurorehabil. Neural Repair* 26, 976–987. doi: 10.1177/1545968312437940

Teasell, R., Bitensky, J., Foley, N., and Bayona, N. A. (2005). Training and stimulation in post stroke recovery brain reorganization. *Top Stroke Rehabil.* 12, 37–45. doi: 10.1310/e893-m0pr-njej-1gxm

Thabit, M. N., Ueki, Y., Koganemaru, S., Fawi, G., Fukuyama, H., and Mima, T. (2010). Movement-related cortical stimulation can induce human motor plasticity. *J. Neurosci.* 30, 11529–11536. doi: 10.1523/JNEUROSCI.1829-10.2010

Ueki, Y., Mima, T., Kotb, M. A., Sawada, H., Saiki, H., Ikeda, A., et al. (2006). Altered plasticity of the human motor cortex in Parkinson's disease. *Ann. Neurol.* 59, 60–71. doi: 10.3410/f.7018.349452

Verheyden, G., Nieuwboer, A., De Wit, L., Thijs, V., Dobbelaere, J., Devos, H., et al. (2008). Time course of trunk, arm, leg and functional recovery after ischemic stroke. *Neurorehabil. Neural Repair* 22, 173–179. doi: 10.1177/1545968307305456

Wang, R. Y., Tseng, H. Y., Liao, K. K., Wang, C. J., Lai, K. L., and Yang, Y. R. (2012). rTMS combined with task-oriented training to improve symmetry of interhemispheric corticomotor excitability and gait performance after stroke: a randomized trial. *Neurorehabil Neural Repair* 26, 222–230. doi: 10.1177/1545968311423265

Ward, N. S., Brown, M. M., Thompson, A. J., and Frackowiak, R. S. (2003a). Neural correlates of motor recovery after stroke: a longitudinal fMRI study. *Brain* 126, 2476–2496. doi: 10.1093/brain/awg245

Ward, N. S., Brown, M. M., Thompson, A. J., and Frackowiak, R. S. (2003b). Neural correlates of outcome after stroke: a cross-sectional fMRI study. *Brain* 126, 1430–1448. doi: 10.1093/brain/awg145

Ward, N. S., Brown, M. M., Thompson, A. J., and Frackowiak, R. S. (2004). The influence of time after stroke on brain activations during a motor task. *Ann. Neurol.* 55, 829–834. doi: 10.1002/ana.20099

Ward, N. S., and Cohen, L. G. (2004). Mechanisms underlying recovery of motor function after stroke. *Arch. Neurol.* 61, 1844–1848. doi: 10.1001/archneur.61.12.1844

Ward, N. S., Newton, J. M., Swayne, O. B., Lee, L., Thompson, A. J., Greenwood, R. J., et al. (2006). Motor system activation after subcortical stroke depends on corticospinal system integrity. *Brain* 129, 809–819. doi: 10.1093/brain/awl002

Woldag, H., Stupka, K., and Hummelsheim, H. (2010). Repetitive training of complex hand arm movements with shaping is beneficial for motor improvement in patients after stroke. *J. Rehabil. Med.* 42, 582–587. doi: 10.2340/16501977-0558

Zaehle, T., Rach, S., and Herrmann, C.S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One* 5:e13766. doi: 10.1371/journal.pone.0013766

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

PERSPECTIVE
published: 15 November 2016
doi: 10.3389/fnsys.2016.00082

A Framework for Combining rTMS with Behavioral Therapy

*K. Zoe Tsagaris[1], Douglas R. Labar[1,2] and Dylan J. Edwards[1,2]**

[1] Non-Invasive Brain Stimulation and Human Motor Control Laboratory, Burke Medical Research Institute, White Plains, NY, USA, [2] Department of Neurology, New York Presbyterian, Weill Cornell Medicine, New York, NY, USA

Upon its inception, repetitive transcranial magnetic stimulation (rTMS) was delivered at rest, without regard to the potential impact of activity occurring during or around the time of stimulation. rTMS was considered an experimental intervention imposed on the brain; therefore, the myriad features that might suppress or enhance its desired effects had not yet been explored. The field of rTMS has since grown substantially and therapeutic benefits have been reported, albeit with modest and inconsistent improvements. Work in this field accelerated following approval of a psychiatric application (depression), and it is now expanding to other applications and disciplines. In the last decade, experimental enquiry has sought new ways to improve the therapeutic benefits of rTMS, intended to enhance underlying brain reorganization and functional recovery by combining it with behavioral therapy. This concept is appealing, but poorly defined and requires clarity. We provide an overview of how combined rTMS and behavioral therapy has been delineated in the literature, highlighting the diversity of approaches. We outline a framework for study design and reporting such that the effects of this emerging method can be better understood.

Keywords: repetitive transcranial magnetic stimulation (rTMS), non-invasive brain stimulation, neurological rehabilitation, behavioral interventions, therapy

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Patrizia Silvia Bisiacchi, University of Padua, Italy
Rachel M. Sherrard, Pierre-and-Marie-Curie University, France

****Correspondence:***
Dylan J. Edwards
dje2002@med.cornell.edu

Received: *30 March 2016*
Accepted: *27 September 2016*
Published: *15 November 2016*

Citation:
Tsagaris KZ, Labar DR and Edwards DJ (2016) A Framework for Combining rTMS with Behavioral Therapy. Front. Syst. Neurosci. 10:82. doi: 10.3389/fnsys.2016.00082

INTRODUCTION

The brain is never at rest; the *default-mode network* comprised of coherent and connected brain networks (cingulate cortices, inferior parietal, and medial prefrontal regions), operative at times of behavioral rest, has been examined through the use of blood oxygenation level-dependent (BOLD) signals. Functional MRI testing has shown the default-mode network is active at rest and in the presence of volitional behavior (e.g., repetitive task practice), decreases in activation. This deactivation may allow for associated activity-dependent plasticity in other parts of the motor system. Because of the default-mode network's activation/deactivation patterns, it appears the motor cortex does not work in isolation during volitional behavior, motor learning, recovery and reorganization (Sanes and Donoghue, 2000; Gusnard and Raichle, 2001; Damoiseaux et al., 2006). Instead, the motor cortex injury-response functional reorganization appears to occur through use-dependent alteration of outputs, which result from behavioral experiences, such as repetitive task practice (Nudo et al., 2001). Interventions promoting recovery should attend to this complex dynamic process, and likely utilize a multi-faceted approach for optimal outcome.

Background

The initial application of spatially targeted non-invasive brain stimulation (NIBS) as treatment to improve function was not temporally linked to meaningful voluntary brain activity; instead was delivered in isolation of standard therapy practice techniques (Amassian and Maccabee, 2006). Such applications would seem to tap into only a fraction of the complex multi-faceted systems changes involved in learning and memory. Influencing brain areas remote from, but functionally connected to, a primary target, and influencing brain activity at critical time periods relative to practice and therapy timing, may be important additional considerations in this domain.

Hardwired existing pathways (e.g., skilled movement, speech, or executive function) may have lower threshold for activation by transcranial magnetic stimulation (TMS), and therefore be preferentially predisposed to modulation by NIBS. For example, in the motor system, Rothwell et al. (1987) studied a healthy population at rest and found the largest electromyographic (EMG) responses in distal muscles (which have the finest movements associated with them) versus lower EMG responses in proximal muscles. Furthermore, upon simultaneous voluntary contraction, the EMG response latency to brain TMS shortens, becomes larger, and the threshold is lower. Therefore, if voluntary activation is absent or decreased, such as in a diseased brain state, the modulation possible with NIBS may be altered. Conversely, TMS may affect a patient's ability to produce a voluntary motor activation. Thus the quality and duration of responses seem likely to be influenced by the interactions of interdependent complex systems. The efficacy of stand-alone NIBS is influenced by its stimulation variables, which include: frequency, intensity, coil positioning, stimulation site, and number of sessions delivered (Nollet et al., 2003; Goetz et al., 2016). Therefore, TMS protocols may be adapted to modulate the motor response through excitatory or depressive methods dependent on stimulation parameters, providing opportunity for custom programs based on the population, person, and pathology.

rTMS and Behavioral Intervention: Current Focus

One contemporary approach is to link NIBS with behavioral techniques, in hopes of producing more robust and durable outcomes. The logic for doing so stems from the idea that effects of repetitive TMS (rTMS) and behavioral therapy will sum or that rTMS will enhance, or consolidate, the effects of therapy. For instance, Mills and Schubert (1995) suggest TMS during sustained voluntary, tonic activity may increase synchronicity of common input fibers and motor neurons. This improved synchrony may promote increased motor cortex plasticity, compared to one intervention alone. Finding the optimal pairing of intensity, duration, frequency, and site, for both rTMS and therapy, would be important for the strongest enhancement, but also to avoid maladaptive plasticity.

Thus one can learn from analyzing the variety of approaches that have been employed previously and assess outcome. In the present paper we aimed to: (a) outline what is meant by *combined therapy* in the context of historical literature [this is examining how traditional therapies (occupational, speech, physical, cognitive-behavioral, or task practice) are combined with rTMS either on or off-line]; (b) provide a snapshot of the literature showing the diverse range of approaches, and outcomes; and (c) propose a framework for how combined therapy should be reported in the literature moving forward.

Most *combined therapy* studies have been on stroke survivors to date and have been intuitively and practically based (Supplementary Table S1). For example, time considerations such as equipment practicality, busy clinical setting, staffing, training, and availability, may affect feasibility of temporally combined therapies. Additionally, the participant's cognitive or physical state during rTMS delivery is rarely reported in the literature but may influence intervention efficacy (e.g., patient engaged in conversation, use of mobile device, listening to music). Despite increasing application of TMS in the field of psychiatry and FDA approval for refractory depression, very little has been done in examining the combination of rTMS with behavioral interventions, such as cognitive behavioral therapy (Micoulaud-Franchi et al., 2013; *note French language*). Instead, most commonly, papers describe using rTMS in isolation for patients with medication-resistant depression (Lam et al., 2008; Slotema et al., 2010). It would be of considerable interest for systematic research on *combined therapy* to expand beyond the motor function domain, and study brain processes such as depression or learning in the future. Hopefully this would lead to a better understanding of broader principles concerning the effects of rTMS as an adjunct to older, traditional, well-accepted therapy approaches.

Cumulative Effect

Research to determine the cumulative effect and duration of results of NIBS intervention is ongoing. This cumulative effect was examined in a healthy population by Baumer et al. (2003) who found repeated sessions of rTMS (two trains of inhibitory, sub-motor threshold rTMS over the pre-motor cortex) delivered within 24 h, or no greater than 7 days later (consecutive days), induced plastic changes of intrinsic motor cortex excitability. Khedr et al. (2006) and Lomarev et al. (2006) found a cumulative effect of rTMS in individuals with Parkinson's Disease (eight sessions of 25 Hz rTMS over 4 weeks) which lasted 1 month or more. It appears that multiple sessions of rTMS will lead to a cumulative effect, identified as regions of sustained membrane polarization (Pell et al., 2011). With this effect it may be possible to build on neuromodulatory changes in subsequent sessions to promote recovery or slow disease progression. Similarly, in behavioral therapy, efficacy of treatment may be affected by factors including: duration, intensity, type of intervention, and modality used. These therapeutic variables, specifically intensity and duration of therapy, may carry varying levels of importance dependent on individual functional status, though there is still much debate regarding this (Winstein et al., 2016). Thus there are striking similarities between NIBS and traditional therapy variables, in that frequency, intensity, site or system, and duration of the intervention affect outcome.

LITERATURE SEARCH

The intent of this search was to identify and summarize published studies combining rTMS with behavioral intervention for clinical benefit. Therefore, we have limited the search to a neurological patient population. All articles were found through the PubMed database and excluded: non-English, case studies, drug studies, transcranial direct current stimulation (tDCS) or other non-invasive stimulation methods, and/or healthy subjects. The acronym *rTMS* was used in combination with the following search terms (relevant/hits): physical therapy (23/267), occupational therapy (8/35), speech therapy (10/54), behavioral intervention (0/95), task training (2/23), motor practice (3/42), motor training (3/50), rehabilitation (8/232), cognition (0/144), cognitive rehabilitation (0/24), cognitive behavioral therapy (2/144), CBT (0/7), cognitive training (0/19). A summary of hits for tDCS in combination with the above search terms is located in Supplementary Figure S1, however, will not be further reviewed in this paper.

LITERATURE ANALYSIS

The majority of rTMS papers report application of the full stimulation protocol at rest, i.e., not during associated therapy (37/50). A smaller proportion of experimental studies used an interleaved approach of combined therapy, performing the therapeutic task during inter-train intervals (5/50). Less than twenty percent of studies fell into the following categories; unspecified timing (2/50), used high or low frequency dependent on experimental group (3/50), delivered rTMS and therapy simultaneously (2/50) or completed rTMS and therapy on different days (1/50).Within these experimental studies, there was great diversity in the stimulation and therapy variables used for each diagnosis within the neurological population (**Figure 1**). Despite differences, the majority of papers show improvement in clinical outcomes. However, vague and inconsistent reporting of the combined intervention, as well as diverse approaches, impede advancement of understanding toward optimizing intervention and maximizing clinical efficacy. Elements of rTMS application that varied most commonly between studies include; rTMS timing relative to therapy, and either inhibitory (low-frequency), or excitatory (high-frequency) rTMS (Supplementary Table S1). Detailed reporting and standardization for both rTMS and therapy characteristics is a strategy for taking into consideration variations between clinicians, locations, resources, and type of intervention.

Stimulation Targeting

Of studies investigating chronic motor impairment following stroke (24/50), all but one targeted the primary motor cortex, the exception targeted the somatosensory cortex (Supplementary Table S1, Study #18). The majority of these studies targeted the unaffected hemisphere (17/24). The remaining studies stimulated the affected hemisphere (5/24; Supplementary Table S1, Study #5, 16, 18, 20, 23), or stimulated bilaterally (3/24; Supplementary Table S1, Study #6, 9, 13). With regards to pulse frequency in this population, 14/24 stimulated with low, 7/24 with high (Supplementary Table S1, Study # 5, 14, 16, 18, 20, 23, 26), and 3/24 alternated between high-frequency stimulation (affected hemisphere) and low-frequency (unaffected hemisphere).

All papers (6/50) addressing motor impairment in a sub-acute stroke population targeted the primary motor cortex. Three studies stimulated the unaffected hemisphere at low-frequency (Supplementary Table S1, Study #33, 34, 35), 1 stimulated the affected hemisphere at high-frequency (Supplementary Table S1, Study #32), and 1 stimulated both hemispheres (unaffected at low-frequency, affected at high-frequency) dependent on treatment group (Supplementary Table S1, Study #31). Cha and Kim (2016) stimulated the cortical representation of the first right dorsal interosseous muscle at a low-frequency in their study examining unilateral neglect and motor control.

Additional studies in the stroke population were completed in acute stroke [>1 month post onset (Emara et al., 2010)] or in sub-acute and chronic patients [3+ months post onset (Chang et al., 2012)]. In the acute population patients were stimulated at high-frequency (affected motor cortex) or low-frequency (unaffected). Chang et al. (2012) stimulated the affected primary motor cortex at high-frequency. Other motor impairment studies were completed in patients with congenital hemiparesis (children), Parkinson's disease, and hand dystonia (Supplementary Table S1, Study # 1, 2, 3, respectively). Gillick et al. (2014) stimulated the unaffected primary motor cortex with priming rTMS (high then low-frequency) for children with congenital hemiparesis. Yang et al. (2013) stimulated the contralateral primary motor cortex at high-frequency to "more affected side" in patients with Parkinson's disease. Kimberley et al. (2015) stimulated the unaffected pre-motor cortex at low-frequency in patients with hand dystonia. One study (Lim et al., 2010) studied hemispatial neglect and stimulated the left parietal area (P5; affected side for all participants) at low-frequency.

Studies examining speech impairments following stroke ($n = 11$) were completed in sub-acute (<6 months post-onset; 6/11) and chronic populations (>6 months post-onset; 5/11). Within the speech domain, Momosaki et al. (2014) studied oral motor control and stimulated two times daily (low then high-frequency) to the pharyngeal muscles representation. Of studies examining aphasia ($n = 10$), four stimulated Broca's Area (i.e., pars triangularis); 3/4 delivered a low-frequency (Supplementary Table S1, Study #40, 42, 46), while 1/4 stimulated with priming rTMS [high then low-frequency (Khedr et al., 2014)]. Abo et al. (2012) differentially defined the stimulation target based on aphasia-type, as determined by fMRI activation (inferior frontal gyrus in non-fluent aphasia and superior temporal gyrus in fluent aphasia) and stimulated at low-frequency.

Studies targeting cognitive impairment were completed in Alzheimer's (2/3), and chronic stroke populations (1/3). In the Alzheimer's studies, high-frequency stimulation was delivered to cortical targets including: Broca's Area, Wernicke's Area, right dorsolateral prefrontal cortex, and left parietal somatosensory association cortex (two targets stimulated one day, third target on another) (Supplementary Table S1, Study #49, 50). Park and Yoon (2015) targeted the left prefrontal cortex in stroke, the affected hemisphere for all participants, at high-frequency.

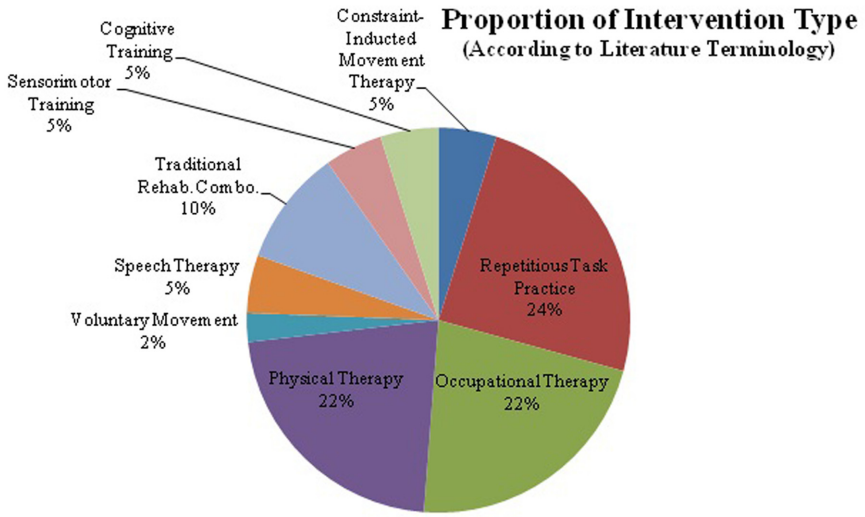

FIGURE 1 | The majority of research in repetitive transcranial magnetic stimulation (rTMS) and behavioral intervention has been completed with rTMS in conjunction with motor training. rTMS paired with speech therapy, cognitive training, and sensorimotor training account for only 30%. rTMS in conjunction with cognitive therapies has not been widely studied, despite FDA approval for use in refractory depression. This graph shows the distribution of behavioral interventions used in conjunction with rTMS. Despite there being a fairly wide variety of interventions utilized there are great differences in protocol design and stimulation parameters, which makes comparing protocols or drawing firm conclusions difficult.

Thus no clearly superior targeting strategy has emerged. Stimulation of a primary lesion area, stimulation of other areas with significant network connections to a primary lesion area, and stimulation of a focal area in the presence of a widespread brain disease process, all seem effective.

Outcomes with Respect to Timing

The relative timing of combined therapy has not been well explored, and is one of the most poorly reported variables in rTMS studies; however, none of the papers examined in this review reported a negative response, regardless of timing. In papers examining rTMS prior to behavioral intervention, 86.5% (32/37) cited a positive response, while 5/37 (Supplementary Table S1, Study #3, 20, 28, 35, 42) report a neutral response. The second most frequent approach (5/50) involved alternating protocols (behavioral intervention during the inter-train interval), all of which indicate a positive result in outcome (Supplementary Table S1, Study #5, 23, 30, 31, 32). Out of the remaining 20% of identified studies, 7/8 reported a positive response (Supplementary Table S1, Study #1, 10, 16, 26, 46, 49, 50), while 1/10 reported a neutral response (Supplementary Table S1, Study #48). Thus, as in the case of stimulation targeting, discussed above, no clearly superior strategy for temporal pairing of rTMS and behavioral therapy has emerged. However, firm conclusions cannot be drawn due to small sample sizes and need for additional randomized, controlled clinical trials.

For future research and reporting, we propose the temporal relationship of rTMS application with behavioral intervention be defined as: *concurrent* (rTMS being applied at the same time as the behavior is expressed); *sequential* (one intervention follows the other), *interleaved* (rTMS trains are alternating with behavioral expression/repetition) (**Figure 2**).

One could consider an ongoing pharmacological intervention paired with rTMS a concurrent application of combined therapy, and studies are underway of this nature. Sequential stimulation can occur at numerous time points prior to or following therapy (e.g., volitional activity), ranging from seconds to days. For instance, pulses can be delivered in an event-triggered manner, perhaps with an electroencephalogram (EEG) defined trigger; where important EEG changes known within the neural systems are presently under investigation. Therapeutic rTMS could plausibly be delivered seconds before a task through a clinician prompted visual or verbal cue to promote cognitive effort or movement. Indeed, a single case with positive outcome was reported in the literature for depression where rTMS was combined with a form of cognitive behavioral therapy; here, the cognitive effort was performed in between trains of rTMS – so in this case, combined, but alternating (Vedeniapin et al., 2010). Behavioral intervention can be initiated within minutes following stimulation through use of a defined time window to ensure consistency [i.e., therapy starts 5–10 min after rTMS completion, as in clinical trial NCT02089464 (Nexstim Ltd, 2015)]. Stimulation can be delivered within hours or days of therapeutic intervention (i.e., rTMS in morning, therapy in the afternoon or next day). Optimal timing may vary depending on diagnosis and behavioral intervention type, and further research is needed in this area, as 74% of papers delivered rTMS before behavioral intervention (37/50).

While the physiologic effects of rTMS and behavioral therapy are likely different; a key commonality in both is that after-effects have been linked to adaptive behavioral response, and attributed to lasting modification of synaptic strength in cortical networks subserving the behavior (Butefisch et al., 1995; Silasi and Murphy, 2014), which can be local (e.g., primary motor cortex), or distant

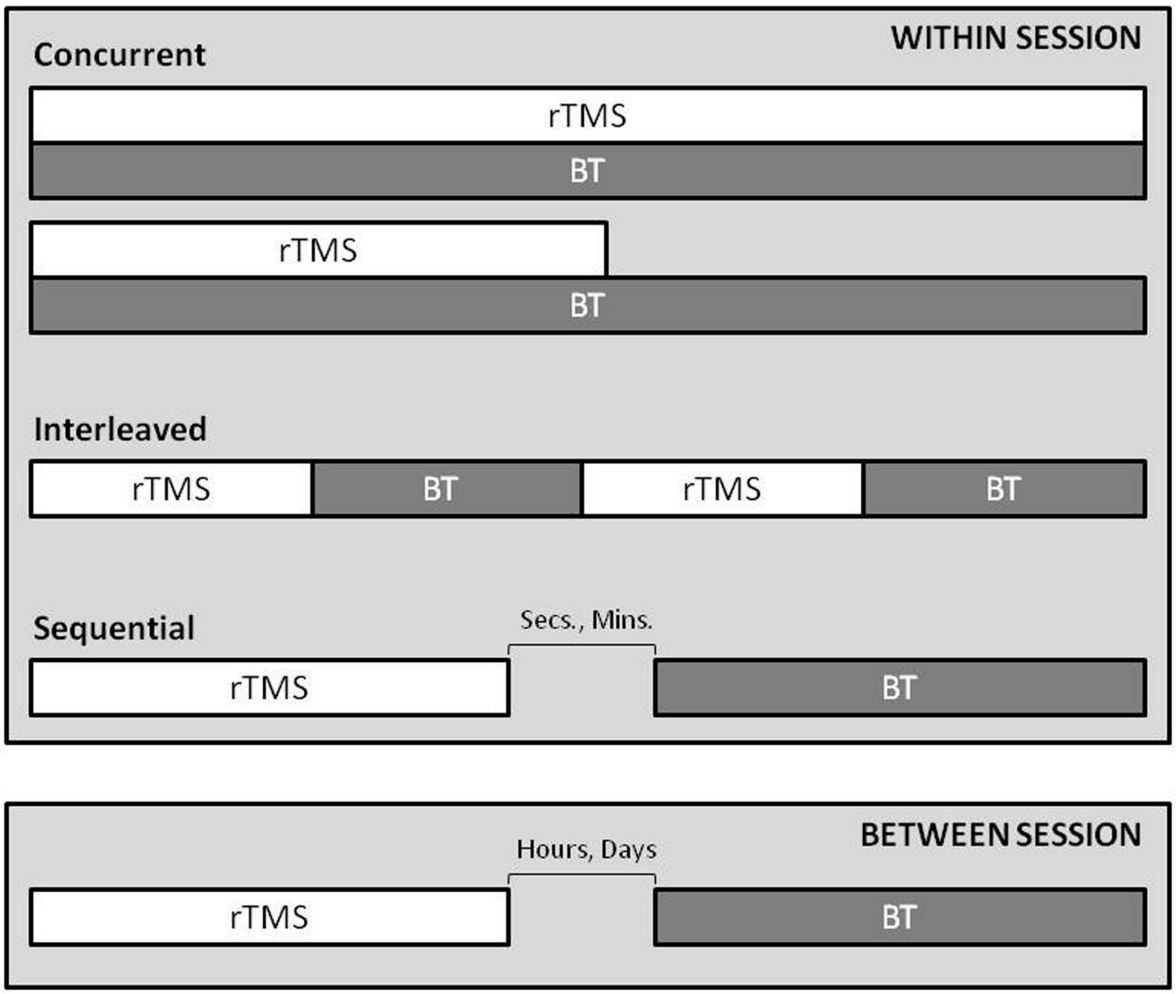

FIGURE 2 | A conceptual guide for timing of rTMS and behavioral therapy. The within session and between session temporal relationship, influences *during* and *after-effect* interactions, depending on the time-course of combination. The relationship can be defined as; 1. *concurrent*, rTMS is applied at the same time as the behavior is expressed (reporting should include if one intervention outlasts the other), 2. *interleaved*, rTMS trains are alternating with behavioral expression/repetition, 3. *sequential,* one intervention follows the other. Note: Separation of the two techniques by hours or days may not harness the interactions of short term after effects of each.

through functionally connected networks. The physiological interaction of brain stimulation and discrete voluntary behaviors has been well reported experimentally in healthy subjects (Iyer et al., 2003; Daskalakis et al., 2006; Buch et al., 2011), with striking interactive effects, including augmentation, cancelation, and reversal of effect, depending on the circumstances of the interaction. In the context of the present review, the data suggest a more uniform positive response to intervention, which may be troubling. The authors suggest a framework for reporting research methods in studies examining paired rTMS and behavioral interventions (Supplementary Table S2).

rTMS and Behavioral Therapy in Other Functional Domains and Adverse Events

Currently there is a lack of research on combined applications studying functional domains outside of motor and speech. In the future, rTMS may be a promising approach for increasing the efficacy of currently accepted, traditional behavioral interventions. There were no serious adverse events reported in our reviewed studies, and only mild headaches were reported in a few papers.

CONCLUSION

Combined therapy of rTMS paired with behavioral intervention has gained traction in the scientific field. Historically, rTMS was delivered in isolation, yet with positive results. Researchers are now examining how rTMS may be used as an adjuvant to more traditional therapies (e.g., physical, occupational, speech, or cognitive therapies) to maximize the benefit of both interventions. To date, the majority of research has

been completed in the motor-domain of a stroke population and has examined delivering rTMS prior to the behavioral intervention. Of the studies reviewed, combined therapy appears to be safe, since only minor adverse effects (primarily mild headache) were reported in a few papers. Further research is needed to examine the optimal pairing of rTMS and behavioral intervention. Focused attention to all rTMS parameters and timing of stimulation is essential to allow for study replication and data interpretation. Similarly, therapy-related specifications must be clearly reported and standardized. In order to determine optimal combined therapy, larger sample sizes and randomized, controlled clinical trials are needed to account for variability among individuals and conditions.

AUTHOR CONTRIBUTIONS

KT contributed the acquisition of data and drafting of manuscript. DL and DE contributed study conception and design, as well as critical revision.

FUNDING

DE is supported by National Institute of Child Health and Human Development (NICHD) of the NIH (award number R01HD069776).

ACKNOWLEDGMENTS

The authors would like to acknowledge Daniel Press MD for insight in psychiatric application of rTMS, and Gary Thickbroom Ph.D. for helpful comments during manuscript development.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnsys.2016.00082/full#supplementary-material

REFERENCES

Abo, M., Kakuda, W., Watanabe, M., Morooka, A., Kawakami, K., and Senoo, A. (2012). Effectiveness of low-frequency rTMS and intensive speech therapy in poststroke patients with aphasia: a pilot study based on evaluation of fMRI in relation to type of aphasia. *Eur. Neurol.* 68, 199–208. doi: 10.1159/000338773

Amassian, V., and Maccabee, P. (2006). "Transcranial magnetic stimulation," in *Proceeding of the 28th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 2006,* New York, NY, 1620–1623. doi 10.1109/IEMBS.2006.259398.

Avenanti, A., Coccia, M., Ladavas, E., Provinciali, L., and Ceravolo, M. G. (2012). Low-frequency rTMS promotes use-dependent motor plasticity in chronic stroke: a randomized trial. *Neurology* 78, 256–264. doi: 10.1212/WNL.0b013e3182436558

Barros Galvao, S., dos Santos, R. B., dos Santos, P., Cabral, M. E., and Monte-Silva, K. (2014). Efficacy of coupling repetitive transcranial magnetic stimulation and physical therapy to reduce upper-limb spasticity in patients with stroke: a randomized controlled trial. *Arch. Phys. Med. Rehabil.* 95, 222–229. doi: 10.1016/j.apmr.2013.10.023

Baumer, T., Lange, R., Liepert, J., Weiller, C., Siebner, H. R., Rothwell, J. C., et al. (2003). Repeated premotor rTMS leads to cumulative plastic changes of motor cortex excitability in humans. *Neuroimage* 20, 550–560. doi: 10.1016/S1053-8119(03)00310-0

Bentwich, J., Dobronevsky, E., Aichenbaum, S., Shorer, R., Peretz, R., Khaigrekht, M., et al. (2011). Beneficial effect of repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease: a proof of concept study. *J Neural. Transm. (Vienna)* 118, 463–471. doi: 10.1007/s00702-010-0578-1

Brodie, S. M., Meehan, S., Borich, M. R., and Boyd, L. A. (2014). 5 Hz repetitive transcranial magnetic stimulation over the ipsilesional sensory cortex enhances motor learning after stroke. *Front. Hum. Neurosci.* 8:143. doi: 10.3389/fnhum.2014.00143

Buch, E. R., Johnen, V. M., Nelissen, N., O'Shea, J., and Rushworth, M. F. S. (2011). Noninvasive associative plasticity induction in a corticocortical pathway of the human brain. *J. Neurosci.* 31, 17669–17679. doi: 10.1523/JNEUROSCI.1513-11.2011

Butefisch, C., Hummelsheim, H., Denzler, P., and Mauritz, K. (1995). Repetitive training of isolated movements improves the outcome of motor rehabilitation of the centrally paretic hand. *J. Neurol. Sci.* 130, 59–68. doi: 10.1016/0022-510X(95)00003-K

Cha, H. G., and Kim, M. K. (2016). Effects of repetitive transcranial magnetic stimulation on arm function and decreasing unilateral spatial neglect in subacute stroke: a randomized controlled trial. *Clin. Rehabil.* 30, 649–656. doi: 10.1177/0269215515598817

Chang, W. H., Kim, Y., Bang, O., Kim, S. T., Park, Y. H., and Lee, P. K. W. (2010). Long-term effects of rTMS on motor recovery in patients after subacute stroke. *J. Rehabil Med.* 42, 758–764. doi: 10.2340/16501977-0590

Chang, W. H., Kim, Y. H., Yoo, W. K., Goo, K. H., Park, C. H., Kim, S. T., et al. (2012). rTMS with motor training modulates cortico-basal ganglia-thalamocortical circuits in stroke patients. *Restor. Neurol. Neurosci.* 30, 179–189. doi: 10.3233/RNN-2012-110162

Conforto, A. B., Anjos, S. M., Saposnik, G., Mello, E. A., Nagaya, E. M., Santos, W. Jr., et al. (2012). Transcranial magnetic stimulation in mild to severe hemiparesis early after stroke: a proof of principle and novel approach to improve motor function. *J. Neurol.* 259, 1399–1405. doi: 10.1007/s00415-011-6364-7

Cotelli, M., Fertonani, A., Miozzo, A., Rosini, S., Manenti, R., Padovani, A., et al. (2011). Anomia training and brain stimulation in chronic aphasia. *Neuropsychol. Rehabil.* 21, 717–741. doi: 10.1080/09602011.2011.621275

Damoiseaux, J. S., Rombouts, S., Barkof, F., Scheltens, P., Stam, C., Smith, S., et al. (2006). Consistent resting-state networks across healthy subjects. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12848–12853. doi: 10.1073/pnas.0601417103

Daskalakis, Z. J., Moller, B., Christensen, B. K., Fitzgerald, P. B., Gunraj, C., and Chen, R. (2006). The effects of repetitive transcranial magnetic stimulation on cortical inhibition in healthy human subjects. *Exp. Brain Res.* 174, 403–412. doi: 10.1007/s00221-006-0472-0

Emara, T. H., Moustafa, R. R., ElNahas, N. M., ElGanzoury, A. M., Abdo, T. A., Mohamed, S. A., et al. (2010). Repetitive transcranial magnetic stimulation at 1 Hz and 5 Hz produces sustained improvement in motor function and disability after ischaemic stroke. *Eur. J. Neurol.* 17, 1203–1209. doi: 10.1111/j.1468-1331.2010.03000.x

Gillick, B. T., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., Thomas, W., et al. (2014). Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: a randomized controlled trial. *Dev. Med. Child Neurol.* 56, 44–52. doi: 10.1111/dmcn.12243

Goetz, S. M., Luber, B., Lisanby, S. H., Murphy, D. L., Kozyrkov, I. C., Grill, W. M., et al. (2016). Enhancement of neuromodulation with novel pulse shapes generated by controllable pulse parameter transcranial magnetic stimulation. *Brain Stimul.* 9, 39–47. doi: 10.1016/j.brs.2015.08.013

Gusnard, D. A., and Raichle, M. E. (2001). Searching for a baseline: functional imaging and the resting human brain. *Nat. Rev. Neurosci.* 2, 685–694. doi: 10.1038/35094500

Iyer, M. B., Schleper, N., and Wassermann, E. M. (2003). Priming stimulation enhances depressant effect of low-frequency repetitive transcranial magnetic stimulation. *J. Neurosci.* 23, 10867–10872.

Kakuda, W., Abo, M., Kobayashi, K., Momosaki, R., Yokoi, A., Fukuda, A., et al. (2010). Low-frequency repetitive transcranial magnetic stimulation and intensive occupational therapy for poststroke patients with upper limb hemiparesis: preliminary study of a 15-day protocol. *Int. J. Rehabil. Res.* 33, 339–345. doi: 10.1097/MRR.0b013e32833cdf10

Kakuda, W., Abo, M., Kobayashi, K., Momosaki, R., Yokoi, A., Fakuda, A., et al. (2011). Anti-spastic effect of low-frequency rTMS applied with occupational therapy in post-stroke patients with upper limb hemiparesis. *Brain Inj.* 25, 496–502. doi: 10.3109/02699052.2011.559610

Kakuda, W., Abo, M., Kobayashi, K., Momosaki, R., Yokoi, A., Fukunda, A., et al. (2011). Application of combined 6-Hz primed low frequency rTMS and intensive occupational therapy for upper limb hemiparesis after stroke. *Neurorehabilitation* 29, 365–371. doi: 10.3233/NRE-2011-0714

Kakuda, W., Abo, M., Kobayashi, K., Takagishi, T., Momosaki, R., Yokoi, A., et al. (2011). Baseline severity of upper limb hemiparesis influences the outcome of low-frequency rTMS combined with intensive occupational therapy in patients who have had a stroke. *PM R* 3, 516–522. doi: 10.1016/j.pmrj.2011.02.015

Kakuda, W., Abo, M., Momosaki, R., and Morooka, A. (2011). Therapeutic application of 6 Hz primed low frequency rTMS combined with intensive speech therapy for post-stroke aphasia. *Brain Inj.* 25, 1242–1248. doi: 10.3109/02699052.2011.608212

Kakuda, W., Abo, M., Nobuyoshi, K., Ishikawa, A., Taguchi, K., and Yokoi, A. (2010). Six-day course of repetitive transcranial magnetic stimulation plus occupational therapy for post-stroke patients with upper limb hemiparesis: a case series study. *Disabil. Rehabil.* 32, 801–807. doi: 10.3109/09638280903295474

Kakuda, W., Abo, M., Shimizu, M., Sasanuma, J., Okamoto, T., Yokoi, A., et al. (2012). A multi-center study on low-frequency rTMS combined with intensive occupational therapy for upper limb hemiparesis in post-stroke patients. *J. Neuroeng. Rehabil.* 9:4. doi: 10.1186/1743-0003-9-4

Kakuda, W., Abo, M., Watanabe, S., Momosaki, R., Hashimoto, G., Nakayama, Y., et al. (2013). High-frequency rTMS applied over bilateral leg motor areas combined with mobility training for gait disturbance after stroke: a preliminary study. *Brain Inj.* 27, 1080–1086. doi: 10.3109/02699052.2013.794973

Khedr, E. M., El-Fetoh, N. A., Ali, A. M., El-Hammady, D. H., Khalifa, H., Atta, H., et al. (2014). Dual-hemisphere repetitive transcranial magnetic stimulation for rehabilitation of poststroke aphasia: a randomized, double-blind clinical trial. *Neurorehabil. Neural Repair* 28, 740–750. doi: 10.1177/1545968314521009

Khedr, E. M., Rothwell, J. C., Shawky, O. A., Ahmed, M. A., and Hamdy, A. (2006). Effect of daily repetitive transcranial magnetic stimulation on motor performance in Parkinson's disease. *Mov. Disord.* 21, 2201–2205. doi: 10.1002/mds.21089

Kim, C., Choi, H. E., Jung, H., Lee, B. J., Lee, K. H., and Lim, Y. J. (2014). Comparison of the effects of 1 Hz and 20 Hz rTMS on motor recovery in subacute stroke patients. *Ann. Rehabil. Med.* 38, 585–591. doi: 10.5535/arm.2014.38.5.585

Kim, Y., You, S. H., Ko, M., Park, J., Lee, K., Jang, S. H., et al. (2006). Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. *Stroke* 37, 1471–1476. doi: 10.1161/01.STR.0000221233.55497.51

Kimberley, T. J., Schmidt, R. L. S., Chen, M., Dykstra, D. D., and Buetefisch, C. M. (2015). Mixed effectiveness of rTMS and retraining in the treatment of focal hand dystonia. *Front. Hum. Neurosci.* 9:385. doi: 10.3389/fnhum.2015.00385

Koganemaru, S., Sawamoto, N., Aso, T., Sagara, A., Ikkaku, T., Shimada, K., et al. (2015). Task-specific brain reorganization in motor recovery induced by a hybrid-rehabilitation combining training with brain stimulation after stroke. *Neurosci. Res.* 92, 29–38. doi: 10.1016/j.neures.2014.10.004

Kwon, T. G., Kim, Y., Chang, W., Bang, O., and Shin, Y. (2014). Effective method of combining rTMS and motor training in stroke patients. *Restor. Neurol. Neurosci.* 32, 223–232. doi: 10.3233/RNN-130313

Lam, R. W., Chan, P., Wilkins-Ho, M., and Yatham, L. (2008). Repetitive transcranial magnetic stimulation for treatment-resistant depression: a systematic review and metaanalysis. *Can. J. Psychiatry* 53, 621–631.

Lim, J. Y., Kang, E. K., and Paik, N. J. (2010). Repetitive transcranial magnetic stimulation to hemispatial neglect in patients after stroke: an open-label pilot study. *J. Rehabil. Med.* 42, 447–452. doi: 10.2340/16501977-0553

Lomarev, M. P., Kanchana, S., Bara-Jimenez, W., Iyer, M., Wassermann, E. M., and Hallett, M. (2006). Placebo-controlled study of rTMS for the treatment of Parkinson's disease. *Mov. Disord.* 21, 325–331. doi: 10.1002/mds.20713

Malcolm, M. P., Triggs, W. J., Light, K. E., Gonzalez, R., Wu, S., Reid, K., et al. (2007). Repetitive transcranial magnetic stimulation as an adjunct to constraint-induced therapy: an exploratory randomized controlled trial. *Am. J. Phys. Med. Rehabil.* 86, 707–715. doi: 10.1097/PHM.0b013e31813e0de0

Micoulaud-Franchi, J. A., Richieri, R., Lancon, C., and Vion-Dury, J. (2013). [Interactive rTMS protocols in psychiatry]. *Encephale* 39, 426–431. doi: 10.1016/j.encep.2013.04.009

Mills, K. R., and Schubert, M. (1995). Short term synchronization of human motor units and their responses to transcranial magnetic stimulation. *J. Physiol.* 483(Pt. 2), 511–523. doi: 10.1113/jphysiol.1995.sp020602

Momosaki, R., Abo, M., and Kakuda, W. (2014). Bilateral repetitive transcranial magnetic stimulation combined with intensive swallowing rehabilitation for chronic stroke dysphagia: a case series study. *Case Rep. Neurol.* 6, 60–67. doi: 10.1159/000360936

Nexstim Ltd (2015). *Niche Trial: Navigated Inhibitory rTMS to Contralesional Hemisphere Trial*. Available at: https://clinicaltrials.gov/ct2/show/NCT02089464?term=nexstim&rank=1

Nollet, H., Ham, L. V., Deprez, P., and Vanderstraeten, G. (2003). Transcranial magnetic stimulation: review of the technique, basic principles and applications. *Vet. J.* 166, 28–42. doi: 10.1016/S1090-0233(03)00025-X

Nudo, R., Plautz, E., and Frost, S. (2001). Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 24, 1000–1019. doi: 10.1002/mus.1104

Park, I., and Yoon, J. (2015). The effect of computer-assisted cognitive rehabilitation and repetitive transcranial magnetic stimulation on cognitive function for stroke patients. *J. Phys. Ther. Sci.* 27, 773–776. doi: 10.1589/jpts.27.773

Pell, G. S., Roth, Y., and Zangen, A. (2011). Modulation of cortical excitability induced by repetitive transcranial magnetic stimulation: influence of timing in geometrical parameters and underlying mechanisms. *Prog. Neurobiol.* 93, 59–98. doi: 10.1016/j.pneurobio.2010.10.003

Rabey, J. M., Dobronevsky, E., Aichenbaum, S., Gonen, O., Marton, R. G., and Khaigrekht, M. (2013). Repetitive transcranial magnetic stimulation combined with cognitive training is a safe and effective modality for the treatment of Alzheimer's disease: a randomized, double-blind study. *J. Neural Transm. (Vienna)* 120, 813–819. doi: 10.1007/s00702-012-0902-z

Rose, D. K., Patten, C., McGuirk, T. E., Lu, X., and Triggs, W. J. (2014). Does inhibitory repetitive transcranial magnetic stimulation augment functional task practice to improve arm recovery in chronic stroke? *Stroke Res. Treat.* 2014, 305236. doi: 10.1155/2014/305236

Rothwell, J. C., Thompson, P. D., Day, B. L., Dick, J. P. R., Kachi, T., Cowan, J. M. A., et al. (1987). Motor cortex stimulation in intact man: 1. General characteristics of EMG responses in different muscles. *Brain* 110(Pt. 5), 1173–1190. doi: 10.1093/brain/110.5.1173

Rubi-Fessen, I., Hartmann, A., Huber, W., Fimm, B., Rommel, T., Thiel, A., et al. (2015). Add-on effects of repetitive transcranial magnetic stimulation on subacute aphasia therapy: enhanced improvement of functional communication and basic linguistic skills: a randomized controlled study. *Arch. Phys. Med. Rehabil.* 96, 1935–1944. doi: 10.1016/j.apmr.2015.06.017

Sanes, J. N., and Donoghue, J. P. (2000). Plasticity and primary motor cortex. *Annu. Rev. Neurosci.* 23, 393–415. doi: 10.1146/annurev.neuro.23.1.393

Seniow, J., Bilik, M., Lesniak, M., Waldowski, K., Iwanski, S., and Czlonkowska, A. (2012). Transcranial magnetic stimulation combined with physiotherapy in rehabilitation of poststroke hemiparesis: a randomized, double blind, placebo-controlled study. *Neurorehabil. Neural Repair* 26, 1072–1079. doi: 10.1177/1545968312445635

Seniow, J., Waldowski, K., Lesniak, M., Iwanski, S., Czepiel, W., and Czlonkowska, A. (2013). Transcranial magnetic stimulation combined with speech and language training in early aphasia rehabilitation: a randomized double-blind controlled pilot study. *Top. Stroke Rehabil.* 20, 20–61. doi: 10.1310/tsr2003-250

Silasi, G., and Murphy, T. H. (2014). Stroke and the connectome: how connectivity guides therapeutic intervention. *Neuron* 83, 1354–1368. doi: 10.1016/j.neuron.2014.08.052

Slotema, C. W., Blom, J. D., Hoek, H. W., and Sommer, I. E. C. (2010). Should we expand the toolbox of psychiatric treatment methods to include repetitive transcranial magnetic stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. *J. Clin. Psychiatry* 71, 873–884. doi: 10.4088/JCP.08m04872gre

Takeuchi, N., Tada, T., Toshima, M., Chuma, T., Matsuo, Y., and Ikoma, K. (2008). Inhibition of the unaffected motor cortex by 1 Hz repetitive transcranial magnetic stimulation enhances motor performance and training effect of the paretic hand in patients with chronic stroke. *J. Rehabil. Med.* 40, 298–303. doi: 10.2340/16501977-0181

Thiel, A., Hartmann, A., Rubi-Fessen, I., Anglade, C., Kracht, L., Weiduschat, N., et al. (2013). Effects of noninvasive brain stimulation on language networks and recovery in early poststroke aphasia. *Stroke* 44, 2240–2246. doi: 10.1161/STROKEAHA.111.000574

Vedeniapin, A., Cheng, L., and George, M. S. (2010). Feasibility of simultaneous cognitive behavioral therapy (CBT) and left prefrontal rTMS for treatment resistant depression. *Brain Stimul.* 3, 207–210. doi: 10.1016/j.brs.2010.03.005

Vongvaivanichakul, P., Tretriluxana, J., Bovonsunthonchai, S., Pakaprot, N., and Laksanakorn, W. (2014). Reach-to-grasp training in individuals with chronic stroke augmented by low-frequency repetitive transcranial magnetic stimulation. *J. Med. Assoc. Thai.* 97(Suppl. 7), S45–S49.

Waldowski, K., Seniow, J., Lesniak, M., Iwanski, S., and Czlonkowska, A. (2012). Effect of low-frequency repetitive transcranial magnetic stimulation on naming abilities in early-stroke aphasic patients: a prospective, randomized, double-blind sham-controlled study. *Sci. World J.* 2012: 518568. doi: 10.1100/2012/518568

Wang, C., Hsieh, C., Tsai, P., Wang, C., Lin, F., and Chan, R. (2014). Efficacy of synchronous verbal training during repetitive transcranial magnetic stimulation in patients with chronic aphasia. *Stroke* 45, 3656–3662. doi: 10.1161/STROKEAHA.114.007058

Wang, R., Tseng, H., Liao, K., Wang, C., Lai, K., and Yang, Y. (2012). rTMS combined with task-oriented training to improve symmetry of interhemispheric corticomotor excitability and gait performance after stroke: a randomized trial. *Neurorehabil. Neural Repair* 26, 222–230. doi: 10.1177/1545968311423265

Weiduschat, N., Thiel, A., Rubi-Fessen, I., Hartmann, A., Kessler, J., Merl, P., et al. (2011). Effects of repetitive transcranial magnetic stimulation in aphasic stroke: a randomized controlled pilot study. *Stroke* 42, 409–415. doi: 10.1161/STROKEAHA.110.597864

Winstein, C. J., Wolf, S. L., Dromerick, A. W., Lane, C. J., Nelsen, M. A., Lewthwaite, R., et al. (2016). Effect of task-oriented rehabilitation program on upper extremity recovery following motor stroke: the ICARE randomized clinical trial. *JAMA* 315, 571–581. doi: 10.1001/jama.2016.0276

Yamada, N., Kakuda, W., Kondo, T., Shimizu, M., Mitani, S., and Abo, M. (2013). Bihemispheric repetitive transcranial magnetic stimulation combined with intensive occupational therapy for upper limb hemiparesis after stroke: a preliminary study. *Int. J. Rehabil. Res.* 36, 323–329. doi: 10.1097/MRR.0b013e3283624907

Yang, Y., Tseng, C., Chiou, S., Liao, K., Cheng, S., Lai, K., et al. (2013). Combination of rTMS and treadmill modulates corticomotor inhibition and improves walking in Parkinson Disease: A Randomized Trial. *Neurorehabil. Neural Repair* 27, 79–86. doi: 10.1177/1545968312451915

Zheng, C., Liao, W., and Xia, W. (2015). Effect of combined low-frequency rTMS and virtual reality training on upper limb function in subacute stroke: a double-blind randomized controlled trial. *J. Huazhong Univ. Sci. Technolog. Med. Sci.* 35, 248–254. doi: 10.1007/s11596-015-1419-0

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 23 December 2013
doi: 10.3389/fnsys.2013.00076

"Non-invasive" brain stimulation is not non-invasive

Nick J. Davis[1]* and Martijn G. van Koningsbruggen[2,3]

[1] Department of Psychology, Swansea University, Swansea, UK
[2] Centro Interdipartimentale Mente/Cervello, University of Trento, Rovereto, Italy
[3] Department of Cognitive Sciences, University of Trento, Rovereto, Italy
*Correspondence: n.j.davis@swansea.ac.uk

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Brian D. Earp, University of Oxford, UK
Nadira Faulmüller, University of Oxford, UK

Keywords: TMS, tDCS, non-invasive, neuroethics, safety, human

INTRODUCTION

The functions of the healthy brain can be studied in two main ways. Firstly, the changes in the brain's state can be measured using techniques such as EEG or functional MRI. Secondly, the activity of the brain can be disrupted through the use of brain stimulation. The famous experiments of Wilder Penfield and colleagues in the 1950s showed the power of brain stimulation in people whose brain was exposed in surgery, and highlighted the possibility of inducing changes in the brain's state to demonstrate the involvement of specific brain areas in particular functions (Jasper and Penfield, 1954). Two main techniques are available for human brain stimulation: transcranial magnetic stimulation (TMS) and transcranial current stimulation (tCS). More recently, it has been suggested that TMS and tCS might be used to enhance brain function, as well as to disrupt activity.

These techniques have collectively become known as "non-invasive brain stimulation." We argue that this term is inappropriate and perhaps oxymoronic, as it obscures both the possibility of side-effects from the stimulation, and the longer-term effects (both adverse and desirable) that may result from brain stimulation. We also argue that the established tendency for the effects of TMS and tCS to spread from the target brain area to neighboring areas is in itself contrary to the definition of non-invasiveness. We argue that the traditional definition of an invasive procedure, one which requires an incision or insertion in the body, should be re-examined, and we propose that it be widened to include targeted transcutaneous interventions.

TYPES OF BRAIN STIMULATION

An electric current travelling through a coiled wire creates a magnetic field. This property is used in TMS to create brief magnetic pulses which easily traverse the skull and other matter overlaying the brain. The pulses generate electrical potentials in the brain, depolarizing neurons and thereby triggering action potentials (Di Lazzaro et al., 2004). A single pulse of TMS will have two effects: firstly the generation of action potentials in the targeted brain areas underlying the coil; secondly a refractory "silent period" in those same cells as the ion balance is restored. While the effects of a single TMS may only last on the order of a few milliseconds, multiple pulses may induce long term potentiation or depression in the target cells. For example, trains of pulses delivered at 1 Hz result in reduced excitability in the target area for a prolonged period of time, on the order of tens of minutes after the end of the stimulation. A recent development has been the use of rapid bursts of pulses such as theta-burst stimulation (TBS), which can have opposing effects on excitability depending on the temporal pattern of the bursts (Huang et al., 2005). TMS may be used either "online," to affect the brain during a task, or "offline," to compare task performance after vs. before longer periods of stimulation.

tCS is a term that covers several techniques, principally involving direct or alternating current (tDCS or tACS). In a typical tDCS experiment, the participant performs a task to establish a baseline performance level. Then a pair of electrodes is placed on the head, one (or both) of which overlie a target brain area. The experimenter delivers a small electrical current for around 10–20 min. Following this the participant performs the task a second time to establish whether the stimulation has had an effect on behavior. The effect depends on a number of factors, including current amplitude and duration (higher currents delivered for more time usually induce a greater effect), the polarity of the electrode over the target area (typically the negative electrode, or cathode, will worsen performance while the positive, anodal, electrode will enhance it), and the brain area and task under study (Nitsche and Paulus, 2000, 2001). tACS is less well studied, however the technique offers the possibility of exploring the casual involvement not only of a target brain area, but also of a particular frequency band. For example the beta range (15–35 Hz) is known to be associated with human motor control, however it has only recently been possible to show the causal involvement of beta frequencies in maintaining motor state (Pogosyan et al., 2009; Fuerra et al., 2011). These latter studies used tACS to increase the power of the beta band while the motor system was under study, giving somewhat contradictory results (Davis et al., 2012).

The timescale over which the effects of brain stimulation are seen can vary from milliseconds to weeks. At the briefest level, a single pulse of TMS lasts for 100–200 μs, during which time an electric field is induced in the target area. This is enough to generate action potentials in these target cells, and to induce a refractory silent period following the initial burst. Conversely the instantaneous effects of tCS are under-explored, and much of our knowledge of the effects of the electric field on the brain comes from modeling

studies (e.g., Miranda et al., 2006). Most experimental uses of brain stimulation involve medium-scale effects, which occur on the order of minutes to hours. tDCS experiments exploit the polarizing effect of the electric field on the resting membrane potential, which lasts for around 90 min following 13 min of stimulation (Nitsche and Paulus, 2001). Similarly, the effects of TBS may last up to 1 h (Huang et al., 2005), which can be extended to more than 2 h by slightly adjusting the TBS protocol (Nyffeler et al., 2006).

There is considerable interest in the therapeutic possibilities of brain stimulation. Both TMS and tDCS have shown some success in the treatment of depression (Slotema et al., 2010), stroke (Hummel and Cohen, 2006), and tinnitus (Fregni et al., 2006). Most usefully for clinical applications, certain regimes of brain stimulation may lead to longer-lasting changes in brain function. A particularly effective strategy for generating lasting effects is to apply stimulation in multiple sessions spaced around 24 h apart. This regime makes brain stimulation a possible adjunct therapy for neurological disorders, which can be administered in outpatient clinics, leaving the patient free to return home between stimulation sessions.

SAFETY ISSUES IN BRAIN STIMULATION

No brain stimulation technique is completely free of side-effects. The complications of surgical procedures such as deep brain stimulation (DBS) are well-monitored and well-understood in the context of weighing the potential benefit to the patient, and are considered in terms of short-term and longer-term effects (Beric et al., 2002). While the safety limits for brain stimulation are reasonably well mapped (Nitsche et al., 2003; Bikson et al., 2009) there remain real risks of seizure from TMS and tCS, and scalp burns from tCS, if appropriate care is not taken. However, the greater risk comes from the "known unknowns" of brain stimulation: unplanned effects from build-up of stimulating effects in non-target areas, or from build-up of effects across multiple sessions. This latter risk can also be an advantage, as discussed above, however inducing long-lasting changes in cortical excitability can be dangerous to the participant if not properly controlled. Indeed, many institutions that use brain stimulation insist on a minimum interval between sessions to prevent a build-up of effects.

Brain stimulation of healthy volunteers has recently become an established tool for the study of diverse features of the human brain, from basic neurophysiology (Stagg et al., 2009) to large-scale networks (Polanía et al., 2011). It is clear that brain stimulation is a powerful tool in the hands of neuroscientists, and the use and utility of these techniques will increase as we learn more about their effects on the brain and about the optimum parameters for generating these effects. A key feature of brain stimulation is that the effects of stimulation can greatly outlast the stimulation phase, sometimes up to several weeks after the end of a stimulation session (e.g., Boggio et al., 2007).

COMMON DEFINITIONS OF INVASIVENESS

In opposing the term "non-invasive," we must examine the definition of the term. The common, intuitive definition of the term "non-invasive" implies a procedure where no incision or insertion is made into the body. In most medical contexts this is a sensible distinction; physicians must balance the risks and benefits of invasive and non-invasive procedures for both monitoring (e.g., Shoemaker et al., 1998) and treating (e.g., Medoff, 2008) medical complaints. However, invasiveness is not restricted to this definition alone. For example, the *Oxford English Dictionary* gives two relevant usages for the term "non-invasive": "*Chiefly Med[ical] Esp[ecially] of a neoplasm or microorganism: not spreading into adjacent tissue from an initial site of development or colonization. Also: designating or relating to such a pattern of growth*"; and "*Med[ical] Of a diagnostic or therapeutic procedure: that does not require the insertion of instruments (often including hypodermic needles) through the skin or into a body cavity*" ("non-invasive," Oxford English Dictionary, 3rd Edition, 2003).

The second of these dictionary definitions is what we think of as the intuitive neuroscientific definition of "non-invasiveness." Clearly, in contradistinction to surgical procedures such as DBS or direct cortical stimulation, the application of a coil or electrode to the scalp is non-invasive in the sense that the instrument does not physically enter the body. We believe that transcranial brain stimulation with TMS or tCS fits better with the former definition. Induced currents spread from the point of delivery through the brain (and nearby tissues) to adjacent regions. This spread is large in the case of tCS (Miranda et al., 2006), compared to a relatively focal sphere of stimulation in the clearly invasive procedure of DBS (Butson et al., 2006). This unintended and unwanted current spread is consistent with the dictionary definition's sense of diffusion away from a source region. We would similarly classify novel techniques such as optogenetics as not being non-invasive, since the stimulation (light) must pass through multiple layers of tissue, and possibly beyond, to activate the target cells (leaving aside the problem of introducing photosensitive proteins into the tissue: Fenno et al., 2011).

We do not suggest that invasiveness in its own right should preclude researchers from using a technique; as we have seen, TMS and tCS are safe when used correctly. Referring to brain stimulation techniques as "non-invasive" likely increases the palatability of the techniques to non-expert participants; an important factor in recruitment, as "brain stimulation" already somewhat raises the stakes when recruiting for experiments or trials. We do not suggest that recruitment adverts should advertise "invasive brain stimulation," rather that the use of the term "non-invasive" may create an illusion of comfort in participants' and non-experts' minds that may not be warranted. We therefore propose that TMS and tCS be referred to simply as "brain stimulation," without the potentially misleading qualifier of "non-invasive."

WIDENING ACCESS TO BRAIN STIMULATION

The widening use of brain stimulation has been much discussed recently. In particular, the relatively low cost and ease of manufacture of tDCS has led to something of a movement in so-called "DIY-tDCS" for self-stimulation. Electrical brain stimulation has been suggested as a promising option for improving human

experience in a number of domains, including: numerical skills (Cohen Kadosh et al., 2010; Snowball et al., 2013); sport (Banissy and Muggleton, 2013; Davis, 2013); memory capacity (Hoy et al., 2013); and depression (Nitsche et al., 2009). As positive results trickle out of labs and clinics, the likelihood is that a greater number of people will wish to explore the use of brain stimulation. As technologies improve and become more widespread, the ethical implications of (mis)use of brain stimulation must be considered; this concern has not been thoroughly addressed in relation to brain stimulation (but see Green et al., 1997; Cohen Kadosh et al., 2012).

Recent works have attempted to address the ethical and policy implications of widespread use of enhancing technologies. For example, Fitz and Reiner (2013) propose a stance of "managed technological optimism," whereby stakeholders (including DIY-tDCS developers) take a share in responsibly determining guidelines for using neuroenhancing technology. We see the issue of self-treatment for neurally-mediated disorders as a potentially more serious issue, as people who are not satisfied with physician-delivered treatment seek adjunct treatment with brain stimulation, without clear guidance about proper controls or interactions with existing treatments (Cabrera et al., 2013; Davis et al., 2013). Potential users should be aware that guidelines and principles have been published that address the safe use of brain stimulation techniques (e.g., Green et al., 1997; Rossi et al., 2009; Davis et al., 2013).

CONCLUSIONS

Brain stimulation will continue to develop, to the benefit of scientists and of patients, and we foresee its routine use in clinics. We propose that the term "non-invasive brain stimulation" no longer be used, as the term may mislead non-expert users into the view that the effect of the technique is necessarily mild. Any technique which directly affects brain tissue to generate such powerful acute and long-lasting effects should be treated with the same respect as any surgical technique, and proper safety and ethical guidelines should apply in institutions where brain stimulation is in use. We would draw an analogy between brain stimulation and gamma-knife radiotherapy, which is also "non-invasive" in the sense that no incisions or insertions are made in the person, but clinicians and the public have a healthy and proper respect for the nature of the technique. We propose also that researchers take care to develop good and safe practice for the use of their research, and be mindful that in a climate of wide and open dissemination of scientific results, exciting, and beneficial results will reach well beyond the labs and clinics.

REFERENCES

Banissy, M., and Muggleton, N. (2013). Transcranial direct current stimulation in sports training: potential approaches. *Front. Hum. Neurosci.* 7:129. doi: 10.3389/fnhum.2013.00129

Beric, A., Kelly, P., Rezai, A., Serio, D., Mogilner, A., Zonenshayn, M., et al. (2002). Complications of deep brain stimulation surgery. *Stereotact. Funct. Neurosurg.* 77, 73–78. doi: 10.1159/000064600

Bikson, M., Datta, A., and Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clin. Neurophysiol.* 120, 1033–1034. doi: 10.1016/j.clinph.2009.03.018

Boggio, P. S., Nunes, A., Rigonatti, S. P., Nitsche, M. A., Pascual-Leone, A., and Fregni, F. (2007). Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restor. Neurol. Neurosci.* 25, 123–129.

Butson, C., Maks, C., and McIntyre, C. (2006). Sources and effects of electrode impedance during deep brain stimulation. *Clin Neurophysiol.* 117, 447–454. doi: 10.1016/j.clinph.2005.10.007

Cabrera, L., Evans, E., and Hamilton, R. (2013). Ethics of the electrified mind: defining issues and perspectives on the principled use of brain stimulation in medical research and clinical care. *Brain Topogr.* doi: 10.1007/s10548-10013-10296-10548. [Epub ahead of print].

Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, R108–R111. doi: 10.1016/j.cub.2012.01.013

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R., and Walsh, V. (2010). Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Curr. Biol.* 20, 2016–2020. doi: 10.1016/j.cub.2010.10.007

Davis, N. (2013). Neurodoping: brain stimulation as a performance-enhancing measure. *Sports Med.* 43, 649–653. doi: 10.1007/s40279-013-0027-z

Davis, N., Gold, E., Pascual-Leone, A., and Bracewell, R. (2013). Challenges of proper placebo control for noninvasive brain stimulation in clinical and experimental applications. *Eur. J. Neurosci.* 38, 2973–2977. doi: 10.1111/ejn.12307

Davis, N., Tomlinson, S., and Morgan, H. (2012). The role of beta-frequency neural oscillations in motor control. *J. Neurosci.* 32, 403–404. doi: 10.1523/JNEUROSCI.5106-11.2012

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., et al. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin. Neurophysiol.* 115, 255–266. doi: 10.1016/j.clinph.2003.10.009

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). The development and application of optogenetics. *Annu. Rev. Neurosci.* 34, 389–412. doi: 10.1146/annurev-neuro-061010-113817

Fitz, N., and Reiner, P. (2013). The challenge of crafting policy for do-it-yourself brain stimulation. *J. Med. Ethics.* doi: 10.1136/medethics-2013-101458. [Epub ahead of print].

Fregni, F., Marcondes, R., Boggio, P. S., Marcolin, M. A., Rigonatti, S. P., Sanchez, T. G., et al. (2006). Transient tinnitus suppression induced by repetitive transcranial magnetic stimulation and transcranial direct current stimulation. *Eur. J. Neurol.* 13, 996–1001. doi: 10.1111/j.1468-1331.2006.01414.x

Fuerra, M., Bianco, G., Santarnecchi, E., Del Testa, M., Rossi, A., and Rossi, S. (2011). Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. *J. Neurosci.* 31, 12165–12170. doi: 10.1523/JNEUROSCI.0978-11.2011

Green, R., Pascual-Leone, A., and Wassermann, E. (1997). Ethical guidelines for rTMS research. *IRB* 19, 1–7. doi: 10.2307/3563539

Hoy, K., Emonson, M., Arnold, S., Thomson, R., Daskalakis, Z., and Fitzgerald, P. (2013). Testing the limits: investigating the effect of tDCS dose on working memory enhancement in healthy controls. *Neuropsychologia* 51, 1777–1784. doi: 10.1016/j.neuropsychologia.2013.05.018

Huang, Y., Edwards, M., Rounis, E., Bhatia, K., and Rothwell, J. (2005). Theta burst stimulation of the human motor cortex. *Neuron*, 45, 201–206. doi: 10.1016/j.neuron.2004.12.033

Hummel, F. C., and Cohen, L. G. (2006). Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol.* 5, 708–712. doi: 10.1016/S1474-4422(06)70525-7

Jasper, H., and Penfield, W. (1954). *Epilepsy and the Functional Anatomy of the Human Brain, 2nd Edn.* Boston, MA: Little, Brown and Co.

Medoff, B. (2008). Invasive and noninvasive ventilation in patients with asthma. *Respir. Care* 53, 740–748.

Miranda, P. C., Lomarev, M., and Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clin. Neurophysiol.* 117, 1623–1629. doi: 10.1016/j.clinph.2006.04.009

Nitsche, M., Boggio, P., Fregni, F., and Pascual-Leone, A. (2009). Treatment of depression with transcranial direct current stimulation (tDCS): a review. *Exp. Neurol.* 219, 14–19. doi: 10.1016/j.expneurol.2009.03.038

Nitsche, M., Liebetanz, D., Lang, N., Antal, A., Tergau, F., and Paulus, W. (2003). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin. Neurophysiol.* 114, 2220–2222. doi: 10.1016/S1388-2457(03)00235-9

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans.

Neurology 57, 1899–1901. doi: 10.1212/WNL.57.10.1899

Nyffeler, T., Wurtz, P., Luscher, H.-R., Hess, C., Senn, W., Pflugshaupt, T., et al. (2006). Extending lifetime of plastic changes in the human brain. *Eur. J. Neurosci.* 24, 2961–2966. doi: 10.1111/j.1460-9568.2006.05154.x

Pogosyan, A., Gaynor, L., Eusebio, A., and Brown, P. (2009). Boosting cortical activity at beta-band frequencies slows movement in humans. *Curr. Biol.* 19, 1637–1641. doi: 10.1016/j.cub.2009.07.074

Polanía, R., Paulus, W., Antal, A., and Nitsche, M. (2011). Introducing graph theory to track for neuroplastic alterations in the resting human brain: a transcranial direct current stimulation study. *Neuroimage* 54, 2287–2296. doi: 10.1016/j.neuroimage.2010.09.085

Rossi, S., Hallett, M., Rossini, P., Pascual-Leone, A., and Group, T. S. o. T. C. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Shoemaker, W., Belzberg, H., Wo, C., Milzman, D., Pasquale, M., Baga, L., et al. (1998). Multicenter study of noninvasive monitoring systems as alternatives to invasive monitoring of acutely III emergency patients. *Chest* 114, 1643–1652. doi: 10.1378/chest.114.6.1643

Slotema, C., Blom, J., Hoek, H., and Sommer, I. (2010). Should we expand the toolbox of psychiatric treatment methods to include repetitive transcranial magnetic stimulation (rTMS)? A Meta-Analysis of the Efficacy of rTMS in Psychiatric Disorders. *J. Clin. Psychiatry* 71, 873–884. doi: 10.4088/JCP.08m04872gre

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kineses, Z. T., et al. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J. Neurosci.* 29, 5202–5206. doi: 10.1523/JNEUROSCI.4432-08.2009

Received: 15 October 2013; accepted: 06 December 2013; published online: 23 December 2013.

*Citation: Davis NJ and van Koningsbruggen MG (2013) "Non-invasive" brain stimulation is not non-invasive. Front. Syst. Neurosci. **7**:76. doi: 10.3389/fnsys.2013.00076*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

MINI REVIEW ARTICLE
published: 12 January 2015
doi: 10.3389/fnsys.2014.00246

Biasing neural network dynamics using non-invasive brain stimulation

Martijn E. Wokke[1,2]*, Lotte J. Talsma[1,3] and Marlies E. Vissers[1,3]

[1] *Amsterdam Brain and Cognition, University of Amsterdam, Amsterdam, Netherlands*
[2] *Consciousness, Cognition and Computation Group, Department of Psychology, Université Libre de Bruxelles, Brussels, Belgium*
[3] *Department of Psychology, University of Amsterdam, Amsterdam, Netherlands*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Yoshio Sakurai, Kyoto University, Japan
Estate M. Sokhadze, University of Louisville, USA

****Correspondence:***
Martijn E. Wokke, Consciousness, Cognition and Computation Group, Department of Psychology, Université Libre de Bruxelles, Avenue Franklin Roosevelt 50, 1050 Bruxelles, Belgium
e-mail: martijnwokke@gmail.com

Recently, non-invasive brain stimulation (NBS) has been discovered as a tool to improve human performance on a wide variety of tasks. Although these observations are highly intriguing, the underlying mechanisms of such enhancements are still poorly understood. Here, we argue that in order to advance our understanding of these mechanisms it is necessary to focus on intrinsic network dynamics in the brain. Taking into account well-known network dynamics, increased excitation in one particular network or brain region may necessarily lead to inhibition of an opposing network (and vice versa). As a consequence, observed behavioral improvements due to NBS may emerge from a shift in the balance between (competing) neural networks in the brain, implicating that behavioral enhancement due to stimulation most likely comes with a cost or side effect. We conclude that more elaborate experimental designs are essential for a better understanding of the relationship between network interactions and the behavioral effects of NBS.

Keywords: performance enhancement, neural networks, neuromodulation, TMS, tDCS

INTRODUCTION

In the last decade the use of non-invasive brain stimulation (NBS) techniques in cognitive neuroscience has grown explosively. Especially transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) have proven to be fruitful tools to causally link a wide range of brain regions or neural networks to perception, motor action and higher-level cognition. Moreover, NBS has been welcomed enthusiastically as a method to improve various aspects of human behavior (Luber and Lisanby, 2014). Yet, the full scope and range of the effects of NBS are currently poorly understood. In order to gain a more thorough understanding of the effects caused by stimulation, we will advocate that the observed effects of NBS on brain functioning should be seen in the light of complex interplays between task-relevant and task-irrelevant neural networks in the brain.

Almost three decades ago, TMS was introduced by Barker and colleagues (Barker et al., 1985) as a NBS technique that was able to safely affect brain function in humans (see Rossi et al., 2009). The effect of TMS is based on the principle of electromagnetic induction, in which a rapidly changing magnetic field induces a current in an electrically conducting medium, such as neural tissue. When TMS currents meet the right requirements (e.g., amplitude, duration and frequency, see Wagner et al., 2009) neural function and behavior can be altered, even outlasting the period of stimulation. The application of tDCS in cognitive neuroscientific research was introduced several years later than TMS (Priori et al., 1998; Nitsche and Paulus, 2000), but the popularity of tDCS as a neuromodulatory tool led to a rapidly growing body of research on the effects of tDCS on perception, action and cognition. tDCS can modulate cortical excitability of neural activity by the induction of weak anodal and cathodal electrical currents flowing though the cerebral cortex. The polarity is of great influence on the neuromodulatory effect of tDCS: Where anodal (positive polarization) stimulation in general facilitates cortical excitability of the underlying tissue, cathodal stimulation increases the threshold for neuronal firing (Liebetanz et al., 2002; Krause et al., 2013; however, note that the effect of polarity on neuronal firing may also depend on the state of the targeted brain region during stimulation, see e.g., Krause and Cohen Kadosh, 2014).

Initially, NBS was used to determine whether modulation of neural activity in a particular brain region was able to disrupt performance associated with normal network functioning. However, various studies began to demonstrate that tempering normal network functioning could in fact result in paradoxical improvements of performance (Luber and Lisanby, 2014). Recently, the rise of tDCS and new TMS protocols has highlighted the potential of NBS as a technique that can be used to improve brain functioning in healthy individuals or in patients suffering from neurological or psychiatric illness (Coffman et al., 2014). Although these findings are very intriguing and seem promising for both healthy and clinical populations, the underlying neural mechanisms subserving the augmentation of brain function following TMS and tDCS remains largely elusive.

In this paper we will exclusively focus on two factors that are important when considering the potential benefits and costs of neurostimulation techniques. These factors are not directly

related to parameters (e.g., polarity or frequency) in *generating* a stimulation effect (for an extensive discussion of stimulation parameters in this respect, see e.g., Walsh et al., 2003; Flöel, 2014), but concern a conceptual framework for studying NBS. Firstly, there is ample evidence on facilitatory and inhibitory interactions between different functional networks in the brain (Kinsbourne, 1987; Calautti and Baron, 2003; Fox et al., 2005; Szczepanski and Kastner, 2013), stressing the importance of reckoning the brain as a complex constellation of functional networks. This notion is already widely self-evident in certain domains, such as in research on brain connectivity (e.g., Sporns, 2013) and rehabilitation (Calautti and Baron, 2003), however it currently seems to have little influence in the design and interpretation of NBS experiments and effects. Whereas it is conceivable that stimulation of one particular network or brain region may lead to cognitive benefits that come at the cost of other cognitive processes, studies reporting brain function enhancement typically focus on the effects of stimulation on the targeted process or ability, without co-assessing possible unintended effects on other functions. However, by selectively studying intended behavioral improvements, it is conceivable that cognitive enhancements are commanding the spotlights, while potential costs keep on dancing in the dark. As a consequence, current approaches obscure the value of observed enhancement effects in a broader sense. Secondly, the effects of TMS and tDCS have been shown to be dependent on the state of the probed network (Silvanto et al., 2007; Krause and Cohen Kadosh, 2014). The specific context in which stimulation is applied seems to be critical for the behavioral effect, demonstrating how observed enhancements are emerging from complex, dynamic interactions between internal and external modulations of network activity.

We will first address a small selection of studies demonstrating enhancement of performance by TMS and tDCS in the sensory and higher-level cognitive domain. We will discuss these findings in light of brain network interactions and the modulatory role of the functional state of the stimulated network, in order to illustrate the importance of taking network interactions into account when studying the effects of NBS on behavior.

ENHANCEMENT OF VISUAL PERCEPTION

One of the first studies demonstrating enhancement of sensory processing induced by TMS manipulated visual attention by applying repetitive transcranial magnetic stimulation (rTMS) to the human parietal cortex (Hilgetag et al., 2001). Previously, a great deal of what was known about the neural mechanism of visual attention stemmed from patients suffering from visual neglect. Visual neglect is typically caused by lesions to the posterior parietal (or frontal) cortex, resulting in a deficit in the ability to draw attention towards the visual space contralateral to the lesion. This pattern of findings led to a model of cross-hemispheric competition (Kinsbourne, 1987; Szczepanski and Kastner, 2013), in which the balance in interhemispheric distribution of attentional resources is maintained via mutual inhibition. Interestingly, while researchers are typically focused on contralateral attentional deficits accompanying visual neglect Hilgetag et al. (2001) demonstrated that the induction of a "virtual lesion" to the parietal cortex could actually result in increased ipsilateral visual attention. These findings demonstrate how the intrinsic balance between neural activity across hemispheres is essential for typical perceptual functioning, and how disturbance of this balance can lead to perceptual deficits such as neglect.

Disruption of more subtle network interactions than the interhemispheric interplay discussed above has also been found to affect behavioral performance. This was illustrated in a TMS experiment on visual feature processing (Walsh et al., 1998). Walsh et al. found that disruption of cortical area HMT+/V5, a key region involved in motion processing, impaired performance during a visual search task when motion was the critical target feature. In contrast, disruption of HMT+/V5 resulted in an initially unexpected enhancement of performance when color or form were the essential features of the target. The enhancement of performance caused by the disruption of HMT+/V5 was unexpected, as TMS was considered to induce neural noise, and thereby, to deteriorate normal behavior. The unexpected enhancement was interpreted to be the result of the temporarily reduced competition of (visual) brain areas for limited processing resources, such as energy and communication with other brain regions. Thus, disruption of HMT+/V5 by TMS likely shifted the balance of neural resources towards other visual areas, improving visual perception for features processed in these other brain regions. Recently, we observed additional supporting evidence for a model in which brain regions engage in a "battle for resources" (Wokke et al., 2014). In two experiments, we probed the role of area HMT+/V5 and the object sensitive lateral occipital region (LO) during a figure discrimination task that dominantly relied on HMT+/V5 processing. Disruption of activity in LO and HMT+/V5 led to opposing effects on performance, depending on the stimulation site. Disruption of HMT+/V5 resulted in decreased discriminability, whereas participants' discriminability improved when activity in LO was perturbed. Complementary to the findings by Walsh et al. (1998), we demonstrated that the workings of HMT+/V5 improved during a motion-defined figure discrimination task when we disrupted a cortical region specialized in task-irrelevant properties. These findings provide converging evidence for competitive interactions between extrastriate cortical areas. Such observations of improved performance due to reduced neural activity in task-irrelevant regions, a phenomenon that has been dubbed "addition-by-subtraction" (Luber and Lisanby, 2014), are strong examples of the notion that effects of NBS are established due to the intrinsic interactions of functional brain networks (see **Figure 1**).

IMPROVED COGNITIVE PERFORMANCE

The growing popularity of tDCS has led to a steep rise in the number of studies on enhancement of cognitive functions such as attention, (motor) learning, working memory, and even complex problem solving (Fregni et al., 2005; Cohen Kadosh et al., 2010; Chi and Snyder, 2012; Coffman et al., 2012). For example, there is evidence for a positive effect of tDCS over frontal brain regions on working memory performance. One of the first studies on the effects of tDCS on higher-order cognition used anodal tDCS over

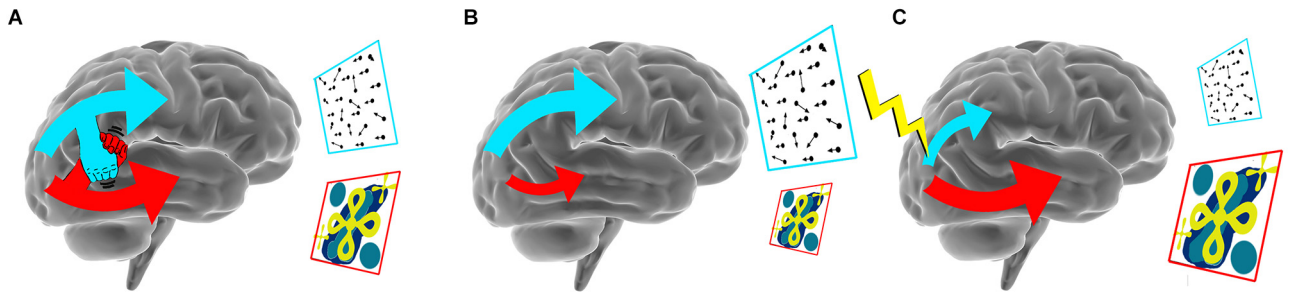

FIGURE 1 | Biasing the balance between networks with NBS. **(A)** A schematic illustration of competing network interactions in the brain during a motion discrimination (blue frame) and a shape discrimination (red frame) task, that both rely on different brain networks. **(B)** When engaging in a motion discrimination task activity in the dorsal network increases, while activity in the ventral stream decreases. **(C)** Disruption of dorsal (visual) activity results in decreased performance in motion direction discrimination while decreased dorsal competition results in enhanced performance on the shape discrimination task.

left prefrontal dorsolateral cortex (lDLPFC) to study its effects on working memory performance (e.g., Fregni et al., 2005). Anodal tDCS over lDLPFC was shown to reduce the number of errors people make on a 3-back working memory task, increasing accuracy of performance. However, there are also studies that fail to demonstrate a positive effect of tDCS on working memory functioning (for a review, see Coffman et al., 2014), complicating the interpretation of observed enhancement effects.

Recently, an elegant study by Iuculano and Cohen Kadosh (2013) revealed detrimental as well as beneficial effects of tDCS on cognition. In this study, participants performed a mathematical training during which tDCS was applied on different sites. Part of the participants received tDCS over posterior parietal cortex (PPC), whereas other participants received stimulation over DLPFC. Results showed that stimulation of the PPC led to increased speed of learning, but to impaired automaticity for the learned materials, whereas the opposite was found to be the case for stimulation over DLPFC. Thus, these findings show that stimulating the brain at different locations may have positive but also disruptive effects on cognitive performance. As suggested by the authors themselves, the eventual benefits and costs that come with a particular stimulation method may be due to corticocortical interactions, and induced shifts between metabolic prioritization. Clearly, this study illustrates the importance of broadening the scope of investigated abilities in order to detect whether behavioral improvements in one domain may come at the expense of another, emphasizing the importance of taking network configurations into account when assessing behavioral effects due to NBS. However, whereas the study by Iuculano and Cohen Kadosh (2013) was directed at comparing stimulation effects on different aspects of a targeted cognitive function (mathematical learning), it would be desirable to pursue this type of research using designs in which one single site is being stimulated, while multiple (cognitive) functions are investigated. This approach would allow one to investigate the extent to which collateral impairments in behavior arise due to intrinsic interactions between functional networks in the brain.

Another important factor to take into account is the state of a network during stimulation (Krause and Cohen Kadosh, 2014). It seems that the initial neural activation state during stimulation determines the behavioral effect of stimulation (Silvanto et al., 2008). When stimulation is applied during performance of a task, its effects have been shown to differ from stimulation during rest (Andrews et al., 2011). Another interesting finding concerning the effect of cognitive state is that application of tDCS over lateral PFC when subjects are not involved in a cognitive task, modulates activity in task-positive as well as task-negative networks (as measured with fMRI; Keeser et al., 2011). Thus, although stimulation effects depend on the currently dominant functional network, these findings show that stimulation effects are not restricted to the currently active network. Therefore, full comprehension of the potential scope of NBS requires a more complete understanding of the extent to which stimulation has an effect on targeted, as well as task-irrelevant and non-targeted networks.

OPPOSING NETWORK DYNAMICS

In the last decade a strong interest in competing network dynamics has been sparked by observed relations between opposed activity levels in task-positive (e.g., attention, fronto-parietal) and task-negative (e.g., default mode) networks and performance on a variety of tasks (Raichle et al., 2001; Fox et al., 2005; Weissman et al., 2006; Kelly et al., 2008; Hampson et al., 2010). Crucially, there seems to be a competitive balance of activity between task-positive and task-negative networks during task performance (Wojciulik and Kanwisher, 1999; McKiernan et al., 2003). During task performance activity increases in regions that are supporting task execution, whereas activity decreases in regions associated with task irrelevant (or task-opposing) processes. A growing amount of studies demonstrate the existence of strong anti-correlations between task-negative networks and task-positive networks (e.g., Fox et al., 2005). These anti-correlations have been shown to relate to performance, such that stronger anti-correlations are predictive of better cognitive performance (Kelly et al., 2008; Hampson et al., 2010). The dichotomy in activity levels observed in different networks during task performance has been suggested to be an intrinsic property of the organization of the brain (Fox et al., 2005).

It is conceivable that an external modulation of the competition between antagonistic networks in the brain could be beneficial in certain circumstances. For instance, in people with disorders such as autism spectrum disorder (ASD), anti-correlations between the default mode network and task-positive regions were found to be less pronounced than in typical subjects (Kennedy et al., 2006). In addition, decreased anti-correlations has been related to ASD symptoms (Anderson et al., 2011). These patterns demonstrate possible detrimental effects of atypical competition between different networks. Interestingly, Josipovic et al. (2012) recently demonstrated that anti-correlations between task-positive and task-negative networks could be differentially modulated depending of the cognitive style during meditation, thus disregarding them as an immutable characteristic of the organization of the brain. Further, the balance between competing networks might be adjusted dynamically to fit currently relevant behavioral goals by regulatory networks involved in top-down control (Spreng et al., 2013). Thus, whereas the interrelatedness of networks might be an inherent property of the functional organization of the brain, the balance between different network states is likely to be flexible and sensitive to top-down control. In light of these findings, NBS could be instrumental by altering the strength of competing interactions between different networks, or dichotomously increase and decrease the amount of synchrony within each network (Peña-Gómez et al., 2012).

Based on findings revealing the dynamic competition between activated and deactivated networks and their effects on behavior, it seems evident that interfering with activity in one network by applying TMS or tDCS necessarily shifts the balance between task relevant and task-irrelevant networks. Taken together with the established functional links between network anti-correlations and healthy and efficient cognitive functioning, this induced balance shift between networks is likely to have an effect on cognitive performance. Therefore, an important venue for future research employing NBS is to examine to what extent the behavioral effects of these methods can be explained by a shift in the balance of activation between different networks, rather than the current approach of solemnly focusing on altered levels of activity within one network. The notion that the balance between competing networks is an important factor may also help to explain the importance of the state of a network during application of tDCS (McKiernan et al., 2003). When taking the interconnectedness of brain networks into account, it becomes trivial that effects of brain stimulation during a state in which different functional networks are anti-correlated differs from stimulation during rest, because of the large differences in the configuration of functional networks (Silvanto et al., 2008).

Furthermore, the principle of improving one cognitive function by suppression of task-irrelevant neural processing, which was observed in TMS research described above, is possibly also at play during application of tDCS. This principle can be understood in terms of the framework proposed by Brem et al. (2014), which holds that the brain functions as a closed energy system. Under this assumption, brain stimulation would always have a "net zero-sum" effect, meaning that enhancement of one domain or function will always have costs for another domain or function. When the brain is stimulated, the distribution of resources over different brain regions or networks is externally modulated. Enhancement or suppression of activity in one neural network will necessarily lead to impairment or enhancement in another network, due to redistribution of available resources. At present we do not know whether brain stimulation exerts its effects by a temporary redistribution of resources, or whether it induces a different type of redistribution in the brain. In order to acquire more insight into the effects of NBS of distribution of neural resources, research on NBS should take the interrelatedness of different network dynamics as a central starting point.

CONCLUSIONS

In the present paper we argued that recent findings of enhancement of brain function should be seen in light of a manipulation of the balance between different functional brain networks, which can result in improved behavior when applied in the right context. However, assuming NBS is biasing the brain towards one particular functional state, this might also have detrimental effects on performance, for example, by hampering flexible transitions between functional networks. It might therefore be misleading to speak of an *enhancement* of brain function, and, alternatively, it might be more appropriate to describe the NBS effects in terms of a *bias shift*.

More research on brain-wide effects of NBS will contribute to an understanding of the way the entire brain is affected by NBS at specific sites, and may yield ways to apply these techniques in more efficient ways. In addition, with a more thorough understanding of NBS effects, brain stimulation should become more applicable outside the realm of specific lab settings. Therefore, in order to move the field of NBS and its (clinical) applications forward, it is essential to extend current studies on the effects of NBS towards investigating the effects of stimulation on task-relevant as well as task-irrelevant functional brain networks.

REFERENCES

Anderson, J. S., Nielsen, J. A., Froehlich, A. L., DuBray, M. B., Druzgal, T. J., Cariello, A. N., et al. (2011). Functional connectivity magnetic resonance imaging classification of autism. *Brain* 134, 3742–3754. doi: 10.1093/brain/awr263

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimul.* 4, 84–89. doi: 10.1016/j.brs.2010.06.004

Barker, A. T., Jalinous, R., and Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet* 325, 1106–1107. doi: 10.1016/s0140-6736(85)92413-4

Brem, A. K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition?. *Neuroimage* 85, 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Calautti, C., and Baron, J. C. (2003). Functional neuroimaging studies of motor recovery after stroke in adults a review. *Stroke* 34, 1553–1566. doi: 10.1161/01.str.0000071761.36075.a6

Chi, R. P., and Snyder, A. W. (2012). Brain stimulation enables the solution of an inherently difficult problem. *Neurosci. Lett.* 515, 121–124. doi: 10.1016/j.neulet.2012.03.012

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85, 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Coffman, B. A., Trumbo, M. C., and Clark, V. P. (2012). Enhancement of object detection with transcranial direct current stimulation is associated with increased attention. *BMC Neurosci.* 13:108. doi: 10.1186/1471-2202-13-108

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R., and Walsh, V. (2010). Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Current Biol.* 20, 2016–2020. doi: 10.1016/j.cub.2010.10.007

Flöel, A. (2014). tDCS-enhanced motor and cognitive function in neurological diseases. *Neuroimage* 85, 934–947. doi: 10.1016/j.neuroimage.2013.05.098

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U S A* 102, 9673–9678. doi: 10.1073/pnas.0504136102

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp. Brain Res.* 166, 23–30. doi: 10.1007/s00221-005-2334-6

Hampson, M., Driesen, N., Roth, J. K., Gore, J. C., and Constable, R. T. (2010). Functional connectivity between task-positive and task-negative brain areas and its relation to working memory performance. *Magn. Reson. Imaging* 28, 1051–1057. doi: 10.1016/j.mri.2010.03.021

Hilgetag, C. C., Théoret, H., and Pascual-Leone, A. (2001). Enhanced visual spatial attention ipsilateral to rTMS-induced 'virtual lesions' of human parietal cortex. *Nat. Neurosci.* 4, 953–957. doi: 10.1038/nn0901-953

Iuculano, T., and Cohen Kadosh, R. (2013). The mental cost of cognitive enhancement. *J. Neurosci.* 33, 4482–4486. doi: 10.1523/jneurosci.4927-12.2013

Josipovic, Z., Dinstein, I., Weber, J., and Heeger, D. J. (2012). Influence of meditation on anti-correlated networks in the brain. *Front. Hum. Neurosci.* 5:183. doi: 10.3389/fnhum.2011.00183

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., et al. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J. Neurosci.* 31, 15284–15293. doi: 10.1523/jneurosci.0542-11.2011

Kelly, A. M., Uddin, L. Q., Biswal, B. B., Castellanos, F. X., and Milham, M. P. (2008). Competition between functional brain networks mediates behavioral variability. *Neuroimage* 39, 527–537. doi: 10.1016/j.neuroimage.2007.08.008

Kennedy, D. P., Redcay, E., and Courchesne, E. (2006). Failing to deactivate: resting functional abnormalities in autism. *Proc. Natl. Acad. Sci. U S A* 103, 8275–8280. doi: 10.1073/pnas.0600674103

Kinsbourne, M. (1987). Mechanisms of unilateral neglect. *Adv. Psychol.* 45, 69–86. doi: 10.1016/s0166-4115(08)61709-4

Krause, B., and Cohen Kadosh, R. (2014). Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation. *Front. Syst. Neurosci.* 8:25. doi: 10.3389/fnsys.2014.00025

Krause, B., Márquez-Ruiz, J., and Kadosh, R. C. (2013). The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance?. *Front. Hum. Neurosci.* 7:602. doi: 10.3389/fnhum.2013.00602

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

Luber, B., and Lisanby, S. H. (2014). Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* 85, 961–970. doi: 10.1016/j.neuroimage.2013.06.007

McKiernan, K. A., Kaufman, J. N., Kucera-Thompson, J., and Binder, J. R. (2003). A parametric manipulation of factors affecting task-induced deactivation in functional neuroimaging. *J. Cogn. Neurosci.* 15, 394–408. doi: 10.1162/089892903321593117

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Peña-Gómez, C., Sala-Lonch, R., Junqué, C., Clemente, I. C., Vidal, D., Bargalló, N., et al. (2012). Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. *Brain Stimul.* 5, 252–263. doi: 10.1016/j.brs.2011.08.006

Priori, A., Berardelli, A., Rona, S., Accornero, N., and Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport* 9, 2257–2260.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U S A* 98, 676–682. doi: 10.1073/pnas.98.2.676

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2009). Safety, ethical considerations and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Silvanto, J., Muggleton, N. G., Cowey, A., and Walsh, V. (2007). Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation. *Eur. J. Neurosci.* 25, 1874–1881. doi: 10.1111/j.1460-9568.2007.05440.x

Silvanto, J., Muggleton, N., and Walsh, V. (2008). State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* 12, 447–454. doi: 10.1016/j.tics.2008.09.004

Sporns, O. (2013). Network attributes for segregation and integration in the human brain. *Curr. Opin. Neurobiol.* 23, 162–171. doi: 10.1016/j.conb.2012.11.015

Spreng, R. N., Sepulcre, J., Turner, G. R., Stevens, W. D., and Schacter, D. L. (2013). Intrinsic architecture underlying the relations among the default, dorsal attention and frontoparietal control networks of the human brain. *J. Cogn. Neurosci.* 25, 74–86. doi: 10.1162/jocn_a_00281

Szczepanski, S. M., and Kastner, S. (2013). Shifting attentional priorities: control of spatial attention through hemispheric competition. *J. Neurosci.* 33, 5411–5421. doi: 10.1523/jneurosci.4089-12.2013

Wagner, T., Rushmore, J., Eden, U., and Valero-Cabre, A. (2009). Biophysical foundations underlying TMS: setting the stage for an effective use of neurostimulation in the cognitive neurosciences. *Cortex* 45, 1025–1034. doi: 10.1016/j.cortex.2008.10.002

Walsh, V., Ellison, A., Battelli, L., and Cowey, A. (1998). Task-specific impairments and enhancements induced by magnetic stimulation of human visual area V5. *Proc. Biol. Sci.* 265, 537–543. doi: 10.1098/rspb.1998.0328

Walsh, V., Pascual-Leone, A., and Kosslyn, S. M. (2003). *Transcranial Magnetic Stimulation: A Neurochronometrics of Mind.* Cambridge, MA: MIT press.

Weissman, D. H., Roberts, K. C., Visscher, K. M., and Woldorff, M. G. (2006). The neural bases of momentary lapses in attention. *Nat. Neurosci.* 9, 971–978. doi: 10.1038/nn1727

Wojciulik, E., and Kanwisher, N. (1999). The generality of parietal involvement in visual attention. *Neuron* 23, 747–764. doi: 10.1016/s0896-6273(01)80033-7

Wokke, M. E., Scholte, H. S., and Lamme, V. A. (2014). Opposing dorsal/ventral stream dynamics during figure-ground segregation. *J. Cogn. Neurosci.* 26, 365–379. doi: 10.1162/jocn_a_00497

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 October 2014; accepted: 15 December 2014; published online: 12 January 2015.

Citation: Wokke ME, Talsma LJ and Vissers ME (2015) Biasing neural network dynamics using non-invasive brain stimulation. Front. Syst. Neurosci. ***8****:246. doi: 10.3389/fnsys.2014.00246*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

OPINION
published: 30 March 2015
doi: 10.3389/fnsys.2015.00054

Direct electric stimulation to increase cerebrovascular function

Victor M. Pulgar[1, 2, 3]*

[1] Biomedical Research and Infrastructure Center, Faculty of Natural and Physical Sciences, Winston-Salem State University, Winston-Salem, NC, USA, [2] Hypertension and Vascular Research Center, Wake Forest School of Medicine, Winston-Salem, NC, USA, [3] Department of Obstetrics and Gynecology, Wake Forest School of Medicine, Winston-Salem, NC, USA

Keywords: brain Stimulation, cerebral blood flow (CBF), neurovascular coupling, functional hyperemia, transcranial direct current stimulation (tDCS)

Various conditions affecting the cerebral vasculature may lead to cumulative damage and thus deterioration of brain function, in what has been called vascular cognitive impairment (Gorelick et al., 2011). Consequently, it makes sense that an increase in glucose and oxygen produced by an increase in blood flow may augment brain function. Since its rediscovery some years ago transcranial direct electric stimulation (tDCS) has attracted interest as potential therapy for patients with neurological impairments. This opinion article aims to succinctly review the mechanisms involved in neurogenic control of the cerebral blood flow (CBF) highlighting the potential of direct electrical stimulation targeting cerebral micro vessels to enhance brain function.

With the highest oxygen consumption than any other organ in the body, the brain utilizes around 20% of the total resting oxygen, making it an organ highly dependent on blood supply (Hossmann, 1994). Moreover, a direct relationship between the development of neurodegenerative diseases and impairment of CBF has been postulated (Farkas and Luiten, 2001).

The appropriate delivery of nutrients and oxygen to the brain tissue is regulated by mechanisms including cerebral autoregulation, vascular reactivity and neurovascular coupling. The autoregulatory properties of cerebral circulation make CBF independent of systemic blood pressure. Therefore, over a physiological range of pressure cerebral arteries relax when systemic pressure decreases and constrict when systemic pressure increases (Heistad and Kontos, 1983). Similarly, reactivity of the brain blood vessels to pH and CO_2 has been suggested to link neuronal metabolic changes to cerebral blood flow.

The Neurovascular Unit

One of the unique characteristics of the brain circulation is the intimate contact between blood vessels, neurons, and glia. Thus, neurons, glia, and vascular cells are structurally and functionally related in what is called the "neurovascular unit" (Iadecola, 2004). Since brain PO_2 is tightly regulated in relation to local brain activity, the neurovascular unit provides a framework for the functional interactions responsible for this concerted regulation. Thereby, functional hyperemia means that blood flow will increase in brain areas with increased activity.

From an anatomical point of view, pial arteries traveling on the surface of the brain are highly innervated with terminals coming from the peripheral nervous system (extrinsic innervation) (Hamel, 2006). These vessels are surrounded by the Virchow-Robin's space which gradually disappears as vessels enter the brain parenchyma. Cerebral arteries entering the brain parenchyma lose extrinsic innervation and come into intimate contact with neuronal and glial cells (intrinsic innervation) (Iadecola, 2004). Functions of these two vascular compartments, macro and micro vessels, involve regulation of global blood supply, as wells as control of local CBF and brain blood barrier permeability respectively.

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Prasad V. Katakam, Tulane University School of Medicine, USA
Hannah Lucy Filmer, University of Queensland, Australia
Justin Schultz Cetas, Oregon Health and Science University, USA

***Correspondence:**
Victor M. Pulgar, vpulgar@wakehealth.edu

Received: *15 January 2015*
Accepted: *13 March 2015*
Published: *30 March 2015*

Citation:
Pulgar VM (2015) Direct electric stimulation to increase cerebrovascular function. Front. Syst. Neurosci. 9:54. doi: 10.3389/fnsys.2015.00054

It is currently accepted that postsynaptic increases in $[Ca^{++}]_i$ due to activation of glutamate receptors during synaptic transmission activate the production of vasoactive mediators.

Several mediators such as neurotransmitters, adenosine, arachidonic acid metabolites, nitric oxide (NO), hydrogen and potassium, have been suggested to mediate increases in CBF (Iadecola, 2004).

Due to their close contact with blood vessels, astrocytes are suggested to play an important role in functional hyperemia. Astrocyte's end-feet surround brain micro capillaries, and may mediate neuron-blood vessel communication and thus neuronal activity-induced blood flow changes (Zonta et al., 2003). Vasodilatory as well as vasoconstrictor activities have been ascribed to glial cells (Metea and Newman, 2006), with an important role for glial eNOS in mediating vasodilatation (Stobart et al., 2013). New evidences also point to astrocytes as relevant components of the recently described "glymphatic pathway," an important mechanism for clearance of solutes from the brain (Iliff et al., 2012). Thus, aquaporin water channels in astrocyte's end feet would couple paravascular pathways for the vectorial convective flow of waste products from arterial toward venous routes, with solutes ultimately clearing the brain through the lymphatic system (Nedergaard, 2013).

The brain endothelium is a highly specialized tissue mediating several physiological functions, such as thrombosis, adhesion, permeability and angiogenesis (Daneman and Prat, 2015). A protective function against cerebral dysfunction has been proposed for the brain vessel's endothelium consistent with the predominant role of endothelial dysfunction in several cerebrovascular diseases. Importantly, *in vivo* experiments have shown that endothelial cell-derived NO mediates cortical hyperemia induced by basal forebrain electrical stimulation (Zhang et al., 1995).

Pericytes, cells located outside of the microvessels in intimate contact with endothelium and astrocyte end-feet, are more frequent on microvessels of the retina and brain and thought to regulate blood flow (Kutcher and Herman, 2009). Pericytes are considered important components of the neurovascular unit as regulators of the brain blood barrier function and also potential mediators of brain vascular dysfunction (Hamilton et al., 2010). Among the properties identified include contraction, hemostasis and angiogenesis. Given their contractile properties, pericytes may act as surrogates of smooth muscle cells in brain microvessels.

Dysfunctional interactions within the neurovascular unit have the potential to lead to brain pathophysiological alterations. Impaired endothelial cell-astrocytes or endothelial cell-pericytes signaling may cause brain blood barrier disruption (Zlokovic, 2008), whereas altered coupling between neuronal activity and vascular responses may contribute to spreading depression (Dreier, 2011).

tDCS and Brain Perfusion

Effects of electrical stimulation on the brain have been known for centuries (Priori, 2003). Work in the rat primary motor region showed that electrical stimulation may increase, decrease, or silence neuron's firing (Bindman et al., 1964; Purpura and McMurtry, 1965). These animal studies showed that anodal stimulation caused depolarization, whereas cathodal stimulation caused hyperpolarization, thus increasing the probability for a neuron to produce an action potential. tDCS has been rediscovered as a non-invasive promising tool to modulate brain activity and as a potential treatment for psychiatric and neurological disorders (Priori, 2003; Filmer et al., 2014). An increasing number of studies have reported that tDCS modulates synaptic transmission by regulating levels of neurotransmitters such as GABA, glutamate, serotonin, and dopamine, among others (Nitsche et al., 2008).

Reports showing that stimulation of cerebellar neurons increased diameter of both adjacent arterioles and the upstream vessels, provided a demonstration of the propagation of vascular responses induced by increased neural activity (Iadecola et al., 1997). Importantly, cerebellar stimulation at the fastigial nucleus, reduced ischemia induced by medial cerebral artery occlusion in rats through NO-mediated hemodynamic mechanisms (Zhang and Iadecola, 1993).

tDCS in humans is performed by applying direct current over the scalp using electrodes and its effects depend on the size, polarity and position of the electrodes, current intensity, duration of stimulation, and tissue properties (DaSilva et al., 2011). Given the intimate relationship between neuronal activity and CBF, it is expected that tDCS will increase brain perfusion, as shown in animal (Han et al., 2014) and human (Zheng et al., 2011) studies. The opinion presented in this article is that in addition to the changes in neuronal-derived metabolites, evidences showing responses to electrical stimulation in non-neuronal cells suggest that tDCS acting on these cells has also the potential to modulate brain perfusion (**Figure 1**). Thus, understanding the vascular effects of tDCS may improve the treatment of diseases associated with vascular dysfunction.

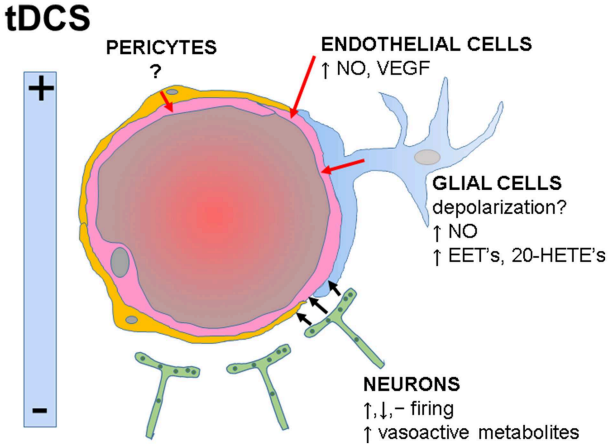

FIGURE 1 | Functional interactions within the neurovascular unit and potential influences of tDCS on specific cell types. tDCS-induced electrical fields may increase, decrease or have no effects on neuronal firing rates. Neuronal-derived metabolites directly or indirectly activate endothelial cells (black arrows), inducing vasodilatation. Potential direct effects of tDCS on glial, endothelial cells, and pericytes are indicated (red arrows). NO, nitric oxide; VEGF, vascular endothelial growth factor; EET's, epoxyeicosatrienoic acids; 20-HETE's, 20-hydroxyeicosatetraeonic acids.

Direct effects of electric stimulation on neurons *in vitro* include alignment of neurites perpendicular to the electric field, increased growth and migration (Pan and Borgens, 2012). In mouse coronal slices, a role for electrical stimulation-induced synaptic plasticity was demonstrated, an effect that may underlie implications of tDCS on motor learning (Fritsch et al., 2010). Results obtained in rat brain slices suggested that electrical stimulation modulates long term potentiation in a polarity-specific manner supporting a regulatory role of tDCS on synaptic plasticity (Ranieri et al., 2012).

Described effects of electrical stimulation on astrocytes *in vitro* include changes in metabolism depending on field polarization and applied voltage (Huang et al., 1997), as well as migration and perpendicular alignment (Pelletier et al., 2014). A theoretical analysis concluded that tDCS has the potential to directly stimulate glial cells since the tDCS-induced changes in membrane potential are similar to the changes induced in astrocytes during neuronal activation (Ruohonen and Karhu, 2012).

The effects of electric stimulation on endothelial cells *in vitro* include the alignment perpendicular to the direction of the electrical field, migration, and elongation (Zhao et al., 2012). These effects are associated with increases in VEGF production, suggesting that electrical stimulation may modulate angiogenesis (Zhao et al., 2012). Conversely, the data from brain slices, including effects on synaptic plasticity (Fritsch et al., 2010) are obtained in the absence of circulation, which may indicate that there is no endothelial contribution to the neuronal effects of electric stimulation. However, endothelial cells in culture exposed to a low physiological electrical field (3.3 mV/mm) showed increased NO production (Trivedi et al., 2013), suggesting a direct route by which electric stimulation may increase brain perfusion. Modeling of the electric properties of the brain suggests that the electric field generated during tDCS in humans is around 1 mV/mm (Neuling et al., 2012) indicating that endothelial cell-dependent responses may be triggered during tDCS.

The proposed role of pericytes in the neurovascular unit suggests that pericytes may transduce signals from neurons to endothelial cells (Hall et al., 2014). Thus, during neuronal activation glutamate release produces prostaglandin E_2 which in turn will induce capillaries vasodilatation by activating K^+ currents in pericytes. These are excitable cells and a direct effect of tDCS on pericyte's membrane potential may hyperpolarize it and induce vasodilatory signals. Whether pericyte-mediated responses to tDCS are playing a role in the tDCS effects remains to be elucidated.

Although general agreement has been observed between animal and human studies (Bennabi et al., 2014), it is necessary to note that stimulating parameters used in animal *in vivo* and in *in vitro* protocols are higher than those used in humans where a maximum current density of $\sim$0.28 A/m^2 is used (Im et al., 2012). In contrast a maximum safe stimulation in rats was reported at 142.9 A/m^2 (Liebetanz et al., 2009).

tDCS and Augmentation of Brain Function

In humans, evidences indicate the potential of tDCS to increase cognitive, motor and memory function. For example, tDCS may enhance gesture comprehension by improving gesture and language integration (Cohen-Maximov et al., 2014), a result especially relevant in cases of autism where patients have difficulties processing symbolic gestures (Baron-Cohen, 1988). Anodal tDCS administered repeatedly facilitates language (Meinzer et al., 2014) and motor skill learning (Zimerman et al., 2013). tDCS has also been shown to produce long-lasting effects on number processing (Cohen Kadosh et al., 2010) and there is increasing interest in the applicability of tDCS for memory enhancement (Bennabi et al., 2014).

However, not all tDCS studies have observed positive effects. Thus, the rate of motor sequence learning is increased by anodal tDCS and decreased by cathodal stimulation, whereas tDCS applied prior to the motor task slowed learning (Stagg et al., 2011). Also cerebellar tDCS has been shown to impair practice-dependent improvement in a working memory task (Ferrucci et al., 2008), whereas tDCS applied to pre-frontal cortex disrupts sensory-motor training (Filmer et al., 2013). Clearly, the research describing the efficacy of tDCS for motor and cognitive improvement is still inconclusive.

Brain blood flow responses to specific tasks may involve cortical and subcortical structures, as seen in the attention-derived effects on flow in both the visual cortex and the lateral geniculate nucleus (O'Connor et al., 2002). Thus, it is relevant to point out that tDCS may also modulate blood flow in subcortical structures (Lang et al., 2005; Nonnekes et al., 2014) demonstrating broader effects of tDCS on CBF.

Recent technical developments applying tDCS simultaneously with electroencephalography and brain blood flow measurements (Dutta et al., 2015) will help to integrate tDCS with modulation of vascular function and ultimately changes in human behavior.

Perspectives and Future Directions

In order to expand direct therapeutic applicability, tDCS needs to overcome the challenges related to inter- and intra-subject variability and parameters of stimulation impacting neuroplasticity (i.e., short *vs.* long term stimulation), among others. Simultaneous determination of vascular signals and cognitive performance during tDCS will help to integrate electrical stimulation with vascular functioning and changes in behavior. The relationships between neuronal and vascular effects are complex and it is proven difficult to differentiate between the effects of electrical stimulation on those two tissues, it is however conceivable that in addition to the vascular effects of neuronal-derived metabolites, direct effects of tDCS on non-neuronal cells especially glial and endothelial cells modulate brain perfusion. Thus, a deeper understanding of the effects tDCS have on non-neuronal members of the neurovascular unit is essential.

Acknowledgments

The author thanks Anne Jeffers for language revision of the article.

References

Baron-Cohen, S. (1988). Social and pragmatic deficits in autism: cognitive or affective? *J. Autism Dev. Disord.* 18, 379–402. doi: 10.1007/BF02212194

Bennabi, D., Pedron, S., Haffen, E., Monnin, J., Peterschmitt, Y., and Van Waes, V. (2014). Transcranial direct current stimulation for memory enhancement: from clinical research to animal models. *Front. Syst. Neurosci.* 8:159. doi: 10.3389/fnsys.2014.00159

Bindman, L. J., Lippold, O. C. J., and Redfearn, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* 172, 369–382. doi: 10.1113/jphysiol.1964.sp007425

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R., and Walsh, V. (2010). Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Curr. Biol.* 20, 2016–2020. doi: 10.1016/j.cub.2010.10.007

Cohen-Maximov, T., Avirame, K., Flöel, A., and Lavidor, M. (2014). Modulation of gestural-verbal semantic integration by tDCS. *Brain Stimul.* doi: 10.1016/j.brs.2014.12.001. [Epub ahead of print]

Daneman, R., and Prat, A. (2015). The Blood-Brain Barrier. *Cold Spring Harb. Perspect. Biol.* 7:a020412. doi: 10.1101/cshperspect.a020412

DaSilva, A. F., Volz, M. S., Bikson, M., and Fregni, F. (2011). Electrode positioning and montage in transcranial direct current stimulation. *J. Vis. Exp.* 51:e2744. doi: 10.3791/2744

Dreier, J. P. (2011). The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. *Nat. Med.* 17, 439–447. doi: 10.1038/nm.2333

Dutta, A., Jacob, A., Chowdhury, S., Das, A., and Nitsche, M. (2015). EEG-NIRS based assessment of neurovascular coupling during anodal transcranial direct current stimulation - a stroke case series. *J. Med. Syst.* 39, 1–9. doi: 10.1007/s10916-015-0205-7

Farkas, E., and Luiten, P. G. M. (2001). Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog. Neurobiol.* 64, 575–611. doi: 10.1016/S0301-0082(00)00068-X

Ferrucci, R., Marceglia, S., Vergari, M., Cogiamanian, F., Mrakic-Sposta, S., Mameli, F., et al. (2008). Cerebellar transcranial direct current stimulation impairs the practice-dependent proficiency increase in working memory. *J. Cogn. Neurosci.* 20, 1687–1697. doi: 10.1162/jocn.2008.20112

Filmer, H. L., Dux, P. E., and Mattingley, J. B. (2014). Applications of transcranial direct current stimulation for understanding brain function. *Trends Neurosci.* 37, 742–753. doi: 10.1016/j.tins.2014.08.003

Filmer, H. L., Mattingley, J. B., Marois, R., and Dux, P. E. (2013). Disrupting prefrontal cortex prevents performance gains from sensory-motor training. *J. Neurosci.* 33, 18654–18660. doi: 10.1523/JNEUROSCI.2019-13.2013

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Gorelick, P. B., Scuteri, A., Black, S. E., DeCarli, C., Greenberg, S. M., Iadecola, C., et al. (2011). Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 42, 2672–2713. doi: 10.1161/STR.0b013e3182299496

Hall, C. N., Reynell, C., Gesslein, B., Hamilton, N. B., Mishra, A., Sutherland, B. A., et al. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508, 55–60. doi: 10.1038/nature13165

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. *J. Appl. Physiol.* 100, 1059–1064. doi: 10.1152/japplphysiol.00954.2005

Hamilton, N. B., Attwell, D., and Hall, C. N. (2010). Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. *Front. Neuroenergetics* 2:5. doi: 10.3389/fnene.2010.00005

Han, C. H., Song, H., Kang, Y. G., Kim, B. M., and Im, C. H. (2014). Hemodynamic responses in rat brain during transcranial direct current stimulation: a functional near-infrared spectroscopy study. *Biomed. Opt. Express* 13, 1812–1821. doi: 10.1364/BOE.5.001812

Heistad, D., and Kontos, H. (1983). *Handbook of Physiology: The Cardiovascular System III.* Bethesda, MD: American Physiological Society.

Hossmann, K. A. (1994). Viability thresholds and the penumbra of focal ischemia. *Ann. Neurol.* 36, 557–565. doi: 10.1002/ana.410360404

Huang, R., Peng, L., and Hertz, L. (1997). Effects of a low-voltage static electric field on energy metabolism in astrocytes. *Bioelectromagnetics* 18, 77–80. doi: 10.1002/(SICI)1521-186X(1997)18:1<77::AID-BEM11>3.0.CO;2-N

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat. Rev. Neurosci.* 5, 347–360. doi: 10.1038/nrn1387

Iadecola, C., Yang, G., Ebner, T., and Chen, G. (1997). Local and propagated vascular responses evoked by focal synaptic activity in cerebellar cortex. *Neurophysiol* 78, 651–659.

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748

Im, C. H., Park, J. H., Shim, M., Chang, W. H., and Kim, Y. H. (2012). Evaluation of local electric fields generated by transcranial direct current stimulation with an extracephalic reference electrode based on realistic 3D body modeling. *Phys. Med. Biol.* 57:2137. doi: 10.1088/0031-9155/57/8/2137

Kutcher, M. E., and Herman, I. M. (2009). The pericyte: cellular regulator of microvascular blood flow. *Microvasc. Res.* 77, 235–246. doi: 10.1016/j.mvr.2009.01.007

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., et al. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *Eur. J. Neurosci.* 22, 495–504. doi: 10.1111/j.1460-9568.2005.04233.x

Liebetanz, D., Koch, R., Mayenfels, S., König, F., Paulus, W., and Nitsche, M. A. (2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clin. Neurophysiol.* 120, 1161–1167. doi: 10.1016/j.clinph.2009.01.022

Meinzer, M., Jähnigen, S., Copland, D. A., Darkow, R., Grittner, U., Avirame, K., et al. (2014). Transcranial direct current stimulation over multiple days improves learning and maintenance of a novel vocabulary. *Cortex* 50, 137–147. doi: 10.1016/j.cortex.2013.07.013

Metea, M. R., and Newman, E. A. (2006). Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J. Neurosci.* 26, 2862–2870. doi: 10.1523/JNEUROSCI.4048-05.2006

Nedergaard, M. (2013). Garbage truck of the brain. *Science* 340, 1529–1530. doi: 10.1126/science.1240514

Neuling, T., Wagner, S., Wolters, C. H., Zaehle, T., and Herrmann, C. S. (2012). Finite element model predicts current density distribution for clinical applications of tDCS and tACS. *Front. Psychiatry* 3:83. doi: 10.3389/fpsyt.2012.00083

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nonnekes, J., Arrogi, A., Munneke, M. A. M., van Asseldonk, E. H. F., Oude Nijhuis, L. B., Geurts, A. C., et al. (2014). Subcortical structures in humans can be facilitated by transcranial direct current stimulation. *PLoS ONE* 9:e107731. doi: 10.1371/journal.pone.0107731

O'Connor, D. H., Fukui, M. M., Pinsk, M. A., and Kastner, S. (2002). Attention modulates responses in the human lateral geniculate nucleus. *Nat. Neurosci.* 5, 1203–1209. doi: 10.1038/nn957

Pan, L., and Borgens, R. B. (2012). Strict perpendicular orientation of neural crest-derived neurons *in vitro* is dependent on an extracellular gradient of voltage. *J. Neurosci. Res.* 90, 1335–1346. doi: 10.1002/jnr.22809

Pelletier, S. J., Lagacé, M., St-Amour, I., Arsenault, D., Cisbani, G., Chabrat, A., et al. (2014). The morphological and molecular changes of brain cells exposed to direct current electric field stimulation. *Int. J. Neuropsychopharmacol.* 18. doi: 10.1093/ijnp/pyu090

Priori, A. (2003). Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clin. Neurophysiol.* 114, 589–595. doi: 10.1016/S1388-2457(02)00437-6

Purpura, D. P., and McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *J. Neurophysiol.* 28, 166–185.

Ranieri, F., Podda, M. V., Riccardi, E., Frisullo, G., Dileone, M., Profice, P., et al. (2012). Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation. *J. Neurophysiol.* 107, 1868–1880. doi: 10.1152/jn.00319.2011

Ruohonen, J., and Karhu, J. (2012). tDCS possibly stimulates glial cells. *Clin. Neurophysiol.* 123, 2006–2009. doi: 10.1016/j.clinph.2012.02.082

Stagg, C. J., Jayaram, G., Pastor, D., Kincses, Z. T., Matthews, P. M., and Johansen-Berg, H. (2011). Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning. *Neuropsychologia* 49, 800–804. doi: 10.1016/j.neuropsychologia.2011.02.009

Stobart, J. L. L., Lu, L., Anderson, H. D. I., Mori, H., and Anderson, C. M. (2013). Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3149–3154. doi: 10.1073/pnas.1215929110

Trivedi, D. P., Hallock, K. J., and Bergethon, P. R. (2013). Electric fields caused by blood flow modulate vascular endothelial electrophysiology and nitric oxide production. *Bioelectromagnetics* 34, 22–30. doi: 10.1002/bem.21741

Zhang, F., and Iadecola, C. (1993). Fastigial stimulation increases ischemic blood flow and reduces brain damage after focal ischemia. *J. Cereb. Blood Flow Metab.* 13, 1013–1019. doi: 10.1038/jcbfm.1993.127

Zhang, F., Xu, S., and Iadecola, C. (1995). Role of nitric oxide and acetylcholine in neocortical hyperemia elicited by basal forebrain stimulation: evidence for an involvement of endothelial nitric oxide. *Neuroscience* 69, 1195–1204. doi: 10.1016/0306-4522(95)00302-Y

Zhao, Z., Qin, L., Reid, B., Pu, J., Hara, T., and Zhao, M. (2012). Directing migration of endothelial progenitor cells with applied DC electric fields. *Stem Cell Res.* 8, 38–48. doi: 10.1016/j.scr.2011.08.001

Zheng, X., Alsop, D. C., and Schlaug, G. (2011). Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. *Neuroimage* 58, 26–33. doi: 10.1016/j.neuroimage.2011.06.018

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L. G., and Hummel, F. C. (2013). Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. *Ann. Neurol.* 73, 10–15. doi: 10.1002/ana.23761

Zlokovic, B. V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57, 178–201. doi: 10.1016/j.neuron.2008.01.003

Zonta, M., Angulo, M. C., Gobbo, S., Rosengarten, B., Hossmann, K. A., and Pozzan, T., et al. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* 6, 43–50. doi: 10.1038/nn980

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

PERSPECTIVE
published: 10 August 2015
doi: 10.3389/fnsys.2015.00107

Bidirectional interactions between neuronal and hemodynamic responses to transcranial direct current stimulation (tDCS): challenges for brain-state dependent tDCS

Anirban Dutta[1,2]*

[1] INRIA (Sophia Antipolis) – CNRS: UMR5506 – Université Montpellier, Montpellier, France, [2] Laboratoire d'Informatique de Robotique et de Microélectronique de Montpellier (LIRMM), CNRS: UMR5506 – Université Montpellier, Montpellier, France

Transcranial direct current stimulation (tDCS) has been shown to modulate cortical neural activity. During neural activity, the electric currents from excitable membranes of brain tissue superimpose in the extracellular medium and generate a potential at scalp, which is referred as the electroencephalogram (EEG). Respective neural activity (energy demand) has been shown to be closely related, spatially and temporally, to cerebral blood flow (CBF) that supplies glucose (energy supply) via neurovascular coupling. The hemodynamic response can be captured by near-infrared spectroscopy (NIRS), which enables continuous monitoring of cerebral oxygenation and blood volume. This neurovascular coupling phenomenon led to the concept of neurovascular unit (NVU) that consists of the endothelium, glia, neurons, pericytes, and the basal lamina. Here, recent works suggest NVU as an integrated system working in concert using feedback mechanisms to enable proper brain homeostasis and function where the challenge remains in capturing these mostly nonlinear spatiotemporal interactions within NVU for brain-state dependent tDCS. In principal accordance, we propose EEG-NIRS-based whole-head monitoring of tDCS-induced neuronal and hemodynamic alterations during tDCS.

Keywords: transcranial direct current stimulation, electroencephalogram, near-infrared spectroscopy, hemo-neural hypothesis, neurovascular coupling

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Victor Manuel Pulgar, Wake Forest School of Medicine, USA
Armando López-Cuevas, Center for Research and Advanced Studies, Mexico

***Correspondence:**
Anirban Dutta, INRIA (Sophia Antipolis) – CNRS: UMR5506 – Université Montpellier, Batiment 5 - 860 Rue de Saint Priest, Montpellier 34095, France
adutta@ieee.org

Received: *02 April 2015*
Accepted: *13 July 2015*
Published: *10 August 2015*

Citation:
Dutta A (2015) Bidirectional interactions between neuronal and hemodynamic responses to transcranial direct current stimulation (tDCS): challenges for brain-state dependent tDCS. Front. Syst. Neurosci. 9:107. doi: 10.3389/fnsys.2015.00107

Challenges in Clinical Translation of Transcranial Brain Stimulation—An Introduction

Transcranial direct current stimulation (tDCS)—an electrically based intervention directed at the central nervous system level—is a promising tool to alter cortical excitability and facilitate neuroplasticity (Nitsche and Paulus, 2011). However, inter-subject variability and intra-subject reliability currently limits clinical translation (Horvath et al., 2014). Indeed, a recent meta-analysis showed that the treatment effects of transcranial brain stimulation in patients with stroke are rather inconsistent across studies and the evidence for

therapeutic efficacy is still uncertain (Raffin and Siebner, 2014). Here, it may be possible to reduce inter-subject variability and improve intra-subject reliability using simultaneous neuroimaging that can objectively quantify the individual brain-state before and during tDCS. Non-invasive neuroimaging techniques that have previously been combined with tDCS include electrophysiological, e.g., electroencephalogram (EEG; Schestatsky et al., 2013) and hemodynamic, e.g., functional magnetic resonance imaging (fMRI; Meinzer et al., 2014) and near-infrared spectroscopy (NIRS; McKendrick et al., 2015) approaches. Here, NIRS presents several advantages relative to fMRI, such as measurement of concentration changes in both oxygenated (HbO_2) and deoxygenated (HHb) hemoglobin, finer temporal resolution, ease of administration and relative insensitivity to movement artifacts. Although fMRI has become the benchmark for *in vivo* imaging of the human brain, in practice, NIRS and EEG are more convenient and less expensive technology than fMRI for simultaneous neuroimaging for brain-state dependent tDCS. However, the challenge remains in modeling whole-head spatiotemporal coupling of neuronal and hemodynamic alterations induced by tDCS where such brain-state dependent tDCS need not only to consider the brain as a dynamical system but also need to consider that its parameters will be inter-individually heterogeneous, dependent on brain injury (and maladaptive plasticity, e.g., reactive gliosis, Buffo et al., 2008), task characteristics (e.g., attention issues) and other factors (Raffin and Siebner, 2014).

Biophysical Models for Capturing Hemodynamic Alterations Induced by tDCS

Neural activity has been shown to be closely related, spatially and temporally, to cerebral blood flow (CBF) that supplies glucose via neurovascular coupling (Girouard and Iadecola, 2006). The hemodynamic response to neural activity can be captured by NIRS, which enables continuous monitoring of cerebral oxygenation and blood volume (Siesler et al., 2008). The regulation of CBF and its spatiotemporal dynamics may be probed with short-duration anodal tDCS which challenges the system with a vasoactive stimulus in order to observe the system response. Based on prior works (Nitsche and Paulus, 2000; Dutta et al., 2015), such short-duration (<1 min) anodal tDCS is postulated to cause no aftereffects and may be used to probe neurovascular coupling (and neurovascular unit, NVU; Jindal et al., 2015b). Here, CBF is increased in brain regions with enhanced neural activity via metabolic coupling mechanisms (Attwell et al., 2010) while cerebral autoregulation mechanisms ensure that the blood flow is maintained during changes of perfusion pressure (Lucas et al., 2010). During such a short-duration anodal tDCS experiment, cerebrovascular reactivity (CVR) can be measured as the change in CBF per unit change in relation to anodal tDCS intensity. Moreover, the rate of change of hemodynamic responses to same tDCS intensity may explain inter-individual differences in tDCS after-effects (Han et al., 2014). Also, phenomological model for metabolic coupling mechanisms (Attwell et al., 2010) can be used to capture CVR that represents the capacity of blood vessels to dilate during anodal tDCS due to neuronal activity-related increased demands of oxygen (Dutta et al., 2013). Here, CVR reflects the capacity of blood vessels to dilate, and is an important marker for brain vascular reserve (Markus and Cullinane, 2001). Indeed pressure–perfusion–cognition relationships may be monitored with the brain vascular reserve (Novak, 2012) where the CVR distributes CBF toward the brain areas in need of increased perfusion due to enhanced neural activity.

Prior work has shown a significant correlation between tDCS current strength and increase in regional CBF in the on-period relative to the pre-stimulation baseline (Zheng et al., 2011). We investigated regional CVR during anodal tDCS by adapting an arteriolar compliance model of the CBF response to a neural stimulus (Behzadi and Liu, 2005). Regional CVR was defined as the coupling between changes in CBF and cerebral metabolic rate of oxygen ($CMRO_2$) during anodal tDCS-induced local brain activation (Leontiev and Buxton, 2007). The complex path from the tDCS-induced change of the synaptic transmembrane current, $u(t)$ (only excitatory effects considered; Molaee-Ardekani et al., 2013) to a change in the concentration of multiple vasoactive agents (such as NO, potassium ions, adenosine), represented by a single vascular flow-inducing vasoactive signal, s, was captured by a first-order Friston's model (Friston et al., 2000). Chander and Chakravarthy (2012) presented a computational model that studied the effect of metabolic feedback on neuronal activity to bridge the gap between measured hemodynamic response and ongoing neural activity. Here, the NVU (see **Figure 1**) consists of the endothelium, glia, neurons, pericytes, and the basal lamina that has been proposed to maintain the homeostasis of the brain microenvironment (Iadecola, 2004). In this connection, the role of lactate as a signaling molecule was described recently (Yang et al., 2014), which supports a (delayed) "reverse" influence in the NVU from the vessel back to neuron via lactate (Chander and Chakravarthy, 2012). Recently, a detailed biophysical model of the brain's metabolic interactions was presented by Jolivet et al. (2015). This not only supported the astrocyte-neuron lactate shuttle (ANLS) hypothesis that the lactate produced in astrocytes (a type of glial cell) can also fuel neuronal activity but it also provided a quantitative mathematical description of the metabolic activation in neurons and glial cells, as well as of the macroscopic measurements obtained during brain imaging. Indeed, this model captured the pattern of neurovascular responses observed in rodents in response to sustained sensory stimulation where CBF only starts to increase above its baseline ∼0.5–1 s after the onset of stimulation (Jolivet et al., 2015). We also found such onset effects (called "initial dip") of anodal tDCS in stroke patients (Dutta et al., 2015). Moreover, Jolivet et al. (2015) highlighted the neuron-astrocyte cross-talk during oscillations linked to blood oxygenation levels (DiNuzzo et al., 2011) where such oscillations also occurred after anodal tDCS-based perturbation of the neuroglial networks in our EEG-NIRS stroke study (Dutta et al., 2015). We therefore postulate that short-duration anodal tDCS can be used to perturb neuroglial networks in health and disease to probe the

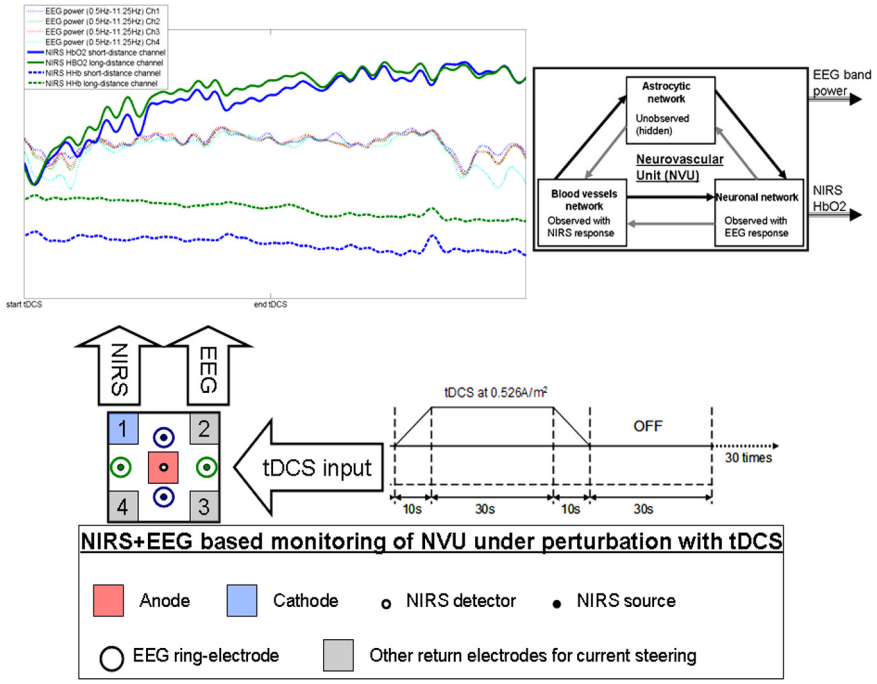

FIGURE 1 | Illustration of the effects of transcranial direct current stimulation (tDCS) from simultaneous recording of electroencephalogram (EEG) and near infra red spectroscopy (NIRS). The colors on the plot corresponds to the sensors. Here, only one anode and one cathode is highlighted for tDCS, however, local current steering based on NIRS-EEG feedback is possible to optimally orient the electric field with multiple return electrodes (Khadka et al., 2015). Here, neurovascular unit (NVU) consists of the endothelium, glia, neurons, pericytes, and the basal lamina in which neurons, astrocytes, and vessels are semi-independent networks operating in tandem. Neuronal network activity drives functional hyperemia via direct effects on the blood vessels network as well as indirect effects via the astrocytic network. Also, the hemodynamics changes can impact neuronal network activity via direct (diffusible messengers, electromechanical and thermal interactions) and indirect (via astrocytic network) pathways.

spatiotemporal dynamics of the NVU based on simultaneous EEG-NIRS neuroimaging (Dutta, 2014; Dutta et al., 2015) and biophysical model (Jolivet et al., 2015) based analysis.

Neural Mass or Field Models for Capturing Neuronal Alterations Induced by tDCS

During neural activity, the electric currents from excitable membranes of brain tissue superimpose at a given location in the extracellular medium and generate a potential, which is referred to as the EEG (Nunez and Srinivasan, 2006). Here, neural mass models (NMM) can provide insights into the neuromodulatory mechanisms underlying alterations of cortical activity induced via tDCS (Molaee-Ardekani et al., 2013). Specifically, the origin of tDCS-induced alterations in the EEG power spectrum was captured using a thalamocortical NMM (Dutta and Nitsche, 2013). The NMM for a single cortical source comprises of four neuronal subpopulations, excitatory pyramidal neurons (ePN), excitatory interneurons (eIN), slow inhibitory interneurons (siIN), and fast inhibitory interneurons (fiIN; Zavaglia et al., 2006). The NMM for the cortical source was coupled with another representing the thalamus (Sotero et al., 2007), which comprises of two neuronal subpopulations—an excitatory thalamocortical (eTCN) and an inhibitory reticular-thalamic (iRT). The basis of our cortical NMM is the Friston model (Moran et al., 2007) that emulates the activity of a cortical area using three neuronal subpopulations, ePN, eIN, and siIN. A population of ePN (output) cells receives inputs from inhibitory and excitatory populations of interneurons via intrinsic connections (intrinsic connections are confined to the cortical sheet). An extrinsic thalamo–cortico–thalamic loop consists of eTCN and iRT in the thalamic NMM (Ursino et al., 2010). Our lumped thalamo–cortico–thalamic network model can be used to simulate the subject-specific EEG power spectral density changes during/following tDCS (Dutta and Nitsche, 2013) by modifying the model parameters (e.g., average gain of synapses, their time constants; Zavaglia et al., 2006). We found that anodal tDCS enhances activity and excitability of the excitatory pyramidal neuron at a population level in a non-specific manner and mu-rhythm desynchronization is generated (Dutta and Nitsche, 2013). The tDCS effects on the population kinetics depend on the direction of cortical current flow determining the relative influence of acute tDCS on the cellular targets responsible for modulation of synaptic efficacy, which are primarily somata and axon terminals (Rahman et al., 2013). Basal and apical dendrites can be concomitantly polarized in opposite directions, and Layer V pyramidal neurons exhibit the highest measured somatic sensitivities to subthreshold fields (Rahman et al., 2013). Therefore, not all neural tissue will be equally affected by a given stimulation protocol which may distinctly

affect neuronal populations/neuronal compartments. Indeed, a recent computational modeling study suggested that tDCS may induce opposing effects on different types of interneurons (Molaee-Ardekani et al., 2013). Here, the excitation vs. inhibition effects (Krause et al., 2013) of tDCS on the population kinetics can produce a whole spectrum of EEG signals within the oscillatory regime of a neural mass model (David and Friston, 2003).

There are several prior works that have shown both "online" effects of tDCS on EEG with EEG performed during tDCS as well as "offline" effects with EEG performed after tDCS. Here, it is important to separate studies where tDCS is applied during a rest state (Ardolino et al., 2005; Zaehle et al., 2011; Spitoni et al., 2013) or an active task state (Matsumoto et al., 2010; Mangia et al., 2014). We computationally found (Dutta and Nitsche, 2013) in concordance with the experimental results of Matsumoto et al. (2010) that tDCS effects on mu-rhythm desynchronization depend on the direction of cortical current flow determining the relative influence of acute tDCS on the cellular targets. Matsumoto et al. (2010) found that tDCS applied over the left primary motor area for 10 min at 1 mA with a 35 cm^2 electrode influenced event-related desynchronization (ERD) during right hand grasping where the mu ERD increased after anodal tDCS and decreased after cathodal tDCS. Here, not only the "local" effects but the "distant" effects of tDCS are also relevant where Polanía et al. (2012) reported that the functional connectivity patterns significantly increased after anodal tDCS (i.e., "offline" effects) over the primary motor cortex where tDCS modulated functional connectivity of cortico-striatal and thalamo-cortical circuits. Notturno et al. (2014) showed spatial diffusion of anodal tDCS (during a motor task) effects where an increment of low alpha band power over the course of pre- and post-stimulation recording sessions was found during motor task that was localized in the sensorimotor and parieto-occipital regions. Indeed, not only the "offline" effects, but changes in functional connectivity patterns may start evolving during tDCS (i.e., "online" effects) as shown by our modeling study (Dutta and Nitsche, 2013). tDCS/EEG co-registration studies have shown that anodal tDCS mostly modulate spontaneous cortical activity in the alpha band where alpha-rhythm states have a significant effect on perceptual learning (Sigala et al., 2014). In fact, more than 60% of the observed inter-subject variability in perceptual learning can be ascribed to ongoing alpha activity where Sigala et al. (2014) highlighted the need for multidisciplinary approaches combining assessment of behavior and multi-scale neuronal activity, active modulation of ongoing brain states and computational modeling to reveal the mathematical principles of the complex neuronal interactions. We therefore postulate that concurrent EEG-NIRS-based neuroimaging of the short-duration tDCS-induced modulation can be analyzed by combining a biophysical model (Jolivet et al., 2015) of the NVU with the computational model (neural mass or field model) of multi-scale neuronal activity of the whole brain (Sigala et al., 2014) to capture the spatiotemporal dynamics of the interactions between the neuronal and hemodynamic responses in health and disease. Here, the challenges remain in ensuring the observability of the NVU with intelligent placement of EEG-NIRS sensors since presence of symmetry in the nonlinear network of NVU (see **Figure 1**) may decrease observability (although networks containing only rotational symmetries remain observable; Whalen et al., 2015).

Bidirectional Interactions Between Neuronal and Hemodynamic Responses to tDCS—A Discussion

In our prior work (Dutta et al., 2015), we found an initial dip in the oxy-hemoglobin concentration and concomitant increase in the mean power spectral density within lower (<12 Hz) EEG frequency band. It was postulated that the immediate need to fuel neuronal energy recovery was via the lactate shuttle (Pellerin and Magistretti, 1994) where blood glucose supply has a longer delay (Gruetter et al., 1996). A detailed biophysical model of the brain's metabolic interactions by Jolivet et al. (2015) also supported the ANLS hypothesis. Moreover, recent works showed that lactate can modulate the activity of primary cortical neurons through a receptor-mediated pathway (Bozzo et al., 2013) and vasomotion rhythms can influence neural firing patterns (Nikulin et al., 2014). Also, lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons, and the action of lactate is mediated by the modulation of NMDA receptor activity (Yang et al., 2014). These dynamic ANLS interactions leave us to question its role in tDCS facilitated neuroplasticity and learning (Suzuki et al., 2011). Also, the spatiotemporal dynamics of the millisecond-to-second-range direct (diffusible messengers, electromechanical and thermal interactions) and seconds-to-tens-of-seconds-range indirect interaction in the NVU following tDCS, i.e., the hemo-neural hypothesis (Moore and Cao, 2008), may at least partially explain the time course of the induction of homeostatic plasticity generated by repeated tDCS of the human motor cortex (Fricke et al., 2011). Fricke et al. (2011) hypothesized a role of L-type voltage-gated Ca^{2+} channels (L-VGCC) in short-term homeostatic plasticity, since tDCS has been shown to induce a long-lasting disturbance of Ca^{2+} homeostasis (Islam et al., 1995) and induce calcium-dependent plasticity (Nitsche et al., 2003). Here, the glial network may have an important role (i.e., spatial buffering) in regulating neural activity by distributing ions (Halnes et al., 2013) in seconds-to-tens-of-seconds-range where an influence of long-lasting disturbance of Ca^{2+} homeostasis via tDCS on the myogenic and the metabolic control of cerebral circulation cannot be excluded. In fact, astrocytes, a sub-type of glia in the central nervous system, can integrate a large number of synapses and can respond to neuronal activity via neurotransmitter-evoked activation of astrocytic receptors (Araque et al., 2001). Indeed, neuronal activity can mobilize internal calcium in astrocytes and the calcium wave in different spatial–temporal dimensions can result in a higher level of brain integration (Volterra et al., 2014) where the evidence for tDCS-induced large scale changes in brain synchronization and topological functional organization has been shown after acute stimulation (Polanía et al., 2011).

Based on these prior works, we recently proposed EEG-NIRS-based monitoring of neurovascular coupling functionality under perturbation with tDCS (Jindal et al., 2015b). Here, neuronal and hemodynamic responses measured with EEG-NIRS neuroimaging can be represented abstractly as the system response of the NVU to tDCS perturbation (see **Figure 1**) where presence of symmetry in the nonlinear network of NVU (see **Figure 1**) may decrease observability (Whalen et al., 2015). Since no real-world network has exact symmetries so with intelligent placement of EEG-NIRS sensors (e.g., to avoid systemic interference; Sood et al., 2015) along with system identification and parameter estimation techniques, it may be possible to track the spatiotemporal change of the states of the NVU. This observer model can then be used to drive multi-electrode tDCS (Dmochowski et al., 2011) for active spatiotemporal modulation of the brain states (e.g., posterior alpha-rhythm). Here, we base our discussions on the recent advances in Kalman filtering approaches to spatiotemporal nonlinear systems (Schiff and Sauer, 2008) and an understanding from group representation theory in controller or observer design by obtaining a modal decomposition into decoupled controllable and uncontrollable (observable and unobservable) subspaces (Whalen et al., 2015). Specifically, Schiff and Sauer (2008) showed the feasibility of unscented Kalman filter (UKF) for recursive estimation of system state for nonlinear systems, including unobserved variables and parameter tracking, in a spatiotemporal model of cortex where such a nonlinear system is controllable using an adaptive feedback electrical field. Here, discretization of the whole-brain detailed biophysical model of NVU (Jolivet et al., 2015), for example with Galerkin methods that are used quite robustly in fluid dynamics, will be necessary where each discrete element corresponds to a volume of tissue imaged as well as stimulated with the EEG-NIRS/tDCS unit (see **Figure 1**). As an alternative to a fundamental NVU model (Jolivet et al., 2015) for the volume of tissue imaged and stimulated with EEG-NIRS/tDCS unit, we tried (Dutta et al., 2015) to find an empirical model where we performed empirical mode decomposition (EMD) and the Hilbert spectrum (Huang et al., 1998) to model the system dynamics and found a negative cross-correlation between one of the intrinsic mode function (IMF) of the HbO_2 time-series and log-transformed mean-power time-course of EEG primarily within 0.5–11.25 Hz frequency band (i.e., one of the EEG IMFs). In principal accordance, for whole-head monitoring, we propose independent component analysis (ICA) to transform multi-channel EEG-NIRS/tDCS unit imaging data to a spatially transformed "virtual channel" (i.e., a spatial filter; Jung et al., 2001). Then, the "virtual channel" activity (e.g., posterior alpha band activity) can be subjected to EMD (i.e., a temporal filter) to reduce the dimension of the observable dynamics (i.e., further observer model reduction) before developing the Kalman filter observer (Schiff and Sauer, 2008) using the IMFs. For EEG-NIRS-based monitoring of NVU under perturbation with tDCS, as shown in the **Figure 2**, the NVU dynamics is captured by the function F and the IMF observations by the function W. The UKF approach should match the nonlinear IMF dynamics up to the second order statistics where

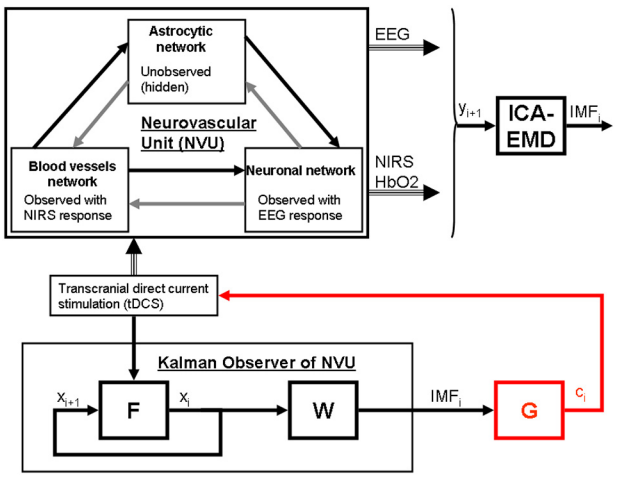

FIGURE 2 | State modulation of the NVU with (tDCS) to facilitate a brain state, e.g., spatiotemporal alpha-rhythm state. F is the function to capture NVU system, W is the function to capture observations, ICA is independent component (IC) analysis is a linear decomposition method to transform EEG—NIRS data collected at single scalp channels to a spatially transformed "virtual channel" (i.e., a spatial filter on multi-channel EEG-NIRS data), empirical mode decomposition (EMD) is empirical model decomposition of the "virtual channel" observations that provide intrinsic mode functions (IMF) for proportional control (gain is G) of individual "virtual channel" activity or IC (e.g., posterior alpha band activity) with tDCS.

the feasibility remains to be tested experimentally in future studies. Furthermore, it may be possible to use the Kalman observer to calculate proportional control (see **Figure 2**) of the brain-state (e.g., cortical excitability; Jindal et al., 2015a) with tDCS. Here, intelligent placement of EEG-NIRS sensors and tDCS effectors is necessary to ensure observability and controllability (Whalen et al., 2015) where Whalen et al. (2015) suggested in general that more direct incoming connections into an observed node lead to higher observability and more direct outgoing connections from a controlled node lead to higher controllability. Furthermore, controllability may be enhanced with multi-modal non-invasive brain stimulation (NIBS), e.g., with direct electrical stimulation (Pulgar, 2015) and photobiostimulation (Gonzalez-Lima and Barrett, 2014), which needs to be investigated.

Towards such brain-state dependent tDCS, the challenges include the nature of observability and controllability in whole-brain complex NVU networks as well as the subtleties of the tDCS interaction with the whole-brain NVU (e.g., based on heterogeneous geometrical characteristics, Molaee-Ardekani et al., 2013) that can also have multi-timescale cross-talk and resulting complex non-linear dynamics (Jolivet et al., 2015) where the spatiotemporal observability and controllability remains to be verified in future studies.

Acknowledgments

Research was conducted within the context of the regional NUMEV funding, Franco-German PHC-PROCOPE 2014

funding, and Franco-Indian INRIA-DST funding. The help and advice received from the German collaborator (Dr. med. Michael A. Nitsche), Indian collaborators (Dr. med. Abhijit Das, Dr. Shubhajit Roy Chowdhury, and Dr. Dipanjan Roy), and the French collaborators (Dr. Mitsuhiro Hayashibe, Dr. Stephane Perrey and Dr. Mark Muthalib) are gratefully acknowledged.

References

Araque, A., Carmignoto, G., and Haydon, P. G. (2001). Dynamic signaling between astrocytes and neurons. *Annu. Rev. Physiol.* 63, 795–813. doi: 10.1146/annurev.physiol.63.1.795

Ardolino, G., Bossi, B., Barbieri, S., and Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *J. Physiol. Lond.* 568, 653–663. doi: 10.1113/jphysiol.2005.088310

Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., Macvicar, B. A., and Newman, E. A. (2010). Glial and neuronal control of brain blood flow. *Nature* 468, 232–243. doi: 10.1038/nature09613

Behzadi, Y., and Liu, T. T. (2005). An arteriolar compliance model of the cerebral blood flow response to neural stimulus. *Neuroimage* 25, 1100–1111. doi: 10.1016/j.neuroimage.2004.12.057

Bozzo, L., Puyal, J., and Chatton, J.-Y. (2013). Lactate Modulates the Activity of Primary Cortical Neurons through a Receptor-Mediated Pathway. *PLoS One* 8:e71721. doi: 10.1371/journal.pone.0071721

Buffo, A., Rite, I., Tripathi, P., Lepier, A., Colak, D., Horn, A.-P., et al. (2008). Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. *Proc. Natl. Acad. Sci. U S A* 105, 3581–3586. doi: 10.1073/pnas.0709002105

Chander, B. S., and Chakravarthy, V. S. (2012). A Computational Model of Neuro-Glio-Vascular Loop Interactions. *PLoS One* 7:e48802. doi: 10.1371/journal.pone.0048802

David, O., and Friston, K. J. (2003). A neural mass model for MEG/EEG: coupling and neuronal dynamics. *Neuroimage* 20, 1743–1755. doi: 10.1016/j.neuroimage.2003.07.015

DiNuzzo, M., Gili, T., Maraviglia, B., and Giove, F. (2011). Modeling the contribution of neuron-astrocyte cross talk to slow blood oxygenation level-dependent signal oscillations. *J. Neurophysiol.* 106, 3010–3018. doi: 10.1152/jn.00416.2011

Dmochowski, J. P., Datta, A., Bikson, M., Su, Y., and Parra, L. C. (2011). Optimized multi-electrode stimulation increases focality and intensity at target. *J. Neural Eng.* 8:046011. doi: 10.1088/1741-2560/8/4/046011

Dutta, A. (2014). "EEG-NIRS based low-cost screening and monitoring of cerebral microvessels functionality," in *International Stroke Conference 2014, At San Diego, Volume: Junior Investigator Session II: Invited Symposium*, San Diego, CA.

Dutta, A., Chowdhury, S. R., Dutta, A., Sylaja, P. N., Guiraud, D., and Nitsche, M. (2013). "A phenomological model for capturing cerebrovascular reactivity to anodal transcranial direct current stimulation," in *6th International IEEE/EMBS Conference on Neural Engineering (NER)*, (San Diego, CA), 827–830.

Dutta, A., Jacob, A., Chowdhury, S. R., Das, A., and Nitsche, M. A. (2015). EEG-NIRS Based Assessment of Neurovascular Coupling During Anodal Transcranial Direct Current Stimulation – a Stroke Case Series. *J. Med. Syst.* 39:205. doi: 10.1007/s10916-015-0205-7

Dutta, A., and Nitsche, M. (2013). "Neural mass model analysis of online modulation of electroencephalogram with transcranial direct current stimulation," in *6th International IEEE/EMBS Conference on Neural Engineering (NER)*, (San Diego, CA), 206–210.

Fricke, K., Seeber, A. A., Thirugnanasambandam, N., Paulus, W., Nitsche, M. A., and Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *J. Neurophysiol.* 105, 1141–1149. doi: 10.1152/jn.00608.2009

Friston, K. J., Mechelli, A., Turner, R., and Price, C. J. (2000). Nonlinear responses in fMRI: the Balloon model, Volterra kernels and other hemodynamics. *NeuroImage* 12, 466–477. doi: 10.1006/nimg.2000.0630

Girouard, H., and Iadecola, C. (2006). Neurovascular coupling in the normal brain and in hypertension, stroke and Alzheimer disease. *J. Appl. Physiol. (1985)* 100, 328–335. doi: 10.1152/japplphysiol.00966.2005

Gonzalez-Lima, F., and Barrett, D. W. (2014). Augmentation of cognitive brain functions with transcranial lasers. *Front. Syst. Neurosci.* 8:36. doi: 10.3389/fnsys.2014.00036

Gruetter, R., Novotny, E. J., Boulware, S. D., Rothman, D. L., and Shulman, R. G. (1996). 1H NMR Studies of Glucose Transport in the Human Brain. *J. Cereb. Blood Flow Metab.* 16, 427–438. doi: 10.1097/00004647-199605000-00009

Halnes, G., Ostby, I., Pettersen, K. H., Omholt, S. W., and Einevoll, G. T. (2013). Electrodiffusive model for astrocytic and neuronal ion concentration dynamics. *PLoS Comput. Biol.* 9:e1003386. doi: 10.1371/journal.pcbi.1003386

Han, C.-H., Song, H., Kang, Y.-G., Kim, B.-M., and Im, C.-H. (2014). Hemodynamic responses in rat brain during transcranial direct current stimulation: a functional near-infrared spectroscopy study. *Biomed. Opt. Express* 5, 1812–1821. doi: 10.1364/BOE.5.001812

Horvath, J. C., Carter, O., and Forte, J. D. (2014). Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Front. Syst. Neurosci.* 8:2. doi: 10.3389/fnsys.2014.00002

Huang, N. E., Shen, Z., Long, S. R., Wu, M. C., Shih, H. H., Zheng, Q., et al. (1998). The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis. *Proc. R. Soc. Lond. Ser. Math. Phys. Eng. Sci.* 454, 903–995. doi: 10.1098/rspa.1998.0193

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in alzheimer's disease. *Nat. Rev. Neurosci.* 5, 347–360. doi: 10.1038/nrn1387

Islam, N., Aftabuddin, M., Moriwaki, A., Hattori, Y., and Hori, Y. (1995). Increase in the calcium level following anodal polarization in the rat brain. *Brain Res.* 684, 206–208. doi: 10.1016/0006-8993(95)00434-r

Jindal, U., Sood, M., Chowdhury, S. R., Das, A., Kondziella, D., and Dutta, A. (2015a). "Corticospinal excitability changes to anodal tDCS elucidated with NIRS-EEG joint-imaging: an ischemic stroke study," in *Conference Proceedings IEEE Engineering in Medicine and Biology Society*, Milan, Italy.

Jindal, U., Sood, M., Dutta, A., and Chowdhury, S. R. (2015b). Development of point of care testing device for neurovascular coupling from simultaneous recording of EEG and NIRS during anodal transcranial direct current stimulation. *IEEE J. Transl. Eng. Health Med.* 3, 1–12. doi: 10.1109/jtehm.2015.2389230

Jolivet, R., Coggan, J. S., Allaman, I., and Magistretti, P. J. (2015). Multi-timescale modeling of activity-dependent metabolic coupling in the neuron-glia-vasculature ensemble. *PLoS Comput. Biol.* 11:e1004036. doi: 10.1371/journal.pcbi.1004036

Jung, T.-P., Makeig, S., McKeown, M. J., Bell, A. J., Lee, T.-W., and Sejnowski, T. J. (2001). Imaging brain dynamics using independent component analysis. *Proc. IEEE Inst. Electr. Electron. Eng.* 89, 1107–1122. doi: 10.1109/5.939827

Khadka, N., Truong, D. Q., and Bikson, M. (2015). Principles of within electrode current steering1. *J. Med. Devices* 9:020947. doi: 10.1115/1.4030126

Krause, B., Márquez-Ruiz, J., and Cohen Kadosh, R. (2013). The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? *Front. Hum. Neurosci.* 7:602. doi: 10.3389/fnhum.2013.00602

Leontiev, O., and Buxton, R. B. (2007). Reproducibility of BOLD, perfusion and CMRO2 measurements with calibrated-BOLD fMRI. *NeuroImage* 35, 175–184. doi: 10.1016/j.neuroimage.2006.10.044

Lucas, S. J. E., Tzeng, Y. C., Galvin, S. D., Thomas, K. N., Ogoh, S., and Ainslie, P. N. (2010). Influence of Changes in Blood Pressure on Cerebral Perfusion and Oxygenation. *Hypertension* 55, 698–705. doi: 10.1161/HYPERTENSIONAHA.109.146290

Mangia, A. L., Pirini, M., and Cappello, A. (2014). Transcranial direct current stimulation and power spectral parameters: a tDCS/EEG co-registration study. *Front. Hum. Neurosci.* 8:601. doi: 10.3389/fnhum.2014.00601

Markus, H., and Cullinane, M. (2001). Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain* 124, 457–467. doi: 10.1093/brain/124.3.457

Matsumoto, J., Fujiwara, T., Takahashi, O., Liu, M., Kimura, A., and Ushiba, J. (2010). Modulation of mu rhythm desynchronization during motor imagery by transcranial direct current stimulation. *J. Neuroeng. Rehabil.* 7:27. doi: 10.1186/1743-0003-7-27

McKendrick, R., Parasuraman, R., and Ayaz, H. (2015). Wearable functional near infrared spectroscopy (fNIRS) and transcranial direct current stimulation (tDCS): expanding vistas for neurocognitive augmentation. *Front. Syst. Neurosci.* 9:27. doi: 10.3389/fnsys.2015.00027

Meinzer, M., Lindenberg, R., Darkow, R., Ulm, L., Copland, D., and Flöel, A. (2014). Transcranial direct current stimulation and simultaneous functional magnetic resonance imaging. *J. Vis. Exp.* e51730. doi: 10.3791/51730

Molaee-Ardekani, B., Márquez-Ruiz, J., Merlet, I., Leal-Campanario, R., Gruart, A., Sánchez-Campusano, R., et al. (2013). Effects of transcranial Direct Current Stimulation (tDCS) on cortical activity: a computational modeling study. *Brain Stimul.* 6, 25–39. doi: 10.1016/j.brs.2011.12.006

Moore, C. I., and Cao, R. (2008). The hemo-neural hypothesis: on the role of blood flow in information processing. *J. Neurophysiol.* 99, 2035–2047. doi: 10.1152/jn.01366.2006

Moran, R. J., Kiebel, S. J., Stephan, K. E., Reilly, R. B., Daunizeau, J., and Friston, K. J. (2007). A neural mass model of spectral responses in electrophysiology. *Neuroimage* 37, 706–720. doi: 10.1016/j.neuroimage.2007.05.032

Nikulin, V. V., Fedele, T., Mehnert, J., Lipp, A., Noack, C., Steinbrink, J., et al. (2014). Monochromatic ultra-slow (∼0.1 Hz) oscillations in the human electroencephalogram and their relation to hemodynamics. *NeuroImage* 97, 71–80. doi: 10.1016/j.neuroimage.2014.04.008

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J. Physiol.* 553, 293–301. doi: 10.1113/jphysiol.2003.049916

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2011). Transcranial direct current stimulation – update 2011. *Restor. Neurol. Neurosci.* 29, 463–492. doi: 10.3233/RNN-2011-0618

Notturno, F., Marzetti, L., Pizzella, V., Uncini, A., and Zappasodi, F. (2014). Local and remote effects of transcranial direct current stimulation on the electrical activity of the motor cortical network. *Hum. Brain Mapp.* 35, 2220–2232. doi: 10.1002/hbm.22322

Novak, V. (2012). Cognition and Hemodynamics. *Curr. Cardiovasc. Risk Rep.* 6, 380–396. doi: 10.1007/s12170-012-0260-2

Nunez, P. L., and Srinivasan, R. (2006). *Electric Fields of the Brain: The Neurophysics of EEG,* 2nd Edn. Oxford: Oxford University Press.

Pellerin, L., and Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. U S A* 91, 10625–10629. doi: 10.1073/pnas.91.22.10625

Polanía, R., Nitsche, M. A., and Paulus, W. (2011). Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. *Hum. Brain Mapp.* 32, 1236–1249. doi: 10.1002/hbm.21104

Polanía, R., Paulus, W., and Nitsche, M. A. (2012). Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Hum. Brain Mapp.* 33, 2499–2508. doi: 10.1002/hbm.21380

Pulgar, V. M. (2015). Direct electric stimulation to increase cerebrovascular function. *Front. Syst. Neurosci.* 9:54. doi: 10.3389/fnsys.2015.00054

Raffin, E., and Siebner, H. R. (2014). Transcranial brain stimulation to promote functional recovery after stroke. *Curr. Opin. Neurol.* 27, 54–60. doi: 10.1097/wco.0000000000000059

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *J. Physiol. Lond.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Schestatsky, P., Morales-Quezada, L., and Fregni, F. (2013). Simultaneous EEG monitoring during transcranial direct current stimulation. *J. Vis. Exp.* e50426. doi: 10.3791/50426

Schiff, S. J., and Sauer, T. (2008). Kalman filter control of a model of spatiotemporal cortical dynamics. *J. Neural Eng.* 5, 1–8. doi: 10.1088/1741-2560/5/1/001

Siesler, H. W., Ozaki, Y., Kawata, S., and Heise, H. M. (2008). *Near-Infrared Spectroscopy: Principles, Instruments, Applications,* 1st Edn. Weinheim: Wiley-VCH.

Sigala, R., Haufe, S., Roy, D., Dinse, H. R., and Ritter, P. (2014). The role of alpha-rhythm states in perceptual learning: insights from experiments and computational models. *Front. Comput. Neurosci.* 8:36. doi: 10.3389/fncom.2014.00036

Sood, M., Jindal, U., Chowdhury, S. R., Das, A., Kondziella, D., and Dutta, A. (2015). "Anterior temporal artery tap to identify systemic interference using short-separation NIRS measurements: a NIRS/EEG-tDCS study," in *Conference Proceedings IEEE Engineering in Medicine and Biology Society*, Milan, Italy.

Sotero, R. C., Trujillo-Barreto, N. J., Iturria-Medina, Y., Carbonell, F., and Jimenez, J. C. (2007). Realistically coupled neural mass models can generate EEG rhythms. *Neural Comput.* 19, 478–512. doi: 10.1162/neco.2007.19.2.478

Spitoni, G. F., Cimmino, R. L., Bozzacchi, C., Pizzamiglio, L., and Di Russo, F. (2013). Modulation of spontaneous alpha brain rhythms using low-intensity transcranial direct-current stimulation. *Front. Hum. Neurosci.* 7:529. doi: 10.3389/fnhum.2013.00529

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

Ursino, M., Cona, F., and Zavaglia, M. (2010). The generation of rhythms within a cortical region: analysis of a neural mass model. *NeuroImage* 52, 1080–1094. doi: 10.1016/j.neuroimage.2009.12.084

Volterra, A., Liaudet, N., and Savtchouk, I. (2014). Astrocyte Ca 2+ signalling: an unexpected complexity. *Nat. Rev. Neurosci.* 15, 327–335. doi: 10.1038/nrn3725

Whalen, A. J., Brennan, S. N., Sauer, T. D., and Schiff, S. J. (2015). Observability and controllability of nonlinear networks: the role of symmetry. *Phys. Rev. X* 5:011005. doi: 10.1103/physrevx.5.011005

Yang, J., Ruchti, E., Petit, J.-M., Jourdain, P., Grenningloh, G., Allaman, I., et al. (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc. Natl. Acad. Sci. U S A* 111, 12228–12233. doi: 10.1073/pnas.1322912111

Zaehle, T., Sandmann, P., Thorne, J. D., Jäncke, L., and Herrmann, C. S. (2011). Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: combined behavioural and electrophysiological evidence. *BMC Neurosci.* 12:2. doi: 10.1186/1471-2202-12-2

Zavaglia, M., Astolfi, L., Babiloni, F., and Ursino, M. (2006). A neural mass model for the simulation of cortical activity estimated from high resolution EEG during cognitive or motor tasks. *J. Neurosci. Methods* 157, 317–329. doi: 10.1016/j.jneumeth.2006.04.022

Zheng, X., Alsop, D. C., and Schlaug, G. (2011). Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. *Neuroimage* 58, 26–33. doi: 10.1016/j.neuroimage.2011.06.018

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 14 March 2014
doi: 10.3389/fnsys.2014.00036

Augmentation of cognitive brain functions with transcranial lasers

F. Gonzalez-Lima* and Douglas W. Barrett

Department of Psychology and Institute for Neuroscience, University of Texas at Austin, Austin, TX, USA
**Correspondence: gonzalezlima@utexas.edu*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Julio C. Rojas, University of Texas Southwestern Medical Center, USA
John Mitrofanis, University of Sydney, Australia

Keywords: cognitive enhancement, cytochrome oxidase, low-level light therapy, brain stimulation, photoneuromodulation

Discovering that transcranial infrared laser stimulation produces beneficial effects on frontal cortex functions such as sustained attention, working memory, and affective state has been groundbreaking. Transcranial laser stimulation with low-power density (mW/cm^2) and high-energy density (J/cm^2) monochromatic light in the near-infrared wavelengths modulates brain functions and may produce neurotherapeutic effects in a nondestructive and non-thermal manner (Lampl, 2007; Hashmi et al., 2010). Barrett and Gonzalez-Lima (2013) provided the first controlled study showing that transcranial laser stimulation improves human cognitive and emotional brain functions. But for the field of low-level light/laser therapy (LLLT), development of a model of how luminous energy from red-to-near-infrared wavelengths modulates bioenergetics began with *in vitro* and *in vivo* discoveries in the last 40 years. Previous LLLT reviews have provided extensive background about historical developments, principles and applications (Rojas and Gonzalez-Lima, 2011, 2013; Chung et al., 2012). The purpose of this paper is to provide an update on LLLT's neurochemical mechanisms supporting transcranial laser stimulation for cognitive-enhancing applications. We will explain first LLLT's action on brain bioenergetics, briefly describe its bioavailability and dose-response, and finish with its beneficial effects on cognitive functions. Although our focus is on prefrontal-related cognitive functions, in principle LLLT should be able to modulate other brain functions. For example, stimulating different brain regions should affect different functions related to sensory and motor systems.

BRAIN BIOENERGETICS

The way that near-infrared lasers and light-emitting diodes (LEDs) interact with brain function is based on bioenergetics, a mechanism that is fundamentally different than that of other brain stimulation methods such as electric and magnetic stimulation. LLLT has been found to modulate the function of neurons in cell cultures, brain function in animals, and cognitive and emotional functions in healthy persons and clinical conditions. Photoneuromodulation involves the absorption of photons by specific molecules in neurons that activate bioenergetic signaling pathways after exposure to red-to-near-infrared light. The 600–1150 nm wavelengths allow better tissue penetration by photons because light is scattered at lower wavelengths and absorbed by water at higher wavelengths (Hamblin and Demidova, 2006). Over 25 years ago, it was found that molecules that absorb LLLT wavelengths are part of the mitochondrial respiratory enzyme cytochrome oxidase in different oxidation states (Karu et al., 2005). Thus, for red-to-near-infrared light, the primary molecular *photoacceptor* of photon energy is cytochrome oxidase (also called cytochrome *c* oxidase or cytochrome *a-a3*) (Pastore et al., 2000).

Therefore, photon energy absorption by cytochrome oxidase is well-established as the primary neurochemical mechanism of action of LLLT in neurons (Wong-Riley et al., 2005). The more the enzymatic activity of cytochrome oxidase increases, the more metabolic energy that is produced via mitochondrial oxidative phosphorylation. LLLT supplies the brain with metabolic energy in a way analogous to the conversion of nutrients into metabolic energy, but with light instead of nutrients providing the source for ATP-based metabolic energy (Mochizuki-Oda et al., 2002). If an effective near-infrared light energy dose is supplied, it stimulates brain ATP production (Lapchak and De Taboada, 2010) and blood flow (Uozumi et al., 2010), thereby fueling ATP-dependent membrane ion pumps, leading to greater membrane stability and resistance to depolarization, which has been shown to transiently reduce neuronal excitability (Konstantinovic et al., 2013). On the other hand, electromagnetic stimulation directly changes the electrical excitability of neurons.

A long-lasting effect is achieved by LLLT's up-regulating the amount of cytochrome oxidase, which enhances neuronal capacity for metabolic energy production that may be used to support cognitive brain functions. In mice and rats, memory has been improved by LLLT (Michalikova et al., 2008; Rojas et al., 2012a) and by methylene blue, a drug that at low doses donates electrons to cytochrome oxidase (Rojas et al., 2012b). Near-infrared light stimulates mitochondrial respiration by donating photons to cytochrome oxidase, because cytochrome oxidase is the main acceptor of photons from red-to-near-infrared light in neurons. By persistently stimulating cytochrome oxidase activity, transcranial LLLT induces post-stimulation up-regulation of the amount of cytochrome

oxidase in brain mitochondria (Rojas et al., 2012a). Therefore, LLLT may lead to the conversion of luminous energy into metabolic energy (during light exposure) and to the up-regulation of the mitochondrial enzymatic machinery to produce more energy (after light exposure).

BIOAVAILABILITY AND HORMETIC DOSE-RESPONSE

The most abundant metalloprotein in nerve tissue is cytochrome oxidase, and its absorption wavelengths are well correlated with its enzymatic activity and ATP production (Wong-Riley et al., 2005). High LLLT bioavailability to the brain *in vivo* has been shown by inducing brain cytochrome oxidase activity transcranially, leading to enhanced extinction memory retention in normal rats (Rojas et al., 2012a) and improved visual discrimination in rats with impaired retinal mitochondrial function (Rojas et al., 2008). Our LLLT studies utilized varied wavelengths (633–1064 nm), daily doses (1–60 J/cm^2), fractionation sessions (1–6), and power densities (2–250 mW/cm^2) that identified effective LLLT parameters for rats and humans.

For example, we tested in rats the effects of different LLLT doses *in vivo* on brain cytochrome oxidase activity, at either 10.9, 21.6, 32.9 J/cm^2, or no LLLT. Treatments were delivered for 20, 40, and 60 min via four 660-nm LED arrays with a power density of 9 mW/cm^2. One day after the LLLT session, brains were extracted, frozen, sectioned, and processed for cytochrome oxidase histochemistry. A 10.9 J/cm^2 dose increased cytochrome oxidase activity by 13.6%. A 21.6 J/cm^2 dose produced a 10.3% increase. A non-significant cytochrome oxidase increase of 3% was found after the highest 32.9 J/cm^{2}dose. Responses of brain cytochrome oxidase to LLLT *in vivo* were characterized by hormesis, with a low dose being stimulatory, while higher doses were less effective.

The first demonstration that LLLT increased oxygen consumption in the rat prefrontal cortex *in vivo* was provided by Rojas et al. (2012a). Oxygen concentration in the cortex of rats was measured using fluorescence-quenching during LLLT at 9 mW/cm^2 and 660 nm. LLLT induced a dose-dependent increase in oxygen consumption of 5% after 1 J/cm^2 and 16% after 5 J/cm^2. Since oxygen is used to form water within mitochondria in a reaction catalyzed by cytochrome oxidase, more cytochrome oxidase activity should lead to more oxygen consumption.

LLLT may offer some advantages over other types of stimulation, because LLLT non-invasively targets cytochrome oxidase, a key enzyme for energy production, with induced expression linked to energy demand. Hence LLLT is mechanistically specific and non-invasive, while transcranial magnetic stimulation may be non-specific, prolonged forehead electrical stimulation may produce muscle spasms, and deep brain or vagus nerve stimulations are invasive.

COGNITIVE AND EMOTIONAL FUNCTIONS

LLLT via commercial low-power sources (such as FDA-cleared laser diodes and LEDs) is a highly promising, affordable, non-pharmacological alternative for improving cognitive function. LLLT delivers safe doses of light energy that are sufficiently high to modulate neuronal functions, but low enough to not result in any damage (Wong-Riley et al., 2005). In 2002, the FDA approved LLLT for pain relief in cases of head and neck pain, arthritis and carpal tunnel syndrome (Fulop et al., 2010). LLLT has been used non-invasively in humans after ischemic stroke to improve neurological outcome (Lampl et al., 2007). It also led to improved recovery and reduced fatigue after exercise (Leal Junior et al., 2010). One LLLT stimulation session to the forehead, as reported by Schiffer et al. (2009), produced a significant antidepressant effect in depressed patients. No adverse side effects were found either immediately or at 2 or 4 weeks after LLLT. Thus, these beneficial LLLT treatments have been found to be safe in humans. Even though LLLT has been regarded as safe and received FDA approval for pain treatment, the use of transcranial lasers for cognitive augmentation should be restricted to research until further controlled studies support this application for clinical use.

We used transcranial laser stimulation to the forehead in a placebo-controlled, randomized study, to influence cognitive tasks related to the prefrontal cortex, including a psychomotor vigilance task (PVT) and a delayed match-to-sample (DMS) memory task (Barrett and Gonzalez-Lima, 2013). The PVT assesses sustained attention, with participants remaining vigilant during delay intervals, and pushing a button when a visual stimulus appears on a monitor. Our laser stimulation targeted prefrontal areas which are implicated in the sustained attentional processes of the PVT (Drummond et al., 2005). Similarly, the DMS task engages the prefrontal cortex as part of a network of frontal and parietal brain regions (Nieder and Miller, 2004).

Healthy volunteers received continuous wave near-infrared light intersecting cytochrome oxidase's absorption spectrum, delivered to the forehead using a 1064 nm low-power laser diode (also known as "cold laser"), which maximizes tissue penetration due to its long wavelength, and has been used in humans for other indications. The power density (or irradiance), 250 mW/cm^2, as well as the cumulative energy density (or fluence), 60 J/cm^2, were the same that showed beneficial psychological effects in Schiffer et al. (2009). This laser exposure produces negligible heat and no physical damage at the low power level used. This laser apparatus is used safely in a clinical setting by the supplier of the laser (Cell Gen Therapeutics, HD Laser Center, Dallas, TX). Reaction time in the PVT was improved by the laser treatment, as shown by a significant pre-post reaction time change relative to the placebo group. The DMS memory task also revealed significant enhancements in measures of memory retrieval latency and number of correct trials, when comparing the LLLT-treated with the placebo group (**Figure 1**). Self-reported positive and negative affective (emotional) states were also measured using the PANAS-X questionnaire before and 2 weeks after laser treatment. As compared to the placebo, treated subjects reported significantly improved affective states. We suggest that this kind of transcranial laser stimulation may serve as a non-invasive and efficacious method to augment cognitive brain functions related to attention, memory, and emotional functions.

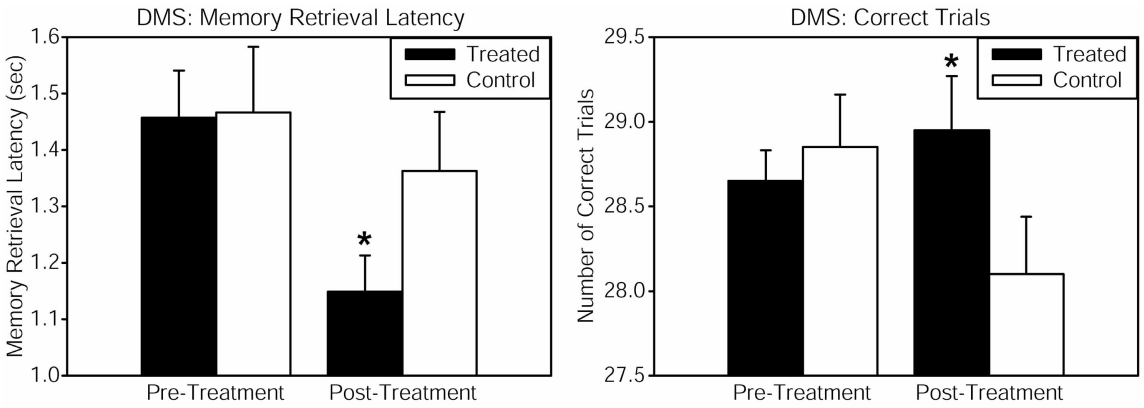

FIGURE 1 | Cognitive performance in the delayed match-to-sample (DMS) memory task was improved after transcranial infrared stimulation to the right forehead. The DMS task involves presentation of a visual stimulus (grid pattern) on a screen. Then the stimulus disappears, and the participant must remember the stimulus through a delay. Then two choices appear, and the participant must decide which of these two is identical to the previous stimulus (the "match"). Treated subjects showed faster memory retrieval (left panel) and increased number of correct trials (right panel) out of 30 trials when attempting to choose the correct grid pattern. The function of frontal cortex regions, implicated in the attentional mode network utilized during this visuospatial memory task, was augmented by the laser treatment. Compared to baseline, this treatment also increased by 5% the oxyhemoglobin concentration of the prefrontal cortex as measured by near-infrared spectroscopy, both during the laser stimulation and during post-treatment DMS performance (in preparation). The data for the treated group consisted of $n = 10$ males and $n = 10$ females; the control group also consisted of $n = 10$ males and $n = 10$ females. *Significant treatment by pre-post score interaction, $p < 0.05$.

LLLT's bioenergetics mechanisms leading to cognitive augmentation may also be at play in its neuroprotective effects (Gonzalez-Lima et al., 2013). LLLT's stimulation of mitochondrial respiration should improve cellular function due to increased metabolic energy, as well as cellular survival after injury, due to the antioxidant effects of increases in cytochrome oxidase and superoxide dismutase (Rojas et al., 2008).

Laser transmittance of the 1064-nm wavelength at the forehead LLLT site was estimated in a post-mortem human specimen, which showed that approximately 2% of the light passed through the frontal bone. This yielded an absorption coefficient of $a = 0.24$, similar to the reported $a = 0.22$ transmittance through cranial bone for this wavelength (Bashkatov and Genina, 2006). Thus, we estimated that about 1.2 J/cm^2 of the 60 J/cm^2 LLLT dose applied reached the surface of the prefrontal cortex. This value is similar to 1 J/cm^2, the peak effective LLLT dose in neuron cultures for increasing cytochrome oxidase activity (Rojas and Gonzalez-Lima, 2011).

CONCLUSIONS

Transcranial absorption of photon energy by cytochrome oxidase, the terminal enzyme in mitochondrial respiration, is proposed as the bioenergetic mechanism of action of LLLT in the brain. Transcranial LLLT up-regulates cortical cytochrome oxidase and enhances oxidative phosphorylation. LLLT improves prefrontal cortex-related cognitive functions, such as sustained attention, extinction memory, working memory, and affective state. Transcranial infrared stimulation may be used efficaciously to support neuronal mitochondrial respiration as a new non-invasive, cognition-improving intervention in animals and humans. This fascinating new approach should also be able to influence other brain functions depending on the neuroanatomical site stimulated and the stimulation parameters used.

REFERENCES

Barrett, D. W., and Gonzalez-Lima, F. (2013). Transcranial infrared laser stimulation produces beneficial cognitive and emotional effects in humans. *Neuroscience* 230, 13–23. doi: 10.1016/j.neuroscience.2012.11.016

Bashkatov, A. N., and Genina, E. A. (2006). Optical properties of human cranial bone in the spectral range from 800 to 2000 nm. *Proc. SPIE* 6163, 616310. doi: 10.1117/12.697305

Chung, H., Dai, T., Sharma, S. K., Huang, Y. Y., Carroll, J. D., and Hamblin, M. R. (2012). The nuts and bolts of low-level laser (light) therapy. *Ann. Biomed. Eng.* 40, 516–533. doi: 10.1007/s10439-011-0454-7

Drummond, S. P., Bischoff-Grethe, A., Dinges, D. F., Ayalon, L., Mednick, S. C., and Meloy, M. J. (2005). The neural basis of the psychomotor vigilance task. *Sleep* 28, 1059–1068.

Fulop, A. M., Dhimmer, S., Deluca, J. R., Johanson, D. D., Lenz, R. V., Patel, K. B., et al. (2010). A meta-analysis of the efficacy of laser phototherapy on pain relief. *Clin. J. Pain* 26, 729–736. doi: 10.1097/AJP.0b013e3181f09713

Gonzalez-Lima, F., Barksdale, B. R., and Rojas J. C. (2013). Mitochondrial respiration as a target for neuroprotection and cognitive enhancement. *Biochem. Pharmacol.* doi: 10.1016/j.bcp.2013.11.010. [Epub ahead of print].

Hamblin, M. R., and Demidova, T. N. (2006). Mechanisms of low level light therapy. *Proc. SPIE* 6140, 1–12. doi: 10.1117/12.646294

Hashmi, J. T., Huang, Y. Y., Osmani, B. Z., Sharma, S. K., Naeser, M. A., and Hamblin, M. R. (2010). Role of low-level laser therapy in neurorehabilitation. *PM R* 2, S292–S305. doi: 10.1016/j.pmrj.2010.10.013

Karu, T. I., Pyatibrat, L. V., Kolyakov, S. F., and Afanasyeva, N. I. (2005). Absorption measurements of a cell monolayer relevant to phototherapy: reduction of cytochrome c oxidase under near IR radiation. *J. Photochem. Photobiol. B* 81, 98–106. doi: 10.1016/j.jphotobiol.2005.07.002

Konstantinovic, L. M., Jelic, M. B., Jeremic, A., Stevanovic, V. B., Milanovic, S. D., and Filipovic, S. R. (2013). Transcranial application of near-infrared low-level laser can modulate cortical excitability. *Lasers Surg. Med.* 45, 648–653. doi: 10.1002/lsm.22190

Lampl, Y. (2007). Laser treatment for stroke. *Expert Rev. Neurother.* 7, 961–965. doi: 10.1586/14737175.7.8.961

Lampl, Y., Zivin, J. A., Fisher, M., Lew, R., Welin, L., Dahlof, B., et al. (2007). Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the neurothera effectiveness and

safety trial-1 (NEST-1). *Stroke* 38, 1843–1849. doi: 10.1161/STROKEAHA.106.478230

Lapchak, P. A., and De Taboada, L. (2010). Transcranial near infrared laser treatment (NILT) increases cortical adenosine-5'-triphosphate (ATP) content following embolic strokes in rabbits. *Brain Res.* 1306, 100–105. doi: 10.1016/j.brainres.2009.10.022

Leal Junior, E. C., Lopes-Martins, R. A., Frigo, L., De Marchi, T., Rossi, R. P., de Godoi, V., et al. (2010). Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. *J. Orthop. Sports Phys. Ther.* 40, 524–532. doi: 10.2519/jospt.2010.3294

Michalikova, S., Ennaceur, A., van Rensburg, R., and Chazot, P. L. (2008). Emotional responses and memory performance of middle-aged CD1 mice in a 3D maze: effects of low infrared light. *Neurobiol. Learn. Mem.* 89, 480–488. doi: 10.1016/j.nlm.2007.07.014

Mochizuki-Oda, N., Kataoka, Y., Cui, Y., Yamada, H., Heya, M., and Awazu, K. (2002). Effects of near-infra-red laser irradiation on adenosine triphosphate and adenosine diphosphate contents of rat brain tissue. *Neurosci. Lett.* 323, 207–210. doi: 10.1016/S0304-3940(02)00159-3

Nieder, A., and Miller, E. K. (2004). A parieto-frontal network for visual numerical information in the monkey. *Proc. Natl. Acad. Sci. U.S.A.* 101, 7457–7462. doi: 10.1073/pnas.0402239101

Pastore, D., Greco, M., and Passarella, S. (2000). Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *Int. J. Radiat. Biol.* 76, 863–870. doi: 10.1080/09553000050029020

Rojas, J. C., and Gonzalez-Lima, F. (2011). Low-level light therapy of the eye and brain. *Eye Brain* 3, 49–67. doi: 10.2147/EB.S21391

Rojas, J. C., Bruchey, A. K., and Gonzalez-Lima, F. (2012a). Low-level light therapy improves cortical metabolic capacity and memory retention. *J. Alzheimers Dis.* 32, 741–752. doi: 10.3233/JAD-2012-120817

Rojas, J. C., Bruchey, A. K., and Gonzalez-Lima, F. (2012b). Neurometabolic mechanisms for memory enhancement and neuroprotection of methylene blue. *Prog. Neurobiol.* 96, 32–45. doi: 10.1016/j.pneurobio.2011.10.007

Rojas, J. C., and Gonzalez-Lima, F. (2013). Neurological and psychological applications of transcranial lasers and LEDs. *Biochem. Pharmacol.* 86, 447–457. doi: 10.1016/j.bcp.2013.06.012

Rojas, J. C., Lee, J., John, J. M., and Gonzalez-Lima, F. (2008). Neuroprotective effects of near-infrared light in an *in vivo* model of mitochondrial optic neuropathy. *J. Neurosci.* 28,13511–13521. doi: 10.1523/JNEUROSCI.3457-08.2008

Schiffer, F., Johnston, A. L., Ravichandran, C., Polcari, A., Teicher, M. H., Webb, R. H., et al. (2009). Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behav. Brain Funct.* 5, 46. doi: 10.1186/1744-9081-5-46

Uozumi, Y., Nawashiro, H., Sato, S., Kawauchi, S., Shima, K., and Kikuchi, M. (2010).Targeted increase in cerebral blood flow by transcranial near-infrared laser irradiation. *Lasers Surg. Med.* 42, 566–576. doi: 10.1002/lsm.20938

Wong-Riley, M. T., Liang, H. L., Eells, J. T., Chance, B., Henry, M. M., Buchmann, E., et al. (2005). Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. *J. Biol. Chem.* 280, 4761–4771. doi: 10.1074/jbc.M409650200

Received: 31 January 2014; accepted: 27 February 2014; published online: 14 March 2014.

Citation: Gonzalez-Lima F and Barrett DW (2014) Augmentation of cognitive brain functions with transcranial lasers. Front. Syst. Neurosci. ***8****:36. doi: 10.3389/fnsys.2014.00036*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 29 April 2014
doi: 10.3389/fnsys.2014.00071

Thinking caps for everyone? The role of neuro-enhancement by non-invasive brain stimulation in neuroscience and beyond

Felix Duecker[1,2]*, Tom A. de Graaf[1,2] and Alexander T. Sack[1,2]

[1] *Department of Cognitive Neuroscience, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, Netherlands*
[2] *Maastricht Brain Imaging Center, Maastricht University, Maastricht, Netherlands*

Edited by:
Manuel Casanova, University of Louisville, USA

Reviewed by:
Casto Rivadulla, Universty of Coruna, Spain
Victor De Lafuente, Universidad Nacional Autónoma de México, Mexico

***Correspondence:**
Felix Duecker, Department of Cognitive Neuroscience, Faculty of Psychology and Neuroscience, Maastricht University, P.O. Box 616, 6200 MD, Maastricht, Netherlands
e-mail: felix.duecker@maastrichtuniversity.nl

Neuro-enhancement by non-invasive brain stimulation (NIBS) has recently made considerable progress, triggering discussions regarding future applications to enhance human performance. We show that neuroscientific research does not aim at improving brain functions *per se*. Instead, neuro-enhancement is a research tool that has great potential to reveal the neural mechanisms underlying perception, cognition, and behavior. We provide instructive examples that showcase the relevance of neuro-enhancement by NIBS in neuroscience. Importantly, we argue that the scientific value of neuro-enhancement critically depends on our understanding of why enhancing effects occur. This is in contrast to applications of neuro-enhancement in other domains, where such knowledge may not be required. We conclude that neuro-enhancement as a therapeutic tool or in healthy people outside of neuroscience should be kept conceptually distinct, as these are separate domains with entirely different motives for enhancing human performance. Consequently, the underlying principles that justify the application of NIBS will be different in each domain and arguments for or against neuro-enhancement in one domain do not necessarily generalize to other domains.

Keywords: neuro-enhancement, mechanisms of enhancement, non-invasive brain stimulation, transcranial magnetic stimulation, transcranial current stimulation

The suggestion to "put on your thinking cap" is generally seen as purely metaphorical. Recent advances of non-invasive brain stimulation (NIBS) techniques have created the prospect of real "thinking caps" that might have the potential to improve perception, cognition, and behavior. Outside the scientific community, these developments have been often interpreted as reflecting the ambition to enhance human performance *per se*, leading to heated debates on applicability, desirability and morality of neuro-enhancement. And indeed, people should carefully consider whether, how, and when the application of NIBS to enhance brain function is appropriate (Farah et al., 2004; Cohen Kadosh et al., 2012). We here argue that in neuroscientific research, the enhancement of brain function serves as a means to an end, that is, it aims at gaining insights into brain function. The scientific value of neuro-enhancement therefore critically depends not on the fact *that* enhancing effects occur, but on our understanding of *why* they occur. This is in contrast to applications of neuro-enhancement in other (non-academic) domains where such knowledge may be not required. In this article, we first explain the relevance of neuro-enhancement by NIBS for neuroscience and showcase how it has produced valuable insights into how the brain works. Then, we outline the different domains that neuro-enhancement by NIBS could be considered for. Rather than making any judgments on the moral or ethical justifications of neuro-enhancement here, we argue that the debate on such matters should be held separately for each of these domains. From this follows that arguments against neuro-enhancement in one domain do not necessarily apply to other domains.

EFFECTS OF NIBS

The growing popularity of NIBS is due to the fact that induced brain changes have been shown to be perceptually, cognitively, and behaviorally relevant. In other words, NIBS can affect everything from low-level vision (Amassian et al., 1989) to attention (Duecker et al., 2013) to social-economic behavior (Knoch et al., 2006). The most common NIBS techniques are transcranial magnetic stimulation (TMS), transcranial current stimulation (tCS) with either direct (tDCS) or alternating (tACS) currents, and transcranial static magnetic stimulation (tSMS). TMS involves the administration of magnetic pulses to localized brain areas. The effects of single pulse TMS are short-lasting and can affect ongoing neuronal processes whereas rhythmic pulse sequences can yield long-lasting effects on the human brain (see e.g., Hallett, 2007 for a TMS primer). In contrast, tCS is applied over larger areas of cortex to send an electrical current through brain matter (see e.g., Paulus, 2011a for a tCS primer). Finally, tSMS exposes the brain to a static magnetic field by positioning a magnet on the head (Oliviero et al., 2011; Paulus, 2011b). Simply speaking, these

techniques produce a combination of excitatory and inhibitory effects at the neuronal level. The polarization of neurons is changed and, depending on the stimulation parameters, regional cortical excitability either increases or decreases. Many different stimulation protocols have been developed over the years and it is common practice to label protocols as either inhibitory or excitatory. It is very tempting to directly relate these effects on cortical excitability to changes in brain function such that excitatory protocols necessarily lead to neuro-enhancement. However, this would be an oversimplification. Whether a particular NIBS protocol will have enhancing or impairing effects on the perceptual, cognitive, or behavioral level will depend not only on excitability changes but also on the functional properties and underlying mechanisms of all brain areas involved, as well as the interactions between them. This is exactly why both neuro-disruption and neuro-enhancement effects are scientifically valuable; because in the proper theoretical framework they allow us to begin teasing apart this functional neuronal architecture. Due to this complexity, we will here not provide an overview of all available NIBS protocols but will instead present mechanisms of neuro-enhancement with instructive examples mostly drawn from the attention literature.

ACCIDENTAL NEURO-ENHANCEMENT

Interestingly, NIBS was initially rarely conceptualized as a neuro-enhancing method but was instead applied with the aim to disrupt brain activity. The idea was to reveal a causal structure-function relationship through a NIBS-induced behavioral deficit, indicating the functional necessity of the stimulated brain area for normal task performance (Sack, 2006). Enhancing effects were very uncommon and their occurrence was rather incidental than purposefully induced. For example, Walsh et al. (1998) showed that TMS over hMT+/V5 lead to perceptual enhancements as reflected by improved performance on a visual search task when motion was either absent or task-irrelevant. Similarly, improvements in cognitive performance have been found in the context of picture naming (Mottaghy et al., 1999) and other language-related processes (Epstein, 1998). As most authors acknowledged, interpretation of these results was difficult because a theoretical framework to explain such findings was often lacking.

PURPOSEFUL NEURO-ENHANCEMENT

Once the potential to enhance brain function by NIBS was recognized, the effects of many different NIBS protocols were explored and, unsurprisingly, excitatory protocols often led to improved performance (Coffman et al., 2014; Luber and Lisanby, 2014). This research line has clearly shown that NIBS is capable of improving various brain functions including perception, attention, memory, and even acquisition of skills that are highly relevant for everyday life such as numerical abilities (Cohen Kadosh et al., 2013) and arithmetic (Snowball et al., 2013). In some sense, this is very similar to the original approach of revealing causal structure-function relationships outlined above. After all, also enhancing effects do imply a functional role of the stimulated brain area in a particular process. However, when the underlying mechanisms that cause an enhancement remain unknown, enhancement results, as exciting as they may be, can be of limited scientific value when not followed-up by further investigations. As will be argued below, NIBS primarily delivers its full potential when embedded in scientific theory. It then produces strong direct evidence regarding the mechanisms underlying brain functions.

INTER-HEMISPHERIC COMPETITION REVEALED WITH NIBS

The application of NIBS has been particularly successful in the context of attention research. Neuroimaging studies had already produced detailed knowledge regarding the brain networks involved in attentional control (Corbetta and Shulman, 2002) but struggled to differentiate between several competing models that were based on lesion studies in neglect patients (Kinsbourne, 1977; Heilman and Abell, 1980; Mesulam, 1981). Because these models were originally developed to explain attentional deficits after brain damage, it was relatively easy to derive hypotheses regarding the consequences of NIBS on attention in healthy people. Interestingly, one of these models also predicted enhancing effects of NIBS, namely Kinsbourne's (1977) "opponent processor" model. It stated that each hemisphere has a natural attention bias to the contralateral side of space. Under normal conditions, the two hemispheres are kept in balance due to inter-hemispheric competition. Whenever an imbalance between the hemispheres occurs, attention will be biased towards one side of space. In the context of neuro-enhancement, there are two important aspects to this model. First, in a situation of inter-hemispheric competition, behavioral enhancement and impairment are predicted to be two sides of the same coin. When the overall attention bias is directed towards one hemifield, processing for stimuli on that side of space will be improved at the expense of impaired processing for stimuli on the other side of space. Specifically, any change in excitability of one hemisphere will always affect its inhibitory influence on the other hemisphere as well, so that the final imbalance between the hemispheres is determined by the interaction between them. Second, such an imbalance can be induced in different ways, either by facilitating or inhibiting one hemisphere. As already explained above, this local excitability change then also affects the other hemisphere where the opposite effect occurs. Thus, this model lends itself perfectly for being tested with NIBS. Hilgetag et al. (2001) were among the first to directly test Kinsbourne's "opponent processor" model. They applied 1 Hz repetitive TMS over right or left parietal cortex and assessed performance on a target detection task. As one might expect with a protocol that decreases cortical excitability, target detection was impaired in the contralateral hemifield. In addition, however, they also observed enhanced target detection in the ipsilateral hemifield, strongly supporting the notion of inter-hemispheric competition. Importantly, these results make perfect sense in the light of Kinsbourne's "opponent processor" model but would be puzzling and less informative if this model did not exist. In other words, in and of itself the enhancement of ipsilateral processing was an interesting trivia. But in the appropriate theoretical framework, the enhancement result became neuro-scientifically valuable. Similarly, and corroborating this finding, Dambeck et al. (2006) found a contralateral impairment of target detection with single-pulse TMS over right or left parietal cortex. Strikingly, when applying TMS over both hemispheres simultaneously, the behavioral effects of unilateral TMS

disappeared and performance was back to normal because the balance between hemispheres remained unchanged. Again, this seemingly paradoxical result is turned into meaningful insights into the mechanisms underlying attention control when linked to the appropriate theoretical framework. As pointed out before, the effects of NIBS are not simply determined by the stimulation protocol but are also a consequence of the functional architecture of the brain. In this sense, these findings are extremely valuable as they can be directly related to competing models of attentional control.

ENTRAINMENT AND PHASE-COUPLING

Thus far, NIBS-induced changes of cortical excitability have been conceptualized as rather static effects. However, it is well-established that rhythmic patterns of neural activity are an essential aspect of information processing in the brain. In the context of attention, the power and phase of alpha-band activity in occipito-parietal regions has repeatedly been related to attentional/perceptual performance (Jensen and Mazaheri, 2010; Klimesch, 2012). Alpha power is negatively correlated with perceptual performance (van Dijk et al., 2008) and lateralized when shifting attention to one hemifield (Händel et al., 2011). It is possible to "entrain" alpha-band activity in the brain using rhythmic sensory stimulation (e.g., de Graaf et al., 2013), but also directly and locally, using non-invasive brain stimulation (e.g., Romei et al., 2010; Thut et al., 2011), in order to investigate frequency-dependent modulations of task performance. Romei et al. (2010) applied a short burst of rhythmic TMS at alpha frequency, or flanker frequencies (theta or beta), prior to stimulus presentation. Only for alpha-frequency stimulation, target visibility in the contralateral visual field was reduced, while it was enhanced in the ipsilateral hemifield. So as above, impairment and enhancement co-occurred due to inter-hemispheric competition, but this time in a frequency-dependent way. Recent studies have pushed even further, demonstrating that not only the frequency of oscillations but also their phase can be essential for neural processing. Polanía et al. (2012) used tACS with electrode patches on frontal and parietal cortex both connected to a third reference electrode on the vertex. Previously acquired EEG results revealed a 0-degree phase lag in synchronized activity in the theta band between frontal and parietal cortex during a working memory task. When frontal and parietal regions were stimulated with an oscillating current pattern at a similar frequency, the phase lag between the frontal and parietal stimulation determined working memory performance. This highly advanced NIBS protocol yielded a fairly simple finding: stimulation of the fronto-parietal network in sync enhanced working memory performance whereas out of sync stimulation impaired working memory performance. This has deep and intriguing implications for our understanding of brain function and, together with the other examples described above, demonstrates how far NIBS has come as a research tool. It can produce neuro-enhancing effects, but its scientific power lies in revealing the neural mechanisms underlying perception, cognition, and behavior. And the growing complexity of NIBS approaches enables increasingly meaningful results, increasingly strong conclusions, and increasingly specific hypotheses about functional brain architecture.

NEURO-ENHANCEMENT BEYOND NEUROSCIENCE

The overview we presented in this article has focused on neuro-enhancement as a research tool, mainly taking brain mechanisms underlying attention as an example, and illustrated how it can produce valuable insights into the neural mechanisms underlying human behavior and cognition. We outlined the various forms neuro-enhancement can take, the various experimental settings underlying them, and the many valuable neuroscientific insights one could gleam from it. Importantly, in the neuroscientific domain neuro-enhancement by NIBS mainly serves its purpose when embedded in theoretical models of the brain. Enhancing effects that lack any explanation are of very limited scientific value and require further attempts to unravel the underlying mechanisms.

Beyond neuroscience, however, the application of NIBS for neuro-enhancement is not necessarily motivated by its scientific value. Instead, enhancing perception, cognition, and behavior could, for some, be considered a goal in itself *irrespective of the underlying mechanisms* that produce such effects. That would be neuro-enhancement as an endgoal, rather than as a means to an end. We therefore suggest that the current debate concerning application of neuro-enhancement should be distinguished for different domains. Specifically, we propose three domains that should be kept separate, at least to some extent, namely neuro-enhancement (a) as a research tool; (b) as a therapeutic tool and (c) applied in healthy people outside of neuroscience.

Note that we certainly do not argue for a "hands-off" approach of scientists to the larger debate on desirability of neuro-enhancement in general. After all, it is undeniable that clinical or non-academic applications of neuro-enhancement stem directly from the efforts in the academic domain. If the science doesn't first develop the tool, there is no tool to be applied outside the scientific setting. What we argue for instead is to have the debate in all domains, but to keep in mind clearly which domain we are discussing. We should keep an open mind to the possibility that the ethical, moral, and practical conclusions that may flow from the larger neuro-enhancement debate will be different for each of the three domains. At present, some neuro-enhancing approaches of NIBS have the potential to be applied as a therapeutic tool in patients, and results so far are promising (Hummel and Cohen, 2006; Miniussi et al., 2008). Still, most neuro-enhancing effects of NIBS appear to be of very limited practical relevance in everyday life. But as the field progresses the possible applications of NIBS will increase. People may have serious concerns about such possible future application of NIBS to healthy human brains in schools, universities, or the workplace. A debate would ensue whether or not society should desire, or even allow, such practices. Should companies be allowed to have their employees wear "thinking caps" to boost performance? Would they even be allowed to demand it from their workers? These are relevant questions that should be discussed by laypeople, government, and scientists. They are extreme examples in a sense, but they allow us to highlight the key point here, which is that the different domains where neuro-enhancement is now or in the future applicable should be considered separately in discussions about neuro-enhancement, its value, its risks, its desirability, its development and its general pursuit. Neuroscientists should participate in this discussion,

contributing their expertise. But laypeople should participate as well, since law- and policy-makers need to develop rules and regulations on the basis of both expert opinion and societal support. But such rules and regulations should, in our view, be specific to the different domains. If the debate takes shape according to these lines, we believe that neuro-enhancement can continue to be of great value for our understanding of the brain, of potential use in clinical and therapeutic environments, and perhaps in the future applied responsibly in non-academic settings.

ACKNOWLEDGMENTS

This work was supported by the European Research Council under the European Union's Seventh Framework Programme (FP7/2007–2013)/ERC Grant agreement (263472, awarded to Alexander T. Sack), and the Netherlands Organization for Scientific Research (451-13-024 to Tom A. de Graaf).

REFERENCES

Amassian, V. E., Cracco, R. Q., Maccabee, P. J., Cracco, J. B., Rudell, A., and Eberle, L. (1989). Suppression of visual perception by magnetic coil stimulation of human occipital cortex. *Electroencephalogr. Clin. Neurophysiol.* 74, 458–462. doi: 10.1016/0168-5597(89)90036-1

Coffman, B. A., Clark, V. P., and Parasuraman, R. (2014). Battery powered thought: enhancement of attention, learning, and memory in healthy adults using transcranial direct current stimulation. *Neuroimage* 85(Pt. 3), 895–908. doi: 10.1016/j.neuroimage.2013.07.083

Cohen Kadosh, R., Dowker, A., Heine, A., Kaufmann, L., and Kucian, K. (2013). Interventions for improving numerical abilities: present and future. *Trends Neurosci. Educ.* 2, 85–93. doi: 10.1016/j.tine.2013.04.001

Corbetta, M., and Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* 3, 201–215. doi: 10.1038/nrn755

Dambeck, N., Sparing, R., Meister, I. G., Wienemann, M., Weidemann, J., Topper, R., et al. (2006). Interhemispheric imbalance during visuospatial attention investigated by unilateral and bilateral TMS over human parietal cortices. *Brain Res.* 1072, 194–199. doi: 10.1016/j.brainres.2005.05.075

de Graaf, T. A., Gross, J., Paterson, G., Rusch, T., Sack, A. T., and Thut, G. (2013). Alpha-band rhythms in visual task performance: phase-locking by rhythmic sensory stimulation. *PLoS ONE* 8, e60035. doi: 10.1371/journal.pone.0060035

Duecker, F., Formisano, E., and Sack, A. T. (2013). Hemispheric differences in the voluntary control of spatial attention: direct evidence for a right-hemispheric dominance within frontal cortex. *J. Cogn. Neurosci.* 25, 1332–1342. doi: 10.1162/jocn_a_00402

Epstein, C. M. (1998). Transcranial magnetic stimulation: language function. *J. Clin. Neurophysiol.* 15, 325–332. doi: 10.1097/00004691-199807000-00004

Farah, M. J., Illes, J., Cook-Deegan, R., Gardner, H., Kandel, E., King, P., et al. (2004). Neurocognitive enhancement: what can we do and what should we do? *Nat. Rev. Neurosci.* 5, 421–425. doi: 10.1038/nrn1390

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* 55, 187–199. doi: 10.1016/j.neuron.2007.06.026

Händel, B. F., Haarmeier, T., and Jensen, O. (2011). Alpha oscillations correlate with the successful inhibition of unattended stimuli. *J. Cogn. Neurosci.* 23, 2494–2502. doi: 10.1162/jocn.2010.21557

Heilman, K. M., and Abell, T. V. D. (1980). Right hemisphere dominance for attention. *Neurology* 30, 327–330. doi: 10.1212/wnl.30.3.327

Hilgetag, C. C., Theoret, H., and Pascual-Leone, A. (2001). Enhanced visual spatial attention ipsilateral to rTMS-induced "virtual lesions" of human parietal cortex. *Nat. Neurosci.* 4, 953–957. doi: 10.1038/nn0901-953

Hummel, F. C., and Cohen, L. G. (2006). Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol.* 5, 708–712. doi: 10.1016/s1474-4422(06)70525-7

Jensen, O., and Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Front. Hum. Neurosci.* 4:186. doi: 10.3389/fnhum.2010.00186

Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, R108–R111. doi: 10.1016/j.cub.2012.01.013

Kinsbourne, M. (1977). Hemi-neglect and hemisphere rivalry. *Adv. Neurol.* 18, 41–49.

Klimesch, W. (2012). Alpha-band oscillations, attention, and controlled access to stored information. *Trends Cogn. Sci.* 16, 606–617. doi: 10.1016/j.tics.2012.10.007

Knoch, D., Pascual-Leone, A., Meyer, K., Treyer, V., and Fehr, E. (2006). Diminishing reciprocal fairness by disrupting the right prefrontal cortex. *Science* 314, 829–832. doi: 10.1126/science.1129156

Luber, B., and Lisanby, S. H. (2014). Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* 85(Pt. 3), 961–970. doi: 10.1016/j.neuroimage.2013.06.007

Mesulam, M. M. (1981). A cortical network for directed attention and unilateral neglect. *Ann. Neurol.* 10, 309–325. doi: 10.1002/ana.410100402

Miniussi, C., Cappa, S. F., Cohen, L. G., Floel, A., Fregni, F., Nitsche, M. A., et al. (2008). Efficacy of repetitive transcranial magnetic stimulation/transcranial direct current stimulation in cognitive neurorehabilitation. *Brain Stimul.* 1, 326–336. doi: 10.1016/j.brs.2008.07.002

Mottaghy, F. M., Hungs, M., Brugmann, M., Sparing, R., Boroojerdi, B., Foltys, H., et al. (1999). Facilitation of picture naming after repetitive transcranial magnetic stimulation. *Neurology* 53, 1806–1812. doi: 10.1212/wnl.53.8.1806

Oliviero, A., Mordillo-Mateos, L., Arias, P., Panyavin, I., Foffani, G., and Aguilar, J. (2011). Transcranial static magnetic field stimulation of the human motor cortex. *J. Physiol.* 589, 4949–4958. doi: 10.1113/jphysiol.2011.211953

Paulus, W. (2011a). Transcranial electrical stimulation (tES – tDCS; tRNS, tACS) methods. *Neuropsychol. Rehabil.* 21, 602–617. doi: 10.1080/09602011.2011.557292

Paulus, W. (2011b). Transcranial static magnetic field stimulation in man: making things as simple as possible? *J. Physiol.* 589, 5917–5918. doi: 10.1113/jphysiol.2011.221655

Polanía, R., Nitsche, M. A., Korman, C., Batsikadze, G., and Paulus, W. (2012). The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr. Biol.* 22, 1314–1318. doi: 10.1016/j.cub.2012.05.021

Romei, V., Gross, J., and Thut, G. (2010). On the role of prestimulus alpha rhythms over occipito-parietal areas in visual input regulation: correlation or causation? *J. Neurosci.* 30, 8692–8697. doi: 10.1523/jneurosci.0160-10.2010

Sack, A. T. (2006). Transcranial magnetic stimulation, causal structure-function mapping and networks of functional relevance. *Curr. Opin. Neurobiol.* 16, 593–599. doi: 10.1016/j.conb.2006.06.016

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L., et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Thut, G., Veniero, D., Romei, V., Miniussi, C., Schyns, P., and Gross, J. (2011). Rhythmic TMS causes local entrainment of natural oscillatory signatures. *Curr. Biol.* 21, 1176–1185. doi: 10.1016/j.cub.2011.05.049

van Dijk, H., Schoffelen, J.-M., Oostenveld, R., and Jensen, O. (2008). Prestimulus oscillatory activity in the alpha band predicts visual discrimination ability. *J. Neurosci.* 28, 1816–1823. doi: 10.1523/jneurosci.1853-07.2008

Walsh, V., Ellison, A., Battelli, L., and Cowey, A. (1998). Task-specific impairments and enhancements induced by magnetic stimulation of human visual area V5. *Proc. Biol. Sci.* 265, 537–543. doi: 10.1098/rspb.1998.0328

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 February 2014; accepted: 10 April 2014; published online: 29 April 2014.
Citation: Duecker F, de Graaf TA and Sack AT (2014) Thinking caps for everyone? The role of neuro-enhancement by non-invasive brain stimulation in neuroscience and beyond. Front. Syst. Neurosci. ***8****:71. doi:10.3389/fnsys.2014.00071*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 08 July 2014
doi: 10.3389/fnsys.2014.00127

Neuroenhancement by noninvasive brain stimulation is not a net zero-sum proposition

Bruce Luber*

Division of Brain Stimulation, Departments of Psychiatry and Behavioral Sciences and Psychology and Neuroscience, Duke University, Durham, NC, USA
**Correspondence: bruce.luber@duke.edu*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Nick J. Davis, Swansea University, UK

Keywords: TMS, zero-sum, cognitive enhancement, noninvasive brain stimulation, plasticity

Overview: Over the last decade, it has become increasingly clear that cognitive enhancement with noninvasive brain stimulation (NBS) is a real phenomenon. Recently, it has been suggested that such enhancements be viewed within the framework of a zero-sum game: that the performance enhancements found with NBS represent a re-allotment of finite processing resources, with the gains in one situation balanced by costs elsewhere. In examining the NBS literature, we have found that about half of reports of NBS enhancements may have been the result of resource reallocation, although it is not clear that a cost can be identified in each situation. Moreover, the other half of reports suggest that NBS can cause not a resource reallocation but an actual addition of resources available. It is suggested here that while it is important to examine whether costs occur with NBS, a more helpful framework from which to understand cognitive enhancements with NBS may be to understand brains as systems designed to continuously enhance their own functions and available resources (through learning, automatizing useful behaviors, etc.), and to view NBS as a means to augment these ongoing processes.

In recent years it has been increasingly apparent that cognitive enhancement via noninvasive brain stimulation (NBS), primarily using magnetic fields (with transcranial magnetic stimulation: TMS) and electric currents (e.g., transcranial direct current stimulation: tDCS), is a real phenomenon. Such enhancements are usually reported as increases in speed, and/or accuracy in the performance of various psychological tasks (McKinley et al., 2012; Luber and Lisanby, 2014). The mechanisms behind these increases in performance are still unclear. Recently, it was suggested that the mechanisms of enhancement could be thought of within a zero-sum framework (Brem et al., 2014).

This idea, which was said to be grounded on the physical principle of conservation of energy in closed systems, can be best expressed using the game theoretical concept of a zero sum game, where the sum of all gains amongst the players is zero: in certain games, if someone wins, someone else must have lost. Brem et al. extended this concept to neural systems: if the system is zero-sum, then gains (in the present case, those achieved via cognitive enhancement) must be balanced by costs (losses in function) somewhere else in the system. To the extent that processing in the brain can be considered zero-sum, there are direct and important implications to any program using NBS- primarily, when considering cognitive enhancement, one should look not only for the gains, but also the losses, and judge the whole with a cost/benefit analysis.

From a practical and ethical point of view, Brem et al. (2014) raised an important issue: whether, in their search for performance enhancement, researchers may not be paying enough attention to potential adverse side effects of NBS, a point recently made by others as well (Davis et al., 2013). However, Brem et al. (2014) was presented as a theoretical framework for interpreting cognitive enhancement effects produced using NBS. In this regard, it should first be mentioned that in no literal sense can the brain be considered a closed system as defined within thermodynamics, so whatever "grounding" was intended by Brem et al. (2014) is at best an analogy. However, a game theoretical approach might be applicable in the context of central executive functions. Many psychological processes exhibit limited capacity. The iconic examples are selective attention (going back to the cocktail party effect: Cherry, 1953) and working memory (e.g., the magic number 7 ± 2: Miller, 1956). Human information processing theories in the 1960s formulated such processing using a computer analogy, with the central executive being like a CPU. In the 1970s, models using resource theories (e.g., Kahneman, 1973), some borrowed from economics (e.g., Navon and Gopher, 1979) were also used to explain limited capacity phenomena. The gist of such modeling is to assume we have a limited set of processing resources under control of a central executive processor which attempts to deploy these resources in an optimal manner to maximize performance. The limited amount of computational capacity to achieve this deployment of resources can be conceptualized as processing power. Differential allotments in resources deployed can be observed in such phenomena as the speed-accuracy trade-off (SATO), where higher accuracy can be achieved by sacrificing greater speed and vice-versa, or in changes in detection accuracy of visual targets depending on how spatial attention is divided or focused in the visual field.

What might be the consequences of NBS enhancement of performance on such a system having a limited capacity

central processor deploying a finite set of resources (say for working memory or attention)? In one report of NBS enhancement, subjects were to detect small rectangles appearing to the left or right visual field, and 1 Hz rTMS (which is thought to temporarily down-regulate cortical excitability, e.g., Chen et al., 1997) increased detection accuracy for ipsilateral stimulation- but at a cost of lower accuracy for items appearing in the contralateral field (Hilgetag et al., 2001). In another experiment, participants were to search an array of objects for a target, defined by some combination of form, color, and motion features (Walsh et al., 1998). When V5, a posterior cortical area central to motion processing, was stimulated with TMS while the visual search array was presented (disrupting motion processing), subjects had enhanced reaction times when the target did not include motion as an essential feature- but at a cost of slowing performance when it did. These two studies demonstrate that one can create enhanced performance with TMS but this can come at a cost for other types of performance.

In a recent review of cognitive enhancement through TMS (Luber and Lisanby, 2014), we termed this sort of phenomenon "addition-by-subtraction," and found 26 instances of it in 62 studies reporting TMS enhancement effects (a more generalized discussion of "enhancement through diminishment" can be found in Earp et al., 2014). We suggested that the addition-by-subtraction mechanism appears to function by disrupting or inhibiting an inessential or less essential but competing part of one or more functional brain networks involved in a task, resulting in temporary network reorganization. This sort of explanation seems in agreement with the zero-sum framework of Brem et al. (2014). However, in many cases of addition-by-subtraction we enumerated it is difficult to identify a cost. For example, in one visual search task, the items in the search array look like tilted "x"s, with the difference between target and distractors being the direction of the tilt. There is a natural learned tendency to identify the items in the array as "x"s and to mentally correct their tilt to the canonical orientation of an x, which makes the search for the item with opposite tilt less efficient. After many trials, individuals learn to overcome this tendency and search more efficiently. On the other hand, subjects who received 1 Hz rTMS to parietal cortex before starting this visual search task are immediately speeded in their performance, presumably because the NBS inhibited the overlearned tendency (Oliveri et al., 2010). Temporarily inhibiting this process so that learning can take place more quickly hardly seems like a cost. On the contrary, inhibiting counterproductive tendencies or processes to make learning more efficient appears to be a promising use of NBS. While NBS did cause a change in how neural resources were used, it is not clear that there was a cost in Oliveri et al. (2010), since what was being temporarily inhibited was a tendency counterproductive to learning.

As presented by Brem et al. (2014), within a zero-sum conceptualization enhancement effects caused by NBS primarily occur via direct or indirect alterations in allotment of neural resources. While close to half of the reports of NBS-caused performance enhancements we found appeared to be of this sort (although not all of those entailed what most would deem a "cost"), more than half did not fit such a framework (Luber and Lisanby, 2014). In these studies, NBS enhanced performance with direct application to a cortical region necessary to processing of the task. Crucially, the best explanation for these enhancements was not that NBS caused greater allotment of neural resources to this region and a loss of resources elsewhere, but that the stimulation in some way added to the resources available to process the task. An example of this is the proposed mechanism of stochastic resonance, in which lower intensities of NBS add enough to neural activity to push it over threshold for detection in some instances, enhancing perceptual sensitivity (Miniussi et al., 2010).

The zero-sum conception thus makes sense when the NBS is causing a change in a limited capacity system to increase processing resources in one part of the system to the detriment of another part. It does not address a situation in which the NBS is actually increasing overall resources and capacity. One useful framework to make the distinction involves diffusion models of perceptual decisions, where there are two primary ways to speed decisions. First, one could lower the decision criterion, which does create costs and benefits: a speed-accuracy trade-off. On the other hand, the size of the individual steps made in the random walk toward the decision boundaries could be made larger by increasing the efficiency of the processing. This increase in processing resources speeds reaction time, but does not entail a cost. This latter mechanism may explain the results when 5 Hz rTMS was applied to lateral occipital complex in sleep deprived subjects, dramatically enhancing reaction time without incurring a cost in accuracy (Luber et al., 2008).

Beyond acute changes caused by NBS, which can in some cases act to increase innate capacity, the great promise for cognitive enhancement lies in more permanent improvements, whether in remediating deficits in neurological or psychiatric patients or in enhancing the skills of healthy individuals. Especially in the latter case, some rudimentary steps have been made toward developing a technology that uses NBS to augment learning. Two manipulations appear to be of importance to creating long-lasting cognitive enhancement: first, repeated NBS sessions to generate a cumulative effect (Thut and Pascual-Leone, 2009), and second, stimulating task-relevant cortical regions while concurrently activating them with task performance, creating Hebbian synergies in neurons directly related to performance (Thickbroom, 2007). For example, four 1-h sessions of concurrent TMS/working memory task performance over the course of 2 days of sleep deprivation resulted in complete remediation of sleep deprivation deficits in working memory, while subjects receiving sham TMS had the normal lapsing and reaction time slowing caused by lack of sleep. This effect lasted at least 18 h beyond the last TMS session (Luber et al., 2013). Research in the NBS field is only struggling through crude beginnings, but as we learn to integrate the optimal pulse waveforms at the optimal sets of frequencies for the right durations and places at the best intensities, timed with the appropriate cognitive tasks, we may learn to

dramatically accelerate the learning of desired skills.

It is in this sense that using a zero-sum framework in the context of NBS cognitive enhancement is not particularly appropriate. Yes, it is important to remember that at any one point in time the capacities of the brain are finite, just as it is essential to understand that the order-generating processes of living organisms occur against the background of the increasing entropic gradient imposed by the second law of thermodynamics. However, a key element of nervous system organization is the way it adapts, remembers and learns at all levels of organization from synapse to cell assembly to systems of nuclei and cortical regions, over the course of seconds, minutes, weeks, and ages. The nervous system is designed to keep redesigning itself, to keep enhancing its capabilities. As one example, much of the brain is organized to continuously automatize its behaviors, to free up the limited-capacity executive processing system designed to deal with novel situations. What before took all of that executive system to deal with later does not occupy it at all as skills are learned (think the first time you tried to drive a car and how effortless it is now). Through such mechanisms as automatization, the brain in essence constantly expands its available resources, despite having a finite processing capacity and resource repertoire at any given instant. Because of this, perhaps the most appropriate framework to develop NBS cognitive enhancement is within a conceptualization of the human brain as the enhancement system it already is. Using NBS to enhance cognition is just another way our brains have found to operate on and improve themselves, this time by using what they are learning about themselves to enhance their operation by direct stimulation from without.

REFERENCES

Brem, A.-K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a zero-sum proposition? *Neuroimage* 85, 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E. M., Hallett, M., et al. (1997). Depression of motor cortex excitability by low frequency transcranial magnetic stimulation. *Neurology* 48, 1398–1403. doi: 10.1212/WNL.48.5.1398

Cherry, E. C. (1953). Some Experiments on the recognition of speech, with one and with two ears. *J. Acoust. Soc. Am.* 25, 975–979. doi: 10.1121/1.1907229

Davis, N. J., Gold, E., Pascual-Leone, A., and Bracewell, R. M. (2013). Challenges of proper placebo control for non-invasive brain stimulation in clinical and experimental applications. *Eur. J. Neurosci.* 38, 2973–2977. doi: 10.1111/ejn.12307

Earp, B. D., Sandberg, A., Kahane, G., and Savulescu, J. (2014). When is diminishment a form of enhancement? Rethinking the enhancement debate in biomedical ethics. *Front. Sys. Neurosci.* 8:12. doi: 10.3389/fnsys.2014.00012

Hilgetag, C. C., Hugo, T., and Pascual-Leone, A. (2001). Enhanced visual spatial attention ipsilateral to rTMS-induced 'virtual lesons' of human parietal cortex. *Nat. Neurosci*. 4, 953–957. doi: 10.1038/nn0901-953

Kahneman, D. (1973). *Attention and Effort*. Englewood Cliffs, NJ: Prentice-Hall.

Luber, B., and Lisanby, S. H. (2014). Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). *Neuroimage* 85, 961–970. doi: 10.1016/j.neuroimage.2013.06.007

Luber, B., Stanford, A. D., Bulow, P., Nguyen, T., Rakitin, B. C., Habeck, C., et al. (2008). Remediation of sleep-deprivation induced visual working memory impairment with fMRI-guided transcranial magnetic stimulation. *Cereb. Cortex* 18, 2077–2085. doi: 10.1093/cercor/bhm231

Luber, B., Steffener, J., Tucker, A., Habeck, C., Peterchev, A. V., Deng, Z.-D., et al. (2013). Extended remediation of sleep deprived-induced working memory deficits using fMRI-guided repetitive transcranial magnetic stimulation. *Sleep* 36, 857–871. doi: 10.5665/sleep.2712

McKinley, R. A., Bridges, N., Walters, C. M., and Nelson, J. (2012). Modulating the brain at work using noninvasive transcranial stimulation. *Neuroimage* 59, 129–137. doi: 10.1016/j.neuroimage.2011.07.075

Miller, G. A. (1956). The magical number seven, plus or minus two: some limits on our capacity for processing information. *Psychol. Rev.* 63, 81–97.

Miniussi, C., Ruzzoli, M., and Walsh, V. (2010). The mechanism of transcranial magnetic stimulation in cognition. *Cortex* 46, 128–130. doi: 10.1016/j.cortex.2009.03.004

Navon, D., and Gopher, D. (1979). On the economy of the human-processing system. *Psychol. Rev*. 86, 214–255.

Oliveri, M., Zhaoping, L., Mangano, G. R., Turriziani, P., Smirni, D., and Cipolotti, L. (2010). Facilitation of bottom-up feature detection following rTMS-interference of the right parietal cortex. *Neuropsychologia* 48, 1003–1010. doi: 10.1016/j.neuropsychologia.2009.11.024

Thickbroom, G. W. (2007). Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. *Exp. Brain Res.* 180, 583–593. doi: 10.1007/s00221-007-0991-3

Thut, G., and Pascual-Leone, A. (2009). A review of combined TMS-EEG studies to characterize lasting effects of repetitive TMS and assess their usefulness in cognitive and clinical neuroscience. *Brain Topogr.* 22, 219–232. doi: 10.1007/s10548-009-0115-4

Walsh, V., Ellison, A., Battelli, L., and Cowey, A. (1998). Task-specific impairments and enhancements induced by magnetic stimulation of human visual area V5. *Proc. Biol. Sci.* 265, 537–543. doi: 10.1098/rspb.1998.0328

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 June 2014; accepted: 19 June 2014; published online: 08 July 2014.

Citation: Luber B (2014) Neuroenhancement by noninvasive brain stimulation is not a net zero-sum proposition. Front. Syst. Neurosci. ***8****:127. doi: 10.3389/fnsys.2014.00127*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

METHODS
published: 06 May 2015
doi: 10.3389/fnsys.2015.00069

Design, fabrication, and packaging of an integrated, wirelessly-powered optrode array for optogenetics application

*Ki Yong Kwon[1], Hyung-Min Lee[2], Maysam Ghovanloo[2], Arthur Weber[3] and Wen Li[1]**

[1] Department of Electrical and Computer Engineering, Michigan State University, East Lansing, MI, USA, [2] School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [3] Department of Physiology, Michigan State University, East Lansing, MI, USA

The recent development of optogenetics has created an increased demand for advancing engineering tools for optical modulation of neural circuitry. This paper details the design, fabrication, integration, and packaging procedures of a wirelessly-powered, light emitting diode (LED) coupled optrode neural interface for optogenetic studies. The LED-coupled optrode array employs microscale LED (μLED) chips and polymer-based microwaveguides to deliver light into multi-level cortical networks, coupled with microelectrodes to record spontaneous changes in neural activity. An integrated, implantable, switched-capacitor based stimulator (SCS) system provides high instantaneous power to the μLEDs through an inductive link to emit sufficient light and evoke neural activities. The presented system is mechanically flexible, biocompatible, miniaturized, and lightweight, suitable for chronic implantation in small freely behaving animals. The design of this system is scalable and its manufacturing is cost effective through batch fabrication using microelectromechanical systems (MEMS) technology. It can be adopted by other groups and customized for specific needs of individual experiments.

Keywords: optrode array, implantable neural interface, optogenetics, microelectromechanical systems, switched-capacitor based stimulators, wireless power transfer

OPEN ACCESS

Edited by:
Ioan Opris, Wake Forest University School of Medicine, USA

Reviewed by:
Stelios M. Smirnakis, Baylor College of Medicine, USA
Oana D. Jurchescu, Wake Forest University School of Medicine, USA

****Correspondence:***
Wen Li, Department of Electrical and Computer Engineering, Michigan State University, 428 S Shaw Ln, RM 2120 Engineering Building, East Lansing, MI 48824, USA
wenli@egr.msu.edu

Received: *31 December 2014*
Accepted: *14 April 2015*
Published: *06 May 2015*

Citation:
Kwon KY, Lee H-M, Ghovanloo M, Weber A and Li W (2015) Design, fabrication, and packaging of an integrated, wirelessly-powered optrode array for optogenetics application. Front. Syst. Neurosci. 9:69. doi: 10.3389/fnsys.2015.00069

Introduction

Optogenetics is an emerging technique that combines optical and genetic tools for light modulation of neural activity. A key advantage of optogenetics in neuroscience is the ability to excite or inhibit specific cell types with millisecond precision and rapid reversibility (Boyden et al., 2005; Zhang et al., 2007; Deisseroth, 2011; Gerits and Vanduffel, 2013; Zalocusky and Deisseroth, 2013). Recently, neural transfection with substantially red-shifted channelrhodopsin or halorhodopsin genes has demonstrated the potential for deep transcranial optogenetic excitation and inhibition non-invasively (Zhang et al., 2008; Lin et al., 2013; Chuong et al., 2014). Recent emphasis on use of optogenetics has created an increased demand for optical devices targeting delivery of light not only to the surface, but also into deeper subregions of the brain. Examples of surface light delivery systems are acousto-optic deflectors (Losavio et al., 2011), digital micro-mirror devices (DMDs; Arrenberg et al., 2010; Zhu et al., 2012), and computer-generated holograms

(CGHs; Reutsky-Gefen et al., 2013). To deliver light to deeper regions of neural tissue, laser- or light-emitting-diode (LED) coupled optical fibers were initially implanted within the brain (Aravanis et al., 2007; Han et al., 2009; Zhang et al., 2010). Recently, more advanced methods, based on micro-/nanofabrication technologies, have been developed to increase the spatial resolution of optogenetic light stimulation of the three-dimensional neural networks of the brain. Such devices include, multichannel silicon oxynitride waveguide arrays (Zorzos et al., 2012), arrayed optical fibers (Royer et al., 2010; Stark et al., 2012), multi-waveguides fabricated on a single substrate (Zorzos et al., 2010), multipoint-emitting optical fibers (Pisanello et al., 2014), optrodes (Wang et al., 2012; Wu et al., 2013), and polymer-based flexible micro-LEDs arrays (Kim et al., 2013).

Despite the aforementioned development of light delivery devices, only limited methods currently are available for use in experiments with freely behaving subjects. These include, laser- or LED-coupled optical fibers, a polymer-based micro-LED array (Kim et al., 2013), and a head-mountable LED system (Iwai et al., 2011). The poor spatial resolution of such systems limits their functionality, and the tethered optical fiber greatly restricts the natural behaviors of the subjects. In addition, since most optogenetic neural probes integrate rigid silica fibers, their mechanical reliability is compromised by optoelectronic integration. As a solution to the current demand for multichannel, bi-directional optogenetic tools, our group has developed a series of flexible, hybrid neural interfacing devices for simultaneous optical modulation and electrical recording of neural activity at multiple levels of cortex. Depending on the interface location, three different devices are available: an Opto-μECoG array for epidural stimulation (Kwon et al., 2012, 2013c), a three dimension (3-D) waveguide array for deep cortical stimulation (Kwon and Li, 2013), and a slanted 3-D waveguide array for multi cortical layers stimulation (Kwon et al., 2013b). Such devices are microfabricated using Parylene-C as the flexible substrate and as the packaging material, thus ensuring biocompatibility while maintaining mechanical compliance (Takeuchi et al., 2005; Rodger et al., 2008; Li et al., 2010, 2011). Using microscale LEDs (μLEDs) as light sources provides several unique advantages compared to the use of external lasers and/or array-based diode lasers, including low power consumption, illumination stability, and fast light switching ability (Mohanty and Thakor, 2013). Integration of individually addressable μLEDs with microwaveguides allows for precise light delivery to the target neurons in individual cortical layers. Furthermore, electronically driven LEDs are particularly suitable for integration with wireless telemetry systems.

Building on the device development, most recently we have implemented a wireless neural interface system. Unlike standard optogenetic approaches that rely on rigid fiber optics tethered to external light sources, our system integrates a microfabricated LED-coupled optrode array with a switched-capacitor stimulator (SCS) and wireless telemetry on a single polymer platform toward a truly untethered bi-directional neural interface for optogenetic application (Kwon et al., 2014a; Lee et al., 2014). This system is flexible, miniaturized and lightweight, and thus suitable for use in small freely behaving animals. The design of our system is scalable and its manufacturing is cost effective, owing to the advantages of batch microfabrication technologies. In this paper, we present the detailed design principle and microfabrication protocols of the slanted LED-coupled optrode array, integration and packaging processes with the wireless system, and demonstration of *in vivo* neural recording and stimulation in rats. The methods described here provide a completely customizable approach for other groups and researchers to design and implement devices for specific needs of individual experiments.

Materials and Methods

Design of the Proposed System

As depicted in **Figure 1**, our wireless neural interfacing system contains three key components: a LED-coupled slanted optrode array, a wireless switched-capacitor based stimulator (SCS), and an inductive link for power transfer. The array has 32 embedded μLED light sources, with 4 × 4 channels per hemisphere on a 2.5 × 2.5 mm^2 flexible substrate to cover the rat visual cortices bilaterally. The μLED chips are coupled to slanted SU-8 microwaveguide cores, fabricated separately on a polydimethylsiloxane (PDMS) substrate. The design of the needle-shaped waveguide is simulated using a numerical ray-tracing method in TracePro (TracePro®, Lambda Research Co., MA, USA), as reported previously in Kwon et al. (2014b). The simulation results suggest that, given the same base size, the illumination range of the optrode is determined by the tip size of the waveguide core. In particular, a small tip results in divergent irradiance and low output light intensity due to the secondary reflection of light beams along the sidewalls of the tapered waveguide. Optrode with a large tip will enable high irradiance with a confined beam shape. For example, the radius of the illumination area increases from ∼0.2 mm to ∼0.6 mm when the tip diameter decreases from 100 μm to 10 μm. On the other hand, a large tip may cause more damage to brain tissue during implantation. Based on the simulation results, we designed the needle-shaped waveguides with various lengths ranging from 300–1500 μm in order to target neurons located in different cortical layers, a tip diameter of 30 μm to facilitate device implantation, and a base diameter of ∼300 μm to match the dimensions of the μLED chip (220 μm (W) × 270 μm (L) × 50 μm (H)). The cladding layer provided with each individual microwaveguide makes it possible to record electrophysiological signals in response to optical stimulation while preventing light leakage through the sidewalls of the tapered microneedles. To minimize the light-induced artifacts during recording, the cladding layer of the waveguide is specifically designed with four layers of an oxide-polymer-metal-polymer sandwich structure, as depicted in **Figure 1B**. Starting from the inner layer, transparent indium tin oxide (ITO) is used as the electrical shielding layer to eliminate photoelectrical artifact without compromising

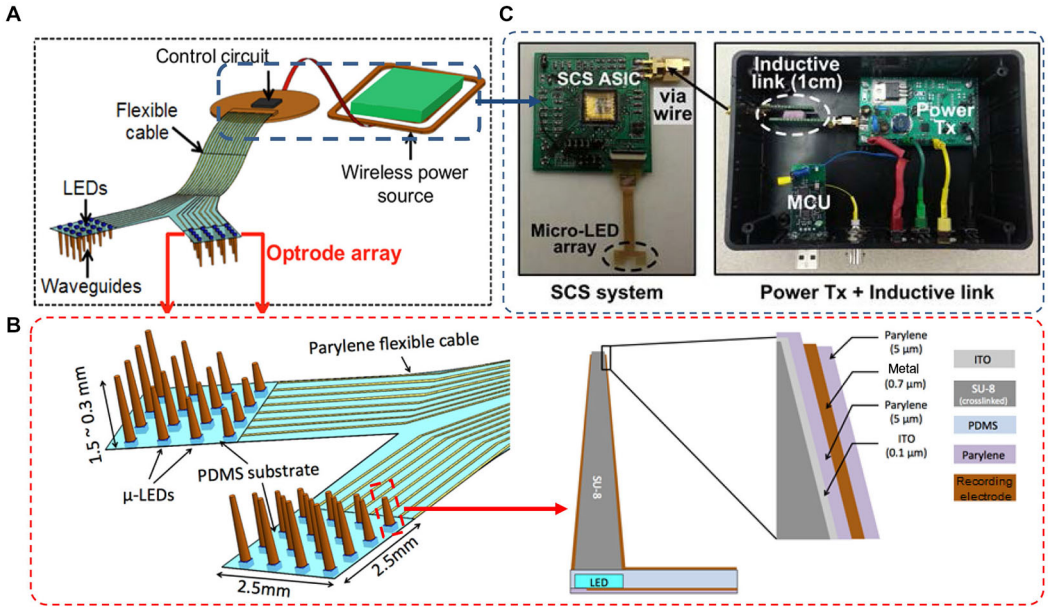

FIGURE 1 | (A) Conceptual illustration of the wireless optrode neural interface. **(B)** Overall design of the LED-coupled optrode array with 32 μLEDs, microwaveguides, and microelectrodes integrated on a Parylene-C substrate. **(C)** The inductively-powered telemetry and switched-capacitor based stimulating (SCS) system used to wirelessly drive the μLED array.

light throughput. After an insulating layer of Parylene-C, an opaque metallic layer is constructed to block out light side-leakage, with the tip being etched to allow light delivery to adjacent neurons expressing optogenetic opsins. This layer also is used for constructing the recording electrodes and leads to interconnect with the peripheral elements. The outermost layer is made of Parylene-C for encapsulation. The tip is selectively etched to expose the electrode side for neural recording.

The optrode array is inductively powered and controlled by a wireless switched-capacitor based stimulation (SCS) system, designed at Georgia Institute of Technology to improve power efficiency over traditional current-controlled stimulators, while being capable of delivering instantaneous high power to the μLEDs (Lee et al., 2013). During optical stimulation, the SCS system charges a small array of storage capacitors directly from the inductive link and periodically discharges them into the μLED array at the stimulation onsets, providing high instantaneous current without burdening the inductive link and system supply. To control stimulation parameters, a custom-designed PC interface wirelessly sends data to the SCS system through the inductive link, while a commercial neural recording system simultaneously detects the evoked neural signals from the same optrode array. For a chronically implantable system, all electronic components can be assembled on a mechanically flexible printed circuit board (PCB) substrate and encapsulated with Parylene-C to improve biocompatibility of the system by protecting the electronics from the corrosive biological environment. We graphically designed the masks of the optrode array and the PCB substrate in the AutoCAD environment (Autodesk Inc.), constructed the optrode array using polymer-based microelectromechanical systems (MEMS) techniques, and assembled the components on the PCB substrate using a customized assembly method. Details of the microfabrication and integration are described in the following sections.

Microfabrication Methods

To reduce fabrication complexity and improve the production yield, the multi-LED array and the slanted optrode array are fabricated and calibrated separately, then bonded together using SU-8 adhesive.

Fabrication of the LED Array

A process flow for making the multi-LED array has been developed previously in Kwon et al. (2013c), based on microfabrication and self-assembly techniques. (a-1) A 3-inch glass carrying wafer was cleaned by sonication in acetone, isopropyl alcohol (IPA), and de-ionized (DI) water (3 mins each), followed by Parylene-C deposition (5 μm thick) in a chemical vapor deposition (CVD) system (PDS2010 Parylene Coater, Specialty Coating System, IN, USA). (a-2) A 0.3 μm copper layer was deposited in a thermal evaporator (Edward Auto306, Edwards, UK) and patterned by wet etching to form contact pads and interconnection leads. A photoresist (PR) mask was selectively patterned to expose only the metal contacts for μLED connections. Copper was used to prove the concept, but can be replaced with gold or other conductive materials to improve chemical resistance and biocompatibility. (a-3) Low melting point (LMP) solder (melting point ~62°C, 144 ALLOY Field's Metal, Rotometals, Inc., CA, USA) was

applied on the contacts. The substrate then was rinsed with acetone, IPA, and DI water to remove the PR layer. (a-4) A PDMS (Sylgard 184, Dow Corning, MI, USA) stamp with the embedded blue μLEDs (Cree® TR2227TM die-form LED, peak wavelength at 460 nm, Cree, NC, USA)[1] was aligned to match the pre-soldered receiver sites on the carrying substrate. Different color LED chips (e.g., green, yellow, or red) can be used for optogenetic inhibition or excitation. (a-5) The substrate with the aligned PDMS stamp was heated on a hot plate at 90°C for 30 s. The PDMS stamp was removed carefully after the substrate had cooled to 40°C, leaving the LEDs attached to the contact pads of the substrate. The substrate then was submerged into an acidic water bath (90°C, pH of 2.0) for 1 min. This step permits fine adjustment of LED alignment and the formation of an electrical connection in a self-assembly manner, utilizing the surface tension of the LMP solder.

The PDMS stamp facilitates the assembly of multiple LEDs on the carrying substrate in a precise and time-efficient way. To fabricate the PDMS stamp, (b-1) a 3-inch glass wafer was first cleaned and went through a dehydration bake. A ∼30 μm SU-8 layer was spun onto the wafer and patterned as the mold for fabricating a PDMS stamp. (b-2) PDMS was poured over the SU-8 mold to form the stamp, which contained cavities matching the shape of the μLED chip. (b-3) After curing the PDMS for 40 min on a 95°C hotplate, the stamp was peeled off the mold. (b-4) Thirty-two LED chips were aligned in the cavities of the stamp, with metal pads facing outward. The assembly method using the PDMS stamp was adapted from Onoe et al. (2009), and the yield of the chip assembly was above 96%.

Fabrication of the Optrode Array and Assembly

As described in **Figure 2**, (1) a 3-inch glass wafer was cleaned and put through a dehydration bake, and a ∼50 μm SU-8 (SU-8 3025, MicroChem Corp, MA, USA) layer was spun onto the wafer and patterned as the mock LEDs; (2) A thin layer of PDMS was spun onto the SU-8 master to create cavities matching the shape of the LED; (3) After the PDMS was cured for 40 min at 95°C, PR was patterned on the PDMS substrate to expose 7 mm-diameter circles, followed by oxygen plasma treatment to convert the exposed hydrophobic areas to hydrophilic ones; (4) After removal of the PR mask, ∼45 μL SU-8 (SU-8 3005) was dispensed on top of the plasma treated PDMS surface using a micropipette; and (5) patterned with a backside droplet backside exposure (DBE) method that utilizes the height variance in a dome-shaped SU-8 structure to create out-of-plane microneedles of varying lengths (Kwon et al., 2013a, 2014b); (6) After SU-8 development, the array was polished by O_2 plasma etching; (7) DC sputtering of a 0.1-μm-thick ITO layer was performed in a Kurt Lesker Axiss PVD System, followed by deposition of 5 μm Parylene-C. Then a 0.2-μm-thick Au layer was deposited in the thermal evaporator, and the tip of the waveguide was chemically etched for light delivery, followed by deposition of 5 μm Parylene-C as a protective layer. The Parylene-C film at the tip of the optrode was removed using reactive-ion etching (RIE); and (8) The optrode array then was released from the glass wafer, and the microneedles with the matched LED cavities were aligned onto the corresponding LEDs. Finally, (9) the LED array and optrode array were bonded together, with a ∼50 μm SU-8 (SU-8 3025) layer spun on the LED array as an adhesive.

System Integration and Packaging

SCS System and Inductive Telemetry

Integrated LED light sources powered by the wireless SCS system enable a truly untethered optogenetic stimulation system. Inductive power transmission across the skin has been a viable solution to provide sufficient power to the implantable medical devices (IMDs), while overcoming size, cost, and longevity constraints of embedded primary batteries. Light sources in optogenetics, however, typically require high instantaneous power to emit sufficient light for optical neural stimulation, which can be a significant limiting factor in conventional IMDs (Wentz et al., 2011). **Figure 3A** shows the conceptual block diagram of a conventional inductively-powered array of LEDs for wireless optogenetics, where a rectifier and a regulator convert AC voltage across a secondary coil, L_2, to DC output voltage to supply a LED driver. Power losses in these stages result in poor overall power efficiency from L_2 to the LED. Moreover, high instantaneous power that flows to the LEDs when they are on, leads to large load variation, which affects the impedance matching with the inductive link. This will significantly increase the required inductive power level, creating safety issues, and degrading the inductive link power efficiency as well as supply voltage for the rest of the IMD. To address these limitations in implantable optogenetics stimulators, the SCS system has been designed and implemented for power-efficient wireless optical stimulation, as shown in **Figure 3B**. The proposed SCS system efficiently charges a bank of storage capacitors, C_S, directly from V_{COIL} through the inductive link without using any rectifiers or regulators, improving the capacitor charging efficiency. Moreover, the charge stored in capacitors is delivered to the load through large switches, efficiently creating high current stimulation pulses.

Integration and Hermetic Package for Chronic Implants

Figure 4 illustrates the process flow for integration and hermetic packaging of a chronic implant on a flexible polyimide PCB substrate for experiments using freely behaving animals. The flexible polyimide PCB substrate was constructed using Pyralux®AP (AP7163E, DuPont) with the following steps: (1) A 3-inch Pyralux® wafer was cut and cleaned, and a 3-μm thick PR layer was spin coated; (2) The circuit design was transferred on to the PR using a lithography technique; (3) The circuit was patterned by wet etching of copper; (4) Through holes were made by a laser cutter; (5) Vias were made by filling the through holes with solder; (6) Solder paste was applied on the contact pads and the SCS chip, and other

[1]Cree LED Datasheet. http://www.cree.com/∼/media/Files/Cree/LED%20Components%20and%20Modules/XLamp/Data%20and%20 Binning/XLampMCE_BL.pdf

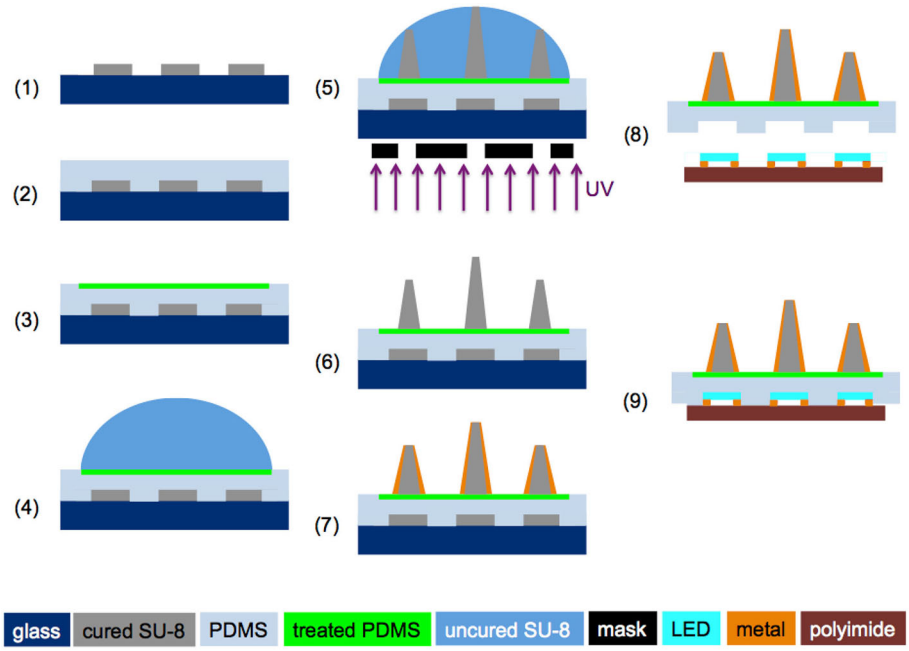

FIGURE 2 | Fabrication process flow for making the slanted optrode array (steps 1–7) and microassembly of the optrode array with the LED array (steps 8 and 9). ©[2014] IEEE. Reprinted, with permission, from Kwon et al. (2014a).

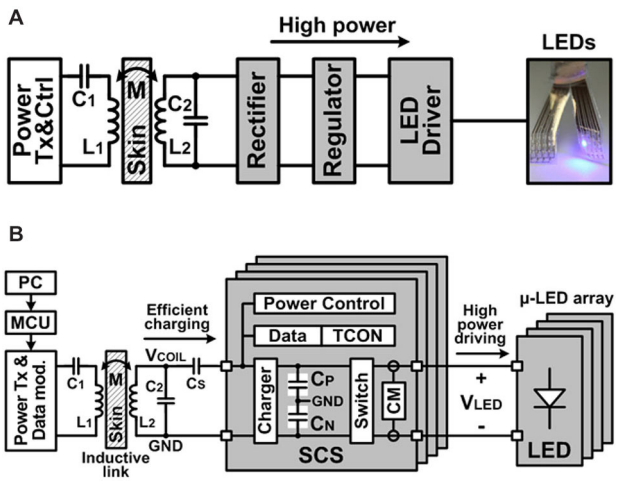

FIGURE 3 | (A) Simplified block diagram of a conventional inductively-powered device combined with an array of LEDs for wireless optogenetics and **(B)** simplified block diagram of the wireless switched-capacitor based stimulating (SCS) system to efficiently drive the μLED-coupled optrode array. ©[2014] IEEE. Reprinted, with permission, from Lee et al. (2014).

surface mount devices (SMDs) were populated on the pads. The circuit was baked at 200°C for 5 min, and extra flux was applied for reflow soldering, if necessary; and (7) Once all components were populated, a thick layer of medical-grade epoxy (200∼500 μm, EP21LVMed, Master Bond) was applied, followed by a 10 μm Parylene-C coating as a biocompatible package.

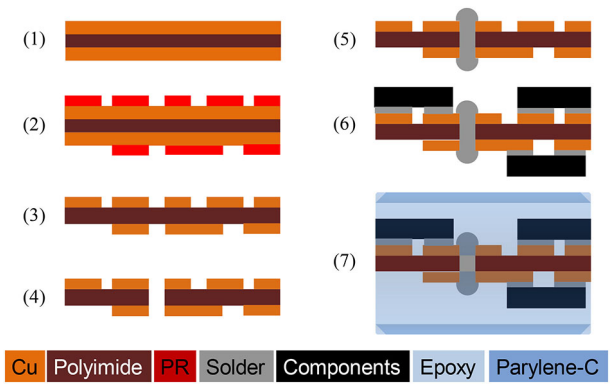

FIGURE 4 | Fabrication process of a hermetically-sealed chronic implant on a flexible polyimide circuit. ©[2014] IEEE. Reprinted, with permission, from Lee et al. (2014).

In Vivo Experiment in Rats

In vivo acute animal experiments have been conducted to demonstrate the ability of the LED-coupled optrode array to simultaneously modulate and record neural activities in the primary visual cortex (V1) of a rat. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Two weeks prior to the experiments, rodent subjects (Sprague-Dawley rats: 250–400 g) had neurons in their V1 transfected with the AAV-hSyn-hChR2(H134R)-mCherry viral vector ($10 \times 10^{11} \sim 10 \times 10^{12}$ vector genome (vg)/mL; UNC Vector Core, NC, USA). During the viral injection, each animal was anesthetized with ketamine/xylazine

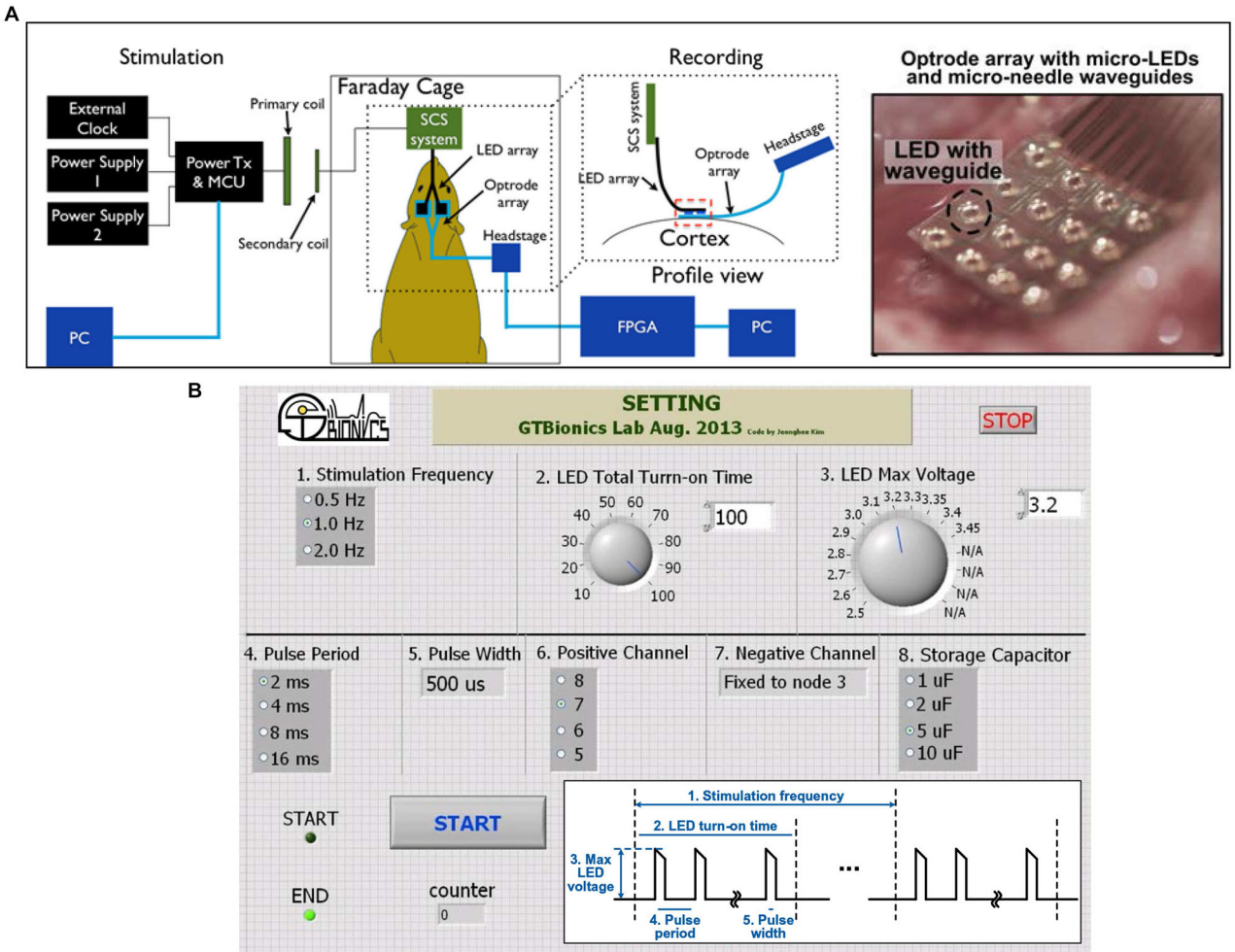

FIGURE 5 | (A) *In vivo* animal experiment setup with the wireless SCS system. **(B)** A SCS system GUI in LabVIEW to wirelessly control the SCS parameters. Inset shows the LED driving voltage waveform and its controllable parameters.

(80 mg/kg: 5 mg/kg i.m.) and anesthesia maintained with ketamine (20 mg/kg i.m.) as needed. The eyes were treated with 1% methylcellulose to prevent corneal drying. Using sterile surgical procedures, a 3–4 cm incision was made in the skin overlying the skull and small holes were drilled through the bone over V1. Using a glass pipette and a pressure injection system, 1.0 μL of the virus solution was injected at 3–4 equidistant locations. Injections occurred over a 2–3 min period, and the glass pipette was left in place for an additional 10 min to allow the vector to diffuse away from the injection site. The cortex then was covered with Gelfoam and the skin overlying the skull was sutured closed. Post-surgery, the animal received pain medication (buprenorphine; 0.05 mg/kg s.c.) and fluids (10–12 cc sterile saline, s.c.) subcutaneously and was placed in a cage on a heating pad until fully recovered, after which the animal was returned to the animal care facility. For device implantation, the animal expressing ChR2 (2–4 weeks after virus injection) was anesthetized using the procedure described above. The anesthetized animal was placed in a stereotaxic apparatus and a small region (4×4 mm^2) of the skull over V1 was removed. The optrode array was surgically implanted over V1, with the LEDs staying on the surface of the cortex and the waveguides inserted in the cortical tissues.

After device implantation, acute experiments were conducted in one transfected animal to validate the functionality of the implanted device. The experimental setup is shown in **Figure 5A**, where the SCS system was used to power and control the 32-channel LED array and a 32-channel headstage (Intan RHD2132) was used to receive and process the neural signals recorded through the optrode. A Faraday cage was used to isolate the animal, the optrode array, the integrated SCS system, and the recording headstage from the environmental interference in order to improve the signal-to-noise ratio of the neural recordings. The SCS system receives wireless power and data through the inductive link and drives μLEDs in the optrode array. The proof-of-concept prototype of the SCS system occupies 3.9×3.9 cm^2 on the PCB, and includes off-chip components for testing and optrode connectors. Key components of this prototype are the SCS chip (5×2.4 mm^2)

and four pairs of 1∼5 μF off-chip storage capacitors (SMD-0402, 1 × 0.5 mm^2 each). The SCS system can be miniaturized further using a single flex-PCB method described previously in **Figure 4**, which can connect directly to the LED array and include the receiver coil, L_2, without connectors or testing components. Storage capacitors can be placed on the opposite side of the SCS chip, minimizing the PCB size for chronically implantable optogenetics. Neural signals were recorded simultaneously from the penetrating electrodes on the same optrode array through an Intan RHD2132 evaluation board and its commercial hardwired recording setup (Intan Technologies, CA, USA).

To selectively turn on the LEDs and facilitate the adjustment of stimulation parameters, a graphical user interface (GUI) has been implemented in the LabVIEW environment (National Instruments, Austin, TX) to send data packets from the PC to the microcontroller unit (MCU) in the power transmitter (Tx) module, as shown in **Figure 5B**. Several parameters of the LED driving signal can be adjusted in this setup: (1) stimulation frequency, 0.5∼2 Hz; (2) LED total turn-on time, 10∼100 ms; (3) LED peak voltage, 2.5∼3.45 V; (4) pulse period, 2∼16 ms; (5) pulse width, 512 μs; (6) positive output connections, 4 channels; (7) negative output connections, fixed; and (8) storage capacitance, 1∼10 μF. While the pulse train periodically turns the LEDs on and off, the effective turn-on time can be simply calculated as (total turn-on time) × (pulse width/pulse period).

Results and Discussions

Fabrication Results

Figure 6 shows the photo images of a LED-coupled optrode array. The electrode-electrolyte interface impedances of the optrode array were measured at 1 kHz using a built-in electrode-impedance-testing circuitry in an Intan evaluation board (RHD2132 and RHD2000-EVAL, Intan Tech. LLC, CA, USA). The impedances of the recording electrodes ranged from 10–500 kΩ, suitable for local field potential (LFP) recordings. The impedance of the optrode can be adjusted by controlling the size of the Parylene-C opening, using the process in **Figures 2–7**.

Power consumption of the μLED at various operating conditions was investigated (**Figure 7A**) to provide useful guidelines for optical stimulation in *in vivo* experiments and future integration of the LED array with wireless power telemetry. The nonlinear current-voltage characteristic of a single μLED was measured with an impedance analyzer (HP 4191A RF, Hewlett Packard, CA, USA). Since a minimum light-energy density of 1 mW/mm^2 must be delivered onto the target area to effectively excite channelrhodopsin-2 (ChR2; Grossman et al., 2010), a minimum input voltage of ∼3.2 V is required to drive a single μLED, resulting in electrical power consumption of 27 mW and average light intensity of 1.4 mW/mm^2 from the waveguide tip. The temperature variation of the LED array was measured using a high definition infrared camera (Delta Therm. HS1570 and its software (DT v.2.19)) with different input voltages (2.7–3.2 V) and activation duration of 100 ms, as shown in **Figure 7B**. Thermal images were taken in air under an environment (at room temperature of 22.7°C) where ventilation was strictly controlled to minimize ambient thermal noise, and the array was suspended in the air during the LED activation. As expected, the localized temperature of the LED increases dramatically as the increase of the input voltage. The relatively high input voltage of the LED (3.2 V) can be attributed to inefficient light coupling between the LED chip and the tapered microwaveguide, which typically has a coupling efficiency of ∼10% for the butt coupling (Keiser, 2008). It is of note that, in **Figure 7B**, the pulse train of 3.2 V and 100 ms resulted in the temperature rise by more than ∼10°C, which may affect neural function and even cause tissue damage. However, this experiment was carried out in air without the thermoregulation effect of the cerebral circulation. To evaluate the safety of our simulation paradigm, we measured the temperature variation of the cortical region close to the implantation site using a thermocouple, when the LED was driven by a continuous train of 3.2 V, 100 ms pulse width, and 2 Hz frequency. Preliminary results show that the maximal temperature rise was approximately 0.1–0.4°C, which satisfies the safety requirement for implantable neuroprosthetics. Comprehensive investigation will be performed in the future to fully characterize the thermal properties of the integrated system.

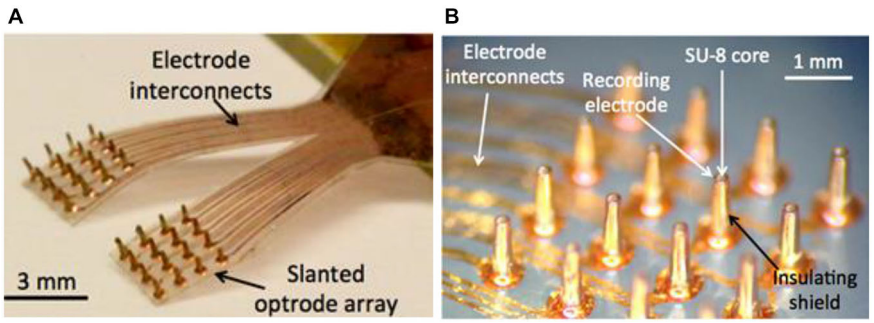

FIGURE 6 | Fabricated prototypes of (A) a 32-channel LED-coupled optrode array; and (B) microscopic image of individual optrodes.

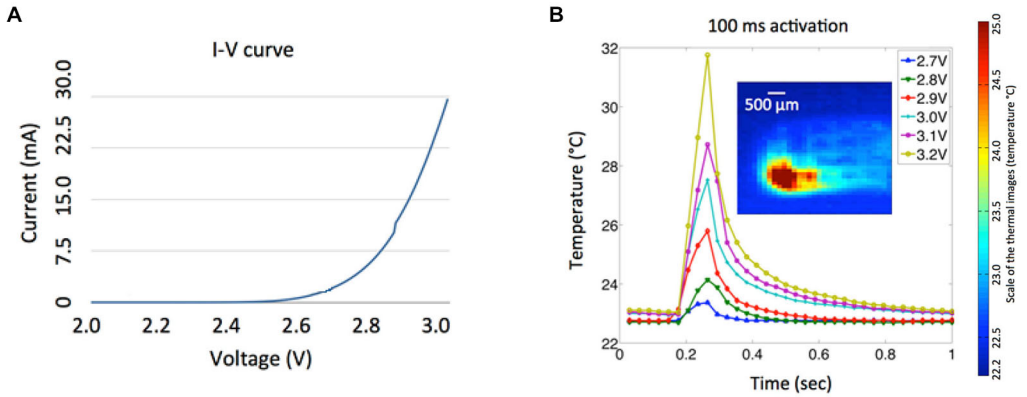

FIGURE 7 | (A) Measured I-V characteristic of a µLED chip. **(B)** Temperature variances of the LED array at various input voltages (2.7–3.2 V) for 100 ms activations. The inset shows the corresponding thermal image of a single LED activation taken at the maximum temperature with 3.2 V input voltage and activation duration of 100 ms.

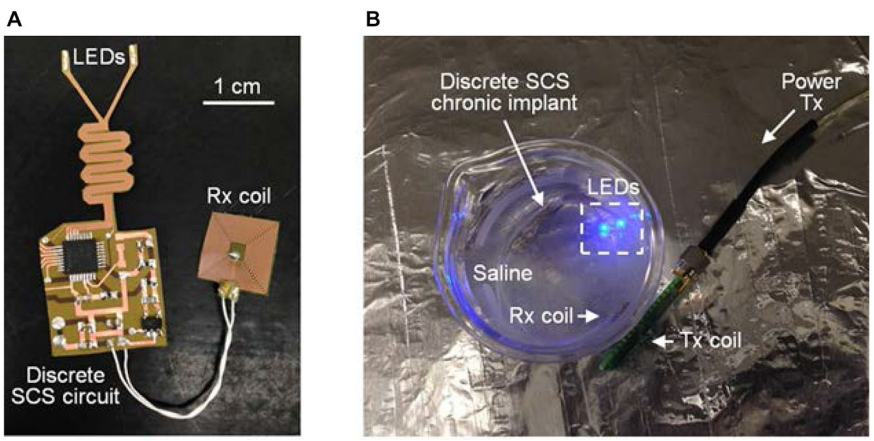

FIGURE 8 | (A) Discrete SCS chronic implant and **(B)** accelerated soak testing in saline with wireless power and data transfer. ©[2014] IEEE. Reprinted, with permission, from Lee et al. (2014).

To evaluate the performance of epoxy-Parylene-C packages and the possible electrical failure of the chronic implant, active accelerated-lifetime soak testing was performed in saline (a solution of 0.90% w/v of NaCl) at a higher than body temperature. For soak testing, a discrete version of the SCS circuit was designed using a MCU (MSP430, Texas Instruments, TX, USA), and constructed using the process described in **Figure 4**. This circuit was programmed to mimic SCS stimulation patterns, once it received wireless power and data through the inductive link. Dimensions and fabrication process of the discrete circuit were identical to the SCS system. Four LEDs (LB QH9G, blue 466 nm, OSRAM, Germany) were integrated on the flexible PCB, and each LED was individually controlled by the MCU. A MEMS-based receiver (Rx) coil was fabricated separately to be placed on the back of the animal and connected to the SCS with flexible wires (392 F high-flex miniature wire, 36 AWG, McMaster-Carr. OH, USA).

Five discrete SCS devices were prepared and immersed in saline at 75°C, as shown in **Figure 8B**. Each individual device was activated by coupling the Rx and Tx coils, and daily the samples were visually monitored for possible device failure. No delamination, major physical corrosion, or performance degradation occurred after 14 days in accelerated lifetime testing conditions. Using the Arrhenius relationship, preliminary results show that the implant lifetime can be the equivalent of 3.5 years at body temperature of 37°C, sufficient for one-year duration requirement in the animal study. Further improvements can be achieved by optimizing temperature and duration of heat treatment for the Parylene/metal thin-film skin or by using additional chemical treatments.

In Vivo Experimental Results

Figure 9 shows the LED driving voltage waveforms, V_{LED}, for optical stimulation with SCS as well as light-induced *in*

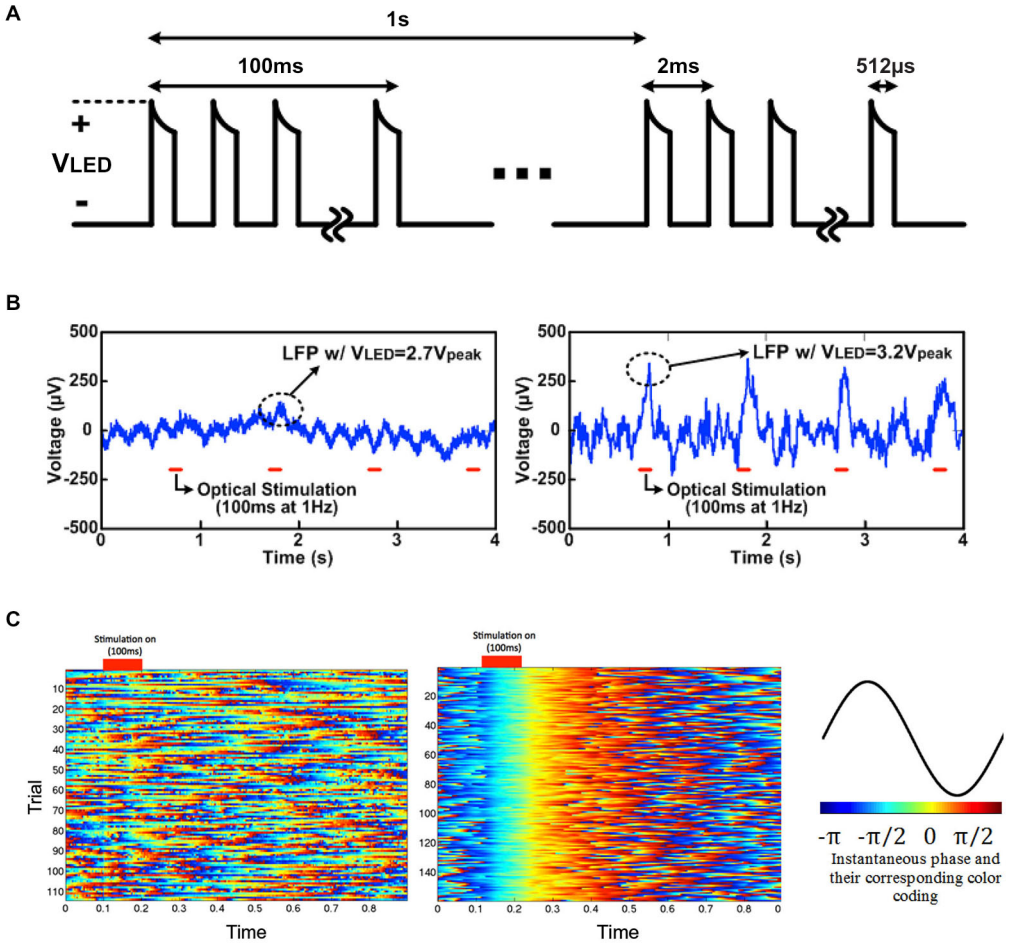

FIGURE 9 | Light-induced LFP recording driven by 2.7 V_{peak} and 3.2 V_{peak} for 100 ms: (A) LED driving voltage, V_{LED}, for *in vivo* optogenetics with SCS, (B) light-induced local field potentials (LFP) with V_{LED} = 2.7 V_{peak} and 3.2 V_{peak}, and (C) instantaneous phase with V_{LED} = 2.7 V_{peak} (top) and 3.2 V_{peak} (bottom) in low frequency band (1–25 Hz), and their corresponding color-coding.

TABLE 1 | Comparison between our LED-coupled optrode array and other laser-coupled optical/electrical devices.

Optical neurostimulation			Electrical recording		Wireless-enabled	Ref
Light source	**#of waveguides**	**Fiber size**	**#of electrodes**	**Electrode size**		
Laser	4 or 8	Tip size: 5~20 μm	8 per probe	~160 μm^2	No	Royer et al. (2010)
Laser	1	50~100 μm	36	50~100 μm	No	Wang et al. (2012)
Laser	1	Tip size: 14~28 μm	8	20 μm × 20 μm	No	Wu et al. (2013)
Laser	7	~400 μm	16	N.A.	No	Pisanello et al. (2014)
LED	32	Base size: 300 μm Tip size: ~30 μm	32	~30 μm	Yes	Our Optrode Array

vivo LFP results. The LFPs below 500 Hz were recorded using the optrode array with microwaveguides in the rat's V1 when the SCS system drove μLEDs with a 512 μs pulse train for 100 ms at 1 Hz and V_{LED} = 2.7 V_{peak} and 3.2 V_{peak}, as shown in **Figure 9A**. For each V_{LED}, in total 150 trials of optical stimulation and simultaneous recording were performed. A clear and stable light-induced neural activity was observed in LFPs (1–500 Hz) in time domain driven by 3.2 V_{peak} input voltage (**Figure 9B**). The high input voltage of 3.2 V_{peak} results in an average irradiance of 1.4 mW/mm^2 at the tip of the waveguide, which is above the minimal irradiance (1 mW/mm^2) required for light-evoked neural responses. Lower input voltage

(for example, 2.7 V_{peak} resulting in the average irradiance of 0.35 mW/mm^2) was also tried, but no significant neural modulation was observed.

To clearly visualize neural oscillations induced by the optrode array, instantaneous phases of the two datasets (with 2.7 V_{peak} and 3.2 V_{peak} input voltages) of light-evoked LFPs (1–25 Hz) were measured based on Hilbert transform. Each trial was truncated to a ∼100 ms pre-stimulation period and an ∼800 ms post-stimulation period. The instantaneous phases of each individual trial (1 s with 100 ms light stimulation), labeled with different colors according to **Figure 9C**, were aligned based on the stimulus ON time and stacked. With 3.2 V_{peak} input voltage, light-evoked neural activity shows strong phase synchronization, while no phase synchrony was observed in neural recording data with 2.7 V_{peak} input voltage. The neural modulation and recording was extremely reliable across over 100 trials during the *in vivo* experiments. The cortically optical stimulation generated almost deterministic phase-locked neuronal oscillations without any latency for ∼0.3 s as validated with a nonparametric test ($p < 0.05$, Wilcoxon signed rank test for each pair of channels). This experiment, while still preliminary, has verified the feasibility of wirelessly powered optical stimulation and simultaneous recording of neural activity via the LED-coupled optrode array.

Discussion and Outlook

Table 1 compares the presented LED-coupled optrode array with some microfabricated, laser-coupled optical/electrical implants reported by other group. As can be seen, our LED-coupled optrode array provides several unique advantages over laser-coupled devices. First, the devices are manufactured using advanced microfabrication techniques, therefore enabling high channel count and fine spatial resolution for optical stimulation and electrical recording. The design of our system is scalable and the developed manufacturing method is cost effective and reliable. In addition, In addition, our approach uses commercially available LED chips coupled to microfabricated SU-8 waveguides, which eliminates the need for tethered optical fibers and thus is suitable for integration with wireless power and data telemetries. While this work is primarily focused on the neural excitation, the wide selection of the LED chips makes it possible to switch the colors of the LED chips to yellow or red for applications in optogenetic neural inhibition.

For chronic implantation the most critical aspects are certainly the aging behavior of the device and the stability of neural recordings. We anticipate that the performance degradation may be caused by two factors: failure of Parylene-C package and aging of SU-8 waveguide cores. The biocompatible package of the current prototype used 10 μm Parylene-C, which has lower moisture permeability over PDMS and polyimide, and is suitable for packaging of chronic neural implants (Loeb et al., 1977; Schmidt et al., 1988; Takeuchi et al., 2005; Rodger et al., 2008). SU-8 for constructing waveguides on implantable neural probes brings along advantages such as flexibility, optical clarity, and compatibility with conventional photolithography techniques. However, since SU-8 is a photosensitive resist, continuous exposure to blue light may cause the degradation of the material flexibility and optical transparency due to cross-linking of the resin (Fiedler et al., 2014), which will lead to increased coupling loss of light along the waveguides. In this paper, the accelerated short-term soak testing of the optrode prototype has shown good reliability and stability of the Parylene-C package. Although we have not observed any performance degradation of the device in short-term experiments, further long-term studies have to be performed to fully characterize the reliability and stability of the optrode array. More detailed investigation is necessary to optimize the fabrication processes, where, for example, the influence of PR exposure and hard baking should be examined carefully.

Conclusions

In conclusion, design, fabrication, and testing procedures of the wireless LED-coupled slanted optrode array for a really untethered bi-directional neural interface were presented. The array was inductively powered and controlled by the wireless SCS system, designed to improve power efficiency. The SCS system for implantable wireless optogenetics provides high instantaneous power through the inductive link to emit sufficient light and evoke neural activities. The LabVIEW PC interface and custom-designed power Tx module provide wireless power and data to the SCS system, while electrodes embedded in the optrode array enable simultaneous neural recording. The self-assembled LED array on a single substrate can reduce manufacturing cost. Acute *in vivo* experiments with optical stimulation and LFP recording have verified the efficacy of our system for wireless optogenetics application.

Acknowledgments

This work was supported by the National Science Foundation under the Award Numbers CBET-1264772 and ECCS-1407880. The authors would like to Dr. Baokang Bi for the help on microfabrication.

References

Aravanis, A. M., Wang, L.-P., Zhang, F., Meltzer, L. A., Mogri, M. Z., Schneider, M. B., et al. (2007). An optical neural interface: *in vivo* control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J. Neural. Eng.* 4, S143–S156. doi: 10.1088/1741-2560/4/3/s02

Arrenberg, A. B., Stainier, D. Y. R., Baier, H., and Huisken, J. (2010). Optogenetic control of cardiac function. *Science* 330, 971–974. doi: 10.1126/science.1195929

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268. doi: 10.3410/f.1026686.335763

Chuong, A. S., Miri, M. L., Busskamp, V., Matthews, G. A. C., Acker, L. C., Sørensen, A. T., et al. (2014). Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat. Neurosci.* 17, 1123–1129. doi: 10.3410/f.718482076.793498459

Deisseroth, K. (2011). Optogenetics. *Nat. Methods* 8, 26–29. doi: 10.1038/nmeth.f.324

Fiedler, E., Haas, N., and Stieglitz, T. (2014). Suitability of SU-8, EpoClad and EpoCore for flexible waveguides on implantable neural probes. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2014, 438–441. doi: 10.1109/EMBC.2014.6943622

Gerits, A., and Vanduffel, W. (2013). Optogenetics in primates: a shining future?. *Trends Genet.* 29, 403–411. doi: 10.1016/j.tig.2013.03.004

Grossman, N., Poher, V., Grubb, M. S., Kennedy, G. T., Nikolic, K., McGovern, B., et al. (2010). Multi-site optical excitation using ChR2 and micro-LED array. *J. Neural. Eng.* 7:016004. doi: 10.1088/1741-2560/7/1/016004

Han, X., Qian, X., Bernstein, J. G., Zhou, H.-H., Franzesi, G. T., Stern, P., et al. (2009). Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. *Neuron* 62, 191–198. doi: 10.1016/j.neuron.2009.03.011

Iwai, Y., Honda, S., Ozeki, H., Hashimoto, M., and Hirase, H. (2011). A simple head-mountable LED device for chronic stimulation of optogenetic molecules in freely moving mice. *Neurosci. Res.* 70, 124–127. doi: 10.1016/j.neures.2011.01.007

Keiser, G. (2008). *Optical Fiber Commn.* 4th Edn. New York: McGraw-Hill Education.

Kim, T. I., Mccall, J. G., Jung, Y. H., Huang, X., Siuda, E. R., Li, Y. H., et al. (2013). Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. *Science* 340, 211–216. doi: 10.1126/science.1232437

Kwon, K. Y., Bi, X., and Li, W. (2013a). Droplet backside exposure for making slanted SU-8 microneedles. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2013, 767–770. doi: 10.1109/EMBC.2013.6609613

Kwon, K. Y., Khomenko, A., Haq, M., and Li, W. (2013b). Integrated slanted microneedle-LED array for optogenetics. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2013, 249–252. doi: 10.1109/EMBC.2013.6609484

Kwon, K. Y., Lee, H.-M., Ghovanloo, M., Weber, A., and Li, W. (2014a). "A wireless slanted optrode array with integrated micro leds for optogenetics," in *Micro Electro Mechanical Systems (MEMS), 2014 IEEE 27th International Conference on* (San Francisco, CA), 813–816.

Kwon, K. Y., and Li, W. (2013). "Integrated multi-LED array with three-dimensional polymer waveguide for optogenetics," in *the IEEE 26th International Conference on Micro Electro Mechanical Systems (IEEE MEMS 2013)* (Taipei), 1017–1020.

Kwon, K. Y., Sirowatka, B., Weber, A., and Li, W. (2012). "Opto-μECoG array: transparent μECoG electrode array and integrated LEDs for optogenetics," in *BioCAS 2012* (Hsinchu), 164–167.

Kwon, K. Y., Sirowatka, B., Weber, A., and Li, W. (2013c). Opto-μECoG array: a hybrid neural interface with transparent μECoG electrode array and integrated LEDs for optogenetics. *IEEE Trans. Biomed. Circuits Syst.* 7, 593–600. doi: 10.1109/TBCAS.2013.2282318

Kwon, K. Y., Weber, A., and Li, W. (2014b). Varying-length polymer microneedle arrays fabricated by droplet backside exposure. *J. Microelectromech. Syst.* 23, 1272–1280. doi: 10.1109/jmems.2014.2352412

Lee, H.-M., Kwon, K. Y., Li, W., and Ghovanloo, M. (2014). A wireless implantable switched-capacitor based optogenetic stimulating system. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2014, 878–881. doi: 10.1109/EMBC.2014.6943731

Lee, H.-M., Park, H., and Ghovanloo, M. (2013). A power-efficient wireless system with adaptive supply control for deep brain stimulation. *IEEE J. Solid-State Circuits* 48, 2203–2216. doi: 10.1109/jssc.2013.2266862

Li, W., Rodger, D. C., Meng, E., Weiland, J. D., Humayun, M. S., and Tai, Y.-C. (2010). Wafer-level parylene packaging with integrated RF electronics for wireless retinal prostheses. *IEEE/ASME J. Microelectromech. Syst.* 19, 735–742. doi: 10.1109/jmems.2010.2049985

Li, W., Rodger, D. C., Pinto, A., Meng, E., Weiland, J. D., Humayun, M. S., et al. (2011). Parylene-based integrated wireless single-channel neurostimulator. *Sens. Actuators. A Phys.* 166, 193–200. doi: 10.1016/j.sna.2010.03.003

Lin, J. Y., Knutsen, P. M., Muller, A., Kleinfeld, D., and Tsien, R. Y. (2013). ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat. Neurosci.* 16, 1499–1508. doi: 10.1038/nn.3502

Loeb, G. E., Bak, M. J., Salcman, M., and Schmidt, E. M. (1977). Parylene as a chronically stable, reproducible microelectrode insulator. *IEEE Trans. Biomed. Eng.* 24, 121–128. doi: 10.1109/tbme.1977.326115

Losavio, B. E., Iyer, V., Patel, S., and Saggau, P. (2011). Acousto-optic laser scanning for multi-site photo-stimulation of single neurons *in vitro*. *J. Neural. Eng.* 7:045002. doi: 10.1088/1741-2560/7/4/045002

Mohanty, S. K., and Thakor, N. V. (2013). Optogenetics: optical methods for cellular control. *Proc. of SPIE* 8586. doi: 10.1117/12.2021444

Onoe, H., Nakai, A., Iwase, E., Matsumoto, K., and Shimoyama, I. (2009). Temperature-controlled transfer and self-wiring for multi-color light-emitting diode arrays. *J. Micromech. Microeng.* 19:075015. doi: 10.1088/0960-1317/19/7/075015

Pisanello, F., Sileo, L., Oldenburg, I. A., Pisanello, M., Martiradonna, L., Assad, J. A., et al. (2014). Multipoint-emitting optical fibers for spatially addressable *in vivo* optogenetics. *Neuron* 82, 1245–1254. doi: 10.1016/j.neuron.2014.04.041

Reutsky-Gefen, I., Golan, L., Farah, N., Schejter, A., Tsur, L., Brosh, I., et al. (2013). Holographic optogenetic stimulation of patterned neuronal activity for vision restoration. *Nat. Commun.* 4:1509. doi: 10.1038/ncomms2500

Rodger, D. C., Fong, A. J., Li, W., Ameri, H., Ahuja, A. K., Gutierrez, C., et al. (2008). Flexible parylene-based multielectrode array technology for high-density neural stimulation and recording. *Sens. Actuators B Chem.* 132, 449–460. doi: 10.1016/j.snb.2007.10.069

Royer, S., Zemelman, B. V., Barbic, M., Losonczy, A., Buzsáki, G., and Magee, J. C. (2010). Multi-array silicon probes with integrated optical fibers: light-assisted perturbation and recording of local neural circuits in the behaving animal. *Eur. J. Neurosci.* 31, 2279–2291. doi: 10.1111/j.1460-9568.2010.07250.x

Schmidt, E. M., Mcintosh, J. S., and Bak, M. J. (1988). Long-term implants of Parylene-C coated microelectrodes. *Med. Biol. Eng. Comput.* 26, 96–101. doi: 10.1007/bf02441836

Stark, E., Koos, T., and Buzsáki, G. (2012). Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals. *J. Neurophysiol.* 108, 349–363. doi: 10.1152/jn.00153.2012

Takeuchi, S., Ziegler, D., Yoshida, Y., Mabuchi, K., and Suzuki, T. (2005). Parylene flexible neural probes integrated with microfluidic channels. *Lab Chip* 5, 519–523. doi: 10.1039/b417497f

Wang, J., Wagner, F., Borton, D. A., Zhang, J. Y., Ozden, I., Burwell, R. D., et al. (2012). Integrated device for combined optical neuromodulation and electrical recording for chronic *in vivo* applications. *J. Neural Eng.* 9:016001. doi: 10.1088/1741-2560/9/1/016001

Wentz, C. T., Bernstein, J. G., Monahan, P., Guerra, A., Rodriguez, A., and Boyden, E. S. (2011). A wirelessly powered and controlled device for optical neural control of freely-behaving animals. *J. Neural. Eng.* 8:046021. doi: 10.1088/1741-2560/8/4/046021

Wu, F., Stark, E., Im, M., Cho, I. J., Yoon, Y. S., Buzsáki, G., et al. (2013). An implantable neural probe with monolithically integrated dielectric waveguide and recording electrodes for optogenetics applications. *J. Neural. Eng.* 10:056012. doi: 10.1088/1741-2560/10/5/056012

Zalocusky, K., and Deisseroth, K. (2013). Optogenetics in the behaving rat: integration of diverse new technologies in a vital animal model. *Optogenetics* 1, 1–17. doi: 10.2478/optog-2013-0001

Zhang, F., Gradinaru, V., Adamantidis, A. R., Durand, R., Airan, R. D., De Lecea, L., et al. (2010). Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat. Protoc.* 5, 439–456. doi: 10.1038/nprot.2009.226

Zhang, F., Prigge, M., Beyrière, F., Tsunoda, S. P., Mattis, J., Yizhar, O., et al. (2008). Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. *Nat. Neurosci.* 11, 631–633. doi: 10.1038/nn.2120

Zhang, F., Wang, L.-P., Brauner, M., Liewald, J. F., Kay, K., Watzke, N., et al. (2007). Multimodal fast optical interrogation of neural circuitry. *Nature* 446, 633–639. doi: 10.1038/nature05744

Zhu, P., Fajardo, O., Shum, J., Zhang Schärer, Y.-P., and Friedrich, R. W. (2012). High-resolution optical control of spatiotemporal neuronal activity patterns in zebrafish using a digital micromirror device. *Nat. Protoc.* 7, 1410–1425. doi: 10.1038/nprot.2012.072

Zorzos, A. N., Boyden, E. S., and Fonstad, C. G. (2010). Multiwaveguide implantable probe for light delivery to sets of distributed brain targets. *Opt. Lett.* 35, 4133–4135. doi: 10.1364/ol.35.004133

Zorzos, A. N., Scholvin, J., Boyden, E. S., and Fonstad, C. G. (2012). Three-dimensional multiwaveguide probe array for light delivery to distributed brain circuits. *Opt. Lett.* 37, 4841–4843. doi: 10.1364/ol.37.004841

Conflict of Interest Statement: Wen Li and Ki Yong Kwon, "Neural Prosthetic Device and Method of Making Same," Pending U.S. Provisional Patent, Application Serial No.: 61/845,106, MSU Ref. No. TEC2013-0092-01Prov -SLW Ref. No. 3000.138PRV, 2014. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

REVIEW
published: 23 November 2015
doi: 10.3389/fnsys.2015.00157

Prospects for Optogenetic Augmentation of Brain Function

Sarah Jarvis and Simon R. Schultz *

Centre for Neurotechnology and Department of Bioengineering, Imperial College London, London, UK

The ability to optically control neural activity opens up possibilities for the restoration of normal function following neurological disorders. The temporal precision, spatial resolution, and neuronal specificity that optogenetics offers is unequalled by other available methods, so will it be suitable for not only restoring but also extending brain function? As the first demonstrations of optically "implanted" novel memories emerge, we examine the suitability of optogenetics as a technique for extending neural function. While optogenetics is an effective tool for altering neural activity, the largest impediment for optogenetics in neural augmentation is our systems level understanding of brain function. Furthermore, a number of clinical limitations currently remain as substantial hurdles for the applications proposed. While neurotechnologies for treating brain disorders and interfacing with prosthetics have advanced rapidly in the past few years, partially addressing some of these critical problems, optogenetics is not yet suitable for use in humans. Instead we conclude that for the immediate future, optogenetics is the neurological equivalent of the 3D printer: its flexibility providing an ideal tool for testing and prototyping solutions for treating brain disorders and augmenting brain function.

Keywords: optogenetics, neural augmentation, neural coding and decoding, neural engineering, neural modulation, neural prosthetics, BMI (brain machine interface)

OPEN ACCESS

Edited by:
Mikhail Lebedev,
Duke University, USA

Reviewed by:
Elena Rusconi,
University College London, UK
Leonardo Sacconi,
University of Florence, Italy
Marco Canepari,
Institut National de la Santé et de la Recherche Médicale, France

***Correspondence:**
Simon R. Schultz
s.schultz@imperial.ac.uk

Received: *28 May 2015*
Accepted: *29 October 2015*
Published: *23 November 2015*

Citation:
Jarvis S and Schultz SR (2015) Prospects for Optogenetic Augmentation of Brain Function. Front. Syst. Neurosci. 9:157. doi: 10.3389/fnsys.2015.00157

1. INTRODUCTION

Combining genetic targeting with optical excitation, optogenetics offers the ability to not only record the activity of large populations of neurons but also manipulate the activity of individual cells. Recording neural activity is achieved by selective expression of activity sensitive fluorophores (such as those from the GCaMP and VSFP protein families, reviewed by Knöpfel, 2012) into neurons, whose activity can then be read out by optical imaging. Manipulation of neural activity can be achieved via the insertion of light-sensitive proteins (opsins) that act as ion channels or pumps into a neuron's membrane and are preferentially controlled by photons of different wavelengths, providing temporal control on the order of milliseconds. Together, this cell type specificity and temporal control results in a tool that can perturb neural circuits with high precision. Since its first application to neural populations in 2005 (Boyden et al., 2005), it has already had substantial impact as a popular technique within the neuroscientific toolkit.

In addition to its use as a research tool, optogenetic stimulation has been suggested as a new approach for neuroprosthetics and treatment of brain disorders. While therapies in these domains have traditionally used electrical or pharmacological techniques, optogenetics has one particular advantage over electrical stimulation in being able to target specific cell classes through gene expression. As a result, specific neuronal populations can in principle be controlled without potential brain-wide, side effects. Likewise, the extremely fine temporal precision it offers on the

scale of milliseconds, due to its optical activation, has the advantage over pharmacology of not only acting immediately but also having no washout time. Together, these characteristics have led to optogenetics to be proposed as a viable approach for improved deep brain stimulation (Kravitz et al., 2010), reinstatement of functionality following spinal cord injury (Alilain et al., 2008) and retinal prostheses (Degenaar et al., 2009; Busskamp et al., 2012), amongst other potential clinical applications in humans, following studies using mice, rats and non-human primates as animal models.

The blurred boundary between restoring function and functional enhancement is present for any biomedical intervention. From improving existing function to the incorporation of new streams of information, augmentation of the central nervous system raises specific challenges, from technical issues that are shared with the development of neural therapies, to the more fundamental difficulty of identifying where and how to best modify existing activity to move to the new neural trajectory. In addition, the ethics of the benefits and unintended consequences of intervening in the brain are substantial, as discussed in a recent report by the Nuffield Council on Bioethics 2013.

Does the usefulness of optogenetics as a tool for probing neural circuits automatically translate to its use for neuroaugmentation? Or instead, is it at best limited to the rapid prototyping of novel approaches for enhancing neural processing, much like 3D printers have accelerated for the development of biomedical devices such as prosthetics (including ears, prosthetics and sockets), with the benefit of allowing easy customization? In this article, we examine how well suited is it as a tool for improving, and not merely probing, neural function. By identifying the advantages optogenetics offers over traditional tools for treatment of dysfunction, as well as the hurdles facing neuroaugmentation, we evaluate the use of optogenetics as a practical tool for neural enhancement.

2. OPTOGENETIC TREATMENT AND NEUROPROSTHETICS

A long-held goal of neuroscience has been to identify the specific roles that various neuronal populations play in neural information processing, in order to develop novel therapeutic approaches for brain disorders. The development of light sensitive tools, including opsins and optically activated G protein–coupled receptors (GPCRs), has provided an exceptional tool with which to dissect out the roles of neuronal populations. Critically, their specificity to target neurons by neuronal class and location is a unique advantage, and the ability to test without inducing irreversible changes allows confirmation without long-term damage. In rodent models, optogenetics has been used for investigating disorders such as Parkinson's Disease (Gradinaru et al., 2010), drug addiction (Witten et al., 2010), epilepsy (Bernstein and Boyden, 2011; Tye and Deisseroth, 2012), post-traumatic stress disorder (Sparta et al., 2013) and obesity (Krashes and Kravitz, 2014), among others. Subsequently, refining our understanding of neuronal processing has led to improving treatments by either refining the stimulation protocol or identifying a different target population.

The potential of optogenetics as a more finely targeted alternative to traditional neuromodulatory treatments, such as deep brain stimulation (DBS), has led to the development of optical DBS in rodent models (Aravanis et al., 2007) and proposals for its use in primates (Han and Boyden, 2007; Han, 2012). More recently, combining optical DBS with online monitoring of state has been possible: By integrating optogenetics with fMRI (Lee et al., 2010; Kahn et al., 2013), thus providing the critical link for evaluating the efficacy of the intervention. Specifically, this has been utilized to evaluate the use of optogenetics for the effective control of epileptic seizures, thus allowing the development of less disruptive interventions for temporal lobe epilepsy than are currently clinically available (Armstrong et al., 2013; Krook-Magnuson et al., 2013).

The ability to either increase or decrease activity is an essential aspect of making defined manipulations of targeted elements of the cortical circuit. However, identifying the optimal opsin for a given effect and target neuronal population is challenging. This is illustrated by the recent history of the development of optogenetic retinal prostheses. Originally, channelrhodopsin-2, an excitatory opsin, was proposed to be used to replace function in the retinal ganglion cells layers in conditions such as Retinitis Pigmentosa (RP) and Macular Degeneration (MD). Despite initially promising reports (reviewed in Busskamp et al., 2012), a later study concluded that channelrhodopsin-2 (ChR2) lacks the necessary sensitivity to make its use in a retinal prosthesis viable (Lagali et al., 2008). Soon after, halorhodopsin, an inhibitory opsin, was instead targeted to the photoreceptors. By changing the targeted circuit element, and sign of the perturbation applied, it was possible to significantly improve performance (Busskamp et al., 2010). Given that the retinal circuit has been mapped and extensively studied, this highlights the difficulty in identifying the optimal population to be targeted for even relatively simple networks.

The prospect of viable optically targeted treatments has been further advanced by the development of opsins that have effects beyond their immediate photoactivation, such as step-function opsins (SFO; Berndt et al., 2009) and stabilized step-function opsins (SSFO; Yizhar et al., 2011) that are able to sustain a photocurrent for longer durations (on the order of 30 min) before deactivation via illumination at another wavelength. These opsins thus offer the potential to alter the balance between excitation and inhibition over long-time scales. By doing so over large cortical areas, they offer the possibility to modulate activity which make them suited for treating conditions that are characterized gain dysfunction, such as depression, anxiety, autism, schizophrenia and attention deficit disorders (Yizhar et al., 2011), while minimizing the need for sustained activation. Independently of other clinical hurdles, the reality of this approach hinges on the stability of SFO and SSFOs to sustain a photocurrent over far longer durations, which drastically limits its potential as a therapy in itself. However, a substantial advantage of these opsins is that the strength of their modulation is dependent on the irradiance, which could provide a effective method by which to evaluate the magnitude of the shift required to restore healthy

neural functioning, thus indirectly assisting in the development of alternative therapies.

A further use of optogenetics exploits its ability for altering neural activity with high temporal precision. By using short, well-timed pulses, it is possible to not only induce short-term plasticity but also induce long-term potentiation and/or depression (Zhang and Oertner, 2006). This suggests the possibility of reprogramming circuits by either strengthening or weakening connections (Gu and Yakel, 2011; Larsen et al., 2014). The same technique has also been applied to promote regrowth following peripheral nerve injury (Li et al., 2011).

Together, these studies demonstrate that optogenetic technology has many useful properties, including specificity for targeting neuronal populations, activation flexibility due to the range of opsins available, and excellent spatiotemporal control. Given this, we now examine how well suited optogenetics is for augmenting brain function.

3. EXTENDING NEURAL FLOW OF INFORMATION THROUGH OPTICAL CONTROL

Neural augmentation technologies aim to enhance the cognitive capacity or sensorimotor function of the brain. The underlying principles here are conceptually similar to those of neuroprosthetics, in that they both involve altering neural activity or neural circuits in order to redefine input-output relationships. However, there is a fundamental difference. Replacing functionality requires only a crude approximation of absent activity, while enhancement can involve either refining existing activity whilst preserving the brain's ability to process existing signals, or alternatively incorporating new streams of information, thus allowing a neuron to sample input from additional stimuli. The difficulty in interfacing with the CNS whilst preserving natural activity can be observed even at the single cell level. In optogenetics, using high levels of illumination lead to optical initiation of an action potential that effectively overrides the neuron's behavior, so that it produces a spike irregardless of its inputs. A subtler approach to integrating additional signals into the nervous system might instead superimpose such signals onto the existing inputs to the neuron, thus allowing the addition of new information streams into neural circuits without necessarily disrupting the processing of existing streams.

The obstacle of effectively blending information in the brain is well-illustrated in the history of the visual prosthesis. Following earlier observations that applying electrical current to the occipital lobe resulted in the perception of phosphenes (Foerster, 1929), the first visual prosthesis was implanted into the cortex (Brindley and Lewin, 1968), preceding the first retinal prosthesis by nearly a decade (Dawson and Radtke, 1977). Despite improvements to electrode design in the decades since, such as increasing the density of cortical electrodes, as well as an ever-increasing understanding of the visual system, cortical visual prostheses have not yet matched the clinical success of retinal prostheses (Eiber et al., 2013), highlighting the difficulties of artificially altering neural activity within the brain. This is despite the presence of organizational features of the visual cortical areas, such as the retinotopic map, providing a topography for mapping information onto the surface of the cortex which could be coopted for brain-machine interfacing purposes. As one progresses synaptically further from the retina, visual information is organized along more complex—and generally less well understood—dimensions. Consequently, the lack of a well-understood topographic map makes neural interfacing with association areas more difficult. This is even more true of higher cortical areas, such as the prefrontal cortex, which receive information from multiple areas. Thus, although information relating to our interaction with the world may be mapped in some kind of ordered fashion onto its surface, our understanding of that map is incomplete and we are therefore currently unable to exploit it for brain augmentation.

However, there is evidence demonstrating that optogenetic manipulation for augmenting simple stimuli is possible. In a recent study, mice were trained to discriminate the location of a bar during whisking (O'Connor et al., 2013). After identifying the coding scheme used within the barrel cortex to encode the location of the bar stimulus, optogenetic perturbations were then applied that would encode, if successful, for the other potential position. The mice reported the bar in the incorrect (virtual) location. While this study was important for the insight it provided into the mechanisms of whisker stimulus encoding, it also provides us with a glimpse into the possibilities provided by illusionary or virtual stimuli. If it is possible to optically signal a false bar position during whisking, then it also hints at the potential of refining sensory inputs based upon additional, non-biological sensors—or potentially even of integrating entirely new sensory inputs into conscious awareness. For instance, it may be possible to augment conscious perception with input from non-visible parts of the electromagnetic spectrum, in order to provide additional sensory capability for working in dangerous environments (**Figure 1A**).

Augmenting sensory function supplies additional input to the relevant sensory cortex and require a detailed topological map of the neural organization. Similarly, motor outputs can be remapped onto robotic manipulators, allowing augmented motor functionality for external actuators. Neuroprostheses for augmenting motor functionality might use open-loop systems, requiring only the reading of signals from motor cortex. However, closed loop neural interfaces have proved to be important for making BMI a completely integrated replacement for motor function (Donoghue, 2002). In the context of optogenetic brain augmentation, this might involve a fully optical system (e.g., reading out signals from motor cortex using optical imaging of a genetically encodable calcium indicator, together with writing feedback signals into, for instance, somatosensory cortex using optical stimulation of opsins). This potentially also opens up the opportunity to improve learning by augmenting the trajectory error, which provides vital feedback during normal motor learning tasks (**Figure 1B**). Studies have shown that for gross movements, both error amplification and offsetting improve the amount and speed of adaptation during motor learning, using protocols normally targeted at

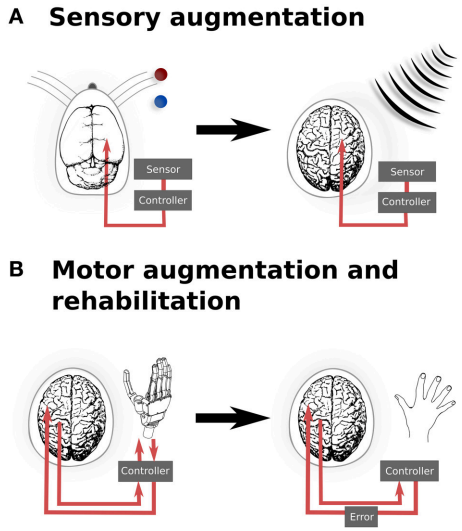

FIGURE 1 | Translation from experimental and existing therapeutics to clinical applications of neural augmentation using optogenetics. (A) Manipulation of somatosensory codes has been achieved in mouse barrel cortex, whereby optogenetically perturbing the sensory code caused the mouse to incorrectly report the location of the real bar as being in the blue position when the correct location was the red position (O'Connor et al., 2013). In a similar manner, directly interfacing into human somatosensory when an external sensor detects signals that are imperceptible for humans, such as electromagnetic radiation, may allow for additional sensory capabilities. **(B)** Currently, BMI applications only offer the possibility of open-loop applications, by reading neural activity to drive interfaces. Optogenetics could potentially extend BMI to provide currently sensory feedback from the prosthetic back, thus closing the feedback loop (left). This could be extended to refine motor control (right), by supplying an additional sensory error which has been proposed to improve motor rehabilitation following stroke (Wei et al., 2005; Celik et al., 2009). Together, these applications highlight the potential to develop applications from both animal models as well as existing applications, such as BMI. Images: prosthetic hand taken from Patent US Patent App. 10/488,008. Human brain (Wikimedia Commons); Mouse brain (Green, 1966).

motor rehabilitation following stroke (Wei et al., 2005; Celik et al., 2009). While these studies have been limited to error augmentation to gross movements, the spatial specificity of optogenetics when applied to topological maps presents the possibility for artificially inducing errors in either a motor-related cortices, to induce a trajectory bias (Wei et al., 2005), or with more difficulty, to generate errors related to subject's knowledge of the current state (Celik et al., 2009).

One requirement for a closed-loop system is the need for optimizing information transfer rates. Motivated by the need for more efficient methods for patients with neuromuscular disorders, much effort has been dedicated to developing classification schemas to optimize information transfer rates (Wolpaw et al., 2002; McFarland et al., 2003). Many BMIs currently use EEG, which is slower and population based, or implanted cortical electrodes, which improve on temporal and spatial resolution for decoding neural activity but lack encoding specificity, particularly for neural stimulation. By replacing this with optogenetics, it becomes possible to record signals with higher temporal and spatial resolution, thereby increasing accuracy, as well as stimulating defined cell types and patterns. This offers the potential of precise decoding of the activity of neural ensembles, which has implications beyond clinical BMI applications. In principle, the provision of a high bit-rate bidirectional interface between the brain and a computer would enable additional computational operations to be outsourced to a computer. This includes, for instance, possibilities ranging from decoding encrypted content using visualized passcodes, through to the external storage of memories (Berger et al., 2011). However, the development of such applications requires human subjects, and is thus unlikely to achieve ethical approval for these applications alone. Yet, as with electrode-based interfaces, such applications of optogenetic brain-machine interfaces may emerge as a by-product of clinical research.

In addition to memory, there is already some evidence that optogenetics can successfully alter cognitive and behavioral processing. In a recent study, it was possible to induce a negative behavior similar to obsessive-compulsive disorder (OCD) by targeting cortico-striatal projections, thus inducing behavior directly (Ahmari et al., 2013). The aim of the study was to test whether hyperstimulation of this pathway in mice would result in OCD-like behavior, which is unable to be tested clinically, but their success demonstrates that optogenetic intervention can change behavioral characteristics—with changes lasting up to weeks after the experiment ended. Similarly, another recent report investigated the role of serotonergic neurons within the dorsal raphe nucleus, which were already known to be involved in signalling reward. Using optogenetics, Miyazaki et al. (2014) established that mice extended their waiting time with a probability that inversely correlated with the delay before serotonergic neurons were optogenetically activated, leading them to conclude that precisely timed stimulation of serotonin neuron correlates with the willingness to wait—a quality they referred to as "patience."

From sensory and motor augmentation, through to modification of cognitive and behavioral traits, optogenetics uniquely has the specificity and precision to affect the neural correlates of these processes and to improve them. Yet for all the promise, this is still hampered by a common limitation: a deep understanding of the topographic mapping of information onto the relevant cortical areas and of the fine-grained neural coding of cognitive signals. Considerable progress in optogenetic augmentation of cognitive capacity will therefore have to wait for a more detailed understanding of "the cognitive neural code" before augmented neural function becomes an achievable reality.

4. CLINICAL CHALLENGES FOR OPTOGENETIC AUGMENTATION

Despite the very clear advantages that optogenetics offers for controlling neural activity, there are also four technical hurdles that exist before its translation to applied clinical use. These are opsin delivery, opsin choice, illumination strategies and optical actuators. Additionally, the ethical and regulatory hurdles raised by augmentation using optogenetics are manifold.

To date, the majority of optogenetic studies use mice, due to the large number of transgenic murine strains available for cellular targeting (e.g., using Cre recombinase, Madisen et al., 2012, and now intersectional genetic targeting approaches, Madisen et al., 2015). Cre recombinase targeting is however inapplicable in humans, thus an alternative method must be used. The most likely remaining candidate for opsin delivery is viral transfection, which uses viruses as carriers, such as adeno-associated viruses (AAV) or lentiviruses. Recent studies have confirmed that opsin delivery via AAV can be performed in species other than mice, including rats (Bass et al., 2010; Witten et al., 2010; Stefanik et al., 2013) and primates (Han, 2012). Furthermore, AAVs have been approved by the FDA for use in clinical trials (1995 for the first application; 2005 for the first application within the brain; Carter, 2005), thus removing a key hurdle for the use of optogenetics in clinical trials. An alternative strategy uses transplantable cells deliver opsins to marked sites (Weick et al., 2010); although this approach can suffer from extended opsin expression time, it raises the intriguing prospect of incorporating optogenetic addressability into new tissue formed by stem cell approaches to brain repair.

Different opsin variants have been shown to modify neuronal firing patterns in different ways. The insertion of CatCh, an excitatory opsin, prevented spikes following an initial volley spiking in fast-spiking cells, while the same cell class demonstrated sustained spiking when another actuating opsin, channelrhodopsin fast receiver (FR), was used instead (Mattis et al., 2012). The improvement of opsin design is ongoing, aiming for faster kinetics, preferred excitation wavelength, increased sensitivity and faster recovery (Lin, 2011). Among the newer generation of opsins are Chronos, a improved channelrhodopsin (Klapoetke et al., 2014), and JAWS, a red-shifted light-driven chloride pump (Chuong et al., 2014). As exhaustively testing all combinations of opsins and neuronal populations is impossible, this issue highlights the importance of good opsin characterization and models for determining suitable opsin-neuron combinations within any given neural circuit. In this, the development of accurate computational models of neurons, circuits (Potjans and Diesmann, 2014) and opsins (Nikolic et al., 2009; Grossman et al., 2013; Nikolic et al., 2013) will be vital for refining the matching of opsin to neuronal population and illumination protocols (Lin, 2011; Mattis et al., 2012; Jarvis et al., 2014).

A more practical concern is the light source, which can either be externally located or implantable. Similarly to the limitations that have troubled other biomedical devices, such as electrical DBS or retinal implants, both external and implantable devices have limitations: respectively, these are the need to open a pathway through the skin, and the need to provide power, either by an implanted battery or by transcutaneous transmission (O'Handley et al., 2008). Further practical limitations apply due to the need to deliver light to structures that can be located deep within the brain. Light penetration through tissue is limited by scattering. This is already limiting in mice (although new redshifted opsins such as ReaChR Lin et al., 2013 and JAWS Chuong et al., 2014, are increasing the achievable penetration depth). This factor becomes a major hurdle upon scaling up to human subjects. One approach to the solution of this problem is to target deep brain structures using penetrating optrodes, analogous to the use of penetrating electrodes in DBS. An additional issue that may arise in larger animals is to ensure the uniform spread of light over a wide area of tissue, i.e., ensuring that the falloff rate for illumination is sufficiently low such that the entirety of targeted areas are illuminated, whilst avoiding tissue damage due to overheating (Han, 2012).

Additionally, it is currently unclear that actuating an entire population of cells will be sufficient to provide useful input to the brain. While population firing rate codes have been demonstrated in some cortical areas (Georgopoulos et al., 1986), even firing rate codes may be "labeled line" codes in which cell identity matters (Montani et al., 2007; Schultz et al., 2009). Processing in some brain circuits has been proposed to occur through precise phase latencies within oscillatory activity (Engel et al., 2001), and the mechanisms underlying coding of cognitive information within the brain are still far from clear. For these latter schemes, whole field illumination of neurons in even a class-specific manner will clearly not be effective. Instead, only a specific subset of cells, such as an orientation column, should be targeted which is currently impossible using whole-field illumination.

This leads directly on to the last significant technical hurdle for clinical optogenetics: how can a large number of neurons within a population be driven with precise spatial, and defined temporal, resolution? The majority of optogenetic experiments to date have employed single photon excitation of opsins, typically supplied by a light emitting diode or fibre-coupled laser. The disadvantage with such approaches is that optogenetic activation is then not spatially limited, and consequently all opsin-expressing cells within an area of tissue are affected (**Figure 2A**). For some optogenetic applications, this may not be sufficient. Instead, it is critical to target subpopulations of neurons, then alternatives are available, such as the use of techniques such as multi-site light emitting diode arrays (Grossman et al., 2010). However, such approaches are in practice limited to the retina or *in vitro* preparations, as they provide negligible to no confinement within the axial plane (**Figure 2B**).

Two further possibilities exist that exploit optical technology to provide precise spatiotemporal patterning. The first is multiphoton excitation (Rickgauer and Tank, 2009), typically 2-photon (2P), which is spatially confined enough to allow the optical activation of individual cells. By using the interaction of two photons of longer wavelengths, which have less scatter, a small point spread function (PSF) is created that is constrained in the axial as well as lateral dimension (**Figure 2C**) and can be relocated in space, thus activating individual neurons throughout the tissue volume. However, as the PSF is typically smaller than the soma, 2P excitation is insufficient to initiate an action potential without scanning within the cell—which in turn reduces the temporal resolution of the technique (Rickgauer and Tank, 2009). Another recent approach uses wavefront shaping methods, such as digital holography or generalized phase contrast (**Figure 2D**), and by manipulating the phase of light with a spatial light modulator, create a defined spatial pattern of light that can similarly scan multiple neurons in a volume of tissue (Papagiakoumou et al., 2010; Oron et al., 2012). Both

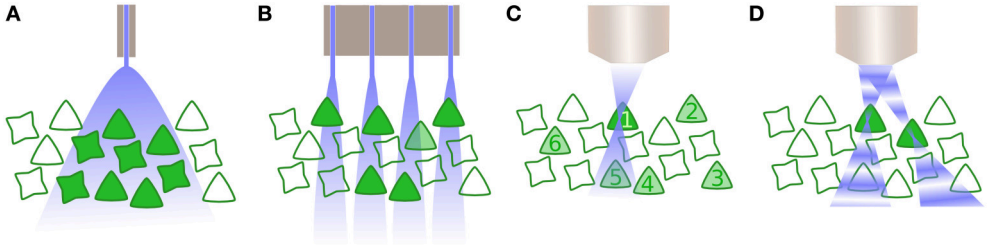

FIGURE 2 | Activating neurons to drive activity. (A) Whole field illumination via diode, either located at the cortical surface or inserted within the tissue. In both instances, all neurons within the illuminated area are activated. **(B)** An array of photodiodes illuminates subpopulations in the lateral plane, illustrated here in combination with opsin specificity for only one class of neuron. However, targeting is not constrained in the axial dimension and likewise drives multiple neurons within a column corresponding to each individual diode. **(C)** Multiphoton excitation results in a spatially constrained point spread function (PSF) in lateral and axial planes. Here, the 2P beam is targeted for the first neuron, whose soma is significantly larger than the PSF thus requiring scanning the neuron and increasing the dwell time. Driving other selected neurons within the population (2–6) is performed sequentially. **(D)** Digital holography utilizes wavefront shaping, in which a spatial light modulator alters the phase of beams in order to create multiple PSFs, allowing multiple neurons to be simultaneously but independently driven.

multiphoton excitation and wavefront shaping are limited at a rate proportional to the population size of the neurons to be targeted. Furthermore, despite being tested *in vivo* as well as *in vitro*, they only exist for head-fixed subjects, thus currently preventing its application for experiments including freely behaving subjects. Yet, as precise spatiotemporal patterning is likely to be necessary to take advantage of the full capabilities of optogenetic manipulation of brain circuitry, particularly where synaptic plasticity mechanisms are involved, this area of technological development is one to watch closely.

In addition to technical hurdles, the regulatory requirements for bringing optogenetics to clinical reality are also substantial. As discussed, the FDA has already cleared the use of AAV-delivered, providing an option for opsin delivery (Carter, 2005). However, opsin expression application in human, in which light-sensitive proteins are inserted into cellular membrane via genetic manipulation, has not been cleared and would likely require similar clearance to other gene therapies. This is compounded with the difficulty in determining the correct levels of opsin expression, as overexpression has been demonstrated to have cytotoxic effects. The FDA has recently cleared an application for optogenetic gene therapy for the treatment of RP, which will allow clinical trials in humans to commence (Francis et al., 2013; RetroSense, 2015). The treatment aims to restore photosensitivity of photoreceptors, bypassing the need for a separate light activation source. This is in contrast to cortical optogenetic application which would additionally require implanted optrodes, placing them in the highest band for both the FDA and EU regulatory approval. In addition to the usual considerations associated with implantable device, implanted optical devices have an additional constraint of minimization of energy via heat lost to prevent tissue damage. Determining the distribution of energy by optical delivery devices has been modeled (Ozden et al., 2013), however althought the FDA have determined power limits for MRI that allow temperature changes of 1°C, there are, as yet, no limits for power limits for chronically implanted optrodes (Ozden et al., 2013). For chronic usage, this may require significantly lower limits for power, thus restricting the range of illumination.

Finally, the ethical considerations that surround neuroenhancement are substantial and have been discussed elsewhere, including in this issue (Clark, 2014; Shook et al., 2014). Many of the same arguments for enhancement of cognitive abilities via pharamacological (Hyman, 2011), transcranial or electrical means are similarly applicable here for optogenetics: the development of a cognitive "arms race"; the question of who is in control of the augmenting device; and safety, both due to unintended consequences of manipulating neural activity as well as from the treament itself. The latter is particularly pertinent for the application of optogenetics, as it is highly invasive, requiring both manipulation of genetic material as well as the subcranial placement of devices to provide optical activation. This raises the ethical cost of optogenetics, such that its capacity for neuroenhancement must be substantially higher in comparison to other treatments to warrant its use preferentially. However, this provides an ethical impasse: how should these critera be tested and developed for optogenetics, while the risk and invasiveness of clinical optogenetics remains high? It may well be that such clinical trials may emerge as a by-product of optogenetic therapies for treatment of existing dysfunction or BMIs, rather than augmentation; or instead that it will ultimately require the use of similar but separate therapies, such as targeted nanoparticles (Carvalho-de Souza et al., 2015) which have the same advantages as optogenetics, but with diminished risks, to make neuroenhancement not only ethically more attractive but also clinically attainable.

5. CONCLUSION

Optogenetics has opened up a variety of new experimental paradigms in neuroscience. Its key advantage lies in the ability to target neuronal populations with precise spatiotemporal activation with immediate and reversible effect, which has been advantageous for untangling the contribution of neuronal populations in neural processing in both anatomical and behavioral studies (Tye and Deisseroth, 2012). These advantages also place optogenetics as a well-suited tool to assist in the

development of neurological therapies and, correspondingly, neural enhancements.

The application of optogenetics as a tool for *direct use* in neural modulation itself is less certain. The technological development of optogenetics is still in progress, with opsin molecular engineering, opsin delivery and optical stimulation techniques advancing rapidly, which will undoubtedly increase the precision of optogenetics and with it, our ability to manipulate neural circuits. However, the translation of optogenetics from research tool to clinical application has additional stipulations, particularly for its use in humans. From the challenges of opsin delivery to the difficulties in optically driving neurons with implantable devices, applying optogenetics outside of research remains a remote possibility in the foreseeable future. Yet in themselves, these elements are not the most significant constraint on the application of optogenetics for neural augmentation, which is instead our understanding of the neural codes that we are attempting to adapt. In the meantime, optogenetics offers a viable possibility for the development of novel neural therapies, by providing a robust capability to technically refine stimulation protocols and evaluate the effect of modulating activity levels of different neuronal populations.

ACKNOWLEDGMENTS

The authors would like to thank Konstantin Nikolic and Stephen Brickley for useful discussions on the uses and limitations of optogenetics, and the EU seventh framework programme (award PIEF-GA-2013-628086) and the BBSRC (awards BB/L018268/1and BB/K001817/1) for financial support.

REFERENCES

Ahmari, S. E., Spellman, T., Douglass, N. L., Kheirbek, M. A., Simpson, H. B., Deisseroth, K., et al. (2013). Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science* 340, 1234–1239. doi: 10.1126/science.1234733

Alilain, W. J., Li, X., Horn, K. P., Dhingra, R., Dick, T. E., Herlitze, S., et al. (2008). Light-induced rescue of breathing after spinal cord injury. *J. Neurosci.* 28, 11862–11870. doi: 10.1523/JNEUROSCI.3378-08.2008

Aravanis, A. M., Wang, L.-P., Zhang, F., Meltzer, L. A., Mogri, M. Z., Schneider, M. B., et al. (2007). An optical neural interface: *in vivo* control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J. Neural Eng.* 4, S143–S156. doi: 10.1088/1741-2560/4/3/s02

Armstrong, C., Krook-Magnuson, E., Oijala, M., and Soltesz, I. (2013). Closed-loop optogenetic intervention in mice. *Nat. Protocols* 8, 1475–1493. doi: 10.1038/nprot.2013.080

Bass, C. E., Grinevich, V. P., Vance, Z. B., Sullivan, R. P., Bonin, K. D., and Budygin, E. A. (2010). Optogenetic control of striatal dopamine release in rats. *J. Neurochem.* 114, 1344–1352. doi: 10.1111/j.1471-4159.2010.06850.x

Berger, T. W., Hampson, R. E., Song, D., Goonawardena, A., Marmarelis, V. Z., and Deadwyler, S. A. (2011). A cortical neural prosthesis for restoring and enhancing memory. *J. Neural Eng.* 8:046017. doi: 10.1088/1741-2560/8/4/046017

Berndt, A., Yizhar, O., Gunaydin, L. a., Hegemann, P., and Deisseroth, K. (2009). Bi-stable neural state switches. *Nat. Neurosci.* 12, 229–234. doi: 10.1038/nn.2247

Bernstein, J. G. and Boyden, E. S. (2011). Optogenetic tools for analyzing the neural circuits of behavior. *Trends Cogn. Sci.* 15, 592–600. doi: 10.1016/j.tics.2011.10.003

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268. doi: 10.1038/nn1525

Brindley, G. S. and Lewin, W. S. (1968). The sensations produced by electrical stimulation of the visual cortex. *J. Physiol.* 196, 479–493. doi: 10.1113/jphysiol.1968.sp008519

Busskamp, V., Duebel, J., Balya, D., Fradot, M., Viney, T. J., Siegert, S., et al. (2010). Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* 329, 413–417. doi: 10.1126/science.1190897

Busskamp, V., Picaud, S., Sahel, J. A., and Roska, B. (2012). Optogenetic therapy for retinitis pigmentosa. *Gene Ther.* 19, 169–175. doi: 10.1038/gt.2011.155

Carter, B. J. (2005). Adeno-associated virus vectors in clinical trials. *Hum. Gene Ther.* 16, 541–550. doi: 10.1089/hum.2005.16.541

Carvalho-de Souza, J. L., Treger, J. S., Dang, B., Kent, S. B., Pepperberg, D. R., and Bezanilla, F. (2015). Photosensitivity of neurons enabled by cell-targeted gold nanoparticles. *Neuron* 86 207–217. doi: 10.1016/j.neuron.2015.02.033

Celik, O., Powell, D., and Malley, M. K. (2009). "Impact of visual error augmentation methods on task performance and motor adaptation," in *Rehabilitation Robotics, 2009. ICORR 2009. IEEE International Conference on* (Kyoto: IEEE). 793–798.

Chuong, A. S., Miri, M. L., Busskamp, V., Matthews, G. A. C., Acker, L. C., Sø rensen, A. T., et al. (2014). Noninvasive optical inhibition with a red-shifted microbial rhodopsin. *Nat. Neurosci.* 17, 1123–1129. doi: 10.1038/nn.3752

Clark, V. P. (2014). The ethical, moral, and pragmatic rationale for brain augmentation. *Front. Syst. Neurosci.* 8:130. doi: 10.3389/fnsys.2014.00130

Dawson, W. W. and Radtke, N. D. (1977). The electrical stimulation of the retina by indwelling electrodes. *Invest. Ophthalmol. Vis. Sci.* 16, 249–252.

Degenaar, P., Grossman, N., Memon, M. A., Burrone, J., Dawson, M., Drakakis, E., et al. (2009). Optobionic vision–a new genetically enhanced light on retinal prosthesis. *J. Neural Eng.* 6:035007. doi: 10.1088/1741-2560/6/3/035007

Donoghue, J. P. (2002). Connecting cortex to machines: recent advances in brain interfaces. *Nat. Neurosci.* 5(Suppl.), 1085–1088. doi: 10.1038/nn947

Eiber, C. D., Lovell, N. H., and Suaning, G. J. (2013). Attaining higher resolution visual prosthetics: a review of the factors and limitations. *J. Neural Eng.* 10:011002. doi: 10.1088/1741-2560/10/1/011002

Engel, A. K., Fries, P., and Singer, W. (2001). Dynamic predictions: oscillations and synchrony in top-down processing. *Nat. Rev. Neurosci.* 2, 704–716. doi: 10.1038/35094565

Foerster, O. (1929). Beiträge zur Pathophysiologie der Sehbahn und der Sehsphäre. *J. Psychol. Neurol. (Leipzig)* 39, 463–485.

Francis, P. J., Mansfield, B., and Rose, S. (2013). Proceedings of the first international optogenetic therapies for vision symposium. *Transl. Vis. Sci. Technol.* 2:4. doi: 10.1167/tvst.2.7.4

Georgopoulos, A. P., Schwartz, A. B., and Kettner, R. E. (1986). Neuronal population coding of movement direction. *Science* 233, 1416–1419.

Gradinaru, V., Zhang, F., Ramakrishnan, C., Mattis, J., Prakash, R., Diester, I., et al. (2010). Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* 141, 154–165. doi: 10.1016/j.cell.2010.02.037

Green, E. L. (ed.). (1966). *Biology of the Laboratory Mouse.* New York, NY: McGraw-Hill Book Co.

Grossman, N., Poher, V., Grubb, M. S., Kennedy, G. T., Nikolic, K., McGovern, B., et al. (2010). Multi-site optical excitation using ChR2 and micro-LED array. *J. Neural Eng.* 7:16004. doi: 10.1088/1741-2560/7/1/016004

Grossman, N., Simiaki, V., Martinet, C., Toumazou, C., Schultz, S. R., and Nikolic, K. (2013). The spatial pattern of light determines the kinetics and modulates

backpropagation of optogenetic action potentials. *J. Comput. Neurosci.* 34, 477–488. doi: 10.1007/s10827-012-0431-7

Gu, Z. and Yakel, J. L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron* 71, 155–165. doi: 10.1016/j.neuron.2011.04.026

Han, X. (2012). Optogenetics in the nonhuman primate. *Prog. Brain Res.* 196, 215–233. doi: 10.1016/B978-0-444-59426-6.00011-2

Han, X. and Boyden, E. S. (2007). Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS ONE* 2:e299. doi: 10.1016/b978-0-444-59426-6.00011-2

Hyman, S. E. (2011). Cognitive enhancement: promises and perils. *Neuron* 69, 595–598.

Jarvis, S. J., Nikolic, K., and Schultz, S. R. (2014). Optical coactivation in cortical cells: reprogramming the excitation-inhibition balancing act to control neuronal gain in abstract and detailed models. *BMC Neurosci.* 15(Suppl. 1):F1. doi: 10.1186/1471-2202-15-S1-F1

Kahn, I., Knoblich, U., Desai, M., Bernstein, J., Graybiel, A. M., Boyden, E. S., et al. (2013). Optogenetic drive of neocortical pyramidal neurons generates fMRI signals that are correlated with spiking activity. *Brain Research* 1511, 33–45. doi: 10.1016/j.brainres.2013.03.011

Klapoetke, N. C., Murata, Y., Kim, S. S., Pulver, S. R., Birdsey-Benson, A., Cho, Y. K., et al. (2014). Independent optical excitation of distinct neural populations. *Nat. Methods* 11, 338–346. doi: 10.1038/nmeth.2836

Knöpfel, T. (2012). Genetically encoded optical indicators for the analysis of neuronal circuits. *Nat. Rev. Neurosci.* 13, 687–700. doi: 10.1038/nrn3293

Krashes, M. J. and Kravitz, A. V. (2014). Optogenetic and chemogenetic insights into the food addiction hypothesis. *Front. Behav. Neurosci.* 8:57. doi: 10.3389/fnbeh.2014.00057

Kravitz, A. V., Freeze, B. S., Parker, P. R. L., Kay, K., Thwin, M. T., Deisseroth, K., et al. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466, 622–626. doi: 10.1038/nature09159

Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltesz, I. (2013). On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat. Commun.* 4, 1376. doi: 10.1038/ncomms2376

Lagali, P. S., Balya, D., Awatramani, G. B., Münch, T. A., Kim, D. S., Busskamp, V., et al. (2008). Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat. Neurosci.* 11, 667–675. doi: 10.1038/nn.2117

Larsen, R. S., Smith, I. T., Miriyala, J., Han, J. E., Corlew, R. J., Smith, S. L., et al. (2014). Synapse-specific control of experience-dependent plasticity by presynaptic NMDA receptors. *Neuron* 83, 879–893. doi: 10.1016/j.neuron.2014.07.039

Lee, J. H., Durand, R., Gradinaru, V., Zhang, F., Goshen, I., Kim, D.-S., et al. (2010). Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. *Nature* 465, 788–792. doi: 10.1038/nature09108

Li, N., Downey, J. E., Bar-Shir, A., Gilad, A. A., Walczak, P., Kim, H., et al. (2011). Optogenetic-guided cortical plasticity after nerve injury. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8838–8843. doi: 10.1073/pnas.1100815108

Lin, J. Y. (2011). A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp. Physiol.* 96, 19–25. doi: 10.1113/expphysiol.2009.051961

Lin, J. Y., Knutsen, P. M., Muller, A., Kleinfeld, D., and Tsien, R. Y. (2013). ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat. Neurosci.* 16, 1499–1508. doi: 10.1038/nn.3502

Madisen, L., Garner, A. R., Shimaoka, D., Chuong, A. S., Klapoetke, N. C., Li, L., et al. (2015). Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron* 85, 942–958. doi: 10.1016/j.neuron.2015.02.022

Madisen, L., Mao, T., Koch, H., Zhuo, J.-m., Berenyi, A., Fujisawa, S., et al. (2012). A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. *Nat. Neurosci.* 15, 793–802. doi: 10.1038/nn.3078

Mattis, J., Tye, K., and Ferenczi, E. (2012). Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat. Methods* 9, 159–172. doi: 10.1038/nmeth.1808

McFarland, D. J., Sarnacki, W. A., and Wolpaw, J. R. (2003). Brain-computer interface (BCI) operation: optimizing information transfer rates. *Biol. Psychol.* 63, 237–251. doi: 10.1016/S0301-0511(03)00073-5

Miyazaki, K., Miyazaki, K., Tanaka, K., Yamanaka, A., Takahashi, A., Tabuchi, S., et al. (2014). Optogenetic activation of dorsal raphe serotonin neurons enhances patience for future rewards. *Curr. Biol.* 24, 2033–2040. doi: 10.1016/j.cub.2014.07.041

Montani, F., Kohn, A., Smith, M. A., and Schultz, S. R. (2007). The role of correlations in direction and contrast coding in the primary visual cortex. *J. Neurosci.* 27, 2338–2348. doi: 10.1523/JNEUROSCI.3417-06.2007

Nikolic, K., Grossman, N., Grubb, M. S., Burrone, J., Toumazou, C., and Degenaar, P. (2009). Photocycles of channelrhodopsin-2. *Photochem. Photobiol.* 85, 400–411. doi: 10.1111/j.1751-1097.2008.00460.x

Nikolic, K., Jarvis, S., Grossman, N., and Schultz, S. (2013). Computational models of optogenetic tools for controlling neural circuits with light. *IEEE Eng. Med. Biol. Soc.* 5, 2–5. doi: 10.1109/embc.2013.6610903

Nuffield Council on Bioethics (2013). *Novel Neurotechnologies: Intervening in the Brain*. London: Nuffield Council on Bioethics.

O'Connor, D. H., Hires, S. A., Guo, Z. V., Li, N., Yu, J., Sun, Q.-Q., et al. (2013). Neural coding during active somatosensation revealed using illusory touch. *Nat. Neurosci.* 16, 958–965. doi: 10.1038/nn.3419

O'Handley, R. C., Huang, J. K., Bono, D, C., and Simon, J. (2008). Improved wireless, transcutaneous power transmission for *in vivo* applications. *Sens. J. IEEE* 8, 57–62.

Oron, D., Papagiakoumou, E., Anselmi, F., and Emiliani, V. (2012). Two-photon optogenetics. *Prog. Brain Res.* 196, 119–143. doi: 10.1016/B978-0-444-59426-6.00007-0

Ozden, I., Wang, J., Lu, Y., May, T., Lee, J., Goo, W., et al. (2013). A coaxial optrode as multifunction write-read probe for optogenetic studies in non-human primates. *J. Neurosci. Methods* 219, 142–154. doi: 10.1016/j.jneumeth.2013.06.011

Papagiakoumou, E., Anselmi, F., Bègue, A., de Sars, V., Glückstad, J., Isacoff, E. Y., et al. (2010). Scanless two-photon excitation of channelrhodopsin-2. *Nat. Methods* 7, 848–854. doi: 10.1038/nmeth.1505

Potjans, T. C. and Diesmann, M. (2014). The cell-type specific cortical microcircuit: relating structure and activity in a full-scale spiking network model. *Cereb. Cortex* 24, 785–806. doi: 10.1093/cercor/bhs358

RetroSense (2015). *Retrosense Therapeutics Lead Gene Therapy Candidate Gets Fda Clearance to Proceed to First-in-human Clinical Trials.* Available online at: http://www.businesswire.com/news/home/20150824005576/en/RetroSense-Therapeutics (Accessed on Augest 24, 2015)

Rickgauer, J. P. and Tank, D. W. (2009). Two-photon excitation of channelrhodopsin-2 at saturation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15025–15030. doi: 10.1073/pnas.0907084106

Schultz, S. R., Kitamura, K., Post-Uiterweer, A., Krupic, J., and Häusser, M. (2009). Spatial pattern coding of sensory information by climbing fiber-evoked calcium signals in networks of neighboring cerebellar Purkinje cells. *J. Neurosci.* 29, 8005–8015. doi: 10.1523/JNEUROSCI.4919-08.2009

Shook, J. R., Galvagni, L., and Giordano, J. (2014). Cognitive enhancement kept within contexts: neuroethics and informed public policy. *Front. Syst. Neurosci.* 8:228. doi: 10.3389/fnsys.2014.00228

Sparta, D. R., Jennings, J. H., Ung, R. L., and Stuber, G. D. (2013). Optogenetic strategies to investigate neural circuitry engaged by stress. *Behav. Brain Res.* 255, 19–25. doi: 10.1016/j.bbr.2013.05.007

Stefanik, M. T., Moussawi, K., Kupchik, Y. M., Smith, K. C., Miller, R. L., Huff, M. L., et al. (2013). Optogenetic inhibition of cocaine seeking in rats. *Addict. Biol.* 18, 50–53. doi: 10.1111/j.1369-1600.2012.00479.x

Tye, K. M. and Deisseroth, K. (2012). Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nat. Rev. Neurosci.* 13, 251–266. doi: 10.1038/nrn3171

Wei, Y., Bajaj, P., Scheidt, R., and Patton, J. (2005). "Visual error augmentation for enhancing motor learning and rehabilitative relearning, " in *Rehabilitation Robotics, 2005. ICORR 2005. 9th International Conference on* (Chicago, IL: IEEE), 505–510.

Weick, J. P., Johnson, M. A., Skroch, S. P., Williams, J. C., Deisseroth, K., and Zhang, S.-C. (2010). Functional control of transplantable human ESC-derived neurons via optogenetic targeting. *Stem cells* 28, 2008–2016. doi: 10.1002/stem.514

Witten, I. B., Lin, S.-C., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., et al. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* 330, 1677–1681. doi: 10.1126/science.1193771

Wolpaw, J. R., Birbaumer, N., McFarland, D. J., Pfurtscheller, G., and Vaughan, T. M. (2002). Brain-computer interfaces for communication and control. *Clin. Neurophysiol.* 113, 767–791. doi: 10.1016/S1388-2457(02)00057-3

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., et al. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477, 171–178. doi: 10.1038/nature10360

Zhang, Y.-P. and Oertner, T. G. (2006). Optical induction of synaptic plasticity using a light-sensitive channel. *Nat. Methods* 4, 139–141. doi: 10.1038/nmeth988

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 20 January 2015
doi: 10.3389/fnsys.2014.00255

The vestibular implant: frequency-dependency of the electrically evoked vestibulo-ocular reflex in humans

Raymond van de Berg[1,2]*, Nils Guinand[3], T. A. Khoa Nguyen[4], Maurizio Ranieri[3], Samuel Cavuscens[3], Jean-Philippe Guyot[3], Robert Stokroos[1], Herman Kingma[1,2] and Angelica Perez-Fornos[3]

[1] *Division of Balance Disorders, Department of Otorhinolaryngology and Head and Neck Surgery, Faculty of Health Medicine and Life Sciences, School for Mental Health and Neuroscience, Maastricht University Medical Center, Maastricht, Netherlands*
[2] *Faculty of Physics, Tomsk State University, Tomsk, Russia*
[3] *Service of Otorhinolaryngology and Head and Neck Surgery, Department of Clinical Neurosciences, Geneva University Hospitals, Geneva, Switzerland*
[4] *Translational Neural Engineering Lab, Center for Neuroprosthetics, Interfaculty Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Bernard Cohen, Mount Sinai School of Medicine, USA
Hans VanDerSteen, Erasmus Medical Center, Netherlands
Stefano Ramat, Università degli Studi di Pavia, Italy
Maria Cervera De La Rosa, Universidad Carlos III de Madrid, Spain

***Correspondence:**
Raymond van de Berg, Department of Otorhinolaryngology and Head and Neck Surgery, Maastricht University Medical Center, Postbus 5800, 6202 AZ Maastricht, Netherlands
e-mail: raymond.vande.berg@mumc.nl

The vestibulo-ocular reflex (VOR) shows frequency-dependent behavior. This study investigated whether the characteristics of the electrically evoked VOR (eVOR) elicited by a vestibular implant, showed the same frequency-dependency. Twelve vestibular electrodes implanted in seven patients with bilateral vestibular hypofunction (BVH) were tested. Stimuli consisted of amplitude-modulated electrical stimulation with a sinusoidal profile at frequencies of 0.5, 1, and 2 Hz. The main characteristics of the eVOR were evaluated and compared to the "natural" VOR characteristics measured in a group of age-matched healthy volunteers who were subjected to horizontal whole body rotations with equivalent sinusoidal velocity profiles at the same frequencies. A strong and significant effect of frequency was observed in the total peak eye velocity of the eVOR. This effect was similar to that observed in the "natural" VOR. Other characteristics of the (e)VOR (angle, habituation-index, and asymmetry) showed no significant frequency-dependent effect. In conclusion, this study demonstrates that, at least at the specific (limited) frequency range tested, responses elicited by a vestibular implant closely mimic the frequency-dependency of the "normal" vestibular system.

Keywords: vestibular implant, vestibular prosthesis, neural prosthesis, bilateral vestibular areflexia, bilateral vestibulopathy, vestibulo-ocular reflex

INTRODUCTION

Bilateral vestibular hypofunction (BVH) is most often a chronic condition in which patients can suffer from blurred vision (oscillopsia), impaired spatial orientation and postural instability (Brandt et al., 2010; van de Berg et al., 2011; Hain et al., 2013). These and other symptoms lead to an important decrease in physical activity, social functioning and vitality that dramatically impact the patients' quality of life (Guinand et al., 2012; Ward et al., 2013). The prognosis of BVH is poor and more than 80% of the patients do not improve (Zingler et al., 2008; McCall and Yates, 2011). Until now, treatment options are limited and with low yield (Porciuncula et al., 2012).

A vestibular implant, in a concept analogous to that of the cochlear implant, has been postulated as a possible therapeutic alternative. This idea is currently investigated by research groups in Europe and the United States. Research both from animal and human studies have demonstrated that electrical stimulation is an effective means to activate the vestibular system (Fridman et al., 2010; Guyot et al., 2011; Lewis et al., 2013; Golub et al., 2014). Considerable research efforts have been devoted to the investigation of the electrically evoked vestibulo-ocular reflex (eVOR). Promisingly, results showed that it is possible to elicit a VOR which corresponds to the plane of the canal which is innervated by the electrically stimulated nerve branch (Gong and Merfeld, 2002; Della Santina et al., 2007; Merfeld et al., 2007; Wall et al., 2007; Fridman et al., 2010; van de Berg et al., 2011; Perez Fornos et al., 2014). Current efforts focus on optimizing stimulation paradigms (Davidovics et al., 2011, 2012), on improving the alignment of the eVOR (Migliaccio et al., 2011; Dai et al., 2013; Davidovics et al., 2013), and on investigating the adaptive properties of the eVOR (Merfeld et al., 2006, 2007; Lewis et al., 2010; Guyot et al., 2011; Dai et al., 2013). Important efforts are also undertaken to improve surgical techniques (Feigl et al., 2009; Dai et al., 2011a; Bierer et al., 2012; Rubinstein et al., 2012; van de Berg et al., 2012) and to solve biomechanical issues (Wall et al., 2003; Hayden et al., 2011; van de Berg et al., 2011; Fridman and Della Santina, 2013).

The Geneva-Maastricht group has recently demonstrated that it is possible to restore the VOR in patients with BVH, using a chronically implanted vestibular implant prototype (Perez Fornos et al., 2014). During these experiments, some frequency dependent effects were observed. Frequency-dependency is a well-known feature of the vestibular system. Gain of the semicircular canals (peak eye velocity/peak head velocity) is high for middle frequencies, but decreases with lower and higher frequencies, consistent with the mechanical properties of the semicircular canals

(Barnes, 1993). Interestingly, these middle frequency movements are often encountered by individuals in daily life, for example during voluntary head movements and locomotor activities (Barnes, 1993; Crane and Demer, 1997). Therefore, it is important to further investigate the frequency dependent behavior of the eVOR and how it compares to the frequency dependent characteristics of the "natural" VOR in healthy subjects. This was the main objective of this study.

MATERIALS AND METHODS

IMPLANTED PATIENTS

Between 2007 and 2013, 11 volunteers with BVH received a vestibular implant prototype consisting of a modified cochlear implant (MED-EL, Innsbruck, Austria) with extra-cochlear branches for vestibular stimulation (Guinand et al., Submitted). The devices, inclusion criteria, and surgical techniques have been described in detail previously (Perez Fornos et al., 2014; Guinand et al., Submitted). Seven of them were available for this study (age 46–68 years; mean age 61.4 years; see **Table 1**). Twelve electrodes at different anatomical locations were tested: four electrodes implanted in the vicinity of the superior ampullary nerve (SAN), three electrodes implanted in the vicinity of the lateral ampullary nerve (LAN) and five electrodes implanted in the vicinity of the posterior ampullary nerve (PAN).

HEALTHY SUBJECTS

Seven age-matched healthy volunteers with a blank history for vestibular disorders were selected for the comparison experiments. These tests involved three men and four women (age 59–69 years; mean age 62.7 years).

ELECTRICAL STIMULATION

Electrical stimulation was delivered exclusively to one vestibular electrode at a time. The activation procedure has been previously described (Guyot et al., 2011; Perez Fornos et al., 2014). Briefly, the generation of bi-directional eye movements (e.g., both leftwards and rightwards for stimulation of the LAN) with unilateral electrical stimulation requires first that a "baseline stimulation" (i.e., constant amplitude) is delivered by the vestibular electrode. Then, up- and down-modulation of this "baseline stimulation" effectively results in the generation of smooth, bi-directional eye movements.

Stimulation consisted of amplitude modulated, charge-balanced, cathodic-first, biphasic pulses (200 μs/phase) presented at a pulse rate of 400 pulses/sec. "Baseline stimulation" amplitudes corresponded to the middle of each patient's dynamic range (see Perez Fornos et al., 2014; Guinand et al., Submitted; for details on the determination of thresholds, upper comfortable level and resulting dynamic range). Modulation strengths for each patient/electrode were selected to correspond to 50–75% of the corresponding dynamic range and were kept constant throughout the experiments. **Figure 1** illustrates this electrical stimulation procedure.

STUDY DESIGN

All tests were conducted in a controlled laboratory setting and performed in complete darkness. All participants (from both groups) were instructed to sit still, look in front of them and keep their eyes open during the trials. If necessary, alerting tasks were given to improve concentration and general level of arousal, in order to obtain as reproducible results as possible.

In order to test the eVOR as specifically as possible without any influences of other inputs like residual vestibular function, the eVOR-experiments were conducted in stationary conditions (e.g., without any head or body movement). Patients sat comfortably in a stationary chair while the implant was activated. Each electrode was separately tested with a fixed sequence of approximately 60 sinusoidal cycles of amplitude modulated electrical stimulation (see details in Section Electrical Stimulation). The strength (i.e., intensity) of modulation was kept constant throughout experimental trials and 3 modulation frequencies (0.5, 1, and 2 Hz) were tested. Lower modulation frequencies were intentionally excluded, since previous investigations (in exactly the same conditions) showed only very small eVOR responses at these frequencies (Perez Fornos et al., 2014). Furthermore, 60-cycle trials at low modulation frequencies below 0.5 Hz resulted in very long sessions, which severely compromised the attention of the patients for the rest of the testing session (Perez Fornos et al., 2014). All tests for a given electrode were performed on the same day.

Modulation of the frequency of the electrical stimulus would correspond in real life to modulation of the frequency of the head velocity stimulus in dynamic situations. Therefore, the eVOR obtained by electrical stimulation in patients with BVH was compared to the "natural" VOR obtained in healthy volunteers during velocity controlled whole body rotations. Healthy volunteers were subjected to 60-cycle trials of horizontal whole body rotations in a custom-made, velocity-controlled rotatory chair (Nystagliner Pro; Erich Jaeger Gmbh). Rotations followed the same sinusoidal

Table 1 | Main characteristics of the tested patients with bilateral vestibular hypofunction.

Subject	Sex	Tested electrode(s)	Age at implantation	Etiology	Year of implantation	Surgical approach
BVH1	M	SAN; LAN	67	DFNA-9	2012	Intra-labyrinthine
BVH2	F	PAN	48	Meningitis	2012	Intra-labyrinthine
BVH3	M	SAN; LAN; PAN	66	DFNA-9	2013	Intra-labyrinthine
BVH4	F	SAN; PAN	68	DFNA-9	2013	Intra-labyrinthine
BVH5	F	SAN; LAN	67	Traumatic	2013	Intra-labyrinthine
BVH6	M	PAN	46	Idiopathic	2008	Extra-labyrinthine
BVH7	M	PAN	68	Idiopathic	2007	Extra-labyrinthine

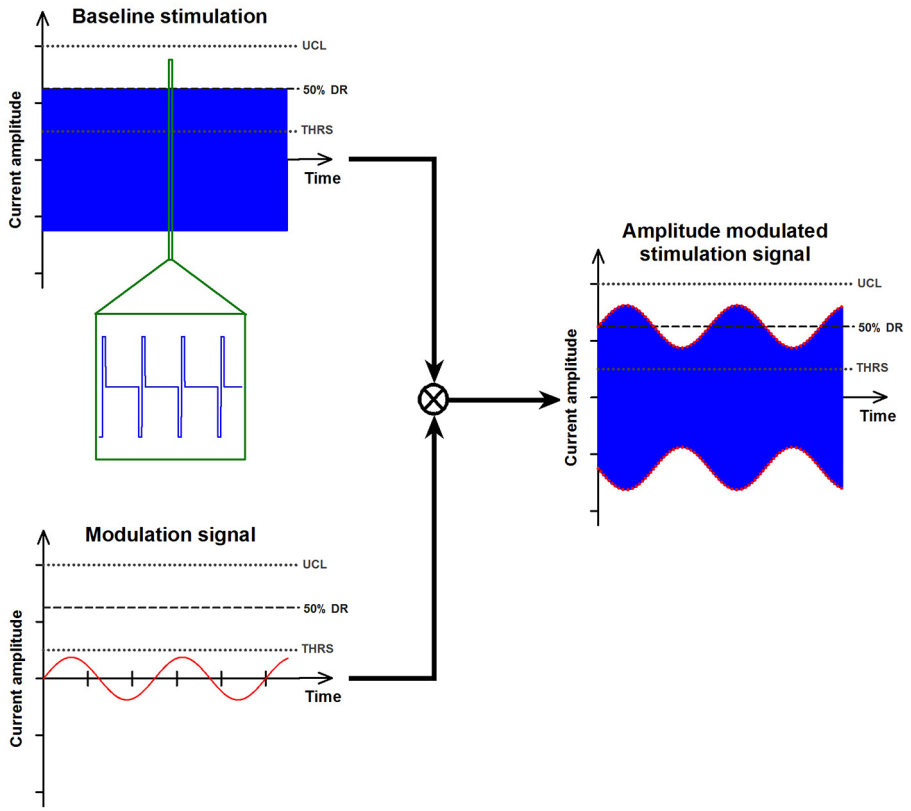

FIGURE 1 | Illustration of the electrical stimulation paradigm. Stimulation was delivered to one vestibular electrode at a time. Patients first received baseline electrical stimulation that consisted of constant amplitude trains of biphasic, cathodic-first pulses (upper-left panel). Baseline stimulation was presented at a fixed pulse rate of 400 pulses per second. Its intensity corresponded to 50% of each electrode's previously measured dynamic range (DR; current range between the vestibular threshold -THRS- and the upper comfortable level -UCL-). Once patients were in "adapted" state, the baseline stimulation could be modulated in amplitude using a signal with a sinusoidal profile (see lower-left panel). The strength (i.e., intensity) of the modulation was kept constant and its frequency was varied between 0.5 and 2 Hz. The right panel shows an example of such an amplitude modulated stimulation signal (blue trace). The envelope of the modulation signal (red dotted lines) has been highlighted for clarity.

profile as electrical stimuli (same frequency range of 0.5, 1, and 2 Hz) and had a peak velocity of 30°/s.

EYE MOVEMENT RECORDING AND ANALYSIS

Bidimensional eye movements (i.e., horizontal and vertical eye position, no torsion) were recorded with the EyeSeeCam system (EyeSeeCam VOG; Munich, Germany) (Bartl et al., 2009; Perez Fornos et al., 2014). Motivation for this choice, as well as the data-processing using cycle-by-cycle analysis and calculation of gain were described previously (Perez Fornos et al., 2014; Guinand et al., Submitted). An example of eye movement data processing is presented in **Figure 2**. Analysis was performed on as many valid cycles (free of saccades and blinks) as possible (minimum 43, maximum 60).

Total peak eye velocity was calculated as the square root of the sums of the squares of horizontal and vertical peak eye velocity. To facilitate the analysis of the frequency-dependent behavior of peak eye velocities of different magnitudes, peak eye velocities per modulation frequency were normalized to the highest measured peak eye velocity per electrode. Angle of the (e)VOR (with respect to the horizontal axis) was defined as the angle between the horizontal and vertical peak eye velocity (Guinand et al., Submitted). The habituation-index was determined by the mean peak eye velocities of the last 10 cycles, divided by the mean peak eye velocities of the first 10 cycles. The asymmetry-index (ratio of excitatory/inhibitory half cycle gain) of the most prominent component (horizontal or vertical) was calculated as (excitatory half cycle gain—inhibitory half cycle gain)/(excitatory half cycle gain + inhibitory half cycle gain) (Dai et al., 2011b) and converted into an absolute value.

STATISTICS

Since normality tests conducted on individual results often failed the normality assumption, individual results (per subject/electrode) are presented as median values, as well as the 25th–75th percentiles. Group results conformed to normal distributions and are therefore presented as mean values ± standard error of the mean (SEM).

Statistics were performed using analysis of variance (ANOVA) modules from IBM SPSS Statistics v22 (IBM Corporation, New York, United States of America). Raw scores were used as input for comparative tests regarding angle, habituation-index and

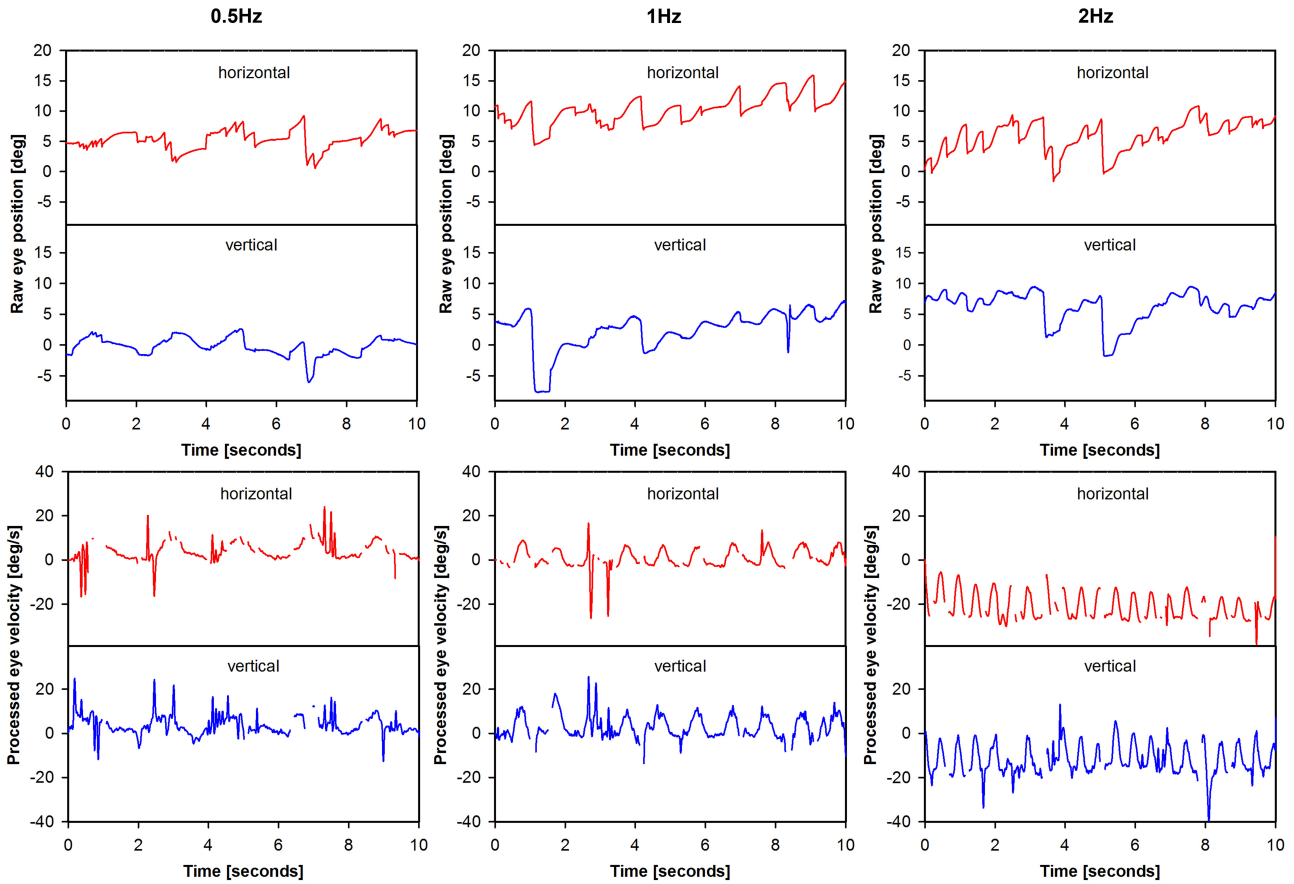

FIGURE 2 | Illustration of eye movement data processing. The figure presents eye movement data tracings for patient BVH1-LAN. Two steps are illustrated for each modulation frequency: raw eye position (e.g., before any processing was performed) and processed eye velocity data.

asymmetry-index. For total peak eye velocity, variance differed between the groups. Therefore, data were first normalized before proceeding to statistical analysis. A significance level of 0.05 was chosen to detect significant differences within and across groups.

ETHICAL CONSIDERATIONS

This study was in accordance with the Declaration of Helsinki (amended version 2013). The testing protocol was approved by the ethical committees of the Maastricht University Medical Center (NL36777.068.11/METC 11-2-031) and the Geneva University Hospital (NAC 11-080).

RESULTS

CHARACTERISTICS OF THE eVOR

The first objective of this paper was to describe the main characteristics of eVOR-responses. Four main characteristics were studied: total peak eye velocity, angle of the eVOR (with respect to the horizontal axis), the habituation-index over 60 cycles, and the asymmetry (ratio of excitatory/inhibitory half cycle gain) of the response.

Total peak eye velocity results obtained per electrode are presented in **Table 2**. Consistent with previous observations (Guinand et al., Submitted), inter-subject variability was high. The medians of the total peak eye velocity for the electrodes ranged from 0.6°/s (BVH4-PAN, 0.5 Hz) up to 21.5°/s (BVH5-SAN, 2 Hz).

To facilitate comparison of the results across patients and across frequencies, total peak eye velocity results were normalized to the highest values per electrode. Individual and pooled results revealed a clear frequency-dependent behavior for the three stimulation sites (**Figure 3**). In general, the lowest peak eye velocities were obtained with a modulation frequency of 0.5 Hz. Peak eye velocities progressively increased with increasing modulation frequency, reaching a maximum at 2 Hz. Note however that interestingly, patient BVH2 showed an opposite behavior (stimulation of the PAN). There was a statistically significant effect of modulation frequency [$F_{(2,\ 27)} = 16.25$, $p < 0.001$] but not for stimulation site. *Post-hoc* pairwise comparisons (Tukey) indicated that the difference in mean normalized peak eye velocities was statistically significant ($p < 0.05$; 0.5 Hz: 0.53 ± 0.08; 1 Hz: 0.72 ± 0.05; 2 Hz: 0.98 ± 0.02).

Figure 4A shows individual angle results (with respect to the horizontal axis) for each stimulation site. Results for each stimulation site were very variable across subjects. No clear effect of modulation frequency could be distinguished either. For example, stimulation of the SAN in BVH3 (dark green triangles in

Table 2 | Medians, 25th percentiles, and 75th percentiles of total peak eye velocity (°/s) for all electrodes, per modulation frequency (perc. = percentile).

Electrode	Frequency (Hz)	Median	25th perc.	75th perc.
BVH1-SAN	0.5	4.2	2.4	5.3
	1	5.4	3.3	7.6
	2	6.9	5.2	9.1
BVH1-LAN	0.5	6.9	5.3	8.6
	1	9.1	7.1	10.4
	2	9.3	7.3	11.4
BVH2-PAN	0.5	8.5	6.4	9.8
	1	8.3	7.8	9.0
	2	6.9	6.2	7.7
BVH3-SAN	0.5	1.1	0.9	1.5
	1	3.9	3.1	5.0
	2	5.6	4.6	7.2
BVH3-LAN	0.5	0.9	0.6	1.2
	1	1.8	1.4	2.5
	2	3.6	2.2	4.8
BVH3-PAN	0.5	2.0	1.5	2.5
	1	5.7	5.2	6.4
	2	8.6	8.0	9.7
BVH4-SAN	0.5	0.8	0.5	1.1
	1	1.4	1.0	1.7
	2	3.6	2.4	4.4
BVH4-PAN	0.5	0.6	0.5	0.8
	1	1.0	0.6	1.3
	2	1.5	1.0	2.3
BVH5-SAN	0.5	12.4	10.8	14.1
	1	13.5	10.8	17.4
	2	21.5	16.6	24.9
BVH5-LAN	0.5	6.8	6.0	8.1
	1	8.0	6.8	9.5
	2	11.1	9.0	12.9
BVH6-PAN	0.5	10.2	8.1	13.5
	1	12.5	10.6	14.7
	2	12.0	9.8	14.6
BVH7-PAN	0.5	4.0	3.5	4.4
	1	4.0	2.8	5.0
	2	5.7	4.6	7.5

Figure 4A) showed, as expected, angles with a predominantly vertical component ranging from 59 to 83°. The eye movement response progressively shifted toward the vertical axis (the angle increased) as modulation frequency increased. However, results for the same stimulation site were completely different in the case of patient BVH5 (purple triangles in **Figure 4A**). Surprisingly, this patient showed median angles with a predominantly horizontal component, ranging from 12 to 14° during stimulation of the SAN. Furthermore, median angles remained relatively stable across modulation frequencies for this patient. Similar inter-subject variability was observed for stimulation of the LAN and the PAN.

Mean results across stimulation sites (gray plots in **Figure 4B**) showed that overall, the stimulation site with the most vertical eVOR response was the PAN (58.6 ± 5.5°). There were only very small differences in angles between stimulation of the SAN and the LAN (respectively 46.8 ± 6.1° and 40.4 ± 7.1°). Differences across modulation frequencies were small, both when each stimulation site was considered separately and when data from all stimulation sites was pooled (black plot in **Figure 4B**). There was no statistically significant main effect of modulation frequency or stimulation site.

Figure 5A shows individual median habituation-indexes per patient and for each stimulation site. Results across subjects and across stimulation sites were again quite variable. Results were very variable from one stimulation site to another in the same patient (e.g., results for patient BVH3, dark green plots in **Figure 5A**). Habituation could also be very different when comparing the same stimulation site between patients (e.g., compare results for stimulation of the PAN, squares in **Figure 5A**).

No clear effect of modulation frequency could be distinguished either. While in some cases habituation seemed to be more important (i.e., indexes became lower) at higher modulation frequencies (e.g., BVH4-SAN, olive green triangles in **Figure 5A**), in other cases the inverse trend was observed (e.g., BVH1-SAN). Mean results across stimulation sites and for all stimulation sites together showed a clearer picture (**Figure 5B**). Habituation-indexes for stimulation of the PAN were in general higher (1.08 ± 0.09), reflecting less adaptation than stimulation of the SAN and the LAN (respectively 0.72 ± 0.11 and 0.78 ± 0.12). This difference was only statistically significant between stimulation of the SAN and the PAN ($p < 0.05$). Another interesting observation from pooled results was that in general, habituation was more important for the 2 Hz modulation frequency than for 0.5 and 1 Hz. However, the effect of modulation frequency, as well as the interaction effect between modulation frequency and stimulation site, were not statistically significant.

Figure 6 displays asymmetry-indexes for each patient and each stimulation site. Values were <0.3 in all cases. The patient showing the most asymmetrical responses was BVH5 (purple plots in **Figure 6A**) and the one with the most symmetrical responses was BVH4 (olive green plots in **Figure 6A**), particularly for stimulation of the SAN. No systematic frequency-dependent behavior was observed in individual results. Group results (**Figure 6B**) confirmed that asymmetry was in general low, and some variability between stimulation sites was also observed. There were no significant effects of modulation frequency or stimulation site. The interaction effect between modulation frequency and stimulation site was not statistically significant either.

THE eVOR vs. THE "NATURAL" VOR

The second goal of this study was to compare the previously described eVOR-characteristics with those of the "natural" VOR

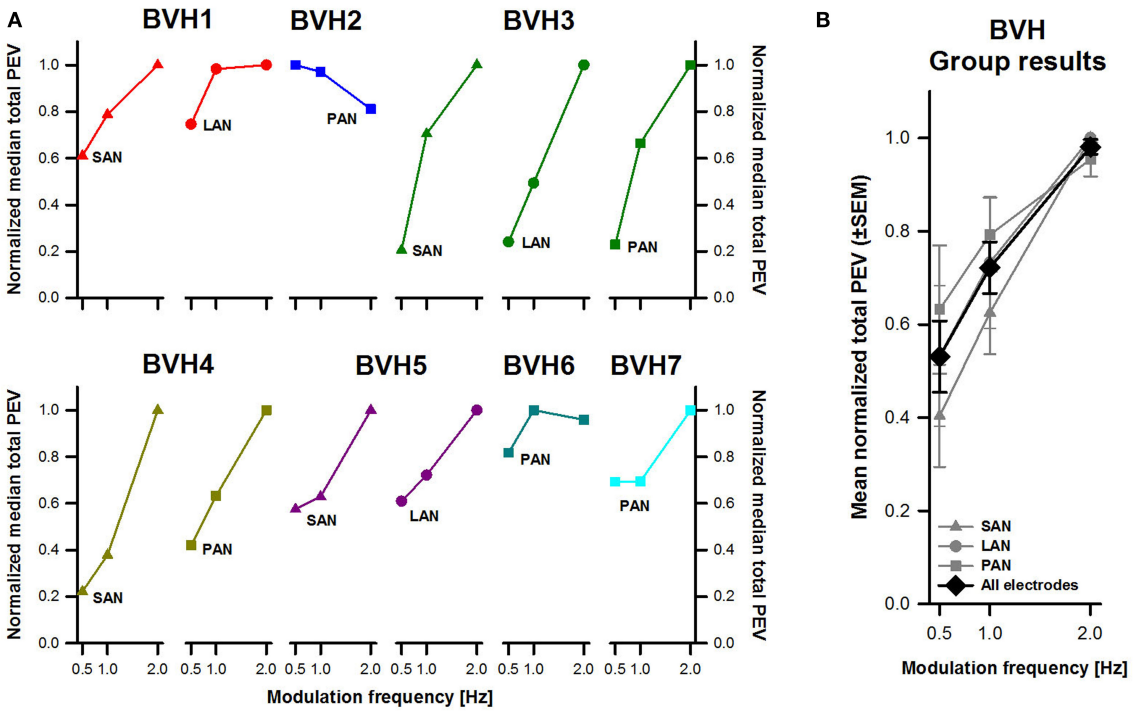

FIGURE 3 | Normalized total peak eye velocity (PEV) vs. modulation frequency. (A) Each panel represents individual results obtained during stimulation for each stimulation site (SAN, LAN, PAN). **(B)** Mean results (±SEM) calculated across patients for each stimulation site (gray plots) and for all electrodes together (black plot).

observed in the group of healthy volunteers. The results of this comparison are summarized in **Figure 7**.

Figure 7A compares the frequency-dependent behavior of the normalized total peak eye velocity of the eVOR to that of the "natural" VOR. From this figure it is clear that both show a strikingly similar frequency-dependent behavior, with the lower peak eye velocities measured at 0.5 Hz (0.53 ± 0.08 for the BVH-group and 0.54 ± 0.05 for the group of healthy volunteers). Peak eye velocities increase progressively with increasing frequency reaching a maximum at 2 Hz (BVH-group: 0.72 ± 0.06 at 1 Hz and 0.98 ± 0.01 at 2 Hz; Group of healthy volunteers: 0.73 ± 0.08 at 1 Hz and 0.99 ± 0.003 at 2 Hz). A Two-Way between-groups ANOVA confirmed a significant effect of frequency [$F_{(2,\ 51)} = 29.39$, $p < 0.001$], but no significant difference between both groups. The interaction between both variables was not significant either.

Figure 7B compares eVOR angles to those of the "natural" VOR. The angles of the "natural" VOR are close to zero (i.e., practically horizontal) and remained relatively stable across modulation frequencies, consistent with the direction of applied whole body rotations. As described previously, mean eVOR angles were much higher, with a predominantly vertical component and also remained relatively stable across modulation frequencies. A Two-Way between-groups ANOVA confirmed a significant difference between both groups [$F_{(1,\ 51)} = 84.91$, $p < 0.001$], but no significant effect of frequency. The interaction effect was not significant either.

Figure 7C compares the habituation-index of the eVOR to that of the "natural" VOR. Habituation-indexes were close to one (i.e., meaning very little adaptation) for both groups at 0.5 and 1 Hz and only slightly decreased at 2 Hz. There was no significant difference between groups or across modulation frequencies. The interaction effect was not significant either.

Finally, **Figure 7D** compares the asymmetry-indexes of the eVOR to those of the "natural" VOR. Mean asymmetry for the eVOR ranged between 0.10 (0.5 Hz) and 0.13 (2 Hz). Mean asymmetry for the "natural" VOR was much lower and close to 0, although values slightly increased at 2 Hz. A Two-Way between-groups ANOVA confirmed a significant difference between both groups [$F_{(1,\ 51)} = 37.64$, $p < 0.001$], but no significant effect of modulation frequency. The interaction effect was not significant either.

DISCUSSION

The goal of this study was to investigate how the characteristics of the eVOR change as a function of modulation frequency in the first group of patients implanted with a vestibular implant prototype, and to compare these results to the "natural" VOR responses obtained in healthy age-matched volunteers.

These results demonstrate that at least in this specific (limited) frequency range, the vestibular implant closely mimics the natural frequency-dependency of the vestibular system. Frequency showed a significant effect on the total peak eye velocity: total peak eye velocity increased with increasing frequency for both groups, without any significant effect between the groups. No significant frequency-dependent changes were observed in angle, habituation-index or asymmetry. This behavior was similar in the eVOR and in the "natural" VOR.

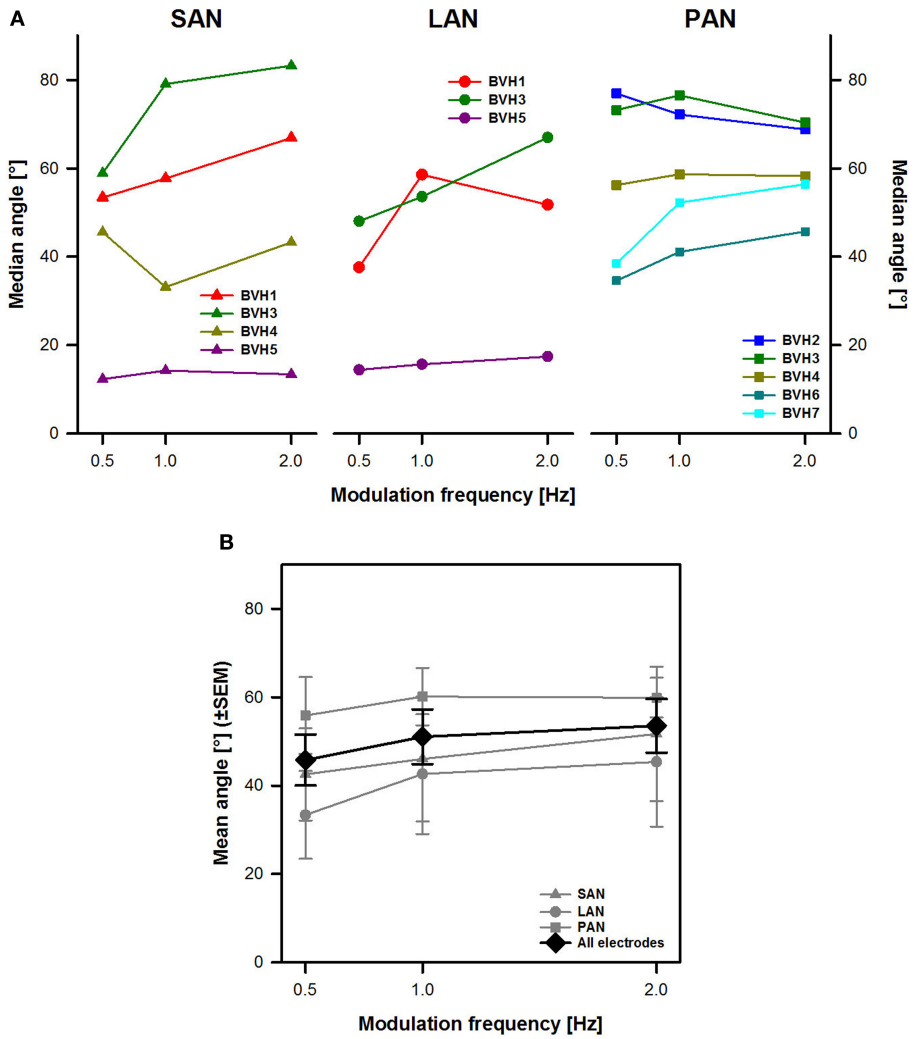

FIGURE 4 | Angle of eye movements vs. modulation frequency. (A) Each panel represents individual results obtained during stimulation for each stimulation site (SAN, LAN, PAN). **(B)** Mean results (±SEM) calculated across patients for each stimulation site (gray plots) and for all electrodes together (black plot).

The increase of peak eye velocity with frequency has already been well documented in normal subjects (Barnes, 1993), but it had never been systematically evaluated in human patients with a vestibular implant. It is reasonable to hypothesize that this effect probably reflects the properties of vestibular afferents, which are the main target of electrical stimulation by a vestibular implant (Goldberg et al., 1984; Kim et al., 2011). However, it cannot be excluded that a small residual population of hair cells and more central connections can contribute to this effect (Aw et al., 2008).

The eVOR angle (with respect to the horizontal axis) was very variable across the BVH-group for the whole tested frequency range. The resulting misalignment has already been described in animals as well as humans and is attributed mainly to current spread or imprecise electrode placement (Fridman et al., 2010; Lewis et al., 2010, 2013; Dai et al., 2011c, 2013; Davidovics et al., 2011; van de Berg et al., 2011; Guinand et al., Submitted). Current spread is particularly relevant in the case of the LAN and the SAN (**Figure 4A**) because of their close anatomical position relative to each other (van de Berg et al., 2012). Many strategies to minimize misalignment have been investigated, such as different stimulus waveforms, precompensation (vector summation), current steering and improving electrode design, but none of these seem totally infallible (Fridman et al., 2010; Dai et al., 2011c, 2013; Davidovics et al., 2013). Fortunately, chronic stimulation experiments in animals have shown that the brain is very adaptive: it is able to significantly improve eVOR alignment, making it possible to develop an ocular response which is aligned with the axis of head motion, even when stimulating the nerve branch of a canal that is orthogonal to the axis of motion. This phenomon is called "cross-axis adaptation." (Lewis et al., 2003, 2010, 2013; Della Santina et al., 2007; Dai et al., 2011c; van de Berg et al., 2011; Guinand et al., Submitted). Therefore, taking the adaptability of the brain into account, it should still be determined to which extent complex stimulation strategies to improve eVOR alignment will have to be incorporated into a device suited for human clinical use.

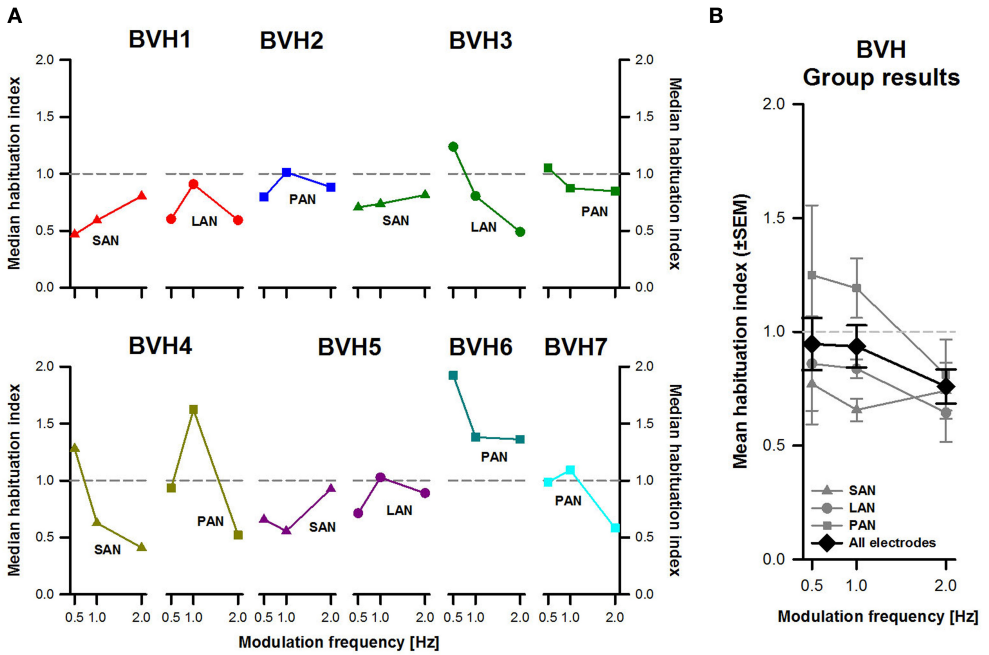

FIGURE 5 | Habituation-index vs. modulation frequency. (A) Each panel represents individual results obtained during stimulation for each stimulation site (SAN, LAN, PAN). **(B)** Mean results (±SEM) calculated across patients for each stimulation site (gray plots) and for all electrodes together (black plot).

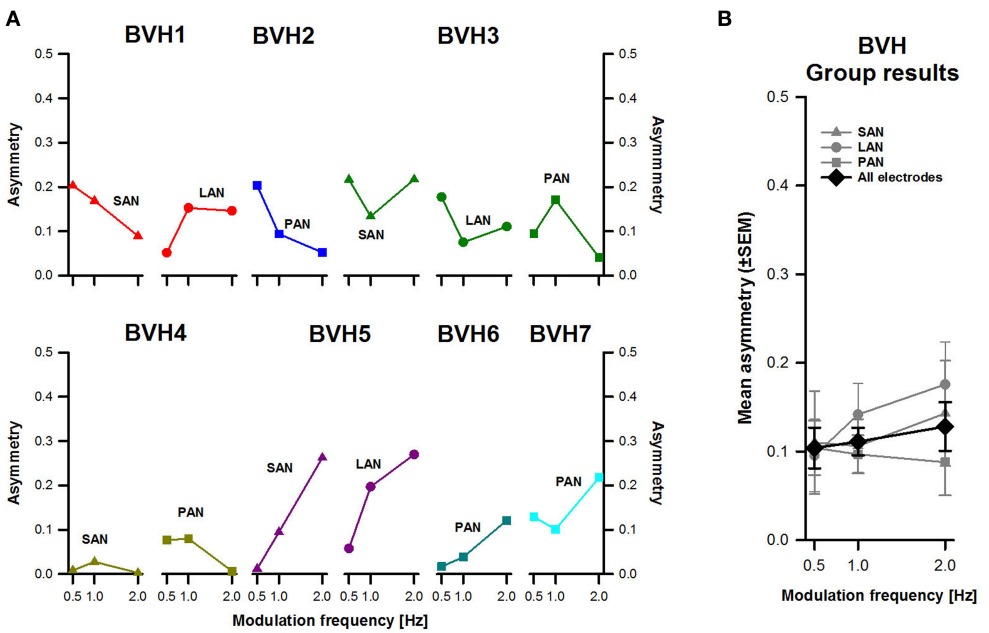

FIGURE 6 | Asymmetry-index vs. modulation frequency. (A) Each panel represents individual results obtained during stimulation for each stimulation site (SAN, LAN, PAN). **(B)** Mean results (±SEM) calculated across patients for each stimulation site (gray plots) and for all electrodes together (black plot).

Repeated exposure to the same sinusoidal stimulus can cause a long-lasting decrease in VOR gain in animals and humans. This habituation can be frequency-specific (Dow and Anastasio, 1999). While significant habituation has been observed for low-frequency stimuli (Buettner et al., 1981; Jäger and Henn, 1981a,b; Dow and Anastasio, 1997, 1999; Clément et al., 2002), repeated stimulation at higher modulation frequencies shows little or no change in VOR gain (Ito et al., 1974; Jäger and Henn,

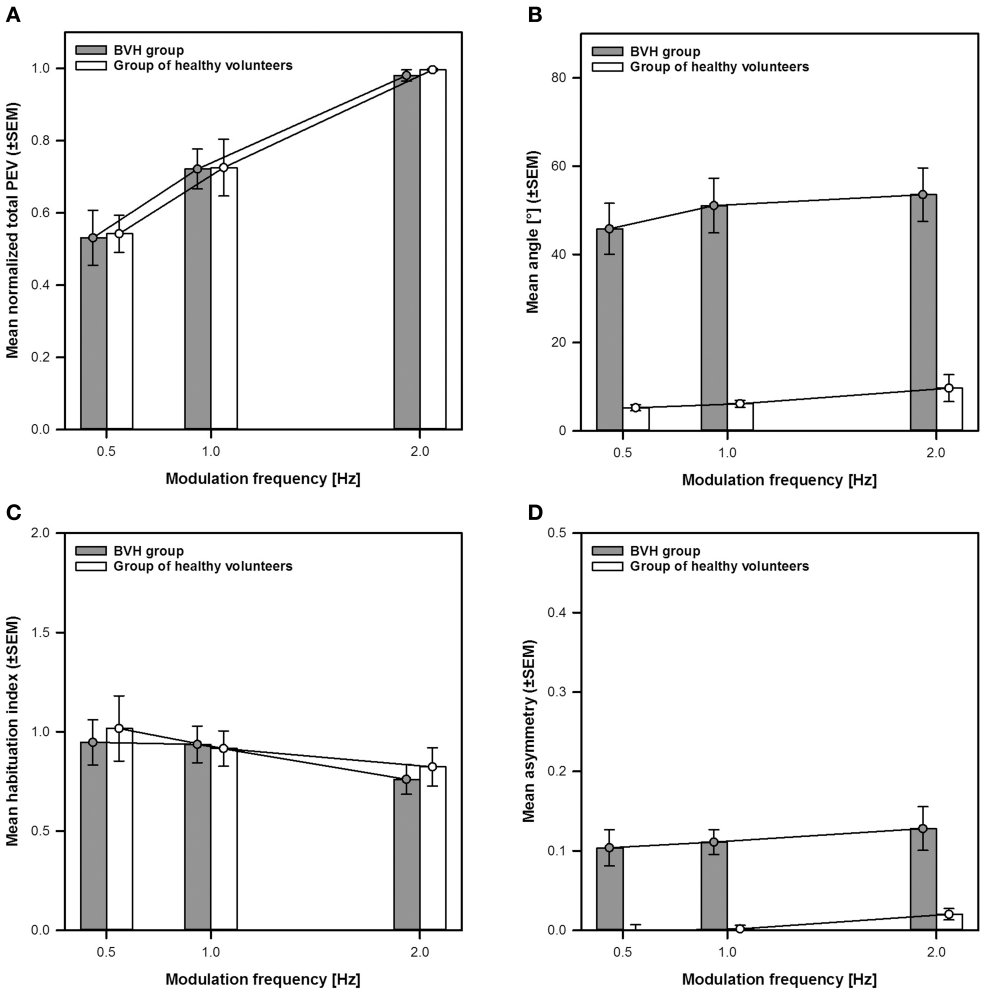

FIGURE 7 | Comparison of the main characteristics of the eVOR to the "natural" VOR. The eVOR is represented as mean group results of the BVH-group (gray bars and circles ±SEM). The "natural" VOR is represented as mean group results of the group of healthy volunteers (white bars and circles ±SEM). **(A)** Normalized total peak eye velocity (PEV) vs. modulation frequency. **(B)** Angle of the VOR responses vs. modulation frequency. **(C)** Habituation-index vs. modulation frequency. **(D)** Asymmetry-index vs. modulation frequency.

1981a; Dow and Anastasio, 1999). Consequently, some authors suggest sinusoidal oscillations should be limited to a few cycles or having a delay between two series of tests (Clément et al., 2002). Other key factors involved in the result of vestibular tests are general level of arousal and instruction set (Wall and Furman, 1989; Weissman et al., 1989; Barnes, 1993; Zee and Leigh, 2006). For example, it is well known and documented that results can be compromised during long testing trials. In this study, low modulation frequencies that would result in long testing times were excluded. Sixty-cycle trials were used and a delay between tests was obeyed. In these testing conditions, no significant habituation was observed. It is therefore reasonable to assume that habituation does not constitute a limiting factor in the tested frequency range.

No significant frequency-dependent changes in asymmetry were observed. However, the BVH-group showed significantly more asymmetry than the group of healthy volunteers. This could be expected, since acute unilateral vestibular stimulation (BVH-group) was compared to bilateral vestibular stimulation (group of healthy volunteers). However, it is interesting to note that the asymmetry index in the BVH-group remained relatively low (maximum 0.27) compared with previous data in unilaterally implanted monkeys (Dai et al., 2013; Guinand et al., Submitted). The major difference between both studies was the level of baseline stimulation used. Baseline stimulation in this study was set supranormally at 50% of the dynamic range, while the animal study used a lower baseline in order to mimic the physiology of normal rhesus monkey vestibular afferent fibers (Sadeghi et al., 2007; Dai et al., 2013). With a supranormal baseline, the decrease in excitatory response is counterbalanced by the increase in inhibitory response, which should improve the symmetry of the response. In other words, using a supranormal baseline corresponding to 50% of the total dynamic range, allows an equal range of stimulation currents to code head movements toward the implanted side and toward the unimplanted side. Consequently, head compensation in all directions should

be enhanced (Davidovics et al., 2012). At this point it is important to point out that it is still not clear yet whether asymmetry will be an issue of clinical relevance for vestibular implants. Results on patients with a unilateral vestibular loss show that response asymmetry is generally well compensated (Curthoys and Halmagyi, 1995; Black et al., 1996; van de Berg et al., 2011). It is therefore reasonable to hypothesize that even an asymmetric eVOR might be enough to restore useful vestibular function.

Knowing the minor frequency effects on angle, habituation and asymmetry could open doors for future research. It allows these eVOR-parameters to be determined at only specific frequencies, without the need for testing the whole frequency range. This is likely to result in more precise measurements, since (1) some modulation frequencies which have specific drawbacks (i.e., low gain for low-frequencies or challenging head stabilization during rotatory tests at higher-frequencies) could be left out of the analysis and (2) time is saved in the already long testing sessions, resulting in improved patients' concentration which has to be optimal for all tests.

The fact that a vestibular implant can closely mimick the "natural" frequency-dependency of the vestibular system, is also a promising finding for device development. Since the VOR is appropriately compensated in a frequency range which is important for every-day activities (Crane and Demer, 1997), there might be no need of implementing complex stimulus processing strategies that consider frequency-dependent characteristics.

ADDITIONAL CONSIDERATIONS

In this study, the threshold for vestibular activation was the current where the first vestibular symptom was reported or observed (Guinand et al., Submitted). This could be a change in nystagmus slow peak eye velocity or a clearly vestibular related sensation that could be below the threshold of activation of the VOR-pathway. This latter case suggests that other pathways can be activated before the VOR-pathway. These sub-VOR-threshold perceptions deserve to be investigated more in the future (Guinand et al., Submitted).

Some healthy volunteers were unable to adequately stabilize their heads at 2 Hz. This resulted in an increase in the vertical peak eye velocity component, which is in accordance with clinical experience that effectively stabilizing the head above a certain rotation frequency becomes challenging. A bite bar could be added to improve head stabilization. However, it was decided not to use a bite bar since we considered that this would impose an additional unnecessary burden to subjects. Furthermore, adding a bite bar would hinder communication with subjects. Both these considerations are particularly relevant for our long, repeated testing sessions performed in complete darkness.

Finally, a potential caveat of this study is that results are based on a small number of subjects. This suggests that results of statistical tests should be interpreted with caution. Nevertheless, the trends reported were similar across subjects and inter-subject variability was smaller than the observed effects. In these conditions, adding more subjects to the study would certainly give more statistical power to the results, but it would not fundamentally change the observed trends.

CONCLUSION

A strong and significant frequency-dependency effect in total peak eye velocity was observed in the tested frequency range (0.5–2 Hz). This effect was comparable to the one observed for the "natural" VOR. (e)VOR-angle, habituation-index and asymmetry showed no significant frequency-dependent effect in any group. This study demonstrates that, at least in the specific (limited) frequency range tested, the vestibular implant closely mimics the natural frequency-dependency of the vestibular system.

ACKNOWLEDGMENTS

We would like to thank Dr. Eric Vuurman for his assistance with the statistical analysis and with the description of statistical details.

REFERENCES

Aw, S. T., Todd, M. J., Aw, G. E., Weber, K. P., and Halmagyi, G. M. (2008). Gentamicin vestibulotoxicity impairs human electrically evoked vestibulo-ocular reflex. *Neurology* 71, 1776–1782. doi: 10.1212/01.wnl.0000335971.43443.d9

Barnes, G. R. (1993). Visual-vestibular interaction in the control of head and eye movement: the role of visual feedback and predictive mechanisms. *Prog. Neurobiol.* 41, 435–472. doi: 10.1016/0301-0082(93)90026-O

Bartl, K., Lehnen, N., Kohlbecher, S., and Schneider, E. (2009). Head impulse testing using video-oculography. *Ann. N.Y. Acad. Sci.* 1164, 331–333. doi: 10.1111/j.1749-6632.2009.03850.x

Bierer, S. M., Ling, L., Nie, K., Fuchs, A. F., Kaneko, C. R. S., Oxford, T., et al. (2012). Auditory outcomes following implantation and electrical stimulation of the semicircular canals. *Hear. Res.* 287, 51–56. doi: 10.1016/j.heares.2012.03.012

Black, F. O., Wade, S. W., and Nashner, L. M. (1996). What is the minimal vestibular function required for compensation? *Am. J. Otol.* 17, 401–409.

Brandt, T., Huppert, T., Hüfner, K., Zingler, V. C., Dieterich, M., and Strupp, M. (2010). Long-term course and relapses of vestibular and balance disorders. *Restor. Neurol. Neurosci.* 28, 69–82. doi: 10.3233/RNN-2010-0504

Buettner, U. W., Henn, V., and Young, L. R. (1981). Frequency response of the vestibulo-ocular reflex (VOR) in the monkey. *Aviat. Space Environ. Med.* 52, 73–77.

Clément, G., Flandrin, J. M., and Courjon, J. H. (2002). Comparison between habituation of the cat vestibulo-ocular reflex by velocity steps and sinusoidal vestibular stimulation in the dark. *Exp. Brain Res.* 142, 259–267. doi: 10.1007/s00221-001-0930-7

Crane, B. T., and Demer, J. L. (1997). Human gaze stabilization during natural activities: translation, rotation, magnification, and target distance effects. *J. Neurophysiol.* 78, 2129–2144.

Curthoys, I., and Halmagyi, G. M. (1995). Vestibular compensation: a review of the oculomotor, neural, and clinical consequences of unilateral vestibular loss. *J. Vestib. Res.* 5, 67–107. doi: 10.1016/0957-4271(94)00026-X

Dai, C., Fridman, G. Y., Chiang, B., Davidovics, N. S., Melvin, T.-A., Cullen, K. E., et al. (2011c). Cross-axis adaptation improves 3D vestibulo-ocular reflex alignment during chronic stimulation via a head-mounted multichannel vestibular prosthesis. *Exp. Brain Res.* 210, 595–606. doi: 10.1007/s00221-011-2591-5

Dai, C., Fridman, G. Y., Chiang, B., Rahman, M. A., Ahn, J. H., Davidovics, N. S., et al. (2013). Directional plasticity rapidly improves 3D vestibulo-ocular reflex alignment in monkeys using a multichannel vestibular prosthesis. *J. Assoc. Res. Otolaryngol.* 14, 863–877. doi: 10.1007/s10162-013-0413-0

Dai, C., Fridman, G. Y., Davidovics, N. S., Chiang, B., Ahn, J. H., and Della Santina, C. C. (2011b). Restoration of 3D vestibular sensation in rhesus monkeys using a multichannel vestibular prosthesis. *Hear. Res.* 281, 74–83. doi: 10.1016/j.heares.2011.08.008

Dai, C., Fridman, G. Y., and Della Santina, C. C. (2011a). Effects of vestibular prosthesis electrode implantation and stimulation on hearing in rhesus monkeys. *Hear. Res.* 277, 204–210. doi: 10.1016/j.heares.2010.12.021

Davidovics, N. S., Fridman, G. Y., Chiang, B., and Della Santina, C. C. (2011). Effects of biphasic current pulse frequency, amplitude, duration, and interphase gap on eye movement responses to prosthetic electrical stimulation of the vestibular nerve. *IEEE Trans. Neural Syst. Rehabil. Eng.* 19, 84–94. doi: 10.1109/TNSRE.2010.2065241

Davidovics, N. S., Fridman, G. Y., and Della Santina, C. C. (2012). Co-modulation of stimulus rate and current from elevated baselines expands head motion encoding range of the vestibular prosthesis. *Exp. brain Res.* 218, 389–400. doi: 10.1007/s00221-012-3025-8

Davidovics, N. S., Rahman, M. A., Dai, C., Ahn, J., Fridman, G. Y., and Della Santina, C. C. (2013). Multichannel vestibular prosthesis employing modulation of pulse rate and current with alignment precompensation elicits improved VOR performance in monkeys. *J. Assoc. Res. Otolaryngol.* 14, 233–248. doi: 10.1007/s10162-013-0370-7

Della Santina, C. C., Migliaccio, A. A., and Patel, A. H. (2007). A multichannel semicircular canal neural prosthesis using electrical stimulation to restore 3-d vestibular sensation. *IEEE Trans. Biomed. Eng.* 54(6 Pt 1), 1016–1030. doi: 10.1109/TBME.2007.894629

Dow, E. R., and Anastasio, T. J. (1997). Induction of periodic alternating nystagmus in intact goldfish by sinusoidal rotation. *Neuroreport* 8, 2755–2759. doi: 10.1097/00001756-199708180-00022

Dow, E. R., and Anastasio, T. J. (1999). Analysis and modeling of frequency-specific habituation of the goldfish vestibulo-ocular reflex. *J. Comput. Neurosci.* 7, 55–70. doi: 10.1023/A:1008967511172

Feigl, G. C., Fasel, J. H., Anderhuber, F., Ulz, H., Rienmüller, R., Guyot, J.-P., et al. (2009). Superior vestibular neurectomy: a novel transmeatal approach for a denervation of the superior and lateral semicircular canals. *Otol. Neurotol.* 30, 586–591. doi: 10.1097/MAO.0b013e3181ab9164

Fridman, G. Y., Davidovics, N. S., Dai, C., Migliaccio, A. A., and Della Santina, C. C. (2010). Vestibulo-ocular reflex responses to a multichannel vestibular prosthesis incorporating a 3D coordinate transformation for correction of misalignment. *J. Assoc. Res. Otolaryngol.* 11, 367–381. doi: 10.1007/s10162-010-0208-5

Fridman, G. Y., and Della Santina, C. C. (2013). "Safe direct current stimulator 2: concept and design," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society* (Osaka), 3126–3129.

Goldberg, J. M., Smith, C. E., and Fernandez, C. (1984). Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. *J. Neurophysiol.* 51, 1236–1256.

Golub, J. S., Ling, L., Nie, K., Nowack, A., Shepherd, S. J., Bierer, S. M., et al. (2014). Prosthetic implantation of the human vestibular system. *Otol. Neurotol.* 35, 136–147. doi: 10.1097/MAO.0000000000000003

Gong, W., and Merfeld, D. M. (2002). System design and performance of a unilateral horizontal semicircular canal prosthesis. *IEEE Trans. Biomed. Eng.* 49, 175–181. doi: 10.1109/10.979358

Guinand, N., Boselie, F., Guyot, J. P., and Kingma, H. (2012). Quality of life of patients with bilateral vestibulopathy. *Ann. Otol. Rhinol. Laryngol.* 121, 471–477. doi: 10.1177/000348941212100708

Guyot, J.-P., Sigrist, A., Pelizzone, M., and Kos, M. I. (2011). Adaptation to steady-state electrical stimulation of the vestibular system in humans. *Ann. Otol. Rhinol. Laryngol.* 120, 143–149. doi: 10.1177/00034894111 2000301

Hain, T. C., Cherchi, M., and Yacovino, D. A. (2013). Bilateral vestibular loss. *Semin. Neurol.* 33, 195–203. doi: 10.1055/s-0033-1354597

Hayden, R., Sawyer, S., Frey, E., Mori, S., Migliaccio, A. A., and Della Santina, C. C. (2011). Virtual labyrinth model of vestibular afferent excitation via implanted electrodes: validation and application to design of a multichannel vestibular prosthesis. *Exp. Brain Res.* 210, 623–640. doi: 10.1007/s00221-011-2599-x

Ito, M., Shiida, T., Yagi, N., and Yamamoto, M. (1974). Visual influence on rabbit horizontal vestibulo-ocular reflex presumably effected via the cerebellar flocculus. *Brain Res.* 65, 170–174. doi: 10.1016/0006-8993(74)90 344-8

Jäger, J., and Henn, V. (1981a). Habituation of the vestibulo-ocular reflex (VOR) in the monkey during sinusoidal rotation in the dark. *Exp. Brain Res.* 41, 108–114. doi: 10.1007/BF00236599

Jäger, J., and Henn, V. (1981b). Vestibular habituation in man and monkey during sinusoidal rotation. *Ann. N.Y. Acad. Sci.* 374, 330–339. doi: 10.1111/j.1749-6632.1981.tb30880.x

Kim, K. S., Minor, L. B., Della Santina, C. C., and Lasker, D. M. (2011). Variation in response dynamics of regular and irregular vestibular-nerve afferents during sinusoidal head rotations and currents in the chinchilla. *Exp. Brain Res.* 210, 643–649. doi: 10.1007/s00221-011-2600-8

Lewis, R. F., Gong, W., Ramsey, M., Minor, L., Boyle, R., and Merfeld, D. M. (2003). Vestibular adaptation studied with a prosthetic semicircular canal. *J. Vestib. Res.* 12, 87–94.

Lewis, R. F., Haburcakova, C., Gong, W., Makary, C., and Merfeld, D. M. (2010). Vestibuloocular reflex adaptation investigated with chronic motion-modulated electrical stimulation of semicircular canal afferents. *J. Neurophysiol.* 103, 1066–1079. doi: 10.1152/jn.00241.2009

Lewis, R. F., Nicoucar, K., Gong, W., Haburcakova, C., and Merfeld, D. M. (2013). Adaptation of vestibular tone studied with electrical stimulation of semicircular canal afferents. *J. Assoc. Res. Otolaryngol.* 14, 331–340. doi: 10.1007/s10162-013-0376-1

McCall, A. A., and Yates, B. J. (2011). Compensation following bilateral vestibular damage. *Front. Neurol.* 2:88. doi: 10.3389/fneur.2011.00088

Merfeld, D. M., Gong, W., Morrissey, J., Saginaw, M., Haburcakova, C., and Lewis, R. F. (2006). Acclimation to chronic constant-rate peripheral stimulation provided by a vestibular prosthesis. *IEEE Trans. Biomed. Eng.* 53, 2362–2372. doi: 10.1109/TBME.2006.883645

Merfeld, D. M., Haburcakova, C., Gong, W., and Lewis, R. F. (2007). Chronic vestibulo-ocular reflexes evoked by a vestibular prosthesis. *IEEE Trans. Biomed. Eng.* 54(6 Pt 1), 1005–1015. doi: 10.1109/TBME.2007.891943

Migliaccio, A. A., Meierhofer, R., and Della Santina, C. C. (2011). Characterization of the 3D angular vestibulo-ocular reflex in C57BL6 mice. *Exp. Brain Res.* 210, 489–501. doi: 10.1007/s00221-010-2521-y

Perez Fornos, A., Guinand, N., van de Berg, R., Stokroos, R., Micera, S., Kingma, H., et al. (2014). Artificial balance: restoration of the vestibulo-ocular reflex in humans with a prototype vestibular neuroprosthesis. *Front. Neurol.* 5:66. doi: 10.3389/fneur.2014.00066

Porciuncula, F., Johnson, C. C., and Glickman, L. B. (2012). The effect of vestibular rehabilitation on adults with bilateral vestibular hypofunction: a systematic review. *J. Vestib. Res.* 22, 283–298. doi: 10.3233/VES-120464

Rubinstein, J. T., Bierer, S., Kaneko, C., Ling, L., Nie, K., Oxford, T., et al. (2012). Implantation of the semicircular canals with preservation of hearing and rotational sensitivity: a vestibular neurostimulator suitable for clinical research. *Otol. Neurotol.* 33, 789–796. doi: 10.1097/MAO.0b013e31825 4ec24

Sadeghi, S. G., Minor, L. B., and Cullen, K. E. (2007). Response of vestibular-nerve afferents to active and passive rotations under normal conditions and after unilateral labyrinthectomy. *J. Neurophysiol.* 97, 1503–1514. doi: 10.1152/jn.00829.2006

van de Berg, R., Guinand, N., Guyot, J.-P., Kingma, H., and Stokroos, R. J. (2012). The modified ampullar approach for vestibular implant surgery: feasibility and its first application in a human with a long-term vestibular loss. *Front. Neurol.* 3:18. doi: 10.3389/fneur.2012.00018

van de Berg, R., Guinand, N., Stokroos, R. J., Guyot, J.-P., and Kingma, H. (2011). The vestibular implant: quo vadis? *Front. Neurol.* 2:47. doi: 10.3389/fneur.2011.00047

Wall, C., and Furman, J. M. (1989). Eyes open versus eyes closed: effect on human rotational responses. *Ann. Otol. Rhinol. Laryngol.* 98, 625–629. doi: 10.1177/000348948909800811

Wall, C., Kos, M. I., and Guyot, J.-P. (2007). Eye movements in response to electric stimulation of the human posterior ampullary nerve. *Ann. Otol. Rhinol. Laryngol.* 116, 369–374. doi: 10.1177/000348940711600509

Wall, C., Merfeld, D. M., Rauch, S. D., and Black, F. O. (2003). Vestibular prostheses: the engineering and biomedical issues. *J. Vestib. Res.* 12, 95–113.

Ward, B. K., Agrawal, Y., Hoffman, H. J., Carey, J. P., and Della Santina, C. C. (2013). Prevalence and impact of bilateral vestibular hypofunction: results from the 2008 US National Health Interview Survey. *JAMA Otolaryngol. Head Neck Surg.* 139, 803–810. doi: 10.1001/jamaoto.2013.3913

Weissman, B. M., DiScenna, A. O., Ekelman, B. L., and Leigh, R. J. (1989). Effect of eyelid closure and vocalization upon the vestibulo-ocular reflex during rotational testing. *Ann. Otol. Rhinol. Laryngol.* 98, 548–550. doi: 10.1177/000348948909800710

Zee, D., and Leigh, J. (2006). "The vestibular-optokinetic system," in *The Neurology of Eye Movements*, eds S. Gilman and W. Herdman (New York, NY: Oxford University Press), 72.

Zingler, V. C., Weintz, E., Jahn, K., Mike, A., Huppert, D., Rettinger, N., et al. (2008). Follow-up of vestibular function in bilateral vestibulopathy. *J. Neurol. Neurosurg. Psychiatry* 79, 284–288. doi: 10.1136/jnnp.2007.122952

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 October 2014; accepted: 29 December 2014; published online: 20 January 2015.

Citation: van de Berg R, Guinand N, Nguyen TAK, Ranieri M, Cavuscens S, Guyot J-P, Stokroos R, Kingma H and Perez-Fornos A (2015) The vestibular implant: frequency-dependency of the electrically evoked vestibulo-ocular reflex in humans. Front. Syst. Neurosci. ***8****:255. doi: 10.3389/fnsys.2014.00255*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

ORIGINAL RESEARCH
published: 10 April 2015
doi: 10.3389/fnsys.2015.00047

Sensitivity to microstimulation of somatosensory cortex distributed over multiple electrodes

*Sungshin Kim[1], Thierri Callier[1], Gregg A. Tabot[2], Francesco V. Tenore[3] and Sliman J. Bensmaia[1,2]**

[1] Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL, USA, [2] Committee on Computational Neuroscience, University of Chicago, Chicago, IL, USA, [3] Research and Exploratory Development Department, Johns Hopkins University Applied Physics Laboratory, Laurel, MD, USA

Meaningful and repeatable tactile sensations can be evoked by electrically stimulating primary somatosensory cortex. Intracortical microstimulation (ICMS) may thus be a viable approach to restore the sense of touch in individuals who have lost it, for example tetraplegic patients. One of the potential limitations of this approach, however, is that high levels of current can damage the neuronal tissue if the resulting current densities are too high. The limited range of safe ICMS amplitudes thus limits the dynamic range of ICMS-evoked sensations. One way to get around this limitation would be to distribute the ICMS over multiple electrodes in the hopes of intensifying the resulting percept without increasing the current density experienced by the neuronal tissue. Here, we test whether stimulating through multiple electrodes is a viable solution to increase the dynamic range of ICMS-elicited sensations without increasing the peak current density. To this end, we compare the ability of non-human primates to detect ICMS delivered through one vs. multiple electrodes. We also compare their ability to discriminate pulse trains differing in amplitude when these are delivered through one or more electrodes. We find that increasing the number of electrodes through which ICMS is delivered only has a marginal effect on detectability or discriminability despite the fact that 2–4 times more current is delivered overall. Furthermore, the impact of multielectrode stimulation (or lack thereof) is found whether pulses are delivered synchronously or asynchronously, whether the leading phase of the pulses is cathodic or anodic, and regardless of the spatial configuration of the electrode groups.

Keywords: neuroprosthetics, intracortical microstimulation, discrimination task, detection performance, non-human primates

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Emilio Salinas, Wake Forest University, USA
Joseph E. O'Doherty, University of California, San Francisco, USA
Christian Klaes, Caltech, USA

***Correspondence:**
Sliman J. Bensmaia, Department of Organismal Biology and Anatomy, University of Chicago, 1027 East 57th Street, Chicago, IL, USA
sliman@uchicago.edu

Received: *22 January 2015*
Accepted: *07 March 2015*
Published: *10 April 2015*

Citation:
Kim S, Callier T, Tabot GA, Tenore FV and Bensmaia SJ (2015) Sensitivity to microstimulation of somatosensory cortex distributed over multiple electrodes. Front. Syst. Neurosci. 9:47. doi: 10.3389/fnsys.2015.00047

Introduction

One approach to restoring sensorimotor function to patients with upper spinal cord injury consists of measuring signals from motor areas of their brains to control anthropomorphic robotic arms (Hochberg et al., 2012; Collinger et al., 2013). However, our ability to use our limbs relies heavily on somatosensory signals, which convey information about the consequences of our movements and about the objects with which we interact. With this in mind, it is necessary not only to re-establish the ability to send commands to the limb but also to restore the ability to receive sensory signals back from the limb. One strategy to restore somatosensation consists of electrically stimulating

neurons in somatosensory cortex through chronically implanted electrode arrays in the hopes of eliciting meaningful tactile and proprioceptive sensations (London et al., 2008; O'Doherty et al., 2011; Berg et al., 2013; Tabot et al., 2013b, 2014; Thomson et al., 2013; Bensmaia and Miller, 2014; Dadarlat et al., 2014). One limitation of intracortical microstimulation (ICMS) is that high levels of current can damage neuronal tissue if the resulting current densities are too high. However, ICMS has been found to have a negligible effect on tissue over a range of current densities (up to about 1.0 mC/cm^2; Rajan et al., unpublished observations), unless it is applied continuously for long periods of time (McCreery et al., 2010). One strategy to expand the dynamic range of elicited sensations without increasing the current density experienced by any one population of neurons, and thus to avoid damaging the brain, is to distribute the injected current over multiple electrodes (Zaaimi et al., 2013). That way, we might be able to achieve a wider dynamic range of sensations without subjecting neurons to higher peak current densities.

To investigate this possibility, we had two non-human primates perform detection or discrimination tasks in a two-alternative forced choice paradigm (**Figure 1A**) to probe their sensitivity to ICMS delivered through one or more electrodes. Electrodes in each group were chosen such that their receptive fields were largely overlapping to ensure that the sensations evoked resulted in tactile sensations that were localized to a single location on the skin (cf. Tabot et al., 2013b). We wished to determine the degree to which stimulation through multiple electrodes (1) reduces the minimum amplitude required to achieve a percept (the absolute threshold) and (2) increases the number of discriminable amplitude increments [just noticeable differences (JNDs)] that can be achieved between absolute threshold and the maximum current per electrode (100 μA).

To these ends, we first investigated whether animals could better detect pulse trains delivered simultaneously through multiple electrodes (2 or 4) than they could the same pulse trains delivered through a single electrode. We also compared the animals' sensitivity to multi-electrode stimulation when pulses were delivered synchronously or asynchronously within each stimulus cycle (**Figure 1B**). Moreover, we probed the effect on sensitivity of polarity (that is, whether the leading phase is cathodic or anodic) and of the spatial configuration of the electrodes on the array (**Figure 1C**). Finally, we investigated how multi-electrode stimulation affects the discriminability of ICMS pulse trains that differ in amplitude. We conclude that multi-electrode stimulation only provides a modest improvement in the dynamic range and does not justify its energetic cost.

Results and Discussion

Effect of Multi-Electrode Stimulation on Detectability

We compared detection performance for ICMS pulse trains delivered through 1, 2, or 4 electrodes with cathodic phase-first current pulses delivered synchronously across electrodes (**Figure 1B**, left). In these experiments, two and four times as much current was delivered in the double and quad conditions as was delivered in the single electrode condition, respectively. We found that the absolute threshold – defined as the ICMS amplitude that yielded 75% detection performance – decreased as the number of stimulated electrodes increased (**Figures 2A,B**;

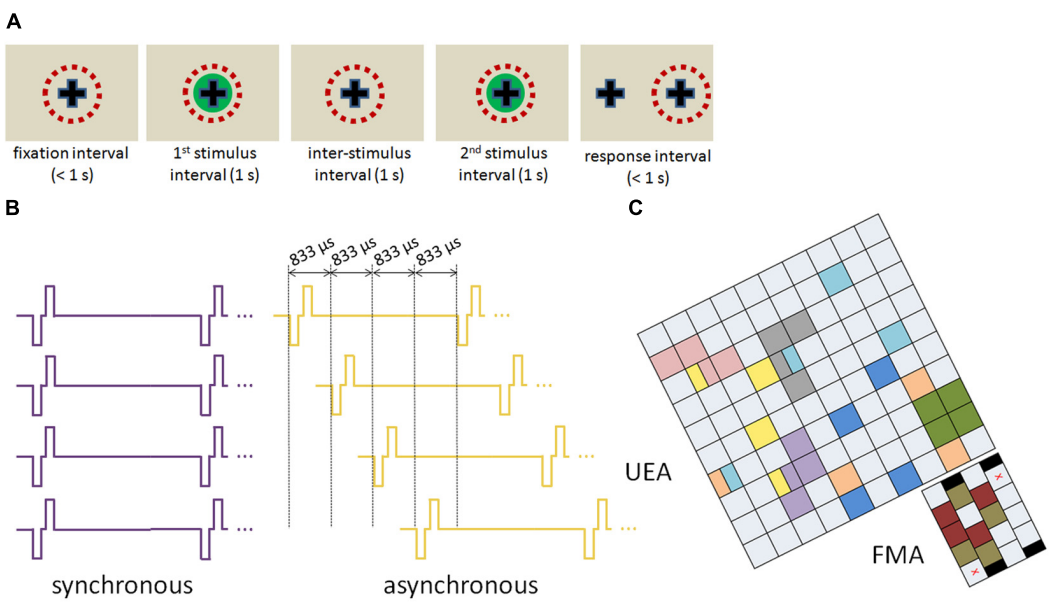

FIGURE 1 | Experimental design. (A) Structure of a trial. The red dashed circle denotes the animal's direction of gaze; on this example trial, the animal responded right. **(B)** Timing of synchronous and asynchronous ICMS for a quad of electrodes. **(C)** Spatial configuration of electrode quads from one monkey. Each square represents an electrode; electrodes that share a color were part of a quad (some electrodes were used in two quads).

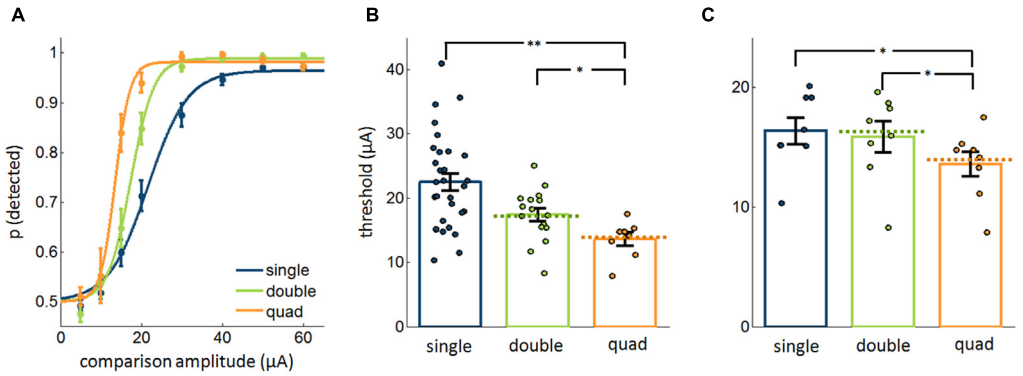

FIGURE 2 | Detectability of ICMS delivered through varying number of electrodes (20,577 trials from 54 electrodes). In these experiments, pulses occurred synchronously (**Figure 1B**, left). **(A)** Psychometric functions, averaged across single electrodes, electrode pairs, and electrode quads. **(B)** Measured and theoretically calculated thresholds. The bars and error bars denote the mean and SEs, respectively, and each point corresponds to a different single electrode, electrode pair, or quad. The dashed lines denote the expected threshold assuming that each electrode independently contributes to detectability. **(C)** Comparison of the most sensitive single electrode, best pair of electrodes, and the quad with pulses delivered synchronously in the multi-electrode conditions (9,520 trials from 24 electrodes). Error bars denote the SE of the mean. $^{*}p < 0.05$, $^{**}p < 0.01$.

Kruskal–Wallis test, $\chi^2_{(2,51)} = 14.8$, $p < 0.001$), as might be expected given that more current was delivered to the brain (see Ghose and Maunsell, 2012; Zaaimi et al., 2013). A *post hoc* analysis revealed that, while single-electrode thresholds were not significantly different from electrode-pair thresholds (rank-sum test, Bonferroni corrected, $p = 0.07$), single thresholds were significantly higher than quad thresholds ($p = 0.003$), and double thresholds were significantly higher than quad thresholds ($p = 0.04$). Furthermore, thresholds measured in the multi-electrode conditions closely matched theoretical predictions based on the assumption that each electrode exerts an independent effect on detectability (signed rank test, $p > 0.5$, dashed lines in **Figure 2B**).

Next, we wished to assess the extent to which stimulation through multiple electrodes improves detectability beyond that achieved through stimulation of the most sensitive electrode. To this end, we compared performance with four electrodes to that with the best (most sensitive) electrode and with the best pair of electrodes in the quad. We found that, while not completely eliminated, the apparent advantage of multi-electrode stimulation was substantially reduced. Indeed, while detection performance remained significantly different between single electrodes and multiple electrodes (Friedman test, $\chi^2_{(2,14)} = 9.25$, $p < 0.01$, **Figure 2C**), the mean difference in threshold between groups was less than 3 μA (mean ± SEM: single vs. double: 0.50 ± 0.50 μA, single vs. quad: 2.76 ± 1.02 μA, double vs. quad: 2.26 ± 0.82 μA, mean ± SEM), representing a decrease of less than 10%. Importantly, the detectability of subthreshold stimuli (5 and 10 μA) did not improve significantly with multiple electrodes (Friedman test, $\chi^2_{(2,30)} = 1.94$, $p = 0.4$), which stands in contrast with previous findings (Zaaimi et al., 2013).

Synchronous vs. Asynchronous Stimulation

In the multi-electrode conditions described above, electrical pulses were delivered synchronously through different electrodes. We wished to determine whether the effect of multi-electrode stimulation on sensitivity might be different when pulses are staggered rather than synchronous. To this end, we repeated the experiments described above, but interleaved trials in which pulses were delivered synchronously across electrodes with trials in which pulses were staggered (**Figure 1B**). First, we found that synchrony did not have a significant overall effect on thresholds [paired *t*-test, $t_{(23)} = 1.03$, $p = 0.31$] (**Figure 3A**), consistent with previous findings in optogenetic experiments with mice (Histed and Maunsell, 2014). Second, asynchronous multi-electrode ICMS had a similar effect on detectability as did its synchronous counterpart, with thresholds decreasing with more electrodes (Friedman test, $\chi^2_{(2,18)} = 14.6$, $p < 0.001$); a *post hoc* analysis revealed significant differences across all three groups (signed rank test, Bonferroni corrected, $p < 0.05$; **Figure 3B**). Additionally, as was the case with synchronous stimulation, measured thresholds were indistinguishable from theoretically estimated thresholds assuming independent contributions of each electrode to detection performance (signed rank test, double: $p = 0.063$, quad: $p > 0.5$). Again, the mean difference in threshold was small (single vs. double: 1.35 ± 0.32 μA, single vs. quad: 2.96 ± 0.43 μA, double vs. quad: 1.61 ± 0.38 μA) and the detectability of subthreshold stimuli did not improve significantly (Friedman test, $\chi^2_{(2,38)} = 1.85$, $p = 0.40$).

Effect of Pulse Polarity on Detectability

Next, we investigated whether changing the polarity of the pulses might modulate how stimulation through multiple electrodes affects detectability. That is, we compared the effect of multi-electrode stimulation when the leading phase was anodic to that when leading phase was cathodic. First, as has been previously shown (Ranck, 1975; Schmidt et al., 1996; Koivuniemi and Otto, 2011), detection thresholds were significantly higher for anodic-first pulses than for cathodic-first pulses [**Figure 4A**; *t*-test: $t_{(70)} = 12.2$, $p < 10^{-18}$]. As was the case for cathodic-first pulses,

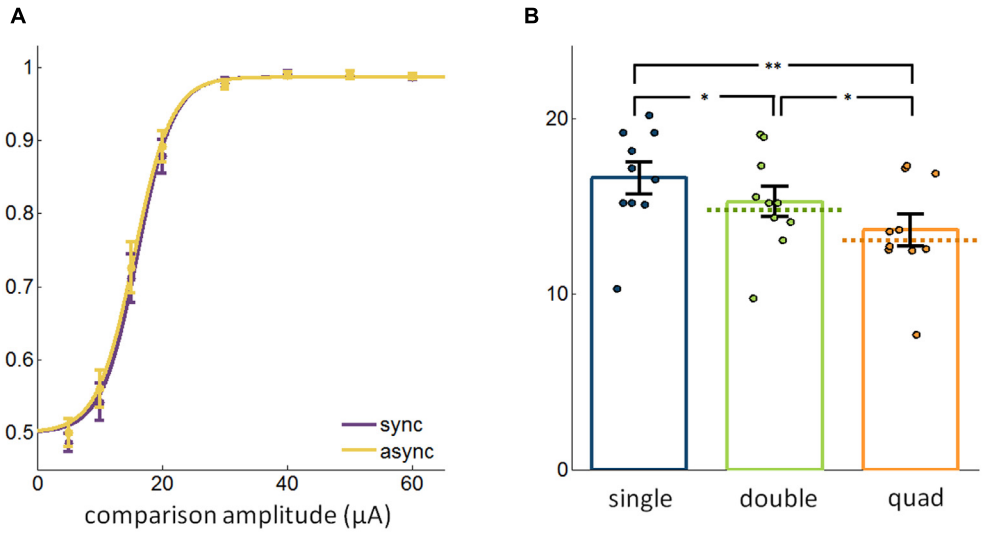

FIGURE 3 | (A) Detection performance with synchronous and asynchronous stimulation (13,202 trials from 48 electrodes). **(B)** Comparison of the most sensitive single electrode, best pair of electrodes, and the quad with pulses delivered asynchronously in the multi-electrode conditions (19,400 trials from 30 electrodes). Error bars denote the SE of the mean. $*p < 0.05$, $**p < 0.01$.

thresholds for anodic-first stimulation decreased as the number of electrodes increased (Kruskal–Wallis test, $\chi^2_{(2,45)} = 6.68$, $p = 0.036$). However, the increase in sensitivity with increasing number of electrodes was eliminated when the best single electrode and the best pair were compared to the quad [Friedman test, $\chi^2_{(2,10)} = 2.33$, $p = 0.31$, single/double/quad: 23.6 ± 1.70 μA/28.9 ± 1.08 μA/27.2 ± 1.64 μA; **Figure 4B**]. Thus, results using multi-electrode ICMS with anodic phase leading did not conform with theoretical predictions based on the assumption of independence.

Effect of Electrode Spacing on Detectability

Electrodes that formed each quad were selected to have largely overlapping receptive fields. In some cases the electrodes were physically adjacent, but in others they were not (**Figure 1C**). We wished to assess whether the spatial configuration of the electrode groups might impact how stimulation through these is combined to culminate in a behavioral outcome. Using ICMS with cathodic phase leading, we found that spatial configuration had no impact on sensitivity to multi-electrode stimulation: threshold decreased as the number of electrodes increased, whether these

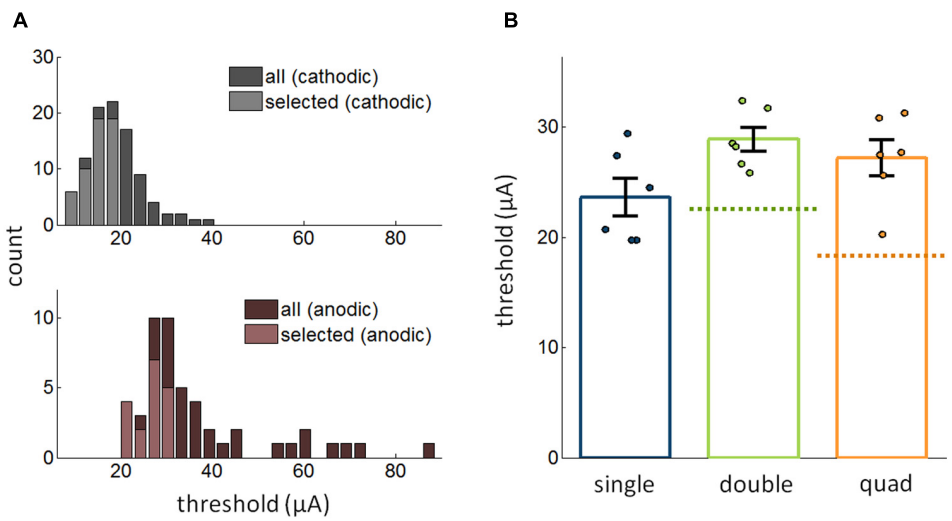

FIGURE 4 | (A) Distribution of thresholds for anodic phase leading and cathodic phase leading ICMS (56,021 trials from 97 electrodes). The distribution of thresholds for the best electrodes is shown in the lighter hue. **(B)** Comparison of the most sensitive single electrode, best pair of electrodes, and the quad with pulses in the multi-electrode conditions with anodic phase leading (12,661 trials from 18 electrodes). Error bars denote the SE of the mean.

were adjacent (Friedman test, $\chi^2_{(2,18)} = 18.2$, $p < 0.001$) or not ($\chi^2_{(2,14)} = 6.75$, $p = 0.03$). In both conditions (adjacent vs. non-adjacent), observed thresholds were consistent with the assumption of independence; that is, these were not significantly different from predicted ones regardless of spatial separation (signed rank test, $p > 0.1$). The effect of separation was similar whether stimulation was presented synchronously or asynchronously, as might be expected from **Figure 3**.

Multi-Electrode Stimulation for Discrimination

Based on results from the detection experiments, we concluded that the detectability of ICMS improves only slightly when stimulation is delivered through multiple electrodes despite the fact that more current is injected. Next, we wished to examine whether the discriminability of ICMS pulse trains differing in amplitude increased when these were delivered through multiple electrodes simultaneously. To this end, we had animals discriminate ICMS that differed in amplitude, with stimuli delivered through single electrodes, pairs, or quads of electrodes. That is, we compared discrimination performance when both stimuli were presented through one, two, or four electrodes. In these experiments, pulses were anodic phase leading. We found that there was no significant difference in discrimination performance across the three conditions with either the 30-μA standard [Friedman test, $\chi^2_{(2,59)} = 0.03$, $p = 0.99$] or the 100-μA standard ($\chi^2_{(2,59)} = 1.12$, $p = 0.57$; **Figure 5**).

Implications for Neuroprosthetics

The results of our detection experiments are consistent with the hypothesis that each electrode exerts an independent effect on sensitivity, except perhaps when the anodic phase leads, where no effect was observed (though the sample was relatively small for that condition). As such, the advantage of multi-electrode stimulation is relatively modest when compared to the "best" electrode, with four electrodes yielding a mean decrease in threshold of less than 10%. There was no effect of multi-electrode stimulation on discrimination, likely reflecting the fact that discrimination is generally less sensitive to stimulus parameters than is detection. The lack of effect on discrimination performance was probably exacerbated by the fact that these experiments were carried out with anodic phase leading.

At first glance, our results seem generally inconsistent with those reported in a previous study (Zaaimi et al., 2013), in which a supra-additive effect of multi-electrode stimulation on sensitivity was reported. Furthermore, in this previous study, the synergistic effects of multi-electrode stimulation were observed even for subthreshold stimuli, which was not the case here. However, in that study, the supra-additive effect was strongest when five or more electrodes were simultaneously stimulated, so perhaps we did not stimulate a sufficient number of electrodes in the present study to observe it. The discrepancy regarding the effect of multi-electrode stimulation on subthreshold stimuli may be attributable to differences in somatosensory areas that were stimulated (areas 3b/1 vs. area 2), in the relevant sensory modalities (tactile vs. proprioceptive), or in the behavioral protocols (one stimulus interval vs. two, 360-ms vs. 1000-ms stimulus duration, etc.).

Whether pulses were delivered synchronously or asynchronously did not affect their detectability, a result that is consistent with previous findings in mice using optogenetic stimulation (Histed and Maunsell, 2014). At peri-threshold, amplitudes, the current may spread to a volume with a radius of 200–300 μm or less (Stoney et al., 1968; Tehovnik et al., 2006; Zaaimi et al., 2013), so the different electrodes may have activated mostly non-overlapping populations of neurons. Even if the fields do interact, it may be that both synchronous and asynchronous stimulation have their respective advantages: with synchronous stimulation, the fields interact, allowing intervening neurons to experience stronger stimulation, thereby increasing their probability of firing; with asynchronous stimulation, neurons experience more continuous stimulation, thereby increasing their probability of firing; the two effects may then be approximately equivalent.

Given its limited effect on sensitivity, stimulation through multiple electrodes is not a very promising way to extend the dynamic range of sensations achievable through ICMS, at

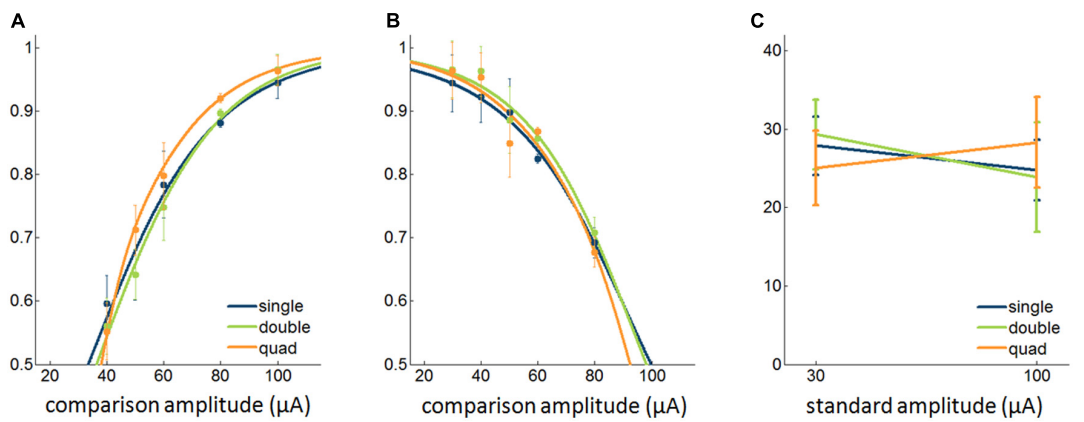

FIGURE 5 | Effect of multi-electrode stimulation on the discriminability of ICMS. (A,B) Psychometric functions with a 30-μA and 100-μA standard, respectively (9,280 trials from 12 sets of electrodes). **(C)** JNDs with different standard amplitudes. Error bars denote the SEM.

least for artificial touch and given current electrode technologies. Indeed, stimulating through the "best" electrode yields nearly equivalent results and requires a fraction of the current as does stimulating through four electrodes. One might argue that, given an independent contribution of each electrode, performance should improve as the number of stimulated electrodes increases. However, more electrodes will likely evoke more diffuse percepts (cf. Tabot et al., 2013b), so gains in dynamic range will be at the expense of spatial localization. On the other hand, for more distributed representations, such as proprioceptive ones, multi-electrode stimulation may be more practical (cf. Zaaimi et al., 2013). As technology develops, and implanted electrodes get closer together, the multi-electrode approach may be viable for touch as well. In the meantime, manipulations of phase width, pulse frequency, and pulse train duration may be more promising avenues to extend the dynamic range (Tabot et al., 2013a; Kim et al., 2014).

Materials and Methods

Animals

Procedures were approved by the University of Chicago Animal Care and Use Committee. Each of two male Rhesus macaques (6 years of age, around 10 kg in weight) was implanted with three electrode arrays: one Utah electrode array (UEA; Blackrock Microsystems, Inc., Salt Lake City, UT, USA) in the hand representation of areas 1 and 2 in the right hemisphere, flanked by two FMAs (Microprobes for Life Science, Gaithersburg, MD, USA) in area 3b (For more detail, see Berg et al., 2013; Tabot et al., 2013b). We mapped the receptive field of each electrode by identifying which areas of skin evoked multiunit activity (monitored through speakers).

Experimental Design

Each trial consisted of two sequentially presented stimulus intervals, one (detection) or both (discrimination) of which contained a stimulus (**Figure 1A**). In the detection task, the animal indicated which of the two stimulus intervals contained the stimulus; in the discrimination task, the animal indicated which of the two intervals contained the more intense stimulus. In both tasks, the animals responded by making a saccade to one of two visual targets. Animals were first trained on these tasks with mechanical indentations delivered to their skin until their performance leveled off. Mechanical stimuli were then replaced with ICMS; importantly, the animals performed at a high level on the very first block of ICMS, suggesting that the ICMS detection and discrimination were very similar to their mechanical counterparts.

Intracortical microstimulation consisted of 1-s long trains of symmetric biphasic pulses with a phase duration of 200 μs, an interphase interval of 53 μs, and a frequency of 300 Hz (**Figure 1B**). In the multi-electrode conditions, ICMS was either delivered synchronously (with all pulses in a given cycle occurring simultaneously) or asynchronously, such that pulses were evenly distributed throughout the cycle (that is, with an interpulse interval of 1667 μs for pairs and 833 μs for quads of electrodes; **Figure 1B**). In each experimental block, trials with a single electrode were interleaved with trials with pairs or quads of electrodes. In all cases, all of the electrodes in a quad had largely overlapping receptive fields on the palmar surface of the hand. Each quad was broken down into pairs, so that we could compare performance with quad stimulation to that with stimulation through electrode pairs or through single electrodes using repeated measures statistics.

In the detection experiments, ICMS amplitude was 5, 10, 15, 20, 30, 40, 50, or 80 μA and varied from trial to trial in pseudorandom order. In the discrimination experiments, the same number of electrodes was used in both intervals of each trial; the animal was comparing ICMS delivered through one, two, or four electrodes. On each trial, the amplitude of the standard stimulus was 30 or 100 μA. The 30-μA standard was paired with comparison stimuli at 40, 50, 60, 80, or 100 μA. The 100-μA standard was paired with comparison stimuli at 30, 40, 50, 60, or 80 μA. The standard stimulus was presented in either the first or the second interval and trials with both standards were interleaved so the animal would have to pay attention to both intervals to perform the task correctly.

Analysis

In the detection task, we estimated the detection threshold as the stimulus amplitude that yielded a performance of 75% correct. Similarly, in the discrimination task, we estimated the JND as the difference between comparison and standard amplitude that yielded a performance of 75% correct. Thresholds and JNDs were estimated using a standard sigmoid function. To compare sensitivity across conditions, we used parametric tests (e.g., *t*-tests) or non-parametric ones (e.g., Kruskal–Wallis test, Friedman test and signed rank test) depending on the sample size and variance of the data.

We also wished to quantify the expected performance if we assume that each electrode independently contributes to perception (cf. Zaaimi et al., 2013):

$$\begin{aligned} P_D &= 1 - (1 - P_{S1})(1 - P_{S2}) \\ P_Q &= 1 - (1 - P_{S1})(1 - P_{S2})(1 - P_{S3})(1 - P_{S4}) \end{aligned} \quad (1)$$

Where P_D and P_Q indicate the probability of detection with pairs and quads of electrodes, respectively, and P_{S1}, P_{S2}, P_{S3}, and P_{S4} denote the detection probability with each of the individual electrodes in the pair or quad. The proportion correct observed in the detection task, P_{obs}, is related to the probability of detection, P_{det}, as follows:

$$P_{\text{obs}} = P_{\text{det}} + 0.5(1 - P_{\text{det}}) \quad (2)$$

So the probability of detection is given by $P_{\text{det}} = 2P_{\text{obs}} - 1$. We computed P_{det} for each electrode and plugged the resulting value into Equation 1 to obtain the theoretical detection probability for double and quad electrodes. Finally, we used Equation 2 to convert the probabilities back to task performance metrics.

Acknowledgments

We would like to thank Lee Miller and Ricardo Ruiz Torres for helpful comments on a previous version of this manuscript. This material is based on work supported by the Defense Advanced Research Projects Agency under Contract N66001-10-C-4056. GT. was supported by National Science Foundation Grant DGE-0903637.

References

Bensmaia, S. J., and Miller, L. E. (2014). Restoring sensorimotor function through intracortical interfaces: progress and looming challenges. *Nat. Rev. Neurosci.* 15, 313–325. doi: 10.1038/nrn3724

Berg, J. A., Dammann, J. F. III, Tenore, F. V., Tabot, G. A., Boback, J. L., Manfredi, L. R., et al. (2013). Behavioral demonstration of a somatosensory neuroprosthesis. *IEEE Trans. Neural Syst. Rehabil. Eng.* 21, 500–507. doi: 10.1109/TNSRE.2013.2244616

Collinger, J. L., Wodlinger, B., Downey, J. E., Wang, W., Tyler-Kabara, E. C., Weber, D. J., et al. (2013). High-performance neuroprosthetic control by an individual with tetraplegia. *Lancet* 381, 557–564. doi: 10.1016/S0140-6736(12)61816-9

Dadarlat, M. C., O'Doherty, J. E., and Sabes, P. N. (2014). A learning-based approach to artificial sensory feedback leads to optimal integration. *Nat. Neurosci.* 18, 138–144. doi: 10.1038/nn.3883

Ghose, K., and Maunsell, J. H. (2012). A strong constraint to the joint processing of pairs of cortical signals. *J. Neurosci.* 32, 15922–15933. doi: 10.1523/JNEUROSCI.2186-12.2012

Histed, M. H., and Maunsell, J. H. (2014). Cortical neural populations can guide behavior by integrating inputs linearly, independent of synchrony. *Proc. Natl. Acad. Sci. U.S.A.* 111, E178–E187. doi: 10.1073/pnas.1318750111

Hochberg, L. R., Bacher, D., Jarosiewicz, B., Masse, N. Y., Simeral, J. D., Vogel, J., et al. (2012). Reach and grasp by people with tetraplegia using a neurally controlled robotic arm. *Nature* 485, 372–375. doi: 10.1038/nature11076

Kim, S. S., Callier, T., Tabot, G. A., Tenore, F. V., and Bensmaia, S. J. (2014). "Discrimination of electrical stimulation to primary somatosensory cortex," in *Proceedings of the Annual Meeting of the Society for Neuroscience*, Washington, DC.

Koivuniemi, A. S., and Otto, K. J. (2011). Asymmetric versus symmetric pulses for cortical microstimulation. *IEEE Trans. Neural Syst. Rehabil. Eng.* 19, 468–476. doi: 10.1109/TNSRE.2011.2166563

London, B. M., Jordan, L. R., Jackson, C. R., and Miller, L. E. (2008). Electrical stimulation of the proprioceptive cortex (area 3a) used to instruct a behaving monkey. *IEEE Trans. Neural Syst. Rehabil. Eng.* 16, 32–36. doi: 10.1109/TNSRE.2007.907544

McCreery, D., Pikov, V., and Troyk, P. R. (2010). Neuronal loss due to prolonged controlled-current stimulation with chronically implanted microelectrodes in the cat cerebral cortex. *J. Neural Eng.* 7:036005. doi: 10.1088/1741-2560/7/3/036005

O'Doherty, J. E., Lebedev, M. A., Ifft, P. J., Zhuang, K. Z., Shokur, S., Bleuler, H., et al. (2011). Active tactile exploration using a brain-machine-brain interface. *Nature* 479, 228–231. doi: 10.1038/nature10489

Ranck, J. B. Jr. (1975). Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* 98, 417–440. doi: 10.1016/0006-8993(75)90364-9

Schmidt, E., Bak, M., Hambrecht, F., Kufta, C., O'rourke, D., and Vallabhanath, P. (1996). Feasibility of a visual prosthesis for the blind based on intracortical micro stimulation of the visual cortex. *Brain* 119, 507–522. doi: 10.1093/brain/119.2.507

Stoney, S. D. Jr., Thompson, W. D., and Asanuma, H. (1968). Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *J. Neurophysiol.* 31, 659–669.

Tabot, G. A., Boback, J. L., and Dammann J. F. III, Tenore, F. V., and Bensmaia, S. J. (2013a). "Restoring touch using a brain interface: dependence of artificial percepts on stimulation parameters," in *Proceedings of the Annual Meeting of the Society for Neuroscience,* San Diego, CA.

Tabot, G. A., Dammann, J. F., Berg, J. A., Tenore, F. V., Boback, J. L., Vogelstein, R. J., et al. (2013b). Restoring the sense of touch with a prosthetic hand through a brain interface. *Proc. Natl. Acad. Sci. U.S.A.* 110, 18279–18284. doi: 10.1073/pnas.1221113110

Tabot, G. A., Kim, S. S., Winberry, J. E., and Bensmaia, S. J. (2014). Restoring tactile and proprioceptive sensation through a brain interface. *Neurobiol. Dis*. doi: 10.1016/j.nbd.2014.08.029 [Epub ahead of print].

Tehovnik, E., Tolias, A., Sultan, F., Slocum, W., and Logothetis, N. (2006). Direct and indirect activation of cortical neurons by electrical microstimulation. *J. Neurophysiol.* 96, 512–521. doi: 10.1152/jn.00126.2006

Thomson, E. E., Carra, R., and Nicolelis, M. A. (2013). Perceiving invisible light through a somatosensory cortical prosthesis. *Nat. commun.* 4: 1482.

Zaaimi, B., Ruiz-Torres, R., Solla, S. A., and Miller, L. E. (2013). Multi-electrode stimulation in somatosensory cortex increases probability of detection. *J Neural Eng.* 10:056013. doi: 10.1088/1741-2560/10/5/056013

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 03 March 2014
doi: 10.3389/fnsys.2014.00030

Augmented memory: a survey of the approaches to remembering more

Christopher R. Madan*

Department of Psychology, University of Alberta, Edmonton, AB, Canada
**Correspondence: cmadan@ualberta.ca*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Mathew E. Diamond, International School for Advanced Studies, Italy
James Bisby, University College London, UK
Ioan Opris, Wake Forest University, USA
Robert E. Hampson, Wake Forest University Health Sciences, USA
Steven P. Wise, Olschefskie Institute for the Neurobiology of Knowledge,USA

Keywords: memory, cognitive enhancement, neuroethics, transcranial stimulation, nootropics, mnemonics

Given that our ability to remember is inherently limited, one "solution" is to artificially enhance memory. Here I discuss four general approaches that have been developed to augment human long-term memory: nootropics agents, brain stimulation, mnemonic strategies, and external aids. The former two have only been recently developed in the field of systems neuroscience, and have become the focus of ethical debate. For example, some ethicists question the propriety of artificial memory enhancement in healthy individuals. As I demonstrate here, all four methods have been considered ethically suspect at one time or another. In medieval times, the use of mnemonics was considered immoral by many, and even the use of written texts as memory aids has been suggested as producing the appearance of knowledge, void of actual knowledge. Here I present a summary of each approach, beginning with those that fall within the scope of systems neuroscience, and discuss considerations critical to each of their respective ethical debates.

NOOTROPICS

Nootropics are pharmacological agents consumed solely for the purpose of cognitive enhancement, sometimes referred to as "cosmetic use." Most nootropics are prescription drugs developed to treat a disorder, but are instead taken off-label for cognitive enhancement. However, nootropics by the broadest definition can also include well-accepted psychoactive compounds including caffeinated drinks and energy drinks. Currently there is some evidence that caffeine can enhance memory (Jarvis, 1993; Hameleers et al., 2000; Borota et al., 2014), however, results are not conclusive (Nehlig, 2010). Active ingredients used in energy drinks, such as taurine and guaraná, can also enhance memory (Alford et al., 2001; Haskell et al., 2007). There is also evidence that nicotine and sage have beneficial effects on memory (Tildsley et al., 2005; Heishman et al., 2010). In general, nootropics can enhance memory encoding, but also may influence retrieval processes.

Numerous drugs are taken off-label for their nootropic properties (see Lannii et al., 2008, for a review). Piracetam is credited as the first nootropic (Winblad, 2005; Winnicka et al., 2005; Margineau, 2011) and has demonstrated memory enhancing effects (Dimond and Brouwers, 1976). Unlike most drugs, piracetam has a very weak affinity to receptors (Winblad, 2005; Margineau, 2011) and its mechanism of action is unclear. Since the initial report of piracetam's memory facilitation in 1976, pharmacology research has attempted to identify other compounds with memory enhancing abilities. Modafinil, marketed as a treatment for sleep disorders, has been found to enhance memory (see Repantis et al., 2010, for a review). Of particular interest, Kohli et al. (2009) found modafinil to enhance both quality and speed of memory. Additionally, memory enhancements were sustained after continued administration. Recent research with ampakines in non-human primates have also yielded promising results (e.g., Porrino et al., 2005). While many other nootropics also exist, such as adderall and ritalin, these drugs do not enhance memory directly, but can effect other cognitive abilities (de Jongh et al., 2008; Lannii et al., 2008).

Recent studies have shown that university students around the world are taking nootropics to improve academic performance (e.g., Eickenhorst et al., 2012; Dietz et al., 2013; Kudlow et al., 2013; Mazanov et al., 2013; Partridge et al., 2013; Sattler and Wiegel, 2013), though prevalence rates vary greatly between studies. Eickenhorst et al. (2012) surveyed students to determine the motivations for using nootropics and found that improving concentration, vigilance, and cognitive potential ranked the highest, though enhancing memory was also a major motive. However, it can be argued that nootropics lead to an uneven playing field, where wealthier individuals, who have access to nootropics, can perform better academically. While the ethics of nootropics is an emerging topic, the consumption of drugs to enhance performance is a time-worn topic within the field of athletics, where such drugs are considered cheating. Additionally, it is unclear what would constitute enhancement versus therapy—consider an older adult with gradually decreasing memory, is it "fair" to use nootropics to perform at the same level as a young adult, or would this be cheating?

BRAIN STIMULATION

Brain regions can be non-invasively stimulated using transcranial magnetic

stimulation (TMS) or transcranial direct current stimulation (tDCS). Briefly, both of these techniques modulate the excitability of neurons in the targeted regions. See Sparing and Mottaghy (2008) for a technical review of TMS and tDCS methodology. As both of these methods have limited depth of penetration, the main memory-related regions (i.e., the medial temporal lobe) cannot be targeted. However, the dorsolateral prefrontal cortex (DLPFC) has been shown to be important to memory encoding and is often the target of TMS or tDCS in memory studies (e.g., Marshall et al., 2004; Gagnon et al., 2011; Javadi and Walsh, 2012; Javadi et al., 2012). As both of these methods are unlikely to globally enhance cognitive function, but instead increase activity in one region while decreasing activity in another (net zero-sum model; Brem et al., 2014), it is important to consider the role of DLPFC in memory. The DLPFC is often associated with attention and working-memory (e.g., Lebedev et al., 2004). Though the aforementioned studies focused on DLPFC stimulation, other regions of the PFC have also been related to memory function (Blumenfeld and Ranganath, 2007). The PFC in general has been associated with several facets of higher-level cognition (see Wood and Grafman, 2003, for a review), with an emphasis on goal planning (Passingham and Wise, 2012). One view of the relation between attention and episodic memory is that information must first be attended to before it can be successfully encoded into memory. Along with this, working memory can serve as an intermediate process between attending to the information and the encoding of it.

To stimulate a brain region using TMS, pulses need to be applied concurrent with the memory task (also see Walsh and Cowey, 2000). As a result, TMS can only be effectively used within a controlled (i.e., laboratory) setting and cannot be readily used as a memory enhancement technique by one's self. In contrast, though not done in conjunction with a memory task, changes in cortical excitability due to tDCS stimulation have been shown to persist 90 minutes after stimulation (Nitsche et al., 2003, 2005), and in some cases can have persisting after-effects even 30 days later (Boggio et al., 2008). Additionally, tDCS devices are becoming available to the public (Nature Editorial, 2013), targeted at improving attention and reaction time in gamers. Of particular concern, this also allows parents to use tDCS in-home with their children to hasten learning (Kadosh et al., 2012), despite the effects of tDCS on development being unclear. Kadosh et al. (2012) suggest that using tDCS to enhance learning may be viewed as cheating as it can confer an "unearned," and thus unfair, advantage to the user. However, hiring a tutor could be similarly unfair as the tutor's guidance would make learning easier.

Memory can also be enhanced through invasive stimulation. Of course, invasive methods cannot be ethically conducted on the same scale and with the same control measures as with non-invasive methods. Hamani et al. (2008) describe a case where a patient was implanted with a deep brain stimulation (DBS) device targeted at the hypothalamus to treat morbid obesity. Through post-operative CT scans, the researchers estimated that the electrodes were located in the hypothalamus, but two were notably proximal to the fornix. Initial stimulation of one electrode evoked an autobiographical memory from decades prior. Of particular relevance, the patient developed enhanced memory function. Hamani et al. found that DBS led to greater activation in the patient's hippocampus and parahippocampal gyrus. Suthana et al. (2012) implanted DBS electrodes in the entorhinal cortex of epilepsy patients and found enhanced spatial memory. Generally, such DBS studies are only conducted with patients that have already been implanted with electrodes for non-memory reasons (e.g., localizing epilepsy foci), but recent successes may soon lead DBS to be used as a treatment for patients with memory impairments. Laxton et al. (2010) implanted DBS electrodes in the fornix in Alzheimer's patients. Stimulation drove activity in entorhinal and hippocampal regions and improved memory.

Recent research in non-human primates has also lead to the development of a neuroprosthetic device that enhances memory through task-specific activity (Hampson et al., 2013). In contrast to DBS, where fixed frequency stimulation is used to activate regions, this neuroprosthetic device is built using a nonlinear systems approach that computes multiple-input-multiple-output (MIMO) associations with CA3 spike trains as

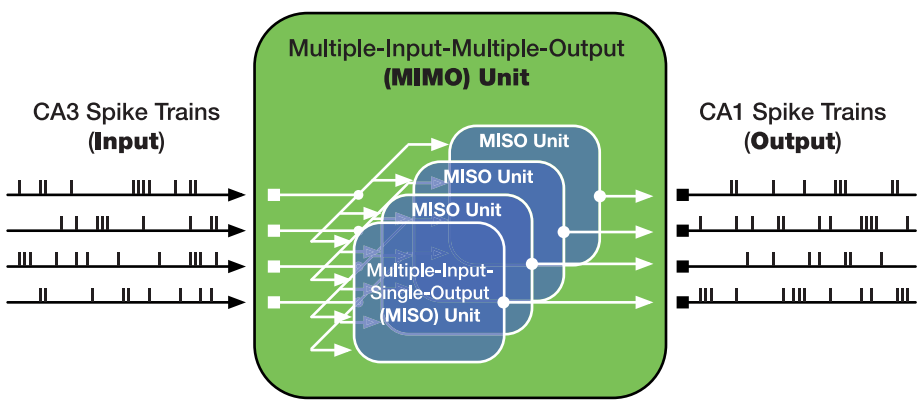

FIGURE 1 | Graphical representation of the mathematical model developed by Berger et al. (2011) to simulate connectivity between CA3 and CA1 of the hippocampus. Figure adapted from Berger et al. (2013).

inputs and CA1 spike trains as outputs (see **Figure 1**; Berger et al., 2011, 2013). More recent developments with this model have allowed for the transference of memories between individuals (Deadwyler et al., 2013). Converging with non-invasive methods, the MIMO device has also been implanted in the PFC and shown to enhance memory (Hampson et al., 2012).

Invasive stimulation techniques involve a myriad of additional ethical issues that are not present with non-invasive methods. For instance, it is unethical to implant stimulation devices purely for research, an upcoming ethical issue will be the option to opt for elective brain implants. As people are already able to opt for cosmetic surgery in the absence of any medical issues, it seems reasonable that one should also be able to elect for cognitive enhancements without a medical need. Along these lines, individuals who get cosmetic surgery can still compete in a beauty pageant without being considered "cheaters." Simply put, assuming no significant risks, should an operation to improve attractiveness be more ethical than improving cognition? That being said, further research is needed before one can ask their family physician for a referral to get a "memory implant."

MNEMONICS

The least controversial approach to enhancing one's memory is to use a strategy (i.e., mnemonics), sometimes referred to as internal aids. Countless strategies exist to improve memory encoding, several of which can be used spontaneously, such as rote repetition, making a sentence or story, imagining the to-be-remembered words, and forming a mnemonic using the first letters of the words (Harris, 1980; Intons-Peterson and Fournier, 1986). Additionally, everyday memory experts such as waiters (Ericsson and Polson, 1988; Bekinschtein et al., 2008), taxi drivers (Maguire et al., 2000), and chess masters (Chase and Simon, 1973; Gobet and Simon, 1996) use more specialized strategies.

For more generalizable strategies it is most useful to focus on individuals who have trained themselves to have superior memory. Maguire et al. (2003) compared superior memorizers, those who placed highly in the World Memory Championships, to controls. Most superior memorizers reported using the method of loci, a strategy first developed by ancient Greeks, and sometimes referred to as a "memory palace." In this strategy, one imagines a familiar environment (usually their home) and walks through this imagined environment, placing the to-be-remembered items at various locations (loci). To recall the items, the individual imagines walking through the environment and sees the items once again (also see Yates, 1966; Raz et al., 2009; Legge et al., 2012; Madan and Singhal, 2012). Importantly, superior memorizers have been found to exhibit differences in functional activations in the hippocampus and retrosplenial cortex (Maguire et al., 2003). Other techniques can also be used to achieve extraordinary memory, such as chunking, where information is hierarchically grouped (e.g., Chase and Simon, 1973). The primary flaw of mnemonics is that effective use often requires extensive practice.

Considering the ethics of mnemonic use, some Christians in the middle ages viewed mnemonics as immoral, considering them to be magic, in part due to their pagan roots (Yates, 1964, 1966). However, others embraced it and used it as a tool for the remembrance of Biblical text.

EXTERNAL AIDS

External aids such as written lists can artificially improve memory (see Harris, 1980, and Intons-Peterson and Fournier, 1986, for a comprehensive list of aids), and are primarily used as retrieval cues. Modern technology has vastly increased the capacity and convenience of external aids, particularly due to the advent of cell phones (Wilson et al., 1999; Wade and Troy, 2001; Svoboda et al., 2012). While the use of external aids is relatively innocuous, it is not free of debate. In *Phaedrus*, Plato (360 BC, 275a) recounts a conversation where Socrates cautions Phaedrus against being too dependent on written texts:

> Trust in writing will make them remember things by relying on marks made by others, from outside themselves, not on their own inner resources, and so writing will make the things they have learnt disappear from their minds. [Writing] is a potion for jogging the memory, not for remembering. You provide your students with the appearance of intelligence, not real intelligence. Because your students will be widely read, though without any contact with a teacher, they will seem to be men of wide knowledge, when they will usually be ignorant.

This passage still rings true and may even be more relevant today. With ready access to the Internet, people have even less reason to remember information directly, instead remembering where to find the information, but not the information itself (Sparrow et al., 2011). However, information stored using external aids is less susceptible to memory biases (e.g., false memories, primacy and recency effects). Future neuroimaging research comparing cued versus uncued memories using external aids may provide additional insight into the neuronal mechanisms of memory.

CONCLUSION

Recent advances in systems neuroscience have provided new approaches to artificially enhancing memory; however, these have not come without controversy. While it is not possible to resolve these debates without further discussion, it is important to acknowledge that although other approaches to artificially enhancing memory appear innocuous now, this has not always been the case. One direction forward is to draw parallels with other fields that have observed similar debates in the past, such as in the case of performance-enhancing drugs for athletes and cosmetic surgery for beauty competitions, and benefit from the discourse that has already surrounded their own ethical disputes.

MEMORY AUGMENTATION AS AN INTERVENTION

While the focus of this article is the use of augmentation to enhance memory in healthy individuals, it is important to acknowledge that these methods should be equally, if not more, beneficial to individuals with diminished memory function (e.g., older adults and Alzheimer's patients). Additionally, it is possible that diminished function can be a form of enhancement (Earp et al., 2014). With respect to impaired memory as an intervention, one such case would be patients with post-traumatic stress disorder.

ACKNOWLEDGMENTS

This work was supported by an Alexander Graham Bell Canada Graduate Scholarship (Doctoral level) from Natural Sciences and Engineering Research Council (NSERC) of Canada.

REFERENCES

Alford, C., Cox, H., and Wescott, R. (2001). The effects of red bull energy drink on human performance and mood. *Amino Acids* 21, 139–150. doi: 10.1007/s007260170021

Bekinschtein, T. A., Cardozo, J., and Manes, F. F. (2008). Strategies of Buenos Aires waiters to enhance memory capacity in a real-life setting. *Behav. Neurol.* 20, 65–70. doi: 10.1155/2008/621964

Berger, T. W., Hampson, R. E., Song, D., Goonawardena, A., Marmarelis, V. Z., and Deadwyler, S. A. (2011). A cortical neural prosthesis for restoring and enhancing memory. *J. Neural Eng.* 8:046017.

Berger, T. W., Song, D., Marmarelis, V. Z., LaCoss, J., Wills, J., Gernhardt, G. A., et al. (2013). "Reverse engineering the brain: a hippocampal cognitive prosthesis for repair and enhancement of memory function," in *Neural Engineering*, ed B. He (New York, NY: Springer), 725–764. doi: 10.1007/978-1-4614-5227-0_18

Blumenfeld, R. S., and Ranganath, C. (2007). Prefrontal cortex and long-term memory encoding: An integrative review of findings from neuropsychology and neuroimaging. *Neuroscientist* 13, 280–291. doi: 10.1177/1073858407299290

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., et al. (2008). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *Int. J. Neuropsychopharmacol.* 11, 249–254. doi: 10.1017/S1461145707007833

Borota, D., Murray, E., Keceli, G., Chang, A., Watabe, J. M., Ly, M., et al. (2014). Post-study caffeine administration enhances memory consolidation in humans. *Nat. Neuro.* 17, 201–203. doi: 10.1038/nn.3623

Brem, A.-K., Fried, P. J., Horvath, J. C., Robertson, E. M., and Pascual-Leone, A. (2014). Is neuroenhancement by noninvasive brain stimulation a net zero-sum proposition? *Neuroimage* 85, 1058–1068. doi: 10.1016/j.neuroimage.2013.07.038

Chase, W. G., and Simon, H. A. (1973). Perception in chess. *Cogn. Psychol.* 4, 55–81. doi: 10.1016/0010-0285(73)90004-2

Deadwyler, S. A., Berger, T. W., Sweatt, A. J., Song, D., Chan, R. H. M., Opris, I., et al. (2013). Donor/recipient enhancement of memory in rat hippocampus. *Front. Syst. Neurosci.* 7:120. doi: 10.3389/fnsys.2013.00120

de Jongh, R., Bolt, I., Scherer, M., and Olivier, B. (2008). Botox for the brain: enhancement of cognition, mood and pro-social behavior and blunting of unwanted memories. *Neurosci. Biobehav. Rev.* 32, 760–776. doi: 10.1016/j.neubiorev.2007.12.001

Dietz, P., Striegel, H., Franke, A. G., Lieb, K., Simon, P., and Ulrich, R. (2013). Randomized response estimates for the 12-month prevalence of cognitive-enhancing drug use in university students. *Pharmacother* 33, 44–50. doi: 10.1002/phar.1166

Dimond, S. J., and Brouwers, E. Y. M. (1976). Increase in the power of human memory in normal man through the use of drugs. *Psychopharmacol* 49, 307–309. doi: 10.1007/BF00426834

Earp, B. D., Sandberg, A., Kahane, G., and Savulescu, J. (2014). When is diminishment a form of enhancement? Rethinking the enhancement debate in biomedical ethics. *Front. Syst. Neurosci.* 8:12. doi: 10.3389/fnsys.2014.00012

Eickenhorst, P., Vitzthum, K., Klapp, B. F., Groneberg, D., and Mache, S. (2012). Neuroenhancement among German university students: Motives, expectations, and relationship with psychoactive lifestyle drugs. *J. Psychoact. Drugs* 44, 418–427. doi: 10.1080/02791072.2012.736845

Ericsson, K. A., and Polson, P. G. (1988). An experimental analysis of the mechanisms of a memory skill. *J. Exp. Psychol. Learn. Mem. Cogn.* 14, 305–316. doi: 10.1037/0278-7393.14.2.305

Gagnon, G., Schneider, C., Grondin, S., and Blanchet, S. (2011). Enhancement of episodic memory in young and healthy adults: a paired-pulse TMS study on encoding and retrieval performance. *Neurosci. Lett.* 488, 138–142. doi: 10.1016/j.neulet.2010.11.016

Gobet, F., and Simon, H. A. (1996). Recall of rapidly presented random chess positions is a function of skill. *Psychon. Bull. Rev.* 3, 159–163. doi: 10.3758/BF03212414

Hamani, C., McAndrews, M. P., Cohn, M., Oh, M., Zumsteg, D., Shapiro, C. M., et al. (2008). Memory enhancement induced by hypothalamic/fornix deep brain stimulation. *Ann. Neurol.* 63, 119–123. doi: 10.1002/ana.21295

Hameleers, P. A. H. M., van Boxtel, M. P. J., Hogervorst, E., Riedel, W. J., Houx, P. J., Buntinx, F., et al. (2000). Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. *Hum. Psychopharmacol. Clin. Exp.* 15, 573–581. doi: 10.1002/hup.218

Hampson, R. E., Gernhardt, G. A., Marmarelis, V., Song, D., Opris, I., Santos, L., et al. (2012). Facilitation and restoration of cognitive function in primate prefrontal cortex by a neuroprosthesis that utilizes minicolumn-specific neural firing. *J. Neural. Eng.* 9:056012. doi: 10.1088/1741-2560/9/5/056012

Hampson, R. E., Song, D., Opris, I., Santos, L. M., Shin, D. C., Gerhardt, G. A., et al. (2013). Facilitation of memory encoding in primate hippocampus by a neuroprosthesis that promotes task-specific neural firing. *J. Neural Eng.* 10:066013. doi: 10.1088/1741-2560/10/6/066013

Harris, J. E. (1980). Memory aids people use: Two interview studies. *Mem. Cogn.* 8, 31–38. doi: 10.3758/BF03197549

Haskell, C. F., Kennedy, D. O., Wesnes, K. A., Milne, A. L., and Scholey, A. B. (2007). A double-blind, placebo-controlled, multi-dose evaluation of the acute behavioural effects of guaraná in humans. *J. Psychopharmacol.* 21, 65–70. doi: 10.1177/0269881106063815

Heishman, S. J., Kleykamp, B. A., and Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacol* 210, 453–469. doi: 10.1007/s00213-010-1848-1

Intons-Peterson, M. J., and Fournier, J. (1986). External and internal memory aids: when and how often do we use them? *J. Exp. Psychol. Gen.* 115, 267–280. doi: 10.1037/0096-3445.115.3.267

Jarvis, M. J. (1993). Does caffeine intake enhance absolute levels of cognitive performance? *Psychopharmacol* 110, 45–52. doi: 10.1007/BF02246949

Javadi, A. H., Cheng, P., and Walsh, V. (2012). Short duration transcranial direct current stimulation (tDCS) modulates verbal memory. *Brain Stimul.* 5, 468–474. doi: 10.1016/j.brs.2011.08.003

Javadi, A. H., and Walsh, V. (2012). Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. *Brain Stimul.* 5, 231–241. doi: 10.1016/j.brs.2011.06.007

Kadosh, R. C., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, R108–R111. doi: 10.1016/j.cub.2012.01.013

Kohli, S., Fisher, S. G., Tra, Y., Adams, M. J., Mapstone, M. E., Wesnes, K. A., et al. (2009). The effect of modafinil on cognitive function in breast cancer survivors. *Cancer* 115, 2605–2616. doi: 10.1002/cncr.24287

Kudlow, P. A., Naylor, K. T., Xie, B., and McIntyre, R. S. (2013). Cognitive enhancement in Canadian medical students. *J. Psychoact. Drugs* 45, 360–365. doi: 10.1080/02791072.2013.825033

Lannii, C., Lenzken, S. C., Pascale, A., Del Vecchio, I., Racchi, M., Pistoia, F., et al. (2008). Cognition enhancers between treating and doping the mind. *Pharmacol. Res.* 57, 196–213. doi: 10.1016/j.phrs.2008.02.004

Laxton, A. W., Tang-Wai, D. F., McAndrews, M. P., Zumsteg, D., Wennberg, R., Keren, R., et al. (2010). A Phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. *Ann. Neurol.* 68, 521–534. doi: 10.1002/ana.22089

Lebedev, M. A., Messinger, A., Kralik, K. D., and Wise, S. P. (2004). Representation of attended versus remembered locations in prefrontal cortex. *PLoS Biol.* 2:e365. doi: 10.1371/journal.pbio.0020365

Legge, E. L. G., Madan, C. R., Ng, E. T., and Caplan, J. B. (2012). Building a memory palace in minutes: equivalent memory performance using virtual versus conventional environments with the method of loci. *Acta Psychol.* 141, 380–390. doi: 10.1016/j.actpsy.2012.09.002

Madan, C. R., and Singhal, A. (2012). Motor imagery and higher-level cognition: four hurdles before research can sprint forward. *Cogn. Process.* 13, 211–229. doi: 10.1007/s10339-012-0438-z

Maguire, E. A., Gadian, D. G., Johnsrude, I. S., Good, C. D., Ashburner, J., Frackowiak, R. S. J., et al. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4398–4403. doi: 10.1073/pnas.070039597

Maguire, E. A., Valentine, E. R., Wilding, J. M., and Kapur, N. (2003). Routes to remembering: the brains behind superior memory. *Nat. Neurosci.* 6, 90–95. doi: 10.1038/nn988

Margineau, D. G. (2011). A weird concept with unusual fate: nootropic drug. *Rev. Quest. Sci.* 182, 33–52.

Marshall, L., Mölle, M., Hallschmid, M., and Born, J. (2004). Transcranial direct current stimulation during sleep improves declarative memory. *J. Neurosci.* 24, 9985–9992. doi: 10.1523/JNEUROSCI.2725-04.2004

Mazanov, J., Dunn, M., Connor, J., and Fielding, M.-L. (2013). Substance use to enhance academic performance among Australian university students. *Perform Enhanc. Health* 2, 110–118. doi: 10.1016/j.peh.2013.08.017

Nature Editorial (2013). Brain blast. *Nature* 498, 271–272. doi: 10.1038/498271b

Nehlig, A. (2010). Is caffeine a cognitive enhancer? *J. Alzheimers Dis.* 20, S85–S94.

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *J. Physiol.* 533, 293–301. doi: 10.1113/jphysiol.2003.049916

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., et al. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *J. Physiol.* 568, 291–303. doi: 10.1113/jphysiol.2005.092429

Partridge, B., Bell, S., Lucke, J., and Hall, W. (2013). Australian university students' attitudes towards the use of prescription stimulants as cognitive enhancers: perceived patterns of use, efficacy and safety. *Drug Alcohol. Rev.* 32, 295–302. doi: 10.1111/dar.12005

Passingham, R. E., and Wise, S. P. (2012). *The Neurobiology of the Prefrontal Cortex: Anatomy, Evolution, and the Origin of Insight*. Cary, NC: Oxford University Press.

Plato (360 BC). *Phaedrus* (R. Waterfield, 2002, Trans.). New York, NY: Oxford University Press.

Porrino, L. J., Daunaus, J. B., Rogers, G. A., Hampson, R. E., and Deadwyler, S. A. (2005). Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. *PLoS Biol.* 3, 1639–1652. doi: 10.1371/journal.pbio.0030299

Raz, A., Packard, M. G., Alexander, G. M., Buhle, J. T., Zhu, H., Yu, S., et al. (2009). A slice of π: an exploratory neuroimaging study of digit encoding and retrieval in a superior memorist. *Neurocase* 15, 361–372. doi: 10.1080/13554790902776896

Repantis, D., Schlattmann, P., Laisney, O., and Heuser, I. (2010). Modafinil and methylphenidate for neuroenhancement in healthy individuals: a systematic review. *Pharmacol. Res.* 62, 187–206. doi: 10.1016/j.phrs.2010.04.002

Sattler, S., and Wiegel, C. (2013). Cognitive test anxiety and cognitive enhancement: the influence of students' worries on their use of performance-enhancing drugs. *Subst. Use Misuse* 48, 220–232. doi: 10.3109/10826084.2012.751426

Sparing, R., and Mottaghy, F. M. (2008). Noninvasive brain stimulation with transcranial magnetic or direct current stimulation (TMS/tDCS)—from insights into human memory to therapy of its dysfunction. *Methods* 44, 329–337. doi: 10.1016/j.ymeth.2007.02.001

Sparrow, B., Liu, J., and Wegner, D. M. (2011). Google effects on memory: cognitive consequences of having information at our fingertips. *Science* 333, 776–778. doi: 10.1126/science.1207745

Suthana, N., Haneef, Z., Stern, J., Mukamel, R., Behnke, E., Knowlton, B., et al. (2012). Memory enhancement and deep-brain stimulation of the entorhinal area. *N. Engl. J. Med.* 366, 502–510. doi: 10.1056/NEJMoa1107212

Svoboda, E., Richards, B., Leach, L., and Mertens, V. (2012). PDA and smartphone use by individuals with moderate-to- severe memory impairment: application of a theory-driven training programme. *Neuropsychol. Rehabil.* 22, 408–427. doi: 10.1080/09602011.2011.652498

Tildsley, N. T. J., Kennedy, D. O., Perry, E. K., Ballard, C. G., Wesnes, K. A., and Scholey, A. B. (2005). Positive modulation of mood and cognitive performance following administration of acute doses of *Salvia lavandulaefolia* essential oil to healthy young volunteers. *Physiol. Behav.* 83, 699–709. doi: 10.1016/j.physbeh.2004.09.010

Wade, T. K., and Troy, J. C. (2001). Mobile phones as a new memory aid: a preliminary investigation using case studies. *Brain Inj.* 15, 305–320. doi: 10.1080/026990501750111256

Walsh, V., and Cowey, A. (2000). Transcranial magnetic stimulation and cognitive neuroscience. *Nat. Rev. Neuro.* 1, 73–79. doi: 10.1038/35036239

Wilson, B. A., Emslie, H., Quirk, K., and Evans, J. (1999). George: learning to live independently with NeuroPage. *Rehabil. Psychol.* 44, 284–296. doi: 10.1037/0090-5550.44.3.284

Winblad, B. (2005). Piracetam: a review of pharmacological properties and clinical uses. *CNS Drug Rev.* 11, 169–182. doi: 10.1111/j.1527-3458.2005.tb00268.x

Winnicka, K., Tomasiak, M., and Bielawksa, A. (2005). Piracetam — an old drug with novel properties? *Acta Pol. Pharm.* 62, 405–409.

Wood, J. N., and Grafman, J. (2003). Human prefrontal cortex: processing and representation perspectives. *Nat. Rev. Neurosci.* 4, 139–147. doi: 10.1038/nrn1033

Yates, F. A. (1964). *Giordano Bruno and the Hermetic Tradition*. London: Routledge.

Yates, F. A. (1966). *The Art of Memory*. London: Routledge.

Received: 12 January 2014; accepted: 10 February 2014; published online: 03 March 2014.

*Citation: Madan CR (2014) Augmented memory: a survey of the approaches to remembering more. Front. Syst. Neurosci. **8**:30. doi: 10.3389/fnsys.2014.00030*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

MINI REVIEW ARTICLE
published: 04 September 2014
doi: 10.3389/fnsys.2014.00159

Transcranial direct current stimulation for memory enhancement: from clinical research to animal models

Djamila Bennabi[1†], Solène Pedron[1†], Emmanuel Haffen[1,2], Julie Monnin[1,2], Yvan Peterschmitt[1] and Vincent Van Waes[1]*

[1] *EA 481 Laboratory of Integrative and Clinical Neuroscience, University of Franche-Comté/SFR FED 4234, Besançon, France*
[2] *INSERM CIC-IT 808 Clinical Investigation Centre for Innovative Technology, University Hospital of Besançon, Besançon, France*

Edited by:
Ioan Opris, Wake Forest University, USA

Reviewed by:
Berthold Langguth, University of Regensburg, Germany
Estate M. Sokhadze, University of Louisville, USA

***Correspondence:**
Vincent Van Waes, EA 481 Laboratory of Integrative and Clinical Neuroscience, University of Franche-Comté - UFR Sciences et Techniques, 2 Place Leclerc, 25030 Besançon, France
e-mail: vincent.van_waes@univ-fcomte.fr

† *Co-first authors.*

There is a growing demand for new brain-enhancing technologies to improve mental performance, both for patients with cognitive disorders and for healthy individuals. Transcranial direct current stimulation (tDCS) is a non-invasive, painless, and easy to use neuromodulatory technique that can improve performance on a variety of cognitive tasks in humans despite its exact mode of action remains unclear. We have conducted a mini-review of the literature to first briefly summarize the growing amount of data from clinical trials assessing the efficacy of tDCS, focusing exclusively on learning and memory performances in healthy human subjects and in patients with depression, schizophrenia, and other neurological disorders. We then discuss these findings in the context of the strikingly few studies resulting from animal research. Finally, we highlight future directions and limitations in this field and emphasize the need to develop translational studies to better understand how tDCS improves memory, a necessary condition before it can be used as a therapeutic tool.

Keywords: tDCS, neuromodulation, prefrontal cortex, cognitive enhancement, memory

Non-invasive neuromodulatory techniques including tDCS have been shown to improve performance on a variety of cognitive domains. tDCS is a painless stimulation method that delivers subthreshold electrical currents to the brain and manipulates the resting membrane potential of cortical neurons (e.g., Stagg and Nitsche, 2011). Since the dorsolateral prefrontal cortex (DLPFC) is a crucial element in the neural network subserving executive functions (i.e., working memory, planning, goal-oriented behavior, attention, and inhibitory control; Wood and Grafman, 2003), targeting this area with neuromodulatory techniques represents a promising therapeutic option for improving cognition. In this mini-review, we summarize data obtained from clinical trials (see **Tables 1A**, **B**) and animal models focusing on tDCS-induced memory enhancement in healthy subjects and in subjects with psychiatric and neurological disorders known to induce mild to severe cognition impairments. Limitations and perspectives are then discussed.

HUMAN STUDIES

tDCS FOR COGNITIVE ENHANCEMENT IN HEALTHY SUBJECTS

To date, most studies conducted in healthy individuals have assessed the effect of tDCS in enhancing verbal and visuospatial components of working memory (WM) and learning processes. Fregni et al. found that online anodal tDCS at 1 mA applied over the left DLPFC enhances accuracy in a three-back letter task compared with cathodal stimulation of the same area or anodal stimulation of the primary motor cortex (Fregni et al., 2005). Based on the same paradigm, Ohn et al. investigated the time-dependency of tDCS and found an increased number of correct responses starting 20 min after the beginning of active stimulation compared to sham, whereas earlier measurements did not reveal any stimulation effects (Ohn et al., 2008). More recently, Lally et al. confirmed these results in a larger cohort, but only when subjects were tested during the stimulation session (online), without a persisting effect 48 h later (Lally et al., 2013). Mulquiney et al. obtained discordant results in a sample of 10 healthy volunteers, with no improvement in accuracy but in speed performance after anodal tDCS (Mulquiney et al., 2011). However, Marshall et al. reported increased reaction time in the same task during both anodal and cathodal bilateral intermittent stimulation over the DLPFC (Marshall et al., 2005). Andrews and collaborators investigated the impact of 1 session of anodal tDCS delivered during a WM task (n-back task) on performances on a subsequent WM task (digit span forward) (Andrews et al., 2011). Upon completion of the n-back task, they observed a significant improvement in performance on the digit span forward task. Berryhill and Jones enhanced WM by application of anodal tDCS over the left or right DLPFC in subjects with a high educational level, whereas tDCS provided no benefit in WM performance to a less educated group (Berryhill and Jones, 2012). Interestingly, Teo et al. found that WM performances are influenced by current strength of anodal tDCS (Teo et al., 2011). Gladwin et al. explored the impact of anodal left DLPFC tDCS on Sternberg task completion when distractor stimuli were presented during the retention period. tDCS improved reaction time only when the incorrect choice had been a distractor suggesting stimulation might have an effect on selective attention. In a subsequent study, they showed that tDCS improves reaction time in an implicit association test

Table 1A | Studies investigating the cognitive effects of tDCS in healthy subjects.

Study					tDCS parameters				Results
Author	**Design**	**n**	**Task**		**A/C**	**I (mA)**	**D (min)**	**E (cm^2)**	
Fregni et al., 2005	Cross over Single blind	15	n-back task	Online	F3/FP2	1	10	35	Improvement in accuracy (more correct responses) No improvement in reaction time
Ohn et al., 2008	Cross over Single blind	15	n-back task	Online Offline	F3/FP2	1	20	35	Increased number of correct responses after 30 min of stimulation with anodal stimulation
Lally et al., 2013	Cross over Double blind	21	n-back task	Online Offline	F3/cheek	1	10	35	Improvement of performance during the first stimulation phase with active stimulation
Mulquiney et al., 2011	Cross over	10	Cogstate Sternberg task	Online Offline	F3/FP2	1	10	35	2-back task: no effects of session or time in accuracy; improvement in reaction time Sternberg task: no effect of session or time
Marshall et al., 2005	Cross over Double blind	12	Modified Sternberg task	Online	F3/F4	0.26	15	64	No improvement in accuracy Slower reaction time after anodal and cathodal tDCS
Andrews et al., 2011	Cross over	10	n-back task Digit span tasks	Online Offline	F3/FP2	1	10	35	Previous application of tDCS during the n-back task resulted in increased performance on digit span forward
Berryhill and Jones, 2012	Cross over	25	n-back task	Offline	F3/cheek F4/cheek	1.5	10	35	Low education group: unchanged or impaired performance High education group: improved performance
Teo et al., 2011	Cross over Double blind	12	n-back task Sternberg task	Online Offline	F3/FP2	1 or 2	20	35	n-back task: decrease reaction time during the last 5 min of 2 mA session. Sternberg task: no difference in reaction time and accuracy between 1 mA, 2 mA, or sham stimulation
Gladwin et al., 2012a	Cross over	14	Sternberg task	Online Offline	F3/FP2	1	10	35	Improvement in reaction time, influence of interference
Gladwin et al., 2012b	Cross over	20	Modified version of the IAT	Offline	F3/FP2	1	10	35	Improvement in reaction time in the congruent rather than in the incongruent condition
Kincses et al., 2004	Cross over	14	PCL	Online	F3/Cz	1	10	35	Improvement of implicit learning by anodal but not cathodal stimulation
Hammer et al., 2011	Cross over Single blind	36	Recognition memory task	Online Offline	F3/FP2	1	30	35	Cathodal stimulation hampered memory performance after errorful learning, whereas anodal stimulation did not alter encoding and memory retrieval
Manenti et al., 2013	Cross over Single blind	64	Episodic memory task	Online	F3/F4	1.5	6	35	Improvement of verbal episodic memory with anodal tDCS applied during the retrieval phase Better performances in young subjects
Zwissler et al., 2014	Cross over Double blind	85	Episodic memory task	Online	F3/contralateral musculus deltoideus	1	15	35	Anodal tDCS increased whereas cathodal stimulation decreased the number of false alarms to lure pictures in subsequent recognition memory testing

Cz, midline central (international 10/20 EEG system); F3, left dorsolateral prefrontal cortex; F4, right dorsolateral prefrontal cortex; FP2, supraorbital right; IAT, Implicit Association Test; PCL, probabilistic classification learning; I, intensity; D, duration; E, electrodes size.

Table 1B | Studies investigating the cognitive effects of tDCS in psychiatric diseases.

Study					tDCS parameters				Results
Author	**Design**	**n**	**Task**		**A/C**	**I (mA)**	**D (min)**	**E (cm^2)**	
DEPRESSION									
Brunoni et al., 2013	Double blind RCT	28 UP	Probabilistic classification learning	Online	F3/F4	2	30	25	No improvement in implicit learning after real stimulation
Fregni et al., 2006	Double blind RCT	18 UP	Digit span forward and backward test	Online	F3/FP2	1	10	35	Improvement in working memory
Oliveira et al., 2013	Double blind RCT	28 UP	n-back task	Online	F3/F4	2	30	25	Enhancement of working memory Increase rate of correct responses Increase ability to discriminate between correct responses and false alarms
Wolkenstein and Plewnia, 2013	Double blind RCT	22 MDD	Delayed- response working memory task	Online	F3/Right upper arm	1	20	35	Enhancement of working memory performance and elimination of attentional bias
Ferrucci et al., 2009	Open label	8 MDD	Sternberg Task Word recognition task Posner paradigm	Offline	F3/F4	2	20	32	Cognitive tasks showed no significant difference between active or sham stimulation
Loo et al., 2012	Double blind RCT	64 MDD	RAVLT, Stroop Test, COWAT, Digit span, SDMT	Offline	F3/F8	2	20	35	Improvement of working memory performances, indexed by the SDMT, after 1 tDCS session No improvement in cognitive performances after 15 sessions
Palm et al., 2012	Double blind RCT	22 MDD	VLMT, RWT LNS_{WAIS}	Offline	F3/FP2	1 or 2	20	35	Cognitive tasks showed no significant difference between active or sham stimulation
SCHIZOPHRENIA									
Vercammen et al., 2011	Single blind Cross over	20	Probabilistic classification learning	Online	F3/FP2	2	20	35	Improvement in implicit learning after real stimulation in a subset of patient
Hoy et al., 2014	Double blind RCT	18	nback	Offline	F3/FP2	1 or 2	20		Improvement in working memory at 2 mA
Goder et al., 2013	Cross over	14	RAVLT	Offline	F3/F4	0–0.3	During sleep	64	Improvement in working memory
ALZHEIMER									
Boggio et al., 2009	Double blind RCT	10	Digit span test Visual recognition Memory task Stroop test	Online	F3/ FP2 T7/FP2	2	30	35	Improvement in working memory after prefrontal and temporal stimulation No effect on digit span and Stroop performance
Cotelli et al., 2014	Double blind RCT	36 (mild to moderate)	Face-name association memory task Memory training	Offline	F3/Right deltoid muscle	2	24		No additive effects of anodal tDCS on memory performance when combined with memory training

(Continued)

Table 1B | Continued

Study					tDCS parameters				Results
Author	**Design**	**n**	**Task**		**A/C**	**I (mA)**	**D (min)**	**E (cm^2)**	
PARKINSON									
Boggio et al., 2006	Double blind RCT	18	n-back task	Online	F3/ FP2	1 or 2	20	35	Improvement in accuracy No improvement in reaction time No effect at 1 mA
Pereira et al., 2013	Cross over	16	Semantic fluency task phonemic task	Offline	F3/FP2 P3-T5/FP2	2	20	35	Improvement in the phonemic fluency task after DLPFC tDCS
POST-STROKE									
Kang et al., 2009	Double blind RCT	10	Go/No-Go	Offline	F3/FP2	2	20	25	Improvement in response accuracy at 1 and 3 h post-stimulation
Jo et al., 2009	Double blind RCT	10	n-back task	Offline	F3/FP2	2	30	35	Improvement in the two-back task after DLPFC tDCS
Park et al., 2013	Double blind RCT	11	Seoul computerized neuropsychological test	Offline	F3/F4	2	30	25	Improvement in attention when combined with cognitive rehabilitation

COWAT, Controlled Oral Word Association Test; F3, left dorsolateral prefrontal cortex (international 10/20 EEG system); F4, right dorsolateral prefrontal cortex; FP2, supraorbital right; F8, lateral aspect of the right orbit; LNS_{WAIS}, Letter Number Sequencing Task of the Wechsler Adult Intelligence Scale; MDD, Major Depressive Disorder; RAVLT, Ray Auditory Verbal Learning Test; RCT, Randomized Controlled Trial; RWT, Regensburg Word Fluency Test; SDMT, Symbol Digit Modalities Test; UP, Unipolar Depression; I, intensity; D, duration; E, electrodes size; VLMT, Verbal Learning Memory Test, P3-T5, left temporo-parietal cortex.

without affecting the subjects' ability to overcome bias (Gladwin et al., 2012a,b).

In addition, tDCS has been recently used as an investigative tool in other memory domains. With regard to implicit memory (probabilistic classification learning), Kincses et al. first demonstrated that anodal tDCS performed over the left DLPFC at 1 mA in healthy volunteers resulted in immediate improvement in implicit learning (Kincses et al., 2004). Hammer et al. showed that cathodal stimulation hampered memory performance after errorful learning, whereas anodal stimulation did not alter encoding and memory retrieval (Hammer et al., 2011). Manenti et al. found that anodal stimulation enhances the long-term episodic memory capacities of young and older subjects with more robust effects in young participants (Manenti et al., 2013). Plewnia and collaborators also reported that tDCS shapes accuracy of episodic memory via polaritiy-specific modulation of false recognition. When applied during encoding of pictures, anodal tDCS increased whereas cathodal stimulation reduced the number of false alarms (i.e., responses to highly similar distracter images) in subsequent recognition memory testing (Zwissler et al., 2014).

tDCS FOR COGNITIVE ENHANCEMENT IN PSYCHIATRIC AND NEUROLOGICAL DISORDERS

Bifrontal tDCS has been shown to prevent procedural learning in depressive states, possibly by inducing a decrease in the activity of the right DLPFC (Brunoni et al., 2013). Beneficial effects of online stimulation applied over the left DLPFC have been reported for working memory, attentional performances, and information processing in depressed patients (Fregni et al., 2006; Oliveira et al., 2013; Wolkenstein and Plewnia, 2013). However, two randomized controlled trials and one open-label trial failed to replicate this finding with offline stimulation, suggesting that multiple tDCS sessions do not have cumulative cognitive enhancing effects (Ferrucci et al., 2009; Loo et al., 2012; Palm et al., 2012).

Only a small number of studies have examined the impact of tDCS on selective cognitive domains altered in schizophrenia. Focusing on working memory, Vercammen et al. reported that a single session of anodal tDCS to the left DLPCF improves probabilistic association learning in a specific subset of schizophrenic patients (Vercammen et al., 2011). These findings were interpreted as an enhancement of DLPFC function primarily in individuals with relatively higher neural and cognitive reserve. Hoy et al. observed the same tDCS effects on a working memory task after a 2 mA stimulation (Hoy et al., 2014). Göder et al. showed improved sleep-associated memory consolidation in patients with schizophrenia when anodal tDCS oscillating at a frequency of 0.75 Hz was applied during sleep (Goder et al., 2013).

Cognitive enhancing properties of tDCS have also been explored in a number of neurological diseases. For example, in Alzheimer disease, Boggio et al. (2009) reported short-term facilitation effects on visual recognition memory after prefrontal and temporal anodal tDCS applied 30 min at 2 mA, with no changes in attention. More recently, Cotelli et al. demonstrated that repeated sessions of anodal tDCS to the left DLFPC plus computerized memory training led to an increase in performance in a face-name association task (Cotelli et al., 2014). However, combined treatment failed to ameliorate the memory performance more

than memory training alone suggesting an absence of effects of tDCS in this paradigm. It has also been shown that a single tDCS session can ameliorate memory deficits in Parkinson's disease. Boggio et al. enhanced WM by application of anodal tDCS over the left DPFC at 2 mA, whereas stimulation with intensities of 1 mA or of other area (motor cortex) provided no benefit in WM performance (Boggio et al., 2006). Pereira et al. found that anodal tDCS (at 2 mA) applied over the left DLPFC enhanced performance and functional connectivity in task-related networks in a verbal fluency task tested during fMRI (Pereira et al., 2013).

Kang et al. reported increased response accuracy in a Go/NoGo task tested 1 and 3 h after anodal stimulation at 2 mA over the DLPFC in 10 patients with post-stroke cognitive decline (MMSE $\leq$ 25) (Kang et al., 2009). Jo et al. also reported that 10 patients with subacute stroke achieved a significant improvement in the accuracy of verbal two-back working memory after receiving the tDCS to the left prefrontal cortex at an intensity of 2 mA for 30 min (Jo et al., 2009). Park et al. found that the concomitant use of anodal tDCS with a computer-assisted cognitive rehabilitation program had a significant effect on improving attention in post-stroke patients with mild-to-moderate cognitive dysfunction (Park et al., 2013).

In spite of the increasing number of clinical studies showing beneficial effects of prefrontal tDCS on the domains of learning and memory, its mechanism of action remains unclear. Recent clinical studies have started to tackle this question (e.g., Keeser et al., 2011a,b; Amadi et al., 2013; Dayan et al., 2013; Palm et al., 2013; Plewnia et al., 2013; Stagg and Johansen-Berg, 2013); however, the cellular mechanisms underlying tDCS will likely require the use of animal models.

ANIMAL MODELS OF tDCS

Animal models provide a powerful tool to identify the mechanisms by which tDCS modulates neural network function to support improved cognition. In rats, tDCS was first used to evaluate the safety limits of cathodal stimulations (Liebetanz et al., 2009) and to map brain activation patterns after tDCS (Takano et al., 2011). In the latter study, the authors observed significantly increased fMRI signal intensities in the frontal cortex and nucleus accumbens of rats after anodal tDCS (of the frontal lobe), suggesting that tDCS induces neuronal activation both in cortical and subcortical areas. To date, few animal studies have addressed the impact of tDCS on learning and memory processes.

tDCS FOR COGNITIVE ENHANCEMENT IN HEALTHY ANIMALS

Similar to humans, the prefrontal cortex (or more generally speaking, the frontal lobe) has been the main target of animal studies for its implication in working memory. In a recent paper, Dockery et al. performed experiments in rats using the Allothetic Place Avoidance Alternation Task (APAAT), a behavioral model of visuospatial working memory and skill learning (Dockery et al., 2011). In this paradigm, a recent memory is engaged by the necessity to remember the location of a to-be-avoided sector (punished by an electric shock), which is alternated daily. tDCS on the frontal lobe (30 min/day before the APAAT task [3 days in total], 200 μA, epicranial electrode: 3.5 mm^2 over the frontal lobe, counter electrode: 10.5 cm^2 placed between the shoulders) had no measurable short-term effect on on-going place avoidance learning. However, in a follow-up session (18 days after the last APAAT session), the rats previously treated with cathodal (but not anodal) tDCS showed significantly more efficient place avoidance and skill retention compared to controls. Other types of memory, such as associative learning processes, can also be affected by tDCS (Marquez-Ruiz et al., 2012). In this case, tDCS was applied to behaving rabbits *via* four silver-ball stimulating electrodes (1 mm in diameter, placed symmetrically above the skull 3 mm from the right S1 vibrissa area on the somatosensory cortex) with a saline-soaked sponge (35 cm^2 surface area) attached to the contralateral ear serving as the counter electrode. The authors found that the acquisition of classical eyeblink conditioning is potentiated or depressed by the simultaneous application of anodal or cathodal tDCS, respectively, when stimulation of the whisker pad was used as a conditioned stimulus. These results suggest that tDCS modulates the sensory perception processes necessary for this type of associative learning (Marquez-Ruiz et al., 2012).

Recently, we have adapted a model of tDCS in mice and tested its validity in a wide range of behavioral paradigms (Pedron et al., 2014). We applied repeated anodal tDCS over the left frontal cortex of the mouse (see, **Figures 1A,B**) and used a 2 $\times$ 20 min/day stimulation paradigm at 200 μA for 5 consecutive days. In agreement with human studies, our data suggest that repeated anodal tDCS improves long-term spatial memory (in the Morris water maze, **Figure 1C**) and working memory (object recognition, **Figure 1D**) without affecting behaviors such as locomotor activity and anxiety-related behaviors (Pedron et al., 2014).

Finally, Marshall and collaborators have investigated the interaction of tDCS with hippocampo-neocortical rhythms and reported that a transcranial slow oscillation stimulation during sleep enhances memory consolidation in rats (anodes: bilaterally over the prefrontal cortex; return electrodes: over the cerebellum; sinusoidal constant current fluctuating between 0 and 5.6 μA at a frequency of 1.5 Hz applied during non-rapid eyes movement sleep) (Binder et al., 2014a,b).

COGNITIVE ENHANCEMENT IN ANIMAL MODELS OF NEUROLOGICAL DISORDERS

To the best of our knowledge, tDCS has yet to be tested for enhancing cognition in animal models of psychiatric disorders, but it has been shown to facilitate recovery from cognitive impairments induced by stroke or status epilepticus in rats. After cerebral ischemia, Yoon et al. employed a cup-shaped anodal stimulation electrode positioned at the ischemic borderline, and a rectangular rubber cathodal electrode (80 $\times$ 60 mm) fixed on the anterior chest (Yoon et al., 2012) to inject a direct current at an intensity of 200 μA for 20 min, once a day for 5 consecutive days. Both early (1 day) and late (1 week after ischemic injury) treatment had a beneficial outcome on cognition (spatial memory evaluated in the Barnes maze test) without exacerbating ischemic volume. Interestingly, this effect was not present 1 day after tDCS, but began to appear 2 weeks after the stimulations and was maximal after 4 weeks. Therapeutic effects of tDCS on cognition were associated with an increase in the

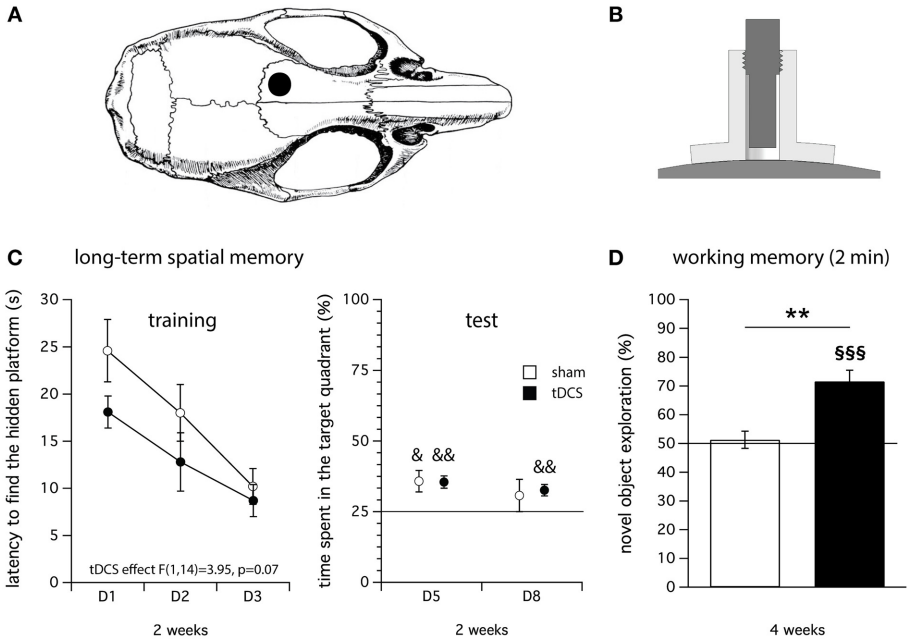

FIGURE 1 | Illustration of the tDCS device used to deliver the electrical stimulation in mice (Pedron et al., 2014) and main results obtained on cognition. (A) The center of the stimulation electrode (anode) is positioned over the left frontal cortex 1 mm anterior to the coronal fissure and 1 mm left of the sagittal fissure (Paxinos and Franklin, 2001). The cathode (rubber-plate electrode, 9.5 cm^2) is placed onto the ventral thorax (not shown). **(B)** Anode: A tubular plastic jacket (internal diameter: 2.1 mm) is surgically fixed onto the skull and filled with saline solution before the stimulation. The stimulation electrode is screwed into the tubular plastic jacket and immersed in the saline solution. Only the saline solution is in contact with the skull. **(C)** Four month-old Swiss female mice were subjected to repeated anodal tDCS for 5 consecutive days (2 × 20 min/day constant current, 0.2 mA). Long-term spatial memory was evaluated 2 weeks after the last stimulation in the Morris water navigation task (training: left; test: right). tDCS significantly improved long-term spatial memory. **(D)** Effect of repeated anodal tDCS on working memory evaluated in an object recognition task (inter-trial interval: 2 min) 4 weeks after the last stimulation. The novel object exploration (score in %) was significantly higher than 50% in the tDCS group, reflecting a better working memory performance compared to sham group for which the score was not significantly different than 50%. $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$ vs. 25%, $^{**}p < 0.01$ vs. sham, $^{\S\S\S}p < 0.001$ vs. 50%; $N = 8$ per group.

expression of Map-2 (a stabilizer of microtubules growth) and Gap-43 (a neuronal growth-promoting gene) in the early treatment group and in the late treatment group, respectively, in both peri-lesional and contra-lesional cortex. Kamida et al. used cathodal tDCS (1.5 mm to the right, 2 mm anterior from bregma; counter electrode: 1 cm needle electrode inserted into the back of the neck, 30 min per day for 2 weeks at 200 μA) to evaluate its effect on seizures and spatial memory deficits following pilocarpine-induced status epilepticus in immature rats (Kamida et al., 2011). Repeated cathodal tDCS reduced seizures, spatial memory impairments, status epilepticus-induced hippocampal cell loss, and supragranular and CA3 mossy fiber sprouting.

POTENTIAL MECHANISMS OF ACTION AND PERSPECTIVES

CANDIDATE MECHANISMS UNDERLYING tDCS ACTION ON COGNITION

To date, it is known that tDCS modifies the resting membrane potential when online and induces prolonged offline after-effects similar to long-term potentiation/depression (Paulus, 2004), considered to be the cellular mechanisms of learning and memory. For example, in humans the long-lasting effects of tDCS (both anodal and cathodal) on the primary motor cortex are suppressed after NMDA-receptor blockade indicating a dependence on glutamatergic activity (Liebetanz et al., 2002). Moreover, previous experiments in rats have shown that anodal polarization directly applied to the cortex has the ability to modulate neural plasticity (i.e., c-Fos activation) *via* activation of NMDA receptors (Islam et al., 1995).

Our team has started to investigate the role of adult neurogenesis as a mechanism involved in tDCS action. Neurogenesis in the hippocampus is of particular interest as tDCS induces both antidepressant effects and enhances cognition in humans and mice, two phenomena critically linked to the generation of new neurons in the adult dentate gyrus (Deng et al., 2010; Eisch and Petrik, 2012). In addition, the time course for the onset of long term tDCS effect on depression-related behavior and on cognition in our animal model (after several weeks, Pedron et al., 2014) is consistent with the delay necessary for newly generated cells in the hippocampus to be functionally integrated (Klempin et al., 2010). Of particular interest is the impact of tDCS on brain-derived neurotrophic factor (BDNF) levels. This growth factor is important for long-term memory (Bekinschtein et al., 2008), is involved in depressive-like behaviors and antidepressant drug action, and can modulate neurogenesis levels (Castren and Rantamaki, 2010; Vithlani et al., 2013). A recent study has shown that BDNF activation is necessary for DCS-induced long-term potentiation in mouse M1 slices

(Fritsch et al., 2010). Enhancement of motor skill acquisition by anodal tDCS also seems to be related to BDNF, as the BDNF val66met polymorphism in humans is associated with decreased proclivity to tDCS-induced benefits on skill learning (Fritsch et al., 2010). Other indirect mechanisms cannot be ruled out, such as the impact of tDCS on cortical blood perfusion (Wachter et al., 2011; Stagg et al., 2013).

LIMITATIONS AND FUTURE DIRECTIONS

One outstanding question in the above-mentioned studies is: where does the current flow? Considering that tDCS has poor spatial resolution on brain tissue, it is important to acknowledge the limitation on the precision with which tDCS is able to target specific areas of the brain. A main issue is the difference between the electrodes used in animals and those used in clinical applications, preventing direct comparisons of current density and voltage distributions between experimental models (higher current densities are often reported in animals). Because in humans the outcome of stimulation depends of the amount of current delivered, it would be necessary to test whether similar dose-response curves occur in animals and attempt stimulation parameters more closely related to clinical studies. Another limiting factor is the considerable protocol variations particularly among animal models. This lack of standardization is deleterious and could contribute to the discrepancy sometimes observed in the literature. The standardization of physical parameters, namely the current density and shape, electrodes size, shape and localization (2 epicranial electrodes vs. 1 epicranial/ 1 outside the skull), the duration and number of stimulations, and the state of animals during the stimulation (awake or anesthetized) would greatly aid in the elucidation of the mechanisms and efficacy of tDCS.

Another often overlooked point is the population on which tDCS is used. The interaction of stimulation polarity, cognitive domain and other intra- and interindividual variables such as anatomic or genetic factors (Plewnia et al., 2013; Kim et al., 2014), personality (Pena-Gomez et al., 2011; Pripfl et al., 2013), cognitive strategy (Berryhill and Jones, 2012) and baseline neuronal activation state (Jacobson et al., 2012) need to be taken into consideration. Likewise, the age at which electrical stimulation occurs (Kessler et al., 2013) might be a key determinants for the physiological and behavioral outcomes of the stimulation. tDCS effects might for example be stronger/different and possibly harmful if applied to the brain during a critical stage of development such as during adolescence when the prefrontal cortex is still not fully mature.

Finally, further basic research is needed to elucidate the duration of the effects of tDCS on memory, which require evaluations at different time-points. The eventual necessity to re-stimulate the brain to maintain the beneficial effects of tDCS has yet to be investigated.

In conclusion, the data reported here are very promising and show that electrical stimulation of the brain is able to improve cognition in humans, in both healthy and in patients with psychiatric or neurological disorders. However, before it can be applied as a therapeutic tool, there is a clear need for method standardization and for a better understanding of its mode of action through the combined use of clinical research and animal models.

ACKNOWLEDGMENT

We would like to warmly thank Adriana Caballero for proofreading and editing the manuscript.

REFERENCES

Amadi, U., Ilie, A., Johansen-Berg, H., and Stagg, C. J. (2013). Polarity-specific effects of motor transcranial direct current stimulation on fMRI resting state networks. *Neuroimage* 88C, 155–161. doi: 10.1016/j.neuroimage.2013.11.037

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimul.* 4, 84–89. doi: 10.1016/j.brs.2010.06.004

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J. I., Goldin, A., et al. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2711–2716. doi: 10.1073/pnas.0711863105

Berryhill, M. E., and Jones, K. T. (2012). tDCS selectively improves working memory in older adults with more education. *Neurosci. Lett.* 521, 148–151. doi: 10.1016/j.neulet.2012.05.074

Binder, S., Berg, K., Gasca, F., Lafon, B., Parra, L. C., Born, J., et al. (2014a). Transcranial slow oscillation stimulation during sleep enhances memory consolidation in rats. *Brain Stimul.* 7, 508–515. doi: 10.1016/j.brs.2014.03.001

Binder, S., Rawohl, J., Born, J., and Marshall, L. (2014b). Transcranial slow oscillation stimulation during NREM sleep enhances acquisition of the radial maze task and modulates cortical network activity in rats. *Front. Behav. Neurosci.* 7:220. doi: 10.3389/fnbeh.2013.00220

Boggio, P. S., Ferrucci, R., Rigonatti, S. P., Covre, P., Nitsche, M., Pascual-Leone, A., et al. (2006). Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. *J. Neurol. Sci.* 249, 31–38. doi: 10.1016/j.jns.2006.05.062

Boggio, P. S., Khoury, L. P., Martins, D. C., Martins, O. E., De Macedo, E. C., and Fregni, F. (2009). Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease. *J. Neurol. Neurosurg. Psychiatr.* 80, 444–447. doi: 10.1136/jnnp.2007.141853

Brunoni, A. R., Zanao, T. A., Ferrucci, R., Priori, A., Valiengo, L., De Oliveira, J. F., et al. (2013). Bifrontal tDCS prevents implicit learning acquisition in antidepressant-free patients with major depressive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 43, 146–150. doi: 10.1016/j.pnpbp.2012.12.019

Castren, E., and Rantamaki, T. (2010). The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. *Dev. Neurobiol.* 70, 289–297. doi: 10.1002/dneu.20758

Cotelli, M., Manenti, R., Brambilla, M., Petesi, M., Rosini, S., Ferrari, C., et al. (2014). Anodal tDCS during face-name associations memory training in Alzheimer's patients. *Front. Aging Neurosci.* 6:38. doi: 10.3389/fnagi.2014.00038

Dayan, E., Censor, N., Buch, E. R., Sandrini, M., and Cohen, L. G. (2013). Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat. Neurosci.* 16, 838–844. doi: 10.1038/nn.3422

Deng, W., Aimone, J. B., and Gage, F. H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* 11, 339–350. doi: 10.1038/nrn2822

Dockery, C. A., Liebetanz, D., Birbaumer, N., Malinowska, M., and Wesierska, M. J. (2011). Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats. *Neurobiol. Learn. Mem.* 96, 452–460. doi: 10.1016/j.nlm.2011.06.018

Eisch, A. J., and Petrik, D. (2012). Depression and hippocampal neurogenesis: a road to remission? *Science* 338, 72–75. doi: 10.1126/science.1222941

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., et al. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. *J. Affect. Disord.* 118, 215–219. doi: 10.1016/j.jad.2009.02.015

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp. Brain Res.* 166, 23–30. doi: 10.1007/s00221-005-2334-6

Fregni, F., Boggio, P. S., Nitsche, M. A., Marcolin, M. A., Rigonatti, S. P., and Pascual-Leone, A. (2006). Treatment of major depression with transcranial direct current stimulation. *Bipolar Disord.* 8, 203–204. doi: 10.1111/j.1399-5618.2006.00291.x

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Gladwin, T. E., Den Uyl, T. E., Fregni, F. F., and Wiers, R. W. (2012a). Enhancement of selective attention by tDCS: interaction with interference in a Sternberg task. *Neurosci. Lett.* 512, 33–37. doi: 10.1016/j.neulet.2012.01.056

Gladwin, T. E., Den Uyl, T. E., and Wiers, R. W. (2012b). Anodal tDCS of dorsolateral prefontal cortex during an Implicit Association Test. *Neurosci. Lett.* 517, 82–86. doi: 10.1016/j.neulet.2012.04.025

Goder, R., Baier, P. C., Beith, B., Baecker, C., Seeck-Hirschner, M., Junghanns, K., et al. (2013). Effects of transcranial direct current stimulation during sleep on memory performance in patients with schizophrenia. *Schizophr. Res.* 144, 153–154. doi: 10.1016/j.schres.2012.12.014

Hammer, A., Mohammadi, B., Schmicker, M., Saliger, S., and Munte, T. F. (2011). Errorless and errorful learning modulated by transcranial direct current stimulation. *BMC Neurosci.* 12:72. doi: 10.1186/1471-2202-12-72

Hoy, K. E., Arnold, S. L., Emonson, M. R., Daskalakis, Z. J., and Fitzgerald, P. B. (2014). An investigation into the effects of tDCS dose on cognitive performance over time in patients with schizophrenia. *Schizophr. Res.* 155, 96–100. doi: 10.1016/j.schres.2014.03.006

Islam, N., Moriwaki, A., Hattori, Y., Hayashi, Y., Lu, Y. F., and Hori, Y. (1995). c-Fos expression mediated by N-methyl-D-aspartate receptors following anodal polarization in the rat brain. *Exp. Neurol.* 133, 25–31. doi: 10.1006/exnr.1995.1004

Jacobson, L., Koslowsky, M., and Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Exp. Brain Res.* 216, 1–10. doi: 10.1007/s00221-011-2891-9

Jo, J. M., Kim, Y. H., Ko, M. H., Ohn, S. H., Joen, B., and Lee, K. H. (2009). Enhancing the working memory of stroke patients using tDCS. *Am. J. Phys. Med. Rehabil.* 88, 404–409. doi: 10.1097/PHM.0b013e3181a0e4cb

Kamida, T., Kong, S., Eshima, N., Abe, T., Fujiki, M., and Kobayashi, H. (2011). Transcranial direct current stimulation decreases convulsions and spatial memory deficits following pilocarpine-induced status epilepticus in immature rats. *Behav. Brain Res.* 217, 99–103. doi: 10.1016/j.bbr.2010.08.050

Kang, E. K., Baek, M. J., Kim, S., and Paik, N. J. (2009). Non-invasive cortical stimulation improves post-stroke attention decline. *Restor. Neurol. Neurosci.* 27, 645–650. doi: 10.3233/RNN-2009-0514

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., et al. (2011a). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J. Neurosci.* 31, 15284–15293. doi: 10.1523/JNEUROSCI.0542-11.2011

Keeser, D., Padberg, F., Reisinger, E., Pogarell, O., Kirsch, V., Palm, U., et al. (2011b). Prefrontal direct current stimulation modulates resting EEG and event-related potentials in healthy subjects: a standardized low resolution tomography (sLORETA) study. *Neuroimage* 55, 644–657. doi: 10.1016/j.neuroimage.2010.12.004

Kessler, S. K., Minhas, P., Woods, A. J., Rosen, A., Gorman, C., and Bikson, M. (2013). Dosage considerations for transcranial direct current stimulation in children: a computational modeling study. *PLoS ONE* 8:e76112. doi: 10.1371/journal.pone.0076112

Kim, J. H., Kim, D. W., Chang, W. H., Kim, Y. H., Kim, K., and Im, C. H. (2014). Inconsistent outcomes of transcranial direct current stimulation may originate from anatomical differences among individuals: electric field simulation using individual MRI data. *Neurosci. Lett.* 564, 6–10. doi: 10.1016/j.neulet.2014.01.054

Kincses, T. Z., Antal, A., Nitsche, M. A., Bartfai, O., and Paulus, W. (2004). Facilitation of probabilistic classification learning by transcranial direct current stimulation of the prefrontal cortex in the human. *Neuropsychologia* 42, 113–117. doi: 10.1016/S0028-3932(03)00124-6

Klempin, F., Babu, H., De Pietri Tonelli, D., Alarcon, E., Fabel, K., and Kempermann, G. (2010). Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front. Mol. Neurosci.* 3:14. doi: 10.3389/fnmol.2010.00014

Lally, N., Nord, C. L., Walsh, V., and Roiser, J. P. (2013). Does excitatory fronto-extracerebral tDCS lead to improved working memory performance? *F1000Research* 2:219. doi: 10.12688/f1000research.2-219.v2

Liebetanz, D., Koch, R., Mayenfels, S., Konig, F., Paulus, W., and Nitsche, M. A. (2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clin. Neurophysiol.* 120, 1161–1167. doi: 10.1016/j.clinph.2009.01.022

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125, 2238–2247. doi: 10.1093/brain/awf238

Loo, C. K., Alonzo, A., Martin, D., Mitchell, P. B., Galvez, V., and Sachdev, P. (2012). Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. *Br. J. Psychiatry* 200, 52–59. doi: 10.1192/bjp.bp.111.097634

Manenti, R., Brambilla, M., Petesi, M., Ferrari, C., and Cotelli, M. (2013). Enhancing verbal episodic memory in older and young subjects after non-invasive brain stimulation. *Front. Aging Neurosci.* 5:49. doi: 10.3389/fnagi.2013.00049

Marquez-Ruiz, J., Leal-Campanario, R., Sanchez-Campusano, R., Molaee-Ardekani, B., Wendling, F., Miranda, P. C., et al. (2012). Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6710–6715. doi: 10.1073/pnas.1121147109

Marshall, L., Molle, M., Siebner, H. R., and Born, J. (2005). Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. *BMC Neurosci.* 6:23. doi: 10.1186/1471-2202-6-23

Mulquiney, P. G., Hoy, K. E., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. *Clin. Neurophysiol.* 122, 2384–2389. doi: 10.1016/j.clinph.2011.05.009

Ohn, S. H., Park, C. I., Yoo, W. K., Ko, M. H., Choi, K. P., Kim, G. M., et al. (2008). Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. *Neuroreport* 19, 43–47. doi: 10.1097/WNR.0b013e3282f2adfd

Oliveira, J. F., Zanao, T. A., Valiengo, L., Lotufo, P. A., Bensenor, I. M., Fregni, F., et al. (2013). Acute working memory improvement after tDCS in antidepressant-free patients with major depressive disorder. *Neurosci. Lett.* 537, 60–64. doi: 10.1016/j.neulet.2013.01.023

Palm, U., Fintescu, Z., Obermeier, M., Schiller, C., Reisinger, E., Keeser, D., et al. (2013). Serum levels of brain-derived neurotrophic factor are unchanged after transcranial direct current stimulation in treatment-resistant depression. *J. Affect. Disord.* 150, 659–663. doi: 10.1016/j.jad.2013.03.015

Palm, U., Schiller, C., Fintescu, Z., Obermeier, M., Keeser, D., Reisinger, E., et al. (2012). Transcranial direct current stimulation in treatment resistant depression: a randomized double-blind, placebo-controlled study. *Brain Stimul.* 5, 242–251. doi: 10.1016/j.brs.2011.08.005

Park, S. H., Koh, E. J., Choi, H. Y., and Ko, M. H. (2013). A double-blind, sham-controlled, pilot study to assess the effects of the concomitant use of transcranial direct current stimulation with the computer assisted cognitive rehabilitation to the prefrontal cortex on cognitive functions in patients with stroke. *J. Korean Neurosurg. Soc.* 54, 484–488. doi: 10.3340/jkns.2013.54.6.484

Paulus, W. (2004). Outlasting excitability shifts induced by direct current stimulation of the human brain. *Suppl. Clin. Neurophysiol.* 57, 708–714. doi: 10.1016/S1567-424X(09)70411-8

Paxinos, G., and Franklin, K. (2001). *The Mouse Brain in Stereotaxic Coordinate, 2nd Edn*. San Diego, CA; London: Academic Press.

Pedron, S., Monnin, J., Haffen, E., Sechter, D., and Van Waes, V. (2014). Repeated transcranial direct current stimulation prevents abnormal behaviors associated with abstinence from chronic nicotine consumption. *Neuropsychopharmacology* 39, 981–988. doi: 10.1038/npp.2013.298

Pena-Gomez, C., Vidal-Pineiro, D., Clemente, I. C., Pascual-Leone, A., and Bartres-Faz, D. (2011). Down-regulation of negative emotional processing by transcranial direct current stimulation: effects of personality characteristics. *PLoS ONE* 6:e22812. doi: 10.1371/journal.pone.0022812

Pereira, J. B., Junque, C., Bartres-Faz, D., Marti, M. J., Sala-Llonch, R., Compta, Y., et al. (2013). Modulation of verbal fluency networks by transcranial direct current stimulation (tDCS) in Parkinson's disease. *Brain Stimul.* 6, 16–24. doi: 10.1016/j.brs.2012.01.006

Plewnia, C., Zwissler, B., Langst, I., Maurer, B., Giel, K., and Kruger, R. (2013). Effects of transcranial direct current stimulation (tDCS) on executive functions: influence of COMT Val/Met polymorphism. *Cortex* 49, 1801–1807. doi: 10.1016/j.cortex.2012.11.002

Pripfl, J., Neumann, R., Kohler, U., and Lamm, C. (2013). Effects of transcranial direct current stimulation on risky decision making are mediated by 'hot' and

'cold' decisions, personality, and hemisphere. *Eur. J. Neurosci.* 38, 3778–3785. doi: 10.1111/ejn.12375

Stagg, C. J., and Johansen-Berg, H. (2013). Studying the effects of transcranial direct-current stimulation in stroke recovery using magnetic resonance imaging. *Front. Hum. Neurosci.* 7:857. doi: 10.3389/fnhum.2013.00857

Stagg, C. J., Lin, R. L., Mezue, M., Segerdahl, A., Kong, Y., Xie, J., et al. (2013). Widespread modulation of cerebral perfusion induced during and after transcranial direct current stimulation applied to the left dorsolateral prefrontal cortex. *J. Neurosci.* 33, 11425–11431. doi: 10.1523/JNEUROSCI.3887-12.2013

Stagg, C. J., and Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *Neuroscientist* 17, 37–53. doi: 10.1177/1073858410386614

Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., and Hironaka, N. (2011). A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neurosci. Lett.* 491, 40–43. doi: 10.1016/j.neulet.2011.01.004

Teo, F., Hoy, K. E., Daskalakis, Z. J., and Fitzgerald, P. B. (2011). Investigating the role of current strength in tDCS modulation of working memory performance in healthy controls. *Front. Psychiatry* 2:45. doi: 10.3389/fpsyt.2011.00045

Vercammen, A., Rushby, J. A., Loo, C., Short, B., Weickert, C. S., and Weickert, T. W. (2011). Transcranial direct current stimulation influences probabilistic association learning in schizophrenia. *Schizophr. Res.* 131, 198–205. doi: 10.1016/j.schres.2011.06.021

Vithlani, M., Hines, R. M., Zhong, P., Terunuma, M., Hines, D. J., Revilla-Sanchez, R., et al. (2013). The ability of BDNF to modify neurogenesis and depressive-like behaviors is dependent upon phosphorylation of tyrosine residues 365/367 in the GABA(A)-receptor gamma2 subunit. *J. Neurosci.* 33, 15567–15577. doi: 10.1523/JNEUROSCI.1845-13.2013

Wachter, D., Wrede, A., Schulz-Schaeffer, W., Taghizadeh-Waghefi, A., Nitsche, M. A., Kutschenko, A., et al. (2011). Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. *Exp. Neurol.* 227, 322–327. doi: 10.1016/j.expneurol.2010.12.005

Wolkenstein, L., and Plewnia, C. (2013). Amelioration of cognitive control in depression by transcranial direct current stimulation. *Biol. Psychiatry* 73, 646–651. doi: 10.1016/j.biopsych.2012.10.010

Wood, J. N., and Grafman, J. (2003). Human prefrontal cortex: processing and representational perspectives. *Nat. Rev. Neurosci.* 4, 139–147. doi: 10.1038/nrn1033

Yoon, K. J., Oh, B. M., and Kim, D. Y. (2012). Functional improvement and neuroplastic effects of anodal transcranial direct current stimulation (tDCS) delivered 1 day vs. 1 week after cerebral ischemia in rats. *Brain Res.* 1452, 61–72. doi: 10.1016/j.brainres.2012.02.062

Zwissler, B., Sperber, C., Aigeldinger, S., Schindler, S., Kissler, J., and Plewnia, C. (2014). Shaping memory accuracy by left prefrontal transcranial direct current stimulation. *J. Neurosci.* 34, 4022–4026. doi: 10.1523/JNEUROSCI.5407-13.2014

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 June 2014; accepted: 13 August 2014; published online: 04 September 2014.

Citation: Bennabi D, Pedron S, Haffen E, Monnin J, Peterschmitt Y and Van Waes V (2014) Transcranial direct current stimulation for memory enhancement: from clinical research to animal models. Front. Syst. Neurosci. ***8****:159. doi: 10.3389/fnsys.2014.00159*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 09 January 2015
doi: 10.3389/fnsys.2014.00243

How to build better memory training games

Jenni Deveau[1], Susanne M. Jaeggi[2,3], Victor Zordan[4], Calvin Phung[4] and Aaron R. Seitz[1]*

[1] *Department of Psychology, University of California, Riverside, Riverside, CA, USA*
[2] *School of Education, University of California, Irvine, Irvine, CA, USA*
[3] *Department of Cognitive Sciences, University of California, Irvine, Irvine, CA, USA*
[4] *Department of Computer Science, University of California, Riverside, Riverside, CA, USA*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Jyoti Mishra, University of California San Francisco, USA
Mariano Sigman, Universidad Torcuato Di Tella, Argentina

****Correspondence:***
Aaron R. Seitz, Department of Psychology, University of California, Riverside, 900 University Ave, Riverside, CA 92521, USA
e-mail: aseitz@ucr.edu

Can we create engaging training programs that improve working memory (WM) skills? While there are numerous procedures that attempt to do so, there is a great deal of controversy regarding their efficacy. Nonetheless, recent meta-analytic evidence shows consistent improvements across studies on lab-based tasks generalizing beyond the specific training effects (Au et al., 2014; Karbach and Verhaeghen, 2014), however, there is little research into how WM training aids participants in their daily life. Here we propose that incorporating design principles from the fields of Perceptual Learning (PL) and Computer Science might augment the efficacy of WM training, and ultimately lead to greater learning and transfer. In particular, the field of PL has identified numerous mechanisms (including attention, reinforcement, multisensory facilitation and multi-stimulus training) that promote brain plasticity. Also, computer science has made great progress in the scientific approach to game design that can be used to create engaging environments for learning. We suggest that approaches integrating knowledge across these fields may lead to a more effective WM interventions and better reflect real world conditions.

Keywords: working memory, video games, brain training, perceptual learning, game design

INTRODUCTION

As long as scientists have explored memory, they have strived, and often failed, to improve it. Most approaches to improve memory implement strategies, such as creating mnemonic devices (for example, the method of loci). However, despite evidence these techniques improve memory *performance*, they do not target underlying memory *processes*, and while they do have some influence on memory systems in the brain (Maguire et al., 2003), they typically fail to broadly generalize to untrained activities (Verhaeghen et al., 1992; Maguire et al., 2003; St Clair-Thompson et al., 2010). Recent research on "brain training" renews promise for improving memory and other cognitive skills. Here, we focus on working memory (WM), a limited-capacity system for storing and manipulating information in a given moment. WM underlies performance in virtually all complex cognitive tasks (Shah and Miyake, 1999). Recent approaches targeting skills related to WM (Klingberg et al., 2002, 2005; Jaeggi et al., 2008, 2010; Anguera et al., 2013; Goldin et al., 2014) have shown generalizing benefits to a wide number of non-trained cognitive tasks that are thought to rely on WM, including executive control and fluid reasoning (c.f. Au et al., 2014; Karbach and Verhaeghen, 2014 for recent meta-analyses).

Here we review recent WM training approaches discussing their strengths and limitations and suggest methods that are based on the principles of perceptual learning (PL) and game design to make them more effective. Off-the-shelf computer games and standard cognitive approaches each contain component properties that can benefit WM. We propose that integrating knowledge from psychology and neuroscience along with the science of video game design could critically inform the development of engaging, cognitively immersive challenges that will more optimally train WM memory processes.

SPAN TRAINING

Span training targets WM capacity (Klingberg et al., 2002, 2005) typically relying on two types of tasks, simple and complex. Simple span tasks present sequences of stimuli that vary in set-size with participants typically reporting the items in (reverse) order of presentation. Research has shown that training on simple span tasks results in transfer in a variety of measures, such as non-trained WM tasks, response inhibition, and even fluid reasoning (Klingberg et al., 2002, 2005; Thorell et al., 2009).

In contrast to simple span tasks, which focus predominantly on WM storage, complex span tasks involve a secondary processing task. An example of a complex span task is Reading Span (Daneman and Carpenter, 1980) where participants judge the semantic content of a series of sentences and then later recall the last word of each sentence in order. Simple and complex span tasks used as interventions are typically adaptive, where the number of items to be recalled increases as training progresses. Adaptive complex span training leads to both near and far transfer in a variety of populations. Chein and Morrison (2010) showed that training on verbal and spatial complex span tasks improves verbal and spatial short-term memory, response inhibition, and reading comprehension in young and older adults (Richmond et al., 2011). In another series of studies, typically developing children (Loosli et al., 2012; Karbach et al., 2014)

and older adults (Buschkuehl et al., 2008) were trained on complex span (see **Figure 1B**) demonstrating improved reading performance (Loosli et al., 2012; Karbach et al., 2014) and improved visual WM and episodic memory (Buschkuehl et al., 2008). Other groups have found similar effects from complex span training in older adults that were maintained several months after training completion (Borella et al., 2010, 2013, 2014).

N-BACK TRAINING

Among the best known WM tasks used for training is the n-back task (Jaeggi et al., 2008; Smith et al., 2009; Buschkuehl and Jaeggi, 2010). Here, participants memorize and constantly update the serial positions "*n* steps back" in a continuous stimulus stream and report whether or not the current stimulus matches the stimulus *n*-items back in a sequence (**Figure 1A**). Task difficulty (equal to level of *n*) is adaptively adjusted in response to participants' performance. N-back training-related improvements are wide-ranging, including non-trained WM functions (e.g., Lilienthal et al., 2013), executive functioning (e.g., Salminen et al., 2012), episodic memory (e.g., Rudebeck et al., 2012), and even fluid reasoning (Stephenson and Halpern, 2013). Effects are observed across the lifespan ranging from typically developing children (Jaeggi et al., 2011) to older adults (Stepankova et al., 2014). Both the amount of training (Stepankova et al., 2014) and achievement on the training task (Jaeggi et al., 2011) have been related to consequent improvements in the untrained fluid intelligence tasks. However, findings of far transfer (e.g., that WM training leads to improved performance on tasks unrelated to the training), are not ubiquitous in the literature (Shipstead et al., 2012). Some of these inconsistencies across studies may be explained by variations in training schedules, outcome measures, or individual differences (Jaeggi et al., 2012, 2014; Shah et al., 2012).

MECHANISMS THAT PROMOTE LEARNING

While there is growing evidence that WM training impacts performance in a wide variety of tasks, the mechanisms driving plasticity in WM systems remain unclear. However, research of PL has identified numerous mechanisms that promote the magnitude and generalization of learning. PL refers to a long lasting improvement in perceptual abilities as a result of experience. Interestingly, key approaches to working WM training, such as extended practice and adaptive procedures (the latter is to use computer algorithms to customize the challenge to each participant), were originally modeled after successful approaches in PL (Klingberg et al., 2002, 2005). Classically, a translational barrier to PL has been its high degree of specificity to trained stimulus features (Fahle, 2005), such as orientation (Fiorentini and Berardi, 1980), retinal location (Karni and Sagi, 1991) or the eye trained (Poggio et al., 1992; Seitz et al., 2009). This specificity to the training task and stimuli mirrors issues that face modern WM training. However, recent research illustrates how to overcome this "curse of specificity" with approaches that integrate many techniques (Deveau et al., 2014a,b) showing greater generalization of learning.

A key question is what mechanisms gate learning? Seitz and Dinse (2007) proposed a model of PL in which mechanisms including attention, reinforcement, optimal stimulation protocols, and multisensory facilitation interact to boost sensory signals over a learning threshold. This model and a host of empirical research on PL demonstrate that learning generalizes best when: (1) a larger set of stimulus features are trained (Xiao et al., 2008; Hung and Seitz, 2014); (2) using multisensory stimuli (Shams and Seitz, 2008); (3) using motivating tasks (Shibata et al., 2009); (4) participants are confident in their performance (Ahissar and Hochstein, 1997); and (5) consistent reinforcement to the training stimuli is used (Seitz and Watanabe, 2009). Combining these approaches increase the magnitude and generality of learning (Deveau et al., 2014a,b). In the following, we review some of the mechanisms that promote PL and discuss how they could be applied to WM training.

ATTENTION AND REINFORCEMENT

Attention refers to a set of mental processes that selectively modulate the processing of relevant information over irrelevant information; attention influences decisions, guides memory processes and our executive functions to direct resources to act upon the world. Numerous studies show that attention gates learning (Shiu and Pashler, 1992; Ahissar and Hochstein, 1993; Schoups et al., 2001; Leclercq and Seitz, 2012b). For example, Schoups et al. (2001) found neuronal plasticity of V1 cells corresponding to attended stimuli but no plasticity for cells with receptive fields overlapping unattended stimuli, suggesting that attention selects what is learned and what is not. A key aspect of WM capacity entails the ability to avoid distraction and is positively correlated with performance on a variety of attention tasks (Engle, 2002; Hutchison, 2007). Furthermore, WM capacity is highly predictive for scholastic achievement (Gathercole et al., 2003) and it is among the major cognitive deficits of children with attention deficit hyperactivity disorder (ADHD; Klingberg et al., 2002). Using casual games found on the Internet, Baniqued et al. (2013) found that playing games focusing on attention/object tracking improved WM abilities, on the other hand, playing games that focused on WM did not improve measures of attention. These and other findings suggest a key role of attention in WM, and that proper engagement of attention during training may be a key factor in WM training success.

Also, reinforcement processes (rewards, punishments, motivation, etc.) have fundamental importance in guiding learning. For example, Seitz et al. (2009) found improved discrimination of orientation stimuli masked in noise after temporal-pairing between a liquid reward and a subliminal presentation of that orientation stimulus. Seitz and Watanabe (2005) suggested a model where learning is gated by reinforcement signals that trigger learning of aspects of the environment (even those for which the organism is not consciously aware) that are predictive or co-vary with the reinforcing event. They suggested that both attention and reinforcement operate in part through the release of neuromodulatory signals in the brain. For example, the orienting of attention, in the direction of the target-arrow, has been linked with the acetylcholine neuromodulatory system (Davidson and Marrocco, 2000). Of interest, cholinergic enhancement through the use of donepezil improves both the attentional processing

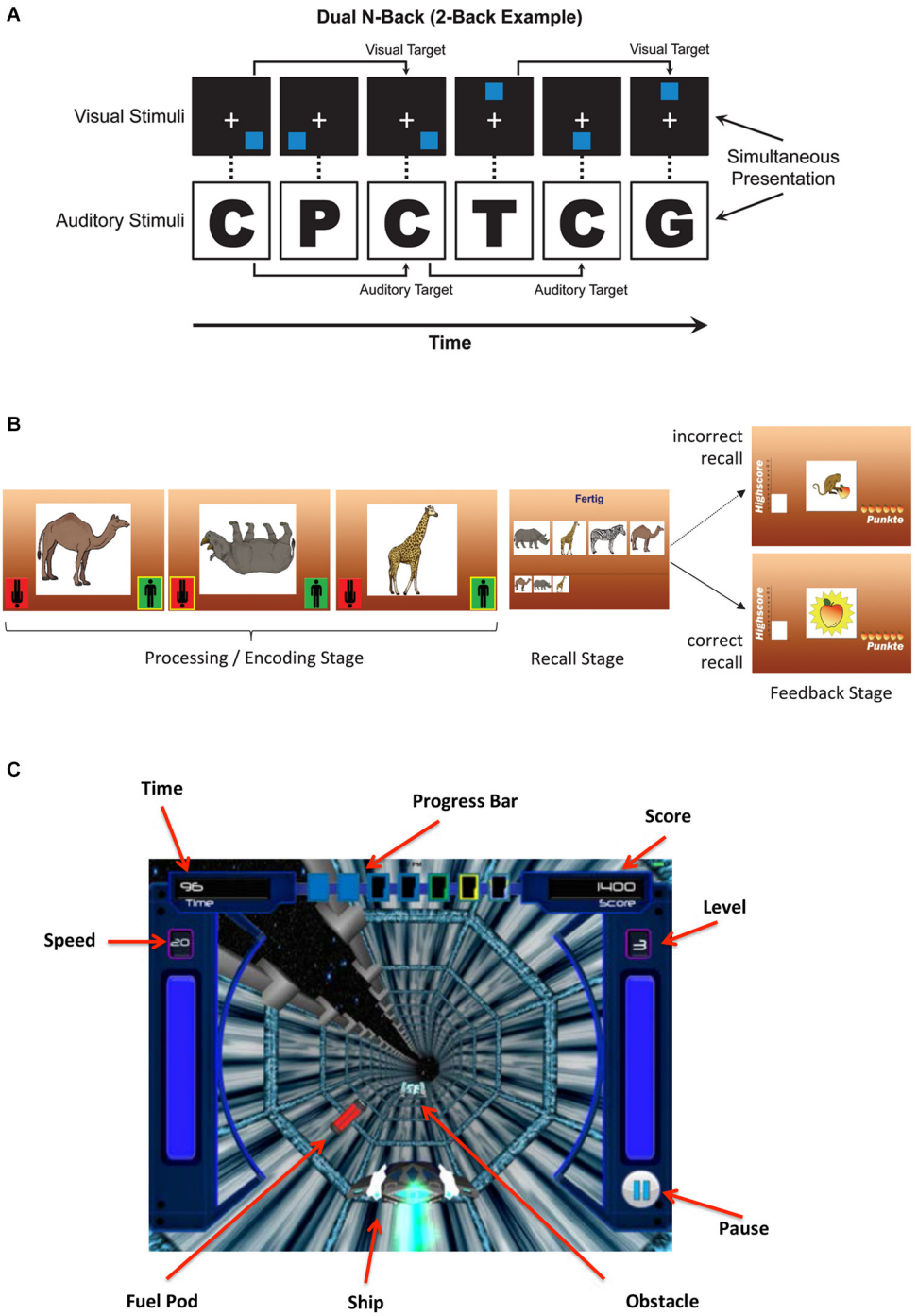

FIGURE 1 | (A) Diagram of an n-back task presented as a 2-back task (Jaeggi et al., 2008, 2010). Here, visual and auditory stimuli are presented simultaneously and participants have to respond to both modality streams independently. **(B)** Diagram of a complex span training task (Buschkuehl et al., 2008; Loosli et al., 2012). Animal pictures are presented sequentially, and participants respond to the orientation of each picture, and then reproduce the sequence in which the animals were presented. **(C)** Schematic of gamified n-back training task.

(Rokem et al., 2010) as well as the magnitude (Rokem and Silver, 2010) and longevity (Rokem and Silver, 2013) of PL. Other neuromodulatory systems, such as dopamine and norepinephrine have also been linked to attention (Posner and Petersen, 1990; Fan et al., 2003), learning (Kilgard and Merzenich, 1998; Bao et al., 2001; Dalley et al., 2001; Blake et al., 2006) and WM (Brehmer et al., 2009; Bellander et al., 2011). These studies suggest that a good training approach should involve the direction of both attention and reinforcement in a coordinated manner to promote learning.

MULTISENSORY FACILITATION

The human brain has evolved to learn and operate optimally in natural environments in which behavior is guided by information integrated across multiple sensory modalities. Crossmodal interactions are ubiquitous in the nervous system and occur even at early stages of perceptual processing (Shimojo and Shams, 2001; Calvert et al., 2004; Schroeder and Foxe, 2005; Ghazanfar and Schroeder, 2006; Driver and Noesselt, 2008). For example, recent research shows that subjects trained with auditory-visual stimuli exhibit a faster rate of learning and a higher degree of improvement than found in subjects trained in silence (Seitz et al., 2006; Kim et al., 2008).

Both memory storage and retrieval involves multiple senses. For example, the smell of a loved one's perfume (olfaction) can invoke memories of their face (vision), and so on. However, existing memory tasks, such as the dual *n*-back, often employ multiple (stimulus) modalities that are not coordinated, which research shows does not promote learning, and may in fact interfere with it (Seitz et al., 2005). Instead, we suggest a different approach where multisensory objects are incorporated into WM training. By defining objects through multi-modal feature sets, each sense can boost learning in the other. For example, an individual with limited visual capabilities will benefit from training utilizing concordant auditory stimuli.

MULTISTIMULUS TRAINING

One way to overcome specificity of learning is by training with multiple stimuli, as demonstrated in PL (Dosher and Lu, 1998; Yu et al., 2004; Xiao et al., 2008; Deveau et al., 2014a,b). For example, the recently developed technique of "double training" found that classically specific learning effects can show broad transfer when more than one stimulus attribute is trained. Xiao et al. (2008) trained participants on a Vernier discrimination task at a specific orientation at a specific location in the visual field, which normally yields location and orientation specific learning (Poggio et al., 1992). However, subsequently training subjects a second orientation at a different spatial location, the training-induced changes for the second orientation transferred to the first location. This data suggests that WM training with a diverse stimulus set might lead to a greater degree of transfer to untrained tasks than training on a narrow set (Estes and Burke, 1953; Schmidt and Bjork, 1992).

Furthermore, PL shows that the arrangement of multiple task elements in space and time can play a pivotal role in determining learning. Poor arrangements, such as when different stimuli are presented in a random order, as opposed to fixed order (Zhang et al., 2008), lead to poor learning (Seitz et al., 2005). Similar rules operate in guiding memory where sudden onsets of task-related stimuli can disrupt memorization of objects paired with those images (Leclercq and Seitz, 2012a). This may explain the recent counter-intuitive finding of WM training where the addition of motivational features in a simple gamification of n-back training led to impaired learning (Katz et al., 2014; see **Figure 2**), by inadvertently leading to greater distraction. We suggest that greater congruence between training stimuli and motivational factors will lead to greater and broader learning from WM training. In the next section we describe how this may be achieved through game design principles.

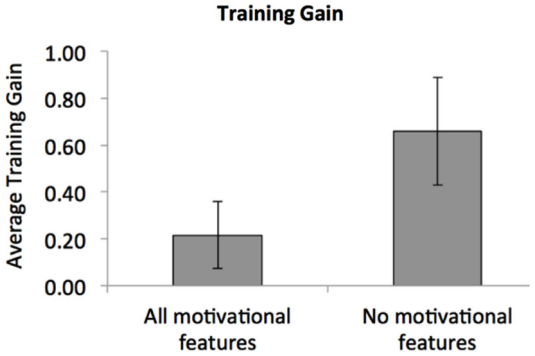

FIGURE 2 | Interference of learning by gamification. Motivational features such as scores, prizes, and scene-changes seem to interfere with learning. Specifically, training with all these features led to a lesser degree of learning compared to training without motivational features over the course of three sessions of *n*-back training (adapted from Katz et al., 2014).

VIDEO GAMES

There are many examples of off-the-shelf video games leading to substantial improvements in a variety of perceptual and cognitive abilities. For example, Green and Bavelier (2003) found that training novices for 10 h on an action video game improved performance on enumeration, useful field of view, and attentional blink tasks when compared to participants trained with a non-action video game. Basak et al. (2008) found that playing a real-time strategy game improved executive control as measured by task switching, visual short-term memory, and reasoning in older adults. Another recent study (Shute et al., 2015) showed that an off-the-shelf video game (Portal 2) led to substantial improvements on measures of problem solving, spatial skills, and persistence (in fact, even more so than training with the popular brain training games of Lumosity). Furthermore, dyslexic children improved reading speed and attentional abilities after playing an action video game (Franceschini et al., 2013). Finally, Goldin et al. (2014) employed several computer games targeting executive control, and found improvements in attention, inhibitory control, and planning, which also translated to school performance (Goldin et al., 2014). Together these studies suggest that video games include important attributes that contribute to learning.

Given the attractive motivational features of video games, recent research in cognitive science is increasingly moving towards adding game-like elements to their assessments. However, without proper design these can impair task performance, and even weaken test quality and learning (Hawkins et al., 2013; Katz et al., 2014). We suggest a better approach is to create training software that will dovetail, and/or implement non-competing concepts from game design that support learning. The video-game field is maturing, proper design rules and constraints are becoming more refined and the practices of coordinated design are becoming better understood and documented (Rabin, 2005). For example, in order to optimally engage players games must establish clear

goals and allow players to realize those goals through meaningful actions (Salen and Zimmerman, 2004). Successful game design has critical aspects that make software engaging, including its mechanics, interaction, visual/sensory experience, and progression (Gee, 2007).

Many game design criteria mirror components found to improve learning from the PL literature, and literature on deliberate practice (Ericsson et al., 1993). For example, consistent reinforcement to training stimuli (Seitz and Watanabe, 2009) maps directly to consistent player feedback, a key part of *player-centric interface design* (Adams, 2009). Adams says (of players) "most critically, they need information about whether their efforts are succeeding or failing, taking them closer to victory or closer to defeat". Likewise, motivating tasks (Shibata et al., 2009) and ensuring subjects are confident of their performance (Ahissar and Hochstein, 1997) are consistent with good game-design principles such as establishing clear goals (Salen and Zimmerman, 2004) and balancing games challenges (related to the adaptive approaches used in PL and WM training) to match player performance (Adams, 2009). Applying video-game techniques purposefully into WM training can inject the cognitive benefits found from off-the-shelf video games into principled cognitive training, while also being fun to play.

INTEGRATING LEARNING AND GAMING PRINCIPLES

Two relevant lines of research have made significant breakthroughs in brain training: (1) Studying incidental benefits of off-the-shelf video games; and (2) Transforming standard cognitive tasks into training tasks. We suggest that the most success will come from integrating knowledge of memory systems with that of brain plasticity and modern game-design principles.

As a first attempt to implement this approach, we created a prototype game that incorporates mechanics of the n-back into an engaging 3D space-themed game[1] (see **Figure 1C**). Typically, the n-back task is very basic, e.g., selecting matches from a grid or a picture series. In contrast, our prototype is a space-themed "collection" game with navigation challenges and obstacles, multi-layered progression through levels, and rich, thematic visual and sound effects. The n-back task is integrated into the game mechanics, where players select the "right" fuel cells while avoiding decoys. Levels are designed to get progressively harder through increasing cognitive challenge (n-level) and other game challenges (such as obstacles). While the game is more difficult and attention is spread over more elements than the conventional n-back, participant's control over their environment is anticipated to increase their engagement with the game.

The game also incorporates principles from PL, where participants are trained on multisensory (auditory and visual) features, where sounds and visuals are designed to facilitate each other, and where attention and reinforcement are carefully sculpted to lead to the best learning. While much work is still required to maximize the game's efficacy, e.g., by incorporating a broader stimulus set, adding other memory tasks, and creating an even more compelling game framework, we put it forward as a first example of how to build such an integrative game. Initial piloting with our prototype indicates participants are engaged in the game and improve performance (n-level) across training sessions. However, more research is needed to make firm conclusions regarding its transfer potential.

In summary, we suggest that more integrative approaches will lead to better learning outcomes. We suggest that the general mechanisms that promote PL are shared across brain regions and will also promote WM. Furthermore, there is enough known about the aspects of conventional video games that lead to positive learning outcomes that these principles can be applied to achieve more effective WM training. Additionally, there are other principles, that were beyond the scope of the present review, such as deliberate practice (Ericsson et al., 1993), and many aspects of healthy lifestyles (Walsh, 2011; Sigman et al., 2014) that also promote cognitive fitness. Integrating these approaches with good design could lead to a more comprehensive impact on WM function that might ultimately transfer to real-world conditions.

[1]https://itunes.apple.com/us/app/recall-the-game/id890271623?mt=8

REFERENCES

Adams, E. (2009). *Fundamentals of Game Design (2nd Edn.)*. Berkeley, CA: New Riders.

Ahissar, M., and Hochstein, S. (1993). Attentional control of early perceptual learning. *Proc. Natl. Acad. Sci. U S A* 90, 5718–5722. doi: 10.1073/pnas.90.12.5718

Ahissar, M., and Hochstein, S. (1997). Task difficulty and the specificity of perceptual learning. *Nature* 387, 401–406. doi: 10.1038/387401a0

Anguera, J. A., Boccanfuso, J., Rintoul, J. L., Al-Hashimi, O., Faraji, F., Janowich, J., et al. (2013). Video game training enhances cognitive control in older adults. *Nature* 501, 97–101. doi: 10.1038/nature12486

Au, J., Sheehan, E., Tsai, N., Duncan, G. J., Buschkuehl, M., and Jaeggi, S. M. (2014). Improving fluid intelligence with training on working memory: a meta-analysis. *Psychon. Bull. Rev.* doi: 10.3758/s13423-014-0699-x. [Epub ahead of print].

Baniqued, P. L., Lee, H., Voss, M. W., Basak, C., Cosman, J. D., Desouza, S., et al. (2013). Selling points: what cognitive abilities are tapped by casual video games? *Acta Psychol. (Amst)* 142, 74–86. doi: 10.1016/j.actpsy.2012.11.009

Bao, S., Chan, V. T., and Merzenich, M. M. (2001). Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412, 79–83. doi: 10.1038/35083586

Basak, C., Boot, W. R., Voss, M. W., and Kramer, A. F. (2008). Can training in a real-time strategy video game attenuate cognitive decline in older adults? *Psychol. Aging* 23, 765–777. doi: 10.1037/a0013494

Bellander, M., Brehmer, Y., Westerberg, H., Karlsson, S., Furth, D., Bergman, O., et al. (2011). Preliminary evidence that allelic variation in the LMX1A gene influences training-related working memory improvement. *Neuropsychologia* 49, 1938–1942. doi: 10.1016/j.neuropsychologia.2011.03.021

Blake, D. T., Heiser, M. A., Caywood, M., and Merzenich, M. M. (2006). Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. *Neuron* 52, 371–381. doi: 10.1016/j.neuron.2006.08.009

Borella, E., Carretti, B., Cantarella, A., Riboldi, F., Zavagnin, M., and De Beni, R. (2014). Benefits of training visuospatial working memory in young-old and old-old. *Dev. Psychol.* 50, 714–727. doi: 10.1037/a0034293

Borella, E., Carretti, B., Riboldi, F., and De Beni, R. (2010). Working memory training in older adults: evidence of transfer and maintenance effects. *Psychol. Aging* 25, 767–778. doi: 10.1037/a0020683

Borella, E., Carretti, B., Zanoni, G., Zavagnin, M., and De Beni, R. (2013). Working memory training in old age: an examination of transfer and maintenance effects. *Arch. Clin. Neuropsychol.* 28, 331–347. doi: 10.1093/arclin/act020

Brehmer, Y., Westerberg, H., Bellander, M., Fürth, D., Karlsson, S., and Backman, L. (2009). Working memory plasticity modulated by dopamine transporter genotype. *Neurosci. Lett.* 467, 117–120. doi: 10.1016/j.neulet.2009.10.018

Buschkuehl, M., and Jaeggi, S. M. (2010). Improving intelligence: a literature review. *Swiss Med. Wkly* 140, 266–272. doi: smw-12852

Buschkuehl, M., Jaeggi, S. M., Hutchison, S., Perrig-Chiello, P., Däpp, C., Müller, M., et al. (2008). Impact of working memory training on memory performance in old-old adults. *Psychol. Aging* 23, 743–753. doi: 10.1037/a0014342

Calvert, G., Spence, C., and Stein, B. E. (2004). *The Handbook of Multisensory Processes.* Cambridge, MA: The MIT Press.

Chein, J. M., and Morrison, A. B. (2010). Expanding the mind's workspace: training and transfer effects with a complex working memory span task. *Psychon. Bull. Rev.* 17, 193–199. doi: 10.3758/PBR.17.2.193

Dalley, J. W., McGaughy, J., O'Connell, M. T., Cardinal, R. N., Levita, L., and Robbins, T. W. (2001). Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. *J. Neurosci.* 21, 4908–4914.

Daneman, M., and Carpenter, P. A. (1980). Individual differences in working memory and reading. *J. Verbal Learn. Verbal Behav.r* 19, 450–466. doi: 10.1016/s0022-5371(80)90312-6

Davidson, M. C., and Marrocco, R. T. (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J. Neurophysiol.* 83, 1536–1549.

Deveau, J., Lovcik, G., and Seitz, A. R. (2014a). Broad-based visual benefits from training with an integrated perceptual-learning video game. *Vision Res.* 99, 134–140. doi: 10.1016/j.visres.2013.12.015

Deveau, J., Ozer, D. J., and Seitz, A. R. (2014b). Improved vision and on field performance in baseball through perceptual learning. *Curr. Biol.* 24, R146–R147. doi: 10.1016/j.cub.2014.01.004

Dosher, B. A., and Lu, Z. L. (1998). Perceptual learning reflects external noise filtering and internal noise reduction through channel reweighting. *Proc. Natl. Acad. Sci. U S A* 95, 13988–13993. doi: 10.1073/pnas.95.23.13988

Driver, J., and Noesselt, T. (2008). Multisensory interplay reveals crossmodal influences on 'sensory-specific' brain regions, neural responses and judgments. *Neuron* 57, 11–23. doi: 10.1016/j.neuron.2007.12.013

Engle, R. W. (2002). Working memory capacity as executive attention. *Curr. Dir. sychol. Sci.* 11, 19–23. doi: 10.1111/1467-8721.00160

Ericsson, K. A., Krampe, R. T., and Tesch-Römer, C. (1993). The role of deliberate practice in the acquisition of expert performance. *Psychol. Rev.* 100, 363–406. doi: 10.1037//0033-295x.100.3.363

Estes, W. K., and Burke, C. J. (1953). A theory of stimulus variability in learning. *Psychol. Rev.* 60, 276–286. doi: 10.1037/h0055775

Fahle, M. (2005). Perceptual learning: specificity versus generalization. *Curr. Opin. Neurobiol.* 15, 154–160. doi: 10.1016/j.conb.2005.03.010

Fan, J., Fossella, J., Sommer, T., Wu, Y., and Posner, M. I. (2003). Mapping the genetic variation of executive attention onto brain activity. *Proc. Natl. Acad. Sci. U S A* 100, 7406–7411. doi: 10.1073/pnas.0732088100

Fiorentini, A., and Berardi, N. (1980). Perceptual learning specific for orientation and spatial frequency. *Nature* 287, 43–44. doi: 10.1038/287043a0

Franceschini, S., Gori, S., Ruffino, M., Viola, S., Molteni, M., and Facoetti, A. (2013). Action video games make dyslexic children read better. *Curr. Biol.* 23, 462–466. doi: 10.1016/j.cub.2013.01.044

Gathercole, S. E., Brown, L., and Pickering, S. J. (2003). Working memory assessments at school entry as longitudinal predictors of national curriculum attainment levels. *Educ. Child Psychol.* 20, 109–122.

Gee, J. P. (2007). *What Video Games have to Teach us about Learning and Literacy: Revised and Updated Edition.* New York, NY: Macmillan.

Ghazanfar, A., and Schroeder, C. E. (2006). Is neocortex essentially multisensory? *Trends Cogn. Sci.* 10, 278–285. doi: 10.1016/j.tics.2006.04.008

Goldin, A. P., Hermida, M. J., Shalom, D. E., Elias Costa, M., Lopez-Rosenfeld, M., Segretin, M. S., et al. (2014). Far transfer to language and math of a short software-based gaming intervention. *Proc. Natl. Acad. Sci. U S A* 111, 6443–6448. doi: 10.1073/pnas.1320217111

Green, C. S., and Bavelier, D. (2003). Action video game modifies visual selective attention. *Nature* 423, 534–537. doi: 10.1038/nature01647

Hawkins, G. E., Rae, B., Nesbitt, K. V., and Brown, S. D. (2013). Gamelike features might not improve data. *Behav. Res. Methods* 45, 301–318. doi: 10.3758/s13428-012-0264-3

Hung, S. C., and Seitz, A. R. (2014). Prolonged training at threshold promotes robust retinotopic specificity in perceptual learning. *J. Neurosci.* 34, 8423–8431. doi: 10.1523/jneurosci.0745-14.2014

Hutchison, K. A. (2007). Attentional control and the relatedness proportion effect in semantic priming. *J. Exp. Psychol. Learn. Mem. Cogn.* 33, 645–662. doi: 10.1037/0278-7393.33.4.645

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U S A* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Shah, P. (2011). Short- and long-term benefits of cognitive training. *Proc. Natl. Acad. Sci. U S A* 108, 10081–10086. doi: 10.1073/pnas.1103228108

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Shah, P. (2012). Cogmed and working memory training—current challenges and the search for underlying mechanisms. *J. Appl. Res. Mem. Cogn.* 1, 211–213. doi: 10.1016/j.jarmac.2012.07.002

Jaeggi, S. M., Buschkuehl, M., Shah, P., and Jonides, J. (2014). The role of individual differences in cognitive training and transfer. *Mem. Cognit.* 42, 464–480. doi: 10.3758/s13421-013-0364-z

Jaeggi, S. M., Studer-Luethi, B., Buschkuehl, M., Su, Y. F., Jonides, J., and Perrig, W. J. (2010). The relationship between n-back performance and matrix reasoning—implications for training and transfer. *Intelligence* 38, 625–635. doi: 10.1016/j.intell.2010.09.001

Karbach, J., Strobach, T., and Schubert, T. (2014). Adaptive working-memory training benefits reading, but not mathematics in middle childhood. *Child Neuropsychol.* doi: 10.1080/09297049.2014.899336. [Epub ahead of print]

Karbach, J., and Verhaeghen, P. (2014). Making working memory work: a meta-analysis of executive-control and working memory training in older adults. *Psychol. Sci.* 25, 2027–2037. doi: 10.1177/0956797614548725

Karni, A., and Sagi, D. (1991). Where practice makes perfect in texture discrimination: evidence for primary visual cortex plasticity. *Proc. Natl. Acad. Sci. U S A* 88, 4966–4970. doi: 10.1073/pnas.88.11.4966

Katz, B., Jaeggi, S., Buschkuehl, M., Stegman, A., and Shah, P. (2014). Differential effect of motivational features on training improvements in school-based cognitive training. *Front. Hum. Neurosci.* 8:242. doi: 10.3389/fnhum.2014.00242

Kilgard, M. P., and Merzenich, M. M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science* 279, 1714–1718. doi: 10.1126/science.279.5357.1714

Kim, R. S., Seitz, A. R., and Shams, L. (2008). Benefits of stimulus congruency for multisensory facilitation of visual learning. *PLoS One* 3:e1532. doi: 10.1371/journal.pone.0001532

Klingberg, T., Fernell, E., Olesen, P. J., Johnson, M., Gustafsson, P., Dahlström, K., et al. (2005). Computerized training of working memory in children with ADHD—a randomized, controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry* 44, 177–186. doi: 10.1097/00004583-200502000-00010

Klingberg, T., Forssberg, H., and Westerberg, H. (2002). Training of working memory in children with ADHD. *J. Clin. Exp. Neuropsychol.* 24, 781–791. doi: 10.1076/jcen.24.6.781.8395

Leclercq, V., and Seitz, A. R. (2012a). Fast task-irrelevant perceptual learning is disrupted by sudden onset of central task elements. *Vision Res.* 61, 70–76. doi: 10.1016/j.visres.2011.07.017

Leclercq, V., and Seitz, A. R. (2012b). The impact of orienting attention in fast task-irrelevant perceptual learning. *Atten. Percept. Psychophys.* 74, 648–660. doi: 10.3758/s13414-012-0270-7

Lilienthal, L., Tamez, E., Shelton, J. T., Myerson, J., and Hale, S. (2013). Dual n-back training increases the capacity of the focus of attention. *Psychon. Bull. Rev.* 20, 135–141. doi: 10.3758/s13423-012-0335-6

Loosli, S. V., Buschkuehl, M., Perrig, W. J., and Jaeggi, S. M. (2012). Working memory training improves reading processes in typically developing children. *Child Neuropsychol.* 18, 62–78. doi: 10.1080/09297049.2011.575772

Maguire, E. A., Valentine, E. R., Wilding, J. M., and Kapur, N. (2003). Routes to remembering: the brains behind superior memory. *Nat. Neurosci.* 6, 90–95. doi: 10.1038/nn988

Poggio, T., Fahle, M., and Edelman, S. (1992). Fast perceptual learning in visual hyperacuity. *Science* 256, 1018–1021. doi: 10.1126/science.1589770

Posner, M. I., and Petersen, S. E. (1990). The attention system of the human brain. *Annu. Rev. Neurosci.* 13, 25–42. doi: 10.1146/annurev.neuro.13.1.25

Rabin, S. (2005). *Introduction to Game Development.* Rockland, MA: Charles River Media.

Richmond, L. L., Morrison, A. B., Chein, J. M., and Olson, I. R. (2011). Working memory training and transfer in older adults. *Psychol. Aging* 26, 813–822. doi: 10.1037/a0023631

Rokem, A., Landau, A. N., Garg, D., Prinzmetal, W., and Silver, M. A. (2010). Cholinergic enhancement increases the effects of voluntary attention but does

not affect involuntary attention. *Neuropsychopharmacology* 35, 2538–2544. doi: 10.1038/npp.2010.118

Rokem, A., and Silver, M. A. (2010). Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. *Curr. Biol.* 20, 1723–1728. doi: 10.1016/j.cub.2010.08.027

Rokem, A., and Silver, M. A. (2013). The benefits of cholinergic enhancement during perceptual learning are long-lasting. *Front. Comput. Neurosci.* 7:66. doi: 10.3389/fncom.2013.00066

Rudebeck, S. R., Bor, D., Ormond, A., O'Reilly, J. X., and Lee, A. C. (2012). A potential spatial working memory training task to improve both episodic memory and fluid intelligence. *PLoS One* 7:e50431. doi: 10.1371/journal.pone.0050431

Salen, K., and Zimmerman, E. (2004). *Rules of Play: Game Design Fundamentals.* (MIT Press).

Salminen, T., Strobach, T., and Schubert, T. (2012). On the impacts of working memory training on executive functioning. *Front. Hum. Neurosci.* 6:166. doi: 10.3389/fnhum.2012.00166

Schmidt, R. A., and Bjork, R. A. (1992). New conceptualizations of practice: common principles in three paradigms suggest new concepts for training. *Psychol. Sci.* 3, 207–217. doi: 10.1111/j.1467-9280.1992.tb00029.x

Schoups, A., Vogels, R., Qian, N., and Orban, G. (2001). Practising orientation identification improves orientation coding in V1 neurons. *Nature* 412, 549–553. doi: 10.1038/35087601

Schroeder, C. E., and Foxe, J. (2005). Multisensory contributions to low-level, 'unisensory' processing. *Curr. Opin. Neurobiol.* 15, 454–458. doi: 10.1016/j.conb.2005.06.008

Seitz, A. R., and Dinse, H. R. (2007). A common framework for perceptual learning. *Curr. Opin. Neurobiol.* 17, 148–153. doi: 10.1016/j.conb.2007.02.004

Seitz, A. R., Kim, R., and Shams, L. (2006). Sound facilitates visual learning. *Curr. Biol.* 16, 1422–1427. doi: 10.1016/j.cub.2006.05.048

Seitz, A. R., Kim, D., and Watanabe, T. (2009). Rewards evoke learning of unconsciously processed visual stimuli in adult humans. *Neuron* 61, 700–707. doi: 10.1016/j.neuron.2009.01.016

Seitz, A., and Watanabe, T. (2005). A unified model for perceptual learning. *Trends Cogn. Sci.* 9, 329–334. doi: 10.1016/j.tics.2005.05.010

Seitz, A. R., and Watanabe, T. (2009). The phenomenon of task-irrelevant perceptual learning. *Vision Res.* 49, 2604–2610. doi: 10.1016/j.visres.2009.08.003

Seitz, A. R., Yamagishi, N., Werner, B., Goda, N., Kawato, M., and Watanabe, T. (2005). Task-specific disruption of perceptual learning. *Proc. Natl. Acad. Sci. U S A* 102, 14895–14900. doi: 10.1073/pnas.0505765102

Shah, P., Buschkuehl, M., Jaeggi, S., and Jonides, J. (2012). Cognitive training for ADHD: the importance of individual differences. *J. Appl. Res. Mem. Cogn.* 1, 204–205. doi: 10.1016/j.jarmac.2012.07.001

Shah, P., and Miyake, A. (1999). "Models of working memory: an introduction," in *Models of Working Memory: Mechanism of Active Maintenance and Executive Control*, eds P. Shah and A. Miyake (New York: Cambridge University Press), 1–26.

Shams, L., and Seitz, A. R. (2008). Benefits of multisensory learning. *Trends Cogn. Sci.* 12, 411–417. doi: 10.1016/j.tics.2008.07.006

Shibata, K., Yamagishi, N., Ishii, S., and Kawato, M. (2009). Boosting perceptual learning by fake feedback. *Vision Res.* 49, 2574–2585. doi: 10.1016/j.visres.2009.06.009

Shimojo, S., and Shams, L. (2001). Sensory modalities are not separate modalities: plasticity and interactions. *Curr. Opin. Neurobiol.* 11, 505–509. doi: 10.1016/s0959-4388(00)00241-5

Shipstead, Z., Hicks, K. L., and Engle, R. W. (2012). Cogmed working memory training: does the evidence support the claims? *J. Appl. Res. Mem. Cogn.* 1, 185–193. doi: 10.1016/j.jarmac.2012.06.003

Shiu, L. P., and Pashler, H. (1992). Improvement in line orientation discrimination is retinally local but dependent on cognitive set. *Percept. Psychophys.* 52, 582–588. doi: 10.3758/bf03206720

Shute, V. J., Ventura, M., and Ke, F. (2015). The power of play: the effects of portal 2 and lumosity on cognitive and noncognitive skills. *Comput. Educ.* 80, 58–67. doi: 10.1016/j.compedu.2014.08.013

Sigman, M., Pñna, M., Goldin, A. P., and Ribeiro, S. (2014). Neuroscience and education: prime time to build the bridge. *Nat. Neurosci.* 17, 497–502. doi: 10.1038/nn.3672

Smith, G. E., Housen, P., Yaffe, K., Ruff, R., Kennison, R. F., Mahncke, H. W., et al. (2009). A cognitive training program based on principles of brain plasticity: results from the Improvement in Memory with Plasticity-based Adaptive Cognitive Training (IMPACT) study. *J. Am. Geriatr. Soc.* 57, 594–603. doi: 10.1111/j.1532-5415.2008.02167.x

St Clair-Thompson, H., Stevens, R., Hunt, A., and Bolder, E. (2010). Improving children's working memory and classroom performance. *Educ. Psychol.* 30, 203–219. doi: 10.1080/01443410903509259

Stepankova, H., Lukavsky, J., Buschkuehl, M., Kopecek, M., Ripova, D., and Jaeggi, S. M. (2014). Dose-response relationship of working memory training and improvements in fluid intelligence: a randomized controlled study in old adults. *Dev. Psychol.* 50, 1049–1059.doi: 10.1037/a0034913

Stephenson, C. L., and Halpern, D. F. (2013). Improved matrix reasoning is limited to training on tasks with a visuospatial component. *Intelligence* 41, 341–357. doi: 10.1016/j.intell.2013.05.006

Thorell, L. B., Lindqvist, S., Bergman Nutley, S., Bohlin, G., and Klingberg, T. (2009). Training and transfer effects of executive functions in preschool children. *Dev. Sci.* 12, 106–113. doi: 10.1111/j.1467-7687.2008.00745.x

Verhaeghen, P., Marcoen, A., and Goossens, L. (1992). Improving memory performance in the aged through mnemonic training: a meta-analytic study. *Psychol. Aging* 7, 242–251. doi: 10.1037//0882-7974.7.2.242

Walsh, R. (2011). Lifestyle and mental health. *Am. Psychol.* 66, 579–592. doi: 10.1037/a0021769

Xiao, L. Q., Zhang, J. Y., Wang, R., Klein, S. A., Levi, D. M., and Yu, C. (2008). Complete transfer of perceptual learning across retinal locations enabled by double training. *Curr. Biol.* 18, 1922–1926. doi: 10.1016/j.cub.2008.10.030

Yu, C., Klein, S. A., and Levi, D. M. (2004). Perceptual learning in contrast discrimination and the (minimal) role of context. *J. Vis.* 4, 169–182. doi: 10.1167/4.3.4

Zhang, J. Y., Kuai, S. G., Xiao, L. Q., Klein, S. A., Levi, D. M., and Yu, C. (2008). Stimulus coding rules for perceptual learning. *PLoS Biol* 6:e197. doi: 10.1371/journal.pbio.0060197

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 November 2014; accepted: 11 December 2014; published online: 09 January 2015.

Citation: Deveau J, Jaeggi SM, Zordan V, Phung C and Seitz AR (2015) How to build better memory training games. Front. Syst. Neurosci. ***8****:243. doi: 10.3389/fnsys.2014.00243*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 02 September 2014
doi: 10.3389/fnsys.2014.00161

Making sense of discrepancies in working memory training experiments: a Monte Carlo simulation

David Moreau*

Department of Psychology, Princeton University, Princeton, NJ, USA
**Correspondence: david.moreau@fulbrightmail.org*

Edited by:
Manuel Casanova, University of Louisville, USA

Reviewed by:
Jose L. Pardo-Vazquez, Fundaçao Champalimaud, Portugal
Matjaž Perc, University of Maribor, Slovenia

Keywords: cognitive training, working memory, individual differences, intelligence, Monte Carlo simulation, random sampling

INTRODUCTION

The idea that working memory training can enhance general cognitive abilities has received a lot of attention in the last few years. Some studies have demonstrated far transfer to other cognitive abilities after training, whereas others have failed to replicate these findings (see Melby-Lervåg and Hulme, 2013, for a meta-analytic review). These setbacks have had unfortunate effects on the field of cognitive training, sometimes raising concerns regarding methodology and analyses in concurrent labs. Although constructive skepticism is often healthy in fields based on peer-review, the current dynamic has created a climate of hostility detrimental to the advancement of science. In this paper, I show that some of these differences can simply emerge from population parameters underlying cognitive growth curves. Based on a Monte Carlo simulation, I demonstrate that unbalanced samples are bound to arise by chance when individuals differ in their ability to learn, and propose a few remedies to circumvent this problem. Finally, I discuss the impact of cognitive training studies in refining theories of cognition and suggest directions for future research.

WORKING MEMORY TRAINING AND INDIVIDUAL DIFFERENCES

Working memory capacity (WMC) is at the core of numerous mental operations, including reasoning, problem solving and decision-making. In line with this idea, recent advances using computational modeling have shown that WMC correlates highly with a wide range of cognitive constructs, including *g* (Süß et al., 2002; Kane et al., 2004), an idea further supported by common neural correlates for WMC and *g*, particularly regions of the prefrontal cortex (Kane and Engle, 2002; Gray et al., 2003). Although a strong correlation between constructs does not guarantee that they will covary with training (Moreau and Conway, 2014), the relationship between WMC and *g* was the starting point for a vast enterprise aimed at increasing the fluid component of intelligence (Gf) via working memory training (Jaeggi et al., 2008). However, since early studies showing improvements in tasks tapping Gf after working memory training (Jaeggi et al., 2008, 2010; Jaušovec and Jaušovec, 2012), others have consistently failed to replicate these findings (Chooi and Thompson, 2012; Harrison et al., 2013; Redick et al., 2013). These contradicting results created a dichotomy between labs interested in the same trend of research but reaching different conclusions. As of today, many would say that the jury is still out concerning the effectiveness of working memory training to improve Gf, and that previous shortcomings need to be addressed.

One factor of particular importance in this debate concerns individual differences in learning curves. This point has been emphasized recently, with researchers suggesting that understanding differential effects is critical to better assess and design cognitive training programs (e.g., Jaeggi et al., 2014). This is a healthy departure from dichotomized claims about the effectiveness of working memory training, illustrating the importance of more nuanced statements—the same training does not work for everyone, and it is critical to determine what components are required for successful transfer and what components need to be adapted to individual needs. Until we can successfully identify these parameters, training programs will yield differential effects that are difficult to predict.

Yet how and to what extent do individual differences influence training outcomes? In the following section, I argue that individual differences in rates of improvement can induce differences in the measured outcomes simply due to random sampling from heterogeneous populations—assuming such a scenario, a non-trivial number of experiments will already be biased at the onset. Regardless of the presence of a true effect of training, sampling errors can obscure comparisons between conditions because of unbalanced samples, which in turns can create the illusion of an effect or of the absence of an effect when in fact the opposite is true.

A MONTE CARLO APPROACH

A Monte Carlo simulation helps to understand this idea. A typical training experiment aims for an *N* of about 20 subjects per cell, so we will consider the case of training experiments with two groups, experimental and control, with a total of 40 subjects. Experimenters usually sample the population until the desired number of subjects is reached, at which point they are randomly assigned to either an experimental or a control group. Recruiting all subjects before random assignment allows starting training at the same time for all, often to provide more control over external factors, and ensuring that group samples are equivalent on the tasks of

interest measured at pre-test. An alternate solution is to assign subjects to a group at the recruiting stage, in order to start training at each subject's earliest convenience. This is often more practical when experimenters have to deal with time constraints, but it can potentially introduce additional confounds. Because the present simulation does not constrain sampling hierarchically, its results will not be influenced by the sampling strategy one might favor.

A simulation with 10,000 draws was performed for each of these scenarios: uniform population, population with two subpopulations, and population with three subpopulations. In the first step of the simulation, all subpopulations were equally represented in the overall population—the only variation allowed in the model was at the random sampling stage.

Assuming the overall population we are sampling from is uniform, dividing a sample into two groups (experimental vs. control) has no effect on how balanced a design is. This is what was is mostly assumed in training studies that are not focused on individual differences, either explicitly or implicitly, because of the nature of the design itself. When assuming such an underlying population, random assignment to experimental conditions never produces unbalanced samples—the population is uniform, and so are the two samples.

Recent evidence, however, suggests different learning curves, or rates of improvement, between individuals, based on distinct neural changes (e.g., Kundu et al., 2013). This is a completely different scenario. Let us assume the general population we are sampling from includes two subpopulations with different rates of improvement (high and low). In this case, a Monte Carlo simulation with 10,000 draws shows that random sampling from the population will yield unbalanced samples 1.74% of the time (**Figure 1A**). This percentage might seem trivial, but when the population includes three subpopulations of learners (high, medium, low), a simulation with 10,000 draws yield unbalanced samples 5.18% of the time (**Figure 1B**). The probability rises quickly when more subpopulations are included in the model: the more individuals differ in their ability to learn, the more likely a training experiment is to be affected by sampling error. In fact, ecological populations are likely to be even more heterogeneous, therefore exacerbating this effect. In training experiments, individual differences matter.

Such sampling errors are substantial, especially given that the model proposed here did not assess the probability for a sample to represent adequately the overall population, but only the probability for two samples drawn from the same population to be equivalent. Moreover, sampling errors and other limitations common with the analyses of training data (e.g., correlated gains and dichotomization; Tidwell et al., 2014) are not mutually exclusive, arguably increasing the risk for experimental confounds. The underlying rationale in training experiments is that sampling error is random, and that it therefore averages with large enough samples. However, despite inferential tools available to estimate the size of sampling error (e.g., standard error), unbalanced samples often go undetected, because no effort is made to test for homogeneity and the distribution of the population is rarely known *a priori*.

One should also note that the percentages presented here are conservative—subpopulations within the overall population were always equally represented in the model, but this is not necessarily the case in real settings. In fact, it is plausible that individuals with different trends of improvement are not equally represented in the overall population. In this case, the probability to draw unbalanced samples rises quickly. In the two-subpopulation scenario, unbalanced overall population with the following ratios (high = 60%; low = 40%) yields unbalanced samples across groups 4.23% of the time with 10,000 draws (**Figure 1C**). In the three-population scenario with the following ratios (high = 55%, medium = 35% and low = 10%), the probability surges to 37.87% (**Figure 1D**). Given an unequal percentage of each subpopulation within the overall population, it can be more accurate to use a stratified sampling method to improve representativeness of the samples; however, this requires knowing *a priori* the percentage of each subpopulation in the overall population to be accurate, which is often difficult in practice.

POTENTIAL REMEDIES

There are a few remedies to circumvent this problem. First, the simulation presented in this paper demonstrates the importance of assessing learning rates before training starts. In this regard, specific tasks could be used to define a growth rate in the ability of interest (e.g., WMC), allowing experimenters to introduce specific constraints in a pseudo-random assignment to groups, such as forcing matched samples. This additional step would ensure balanced samples across experimental conditions at the onset of the study to reduce the potential biases emphasized in this paper. In addition, and because they would be measured before any experimental treatment, initial growth profiles could be used as covariates in the final analyses to refine the interpretation of significant effects.

Testing for differences in learning rates also emphasizes the importance of sample size. All other parameters being equal, a larger sample size increases the probability to detect differences in growth profiles between experimental groups (i.e., power). For example, increasing the sample in the Monte Carlo simulation to 40 subjects per cell allows detecting unbalanced samples in 3.47% of cases in the two-subpopulation scenario (11.05% with unequal subpopulations), and in 5.20% of cases in the three-subpopulation scenario (69.47% with unequal subpopulations). Obviously, the cost of training experiments combined with the desire to publish findings quickly represent strong incentives for experiments with smaller sample size, but the field of cognitive training needs more statistically powered designs to reach more definitive claims.

Finally, predefining a one-sided hypothesis at the onset of a study allows reducing the probabilities defined in previous scenarios by half, since half the time the discrepancies arising spuriously will contradict a specific hypothesis. Preregistering is now made easier by online projects such as the Open Science Framework (http://openscienceframework.org), which keeps time stamps on project submissions and

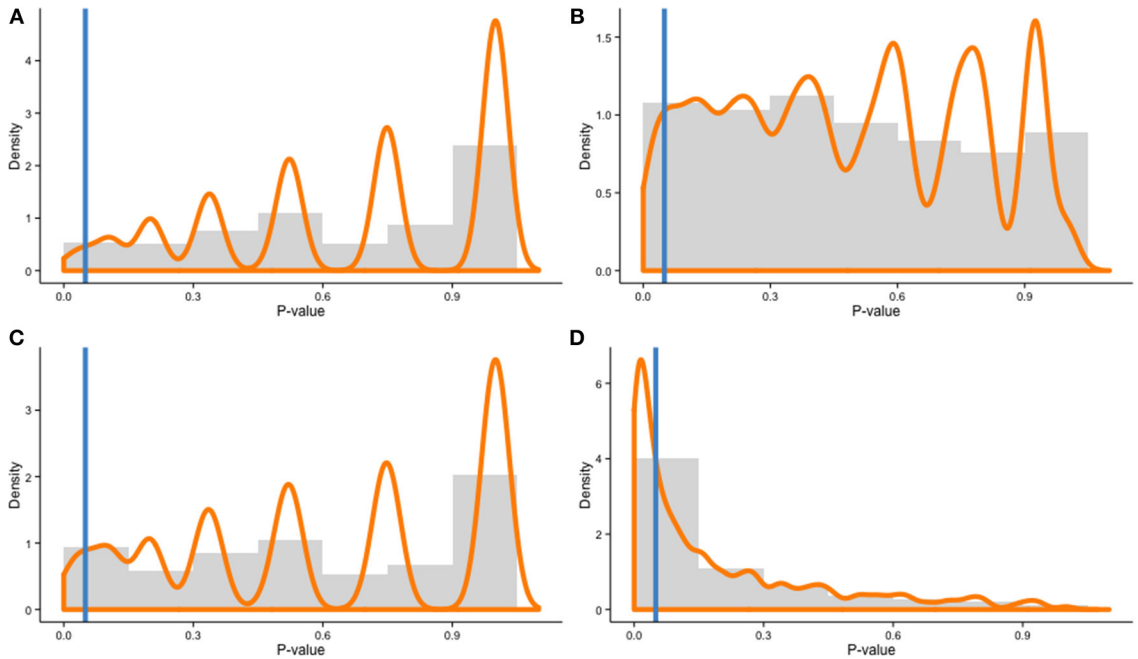

FIGURE 1 | Distributions of χ-squared contingency table test *p*-values for a Monte Carlo simulation with 10,000 draws of two samples ($N = 20$ per cell) in four different scenarios: (A) 2 subpopulations—Equal ratios, (B) 3 subpopulations—Equal ratios, (C) 2 subpopulations—Unequal ratios (60–40%), and (D) 3 subpopulations—Unequal ratios (55–35–10%). Histograms represent distribution frequencies; the orange line depicts density estimates. The blue line represents the threshold for $p = 0.05$ (all *p*-values to the left of the line are significant, indicating unbalanced samples).

allows choosing an appropriate level of privacy for a preregistered project. Furthermore, top-tier journals in psychology and neuroscience are now encouraging such preregistrations, by approving particular designs and hypotheses before an experiment is conducted, therefore guaranteeing publication regardless of the outcome. Clearly, these initiatives reach far beyond solving the problem of ambiguous hypotheses—for example, they also represent a first step toward eradicating the file-drawer problem—and in that regard they should be widely applauded and further encouraged.

CONCLUDING REMARKS

The field of cognitive training is an exciting one, with tremendous potential applications. In this regard, inconsistencies in experimental findings should not be interpreted as failures to advance knowledge but as inevitable consequences of exploring a field still in its infancy. Importantly, I do not argue that sampling error explains *all* discrepancies in working memory training outcomes; rather, the rationale for this opinion piece is to encourage caution when interpreting seemingly incompatible findings. Moreover, the limitation presented herein, as well as its potential remedies, are equally valid to other types of training designs not based on working memory—in fact, I do hope that the paper contributes to an already ongoing shift of focus from general training contents to more individualized programs, taking into account individual differences in cognition. Following this idea serves a dual purpose—it allows designing more effective training programs with applications to clinical and non-clinical populations, particularly important in our aging societies, but also provides suitable environments to test empirical claims and refine current models of cognition.

REFERENCES

Chooi, W.-T., and Thompson, L. A. (2012). Working memory training does not improve intelligence in healthy young adults. *Intelligence* 40, 531–542. doi: 10.1016/j.intell.2012.07.004

Gray, J. R., Chabris, C. F., and Braver, T. S. (2003). Neural mechanisms of general fluid intelligence. *Nat. Neurosci.* 6, 316–322. doi: 10.1038/nn1014

Harrison, T. L., Shipstead, Z., Hicks, K. L., Hambrick, D. Z., Redick, T. S., and Engle, R. W. (2013).Working memory training may increase working memory capacity but not fluid intelligence. *Psychol. Sci.* 12, 2409–2419. doi: 10.1177/0956797613492984

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Jaeggi, S. M., Buschkuehl, M., Shah, P., and Jonides, J. (2014). The role of individual differences in cognitive training and transfer. *Mem. Cogn.* 42, 464–480. doi: 10.3758/s13421-013-0364-z

Jaeggi, S. M., Studer-Luethi, B., Buschkuehl, M., Su, Y.-F., Jonides, J., and Perrig, W. J. (2010). The relationship between n-back performance and matrix reasoning – implications for training and transfer. *Intelligence* 38, 625–635. doi: 10.1016/j.intell.2010.09.001

Jaušovec, N., and Jaušovec, K. (2012). Working memory training: improving intelligence–Changing brain activity. *Brain Cogn.* 79, 96–106. doi: 10.1016/j.bandc.2012.02.007

Kane, M. J., and Engle, R. W. (2002). The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual differences perspective. *Psychon. Bull. Rev.* 9, 637–671. doi: 10.3758/BF03196323

Kane, M. J., Hambrick, D. Z., Tuholski, S. W., Wilhelm, O., Payne, T. W., and Engle, R. W. (2004). The generality of working memory capacity: a latent-variable approach to verbal and visuospatial memory span and reasoning. *J. Exp. Psychol. Gen.* 133, 189–217. doi: 10.1037/0096-3445.133.2.189

Kundu, B., Sutterer, D. W., Emrich, S. M., and Postle, B. R. (2013). Strengthened effective

connectivity underlies transfer of working memory training to tests of short-term memory and attention. *J. Neurosci.* 33, 8705–8715. doi: 10.1523/JNEUROSCI.5565-12.2013

Melby-Lervåg, M., and Hulme, C. (2013). Is working memory training effective? A meta-analytic review. *Dev. Psychol.* 49, 270–291. doi: 10.1037/a0028228

Moreau, D., and Conway, A. R., A (2014). The case for an ecological approach to cognitive training. *Trends Cogn. Sci.* 18, 334–336. doi: 10.1016/j.tics.2014.03.009

Redick, T. S., Shipstead, Z., Harrison, T. L., Hicks, K. L., Fried, D. Z., Hambrick, D. Z., et al. (2013). No evidence of intelligence improvement after working memory training: a randomized, placebo-controlled study. *J. Exp. Psychol. Gen.* 142, 359–379. doi: 10.1037/a0029082

Süß, H.-M., Oberauer, K., Wittmann, W. W., Wilhelm, O., and Schulze, R. (2002). Working memory capacity explains reasoning ability - and a little bit more. *Intelligence* 30, 261–288. doi: 10.1016/S0160-2896(01)00100-3

Tidwell, J. W., Dougherty, M. R., Chrabaszcz, J. R., Thomas, R. P., and Mendoza, J. L. (2014). What counts as evidence for working memory training? Problems with correlated gains and dichotomization. *Psychon. Bull. Rev.* 21, 620–628. doi: 10.3758/s13423-013-0560-7

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 July 2014; accepted: 15 August 2014; published online: 02 September 2014.

Citation: Moreau D (2014) Making sense of discrepancies in working memory training experiments: a Monte Carlo simulation. Front. Syst. Neurosci. ***8****:161. doi: 10.3389/fnsys.2014.00161*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

ORIGINAL RESEARCH
published: 14 September 2015
doi: 10.3389/fnsys.2015.00128

Superior memorizers employ different neural networks for encoding and recall

Johannes Mallow[1], Johannes Bernarding[1,2], Michael Luchtmann[3], Anja Bethmann[4] and André Brechmann[4]*

[1] Institute for Biometry and Medical Informatics, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany, [2] Center for Behavioural Brain Sciences, Otto-von-Guericke University, Magdeburg, Germany, [3] University Clinic for Neurosurgery, Otto-von-Guericke University, Magdeburg, Germany, [4] Special-Lab Non-Invasive Brain Imaging, Leibniz Institute for Neurobiology, Magdeburg, Germany

Superior memorizers often employ the method of loci (MoL) to memorize large amounts of information. The MoL, known since ancient times, relies on a complex process where information to be memorized is bound to landmarks along mental routes in a previously memorized environment. However, functional magnetic resonance imaging data on groups of trained superior memorizer are rare. Based on the memorizing strategy reported by superior memorizers, we developed a scheme of the processes successively employed during memorizing and recalling digits and relate these to brain activation that is specific for the encoding and recall period. In the examined superior memorizers several regions, suggested to be involved in mental navigation and digit-to-word processing, were specifically activated during encoding: bilateral early visual cortex, retrosplenial cortex, left parahippocampus, left visual cortex, and left superior parietal cortex. Although the scheme suggests that some steps during encoding and recall seem to be analog, none of the encoding areas were specifically activated during the recall. Instead, we found strong activation in left anterior superior temporal gyrus, which we relate to recalling the sequential order of the digits, and right motor cortex that may be related to reciting the digits.

Keywords: superior memorizers, method of loci, mental navigation, encoding, recall

OPEN ACCESS

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Beat Meier, University of Bern, Switzerland
Fernand Gobet, University of Liverpool, UK
Andres Rieznik, CONICET, Argentina

****Correspondence:***
Johannes Mallow, Institute for Biometry and Medical Informatics, Medical Faculty, Otto-von-Guericke University, Leipziger Straße 44, Magdeburg, Germany
info@johannes-mallow.de

Received: *27 March 2015*
Accepted: *28 August 2015*
Published: *14 September 2015*

Citation:
Mallow J, Bernarding J, Luchtmann M, Bethmann A and Brechmann A (2015) Superior memorizers employ different neural networks for encoding and recall. Front. Syst. Neurosci. 9:128. doi: 10.3389/fnsys.2015.00128

Introduction

In the last two decades, functional magnetic resonance imaging (fMRI), with its capability to non-invasively localize activated brain areas, led to many studies investigating different aspects of memory-related processes. Although neuroimaging studies on encoding and retrieval have focused on prefrontal cortex (PFC) and the medial temporal lobe (MTL) memory system (Scoville and Milner, 1957; Squire and Zola-Morgan, 1991), several other regions have been found to play important roles as well (Kim, 2011). For one, varying paradigms were used to examine episodic memory, such as successful encoding, successful retrieval, and objective and subjective recollection. A major meta-analysis of the results, with a focus on the use of pictures, words, or faces as memoranda, was published by Spaniol et al. (2009). Furthermore, although enhancing memorization capabilities has always received attention, mind-enhancing techniques appear to be gaining increasing interest. This may be partially due to efforts to counteract a decline in memory capacities of elderly persons, especially those suffering from Alzheimer disease. However,

it remains debatable whether training strategies can have a long-lasting ameliorating effect on memory capabilities. While emotional episodic memories tend to be easy to retain over long periods, abstract data such as numbers, long lists of words, or chemical formulas are usually harder to keep in mind. However, it is well known that trained memory experts ["superior memorizers" (SMs)] can achieve extraordinary results when memorizing long lists of numbers, sequences of images, or other data in a short time. In this study we wanted to compare the brain areas *specifically* activated by SMs using the method of loci (MoL) on digits during encoding with those activated during recall.

The essence of their strategy consists in visualizing simple or abstract data, such as numbers, as concrete objects and subsequently placing them on landmarks along an internal route that was previously memorized. Those landmarks can be actual objects, like a chair or a tree, but also the empty space between two buildings. The far most popular version of this is the MoL (*locus* means *place* in Latin), a technique from ancient art of mnemonics (Yates, 1966). The transformation of the digits into visual objects can be done by using a *phonetic system* (Worthen, 2010). For recall, the internal route is walked through mentally and the objects transformed back into the original data.

Up to now, only one study analyzed the brain networks employed in SMs when encoding data (Maguire et al., 2003) and one studied subjects who received short-term training in the MoL (Kondo et al., 2005). Furthermore, there are only two other studies that investigated how SMs encode and recite digits. Both studies were performed with only one subject (Hu et al., 2009; Raz et al., 2009). However, applying this method to abstract data such as digits is a very complex, poorly described process. For that reason we developed a detailed scheme of the sub-processes of memorizing and recalling digits by using introspective information from superior memorizers. We then used fMRI to identify and compare the brain areas that are selectively activated by trained memorizers when encoding and recalling a sequence of digits while applying a phonetic system and the MoL. Moreover, we present some hypotheses whether the activated regions might be connected to the presumably underlying internal processing chains, which were described in the detailed scheme.

We suggest that the memorizing of digits, while using the MoL in combination with a phonetic system, not only encompasses visuo-spatial and memory-related components, but also recruits several language-related processes such as word generation and internal speech. Thus, in addition to speech- and language-related visuo-spatial areas, auditory brain networks may be activated. We expected to replicate the findings of Maguire et al. (2003), who described brain areas that are active in SMs when encoding items (including digits). However, Maguire et al. (2003) did not analyze the brain areas that were active during the recall phase. Consequently, we did not have a specific hypothesis, except that, since successful retrieval in normal subjects is suggested to be accompanied by processes similar to those involved in encoding, one could expected at least some overlap of brain areas specifically active during recall and encoding (Rugg et al., 2008).

Scheme for Encoding and Recall

According to the first author, who is one of the best trained superior memorizer in the world and expert on the practical use of the mnemonic methods since more than 10 year, during encoding, SMs usually perform an intricate chain of nested language-related, visual-related, and spatial navigation-related processes. Before encoding, SMs choose a previously learned internal route that they are familiar with. The actual encoding process is then a repetition of the process shown in (**Figure 1**). To emphasize which intermediate steps of the process are related to previously learned information, we divided the process into two columns. The encoding process starts with mental visualization of the first landmark from the chosen route. Then, a subset of items (here two or three digits, individual to each superior memorizer) is transformed into letters using a previously learned digit–letter encoding table. Subsequently, these letters are used to generate key words according to previously learned rules. Usually the key words encode objects that can easily be visualized internally (e.g., the digit sequence "5 0 4" corresponds to "lsr" which is expanded to "laser"). A similar approach is described in (Yin et al., 2015). Finally, the imagined objects are placed on the particular landmark along the internal route. To increase efficiency, the connections between the landmarks and placed items are composed into a small story, often within an emotional context. This process is repeated until all numbers are memorized (**Figure 1**). The detailed scheme in **Figure 1** separates the sub-processes reported and confirmed by SMs when initially learning to encode digits using a phonetic system and the MoL. Note that—depending on the performance level and experience of each superior memorizer—the mental effort differs between subjects for the sub-processes involved in translating the digits into words (e.g., recall of the letter table, search for words, and silent speaking of the word). This reportedly requires less effort for experienced memorizers than for naïve subjects or when learning the mnemonic strategies.

Similar to the encoding process, recall (**Figure 2**) begins by mentally visualizing the first landmark from the used route. Now the previously created story or object pops into mind and the memorizer decodes the related key word, using the digit–letter table. After recalling this subset of digits, the memorizer moves on to the next landmark.

According to the first author, the described method seems to be used by almost everybody in the world of ranked memory championships. Anyway there may be other memory experts who uses different approaches, like shown by Richman et al. (1995).

Materials and Methods

Subjects

Eleven SMs (eight male, three female; mean age 30.8 years) and eleven control subjects (six male, five female; mean age 26.3 years) participated in the study. All of the SMs were highly trained in the MoL and have proven their abilities in public competitions of memory performance. They all had perfectly memorized at least 100 digits in 5 min and thus belong to a very exclusive group of SMs worldwide. To give an impression about the group

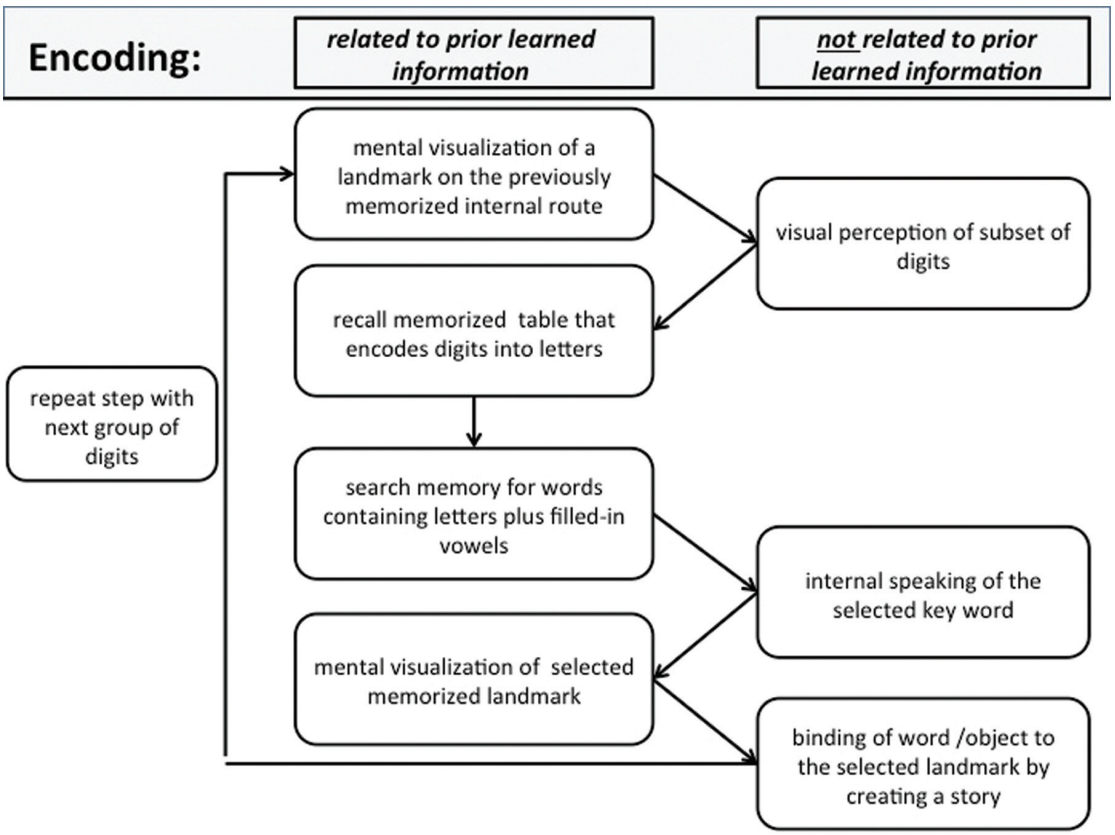

FIGURE 1 | Encoding: schematic view of a typical processing chain when encoding a sequence of digits using the method of loci (MoL). See text for further details.

size, it should be mentioned that at the time of the experiment only about 200 people worldwide had proved that capability. The performance variability for the subjects in that study was between 100 and 320 perfectly memorized digits in 5 min. All subjects confirmed to use the strategy, including the digit–letter transformation and the MoL, as shown in **Figure 1**. The reported time of experience with the methods was between 3 and 8 years. None of the control subjects reported having experience in the MoL or any other mnemonic technique and they where not provided with those strategies or any other method. The data from four SMs and four control subjects had to be excluded from the fMRI analysis due to excessive head motion (>2 mm and/or 2°). This large number of participants that has to be excluded is possibly due to the overt speech response required during the recall and rest condition. The mean age was 32.3 years for the remaining seven SMs (five male, two female, and one left-hander) and 25.1 years for the seven control subjects (four male, three female, and all right-handed). All subjects gave their written informed consent to the study, which was approved by the Local Ethics Committee of the Medical Faculty of the University of Magdeburg in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Experimental Paradigm

The experiment was performed using a block design of eight blocks for the *encoding* condition (60 s duration) and eight blocks for the *attention* condition (20 s duration). During the encoding condition a complete 5×8 matrix of 40 digits was presented visually, and the subjects were asked to memorize as many items as possible (first row from left, then second row, etc.). This *encoding* condition was followed by a 20-s *attention* condition, where a matrix with different digits was visually presented (**Figure 3**). Here, the subjects had to indicate, by pressing one of two buttons, whether or not each digit was even and was marked by exactly two dashes when adding up the dashes below and above the digit. Zero had to be treated as an even number. The attention condition served to stop the encoding process, and thus controlled for both the visual input and attentional load.

These first two blocks were followed by a recall condition (60 s duration) in which the subjects had to recall as many digits as possible and to say the numbers aloud in the same order as they where asked to memorize them (**Figure 4**). The subjects were instructed to not move their head while talking. The sounds were recorded with a dual channel microphone (MR-Confon, Germany) that subtracted the scanner noise, which enabled the experimental supervisor in the monitor room to

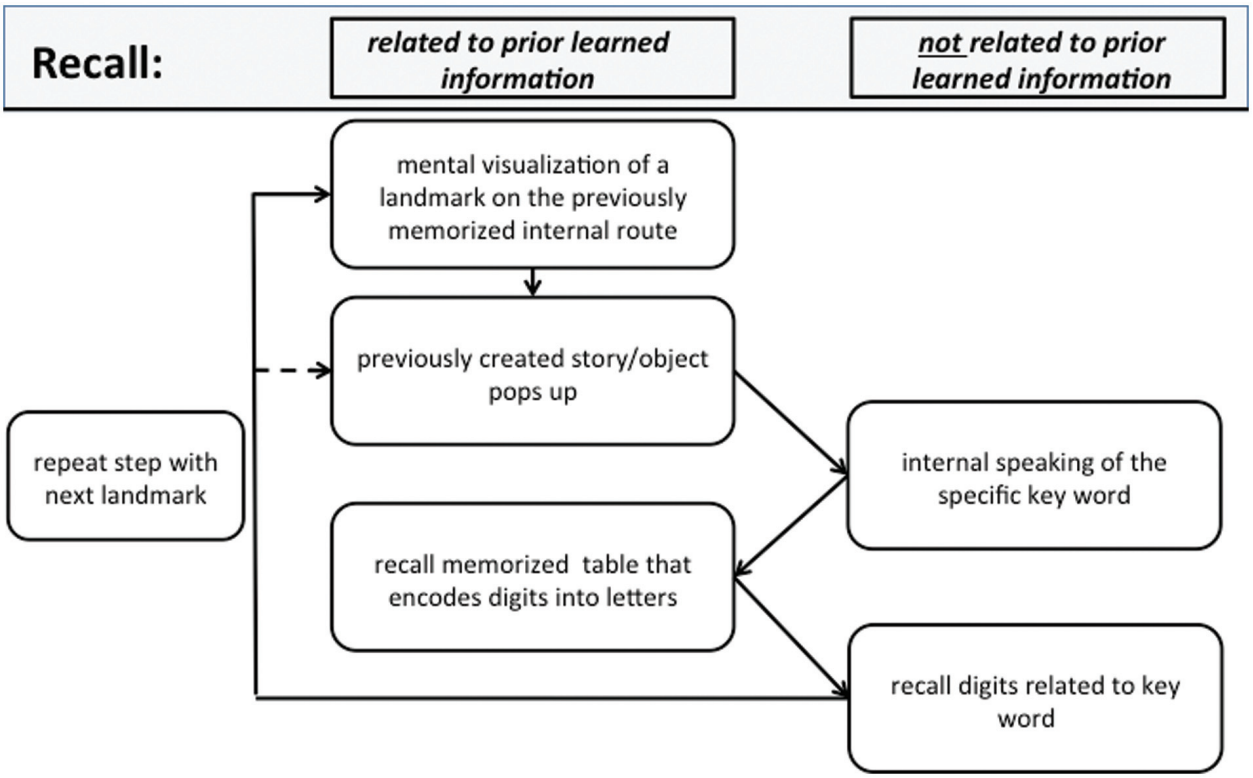

FIGURE 2 | Recall: schematic view of a typical processing chain when recalling a sequence of digits using the MoL. See text for further details.

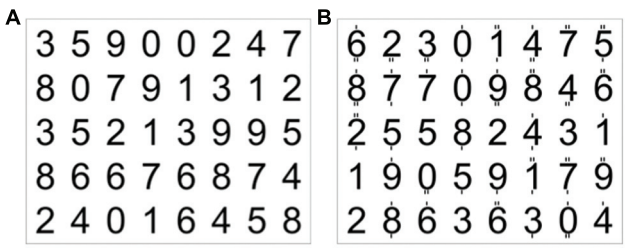

FIGURE 3 | Example of a 5 × 8 matrix of digits (A), which the subject had to encode within 60 s. (B) Digits with dashes on top or below. The subjects had to indicate by button press whether or not each digit was even (zero had to be treated as even) and marked by exactly two dashes (targets in the first row were the 2 and the 0, in the second row 0, 8, 4, 6, and in the third row 4).

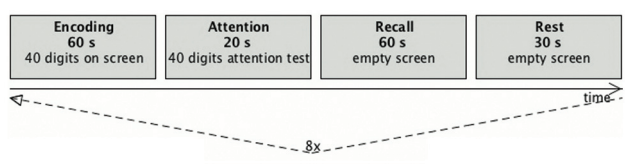

FIGURE 4 | Diagram of the trial scheme. One run consisted of a set of four blocks (encoding, attention, recall, and rest). During the recall and rest condition a fixation cross was presented. After an initial rest phase of 30 s each run was repeated eight times, resulting in 695 data sets.

easily identify the spoken numbers. The recall condition was followed by a 30-s rest condition. Here, the subjects had to recite the alphabet in silence before the next block was started. The visual stimuli were presented by a video projector onto a back-projection screen, which was viewed from inside the scanner through a mirror system. For stimulus presentation and recording of the behavioral responses, the software application *Presentation* (Neurobehavioral Systems, Albany, NY, USA) was used.

Data Acquisition and Data Analysis

Magnetic resonance imaging was carried out using a 3 T Trio MRI scanner (Siemens Medical Solutions) equipped with an eight-channel phased array coil. Anatomical data of the whole brain were acquired with high resolution using a multiplanar rapidly acquired gradient echo (MPRAGE) sequence with 1.0 mm^3 isotropic resolution. For later alignment with the anatomical data an additional inversion-recovery echo-planar imaging (IR-EPI) was acquired prior to each functional run. The IR-EPI exhibited the same geometry as the subsequent functional measurements. 695 volumes were acquired in 23 h 10 min using a gradient-recalled echo-planar imaging (EPI) pulse sequence [echo time (TE) = 30 ms; repetition time (TR) = 2000 ms; flip angle (FA) = 80°; matrix 64 × 64; field of view (FOV) = 19.2 cm; 33 slices of 3 mm, 0.3 mm gap).

The functional data were analyzed with the software system BrainVoyager^{TM}QX (Brain Innovation, Maastricht,

Netherlands). A standard chain of pre-processing steps, slice scan time correction, 3D-motion correction, linear trend removal, filtering with a high-pass of three cycles per scan, and spatial smoothing with a Gaussian filter with 4 mm full width at half maximum was applied. The functional data were projected onto the corresponding IR-EPI images, co-registered with the 3D dataset, and then transformed to Talairach space.

Due to the low number of subjects, a fixed effects analysis of the group data was performed using a conservative significance level of $p < 0.01$ (Bonferroni corrected) with a minimum cluster size of eight voxels. To determine brain regions exhibiting increased activation under specific conditions in SMs as compared to in control subjects, a conjunction analysis was performed that ensures that the resulting brain regions show a positive deflection of the BOLD response during encoding or recall vs. rest and is controlled for general attentional processes (comparison vs. attention condition). Thus, the contrast for the encoding-specific activation in SMs was: *Encode* in SMs vs. *Rest* in SMs AND *Encode* in SMs vs. *Attend* in SMs AND *Encode* in SMs vs. *Encode* in controls. The contrast for the recall-specific activation in SMs was: *Recall* in SMs vs. *Rest* in SMs AND *Recall* in SMs vs. *Attend* in SMs AND *Recall* in SMs vs. *Recall* in controls.

This analysis is rather conservative but it guarantees that the BOLD response during encoding/recall in SMs is significantly stronger. Beside the comparison between encoding in SMs and encoding in controls and the same for recall, two additional conditions must be fulfilled. Firstly, the activation of the SMs during the encoding/recall phases must be significantly stronger than during the attentional control condition of the SMs because we do not want to discuss brain areas which are strongly activated due to general attention differences between the SMs and the control group. Secondly, we only want to consider brain areas that show a positive BOLD response compared to a resting condition in which the SMs only need to recite the alphabet. This is especially important for the identification of areas involved in the recall where overt reciting of numbers is required by the memorizers. All three contrasts must be independently significant at the $p < 0.01$ level.

Results

Task Performance and fMRI Data

For the behavioral analysis we calculated the mean values and standard errors of the mean (SEM). On average, the SMs were capable to recall 34.5 of the 40 digits ($SEM = 2.37$), while the naïve subjects recalled only 10.8 digits ($SEM = 1.58$). The SMs recalled significantly more digits then the controls (t-test: $p < 0.0001$, $t = 7.6$, and effect size: $d > 4$). The mean time for recalling all numbers was 37.1 s (1.2 s/digit; $SEM = 0.15$ s) for SMs, and 24.3 s (2.5 s/digit; $SEM = 0.41$ s) for the naïve subjects. The SMs recalled the digits significantly faster (t-test: $p < 0.01$, $t = 2.86$, and effect size: $d > 1.5$). The difference in overall duration was due to fewer digits memorized by the control group, as well as to a high variation in recall speed. In some cases the number of recalled digits was too small to fill the entire recall interval. In other cases the naïve subjects recalled the numbers so slowly that the duration of the recall phase was used up completely.

Figure 5 shows the results for the conjunction analysis used to detect brain regions that were significantly more activated during the encoding process in SMs than in naïve subjects. It shows that a network of left hemisphere regions is involved in encoding, consisting of left secondary visual areas (BA 18, 19), left medial superior parietal cortex (BA 7), and left middle temporal gyrus (BA 39). The bilaterally activated retrosplenial cortex (BA 30), and posterior parahippocampal gyrus (pHi) also exhibited a very strong lateralization to the left hemisphere. The details are summarized in **Table 1**. To demonstrate the extent of the differences in brain activation between both groups, the time course of the BOLD response during the 60 s of encoding is displayed in **Figure 6** for each brain area, which was found to be significantly stronger in SMs than in naïve control subjects.

None of these regions was significantly activated in the recall condition of the SMs. **Figure 7** shows that the beta values of the recall condition were mostly negative and all significantly

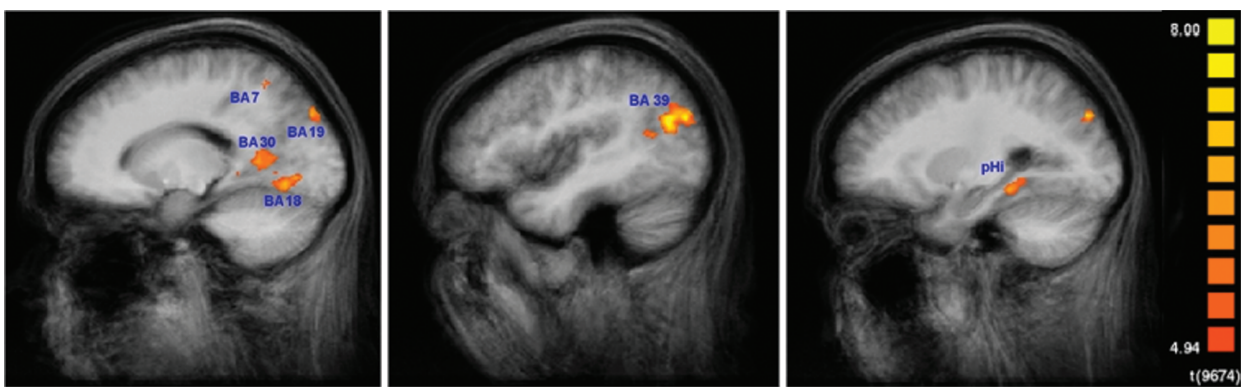

FIGURE 5 | Encoding-specific activation in superior memorizers. Contrast: *Encode* in superior memorizers vs. *Rest* in superior memorizers AND *Encode* in superior memorizers vs. *Attend* in superior memorizers AND *Encode* in superior memorizers vs. *Encode* in controls. Color-encoded activation maps overlaid on group-averaged anatomical images ($p < 0.01$, Bonferroni corrected, minimum cluster size 8 voxel) (left $x = -16$, middle $x = -42$, and right $x = -25$).

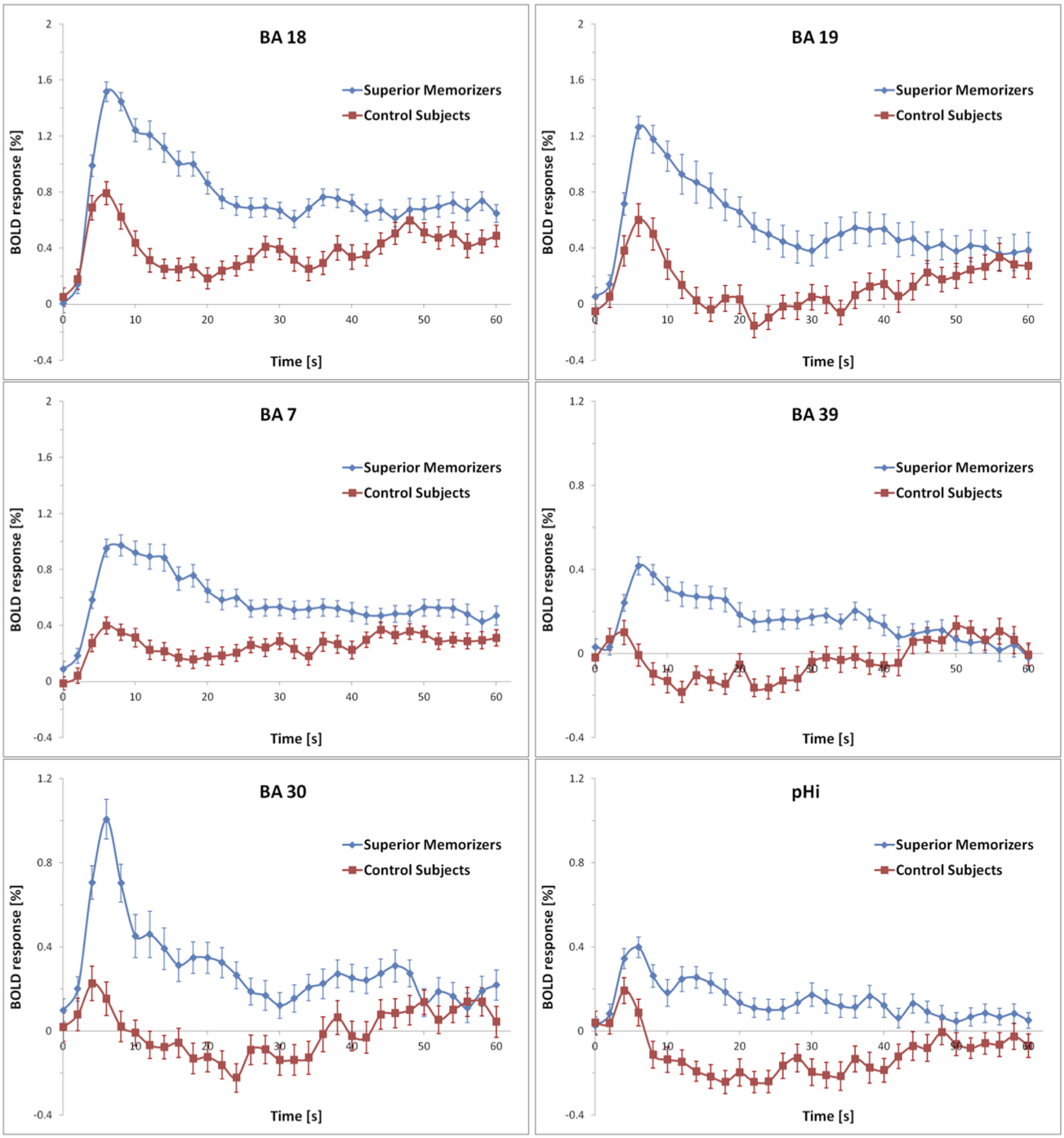

FIGURE 6 | Averaged BOLD responses of the brain areas showing encoding-specific activation in superior memorizers. Time point zero denotes the start of the encoding condition that lasted for 60 s. The baseline (0 % BOLD response) was defined as the average response within 4 s prior to the encoding condition. Note the different scaling of the ordinates. The BOLD response of control subjects is depicted for comparison.

lower than those of the encoding condition (**Figure 7**). Instead, the left anterior superior temporal gyrus (STG) and the right motor cortex were specifically activated (**Figure 8**). The BOLD response during the 60 s when recalling the digits is displayed in **Figure 9**.

Discussion

We now discuss the activated brain regions with respect to the involved processes including information transformation and binding processes of the suggested mnemonic procedure

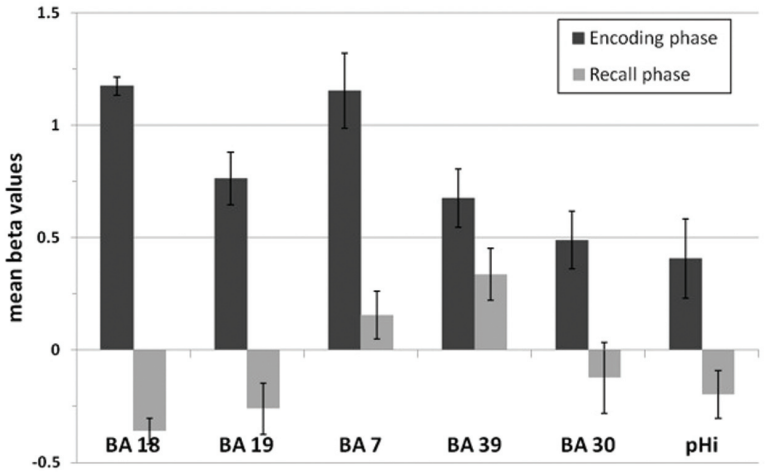

FIGURE 7 | Mean beta values of the encoding-specific areas of superior memorizers shown in Figure 5. Beta values of the encoding phase correspond to the BOLD response of the superior memorizers depicted as blue curve in **Figure 6**. The beta values of the recall phase are all significantly lower than those of the encoding phase (BA 18, BA 19, BA 7, BA 30, and pHi: $p < 0.01$; BA 39: $p < 0.05$; two sides *t*-test).

TABLE 1 | Regions activated to a higher degree in superior memorizers than in naïve controls ($p < 0.01$, Bonferroni corrected, minimum cluster size eight voxels).

Region	Talairach coordinates			BA	Cluster size
	x	*y*	*z*		
Encoding					
Left visual cortex	−13	−67	−3	18	1213
Right visual cortex	10	−72	−2	18	228
Left visual cortex	−20	−81	34	19	615
Left angular gyrus	−40	−65	24	39	2162
Left retrosplenial cortex	−12	−53	8	30	3914
Right retrosplenial cortex	13	−51	8	30	1047
Left superior parietal cortex	−20	−57	52	7	263
Left parahippocampus	−25	−36	−7		449
Recall					
Left anterior superior temporal gyrus	−57	−8	7	22	346
Right motor cortex	50	−8	30	4/6	676

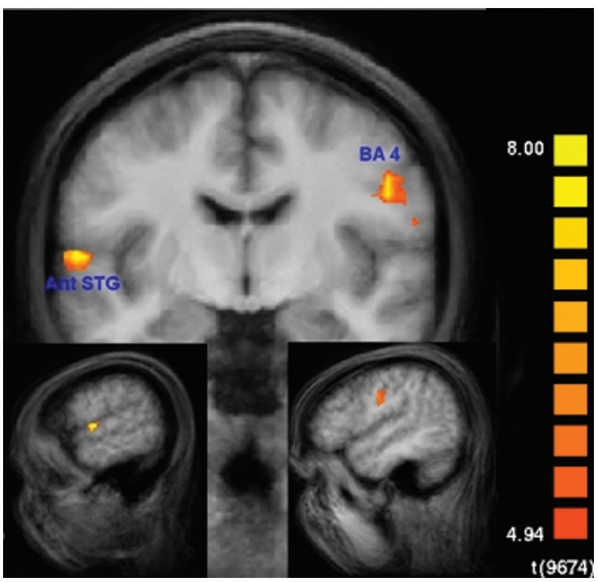

FIGURE 8 | Recall-specific activation in superior memorizers. Left anterior superior temporal gyrus (STG) and right motor cortex are the only regions that are specifically activated during the recall condition in superior memorizers. Contrast: *Recall* in superior memorizers vs. *Rest* in superior memorizers AND *Recall* in superior memorizers vs. *Attend* in superior memorizers AND *Recall* in superior memorizers vs. *Recall* in controls. ($p < 0.01$, Bonferroni corrected, minimum cluster size 8 voxel) (down left $x = -56$, down right $x = 48$, and middle $y = -8$).

(**Figure 1**). The encoding procedure includes the activation of long-term memory, which was found to be characteristic of studies when experts where involved (Guida et al., 2012). This results in mental visualization of a location on the previously memorized internal route. This explains the strong activation in visual brain regions (Johnson and Johnson, 2014). Other contributions to the activation of the visual cortex may come from several sub-processes of translating the digits into visual objects, i.e., in-depth visual perception of digits, recall of the specific letters representing these digits from the previously memorized table, and the search for a correct word containing the letters. Furthermore, several studies also suggest that BA 18 is involved in recollection processes (Spaniol et al., 2009). The fact that SMs use contextual information while applying the MoL supports these findings. Furthermore the use of long-term memory.

The left lateralized effect in BA 19 suggests the involvement of the language-related system when transforming digits into letters and subsequently into words, since a left lateralization of BA 19

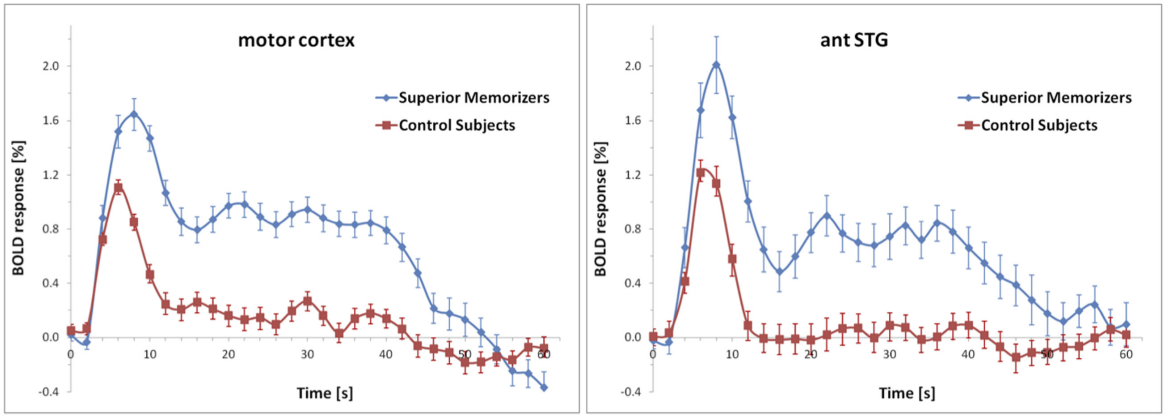

FIGURE 9 | Averaged BOLD responses of the brain areas depicted in Figure 8 showing recall-specific activation in superior memorizers. Time point zero denotes the start of the recall condition that lasted for 60 s. The baseline (0% BOLD response) was defined as the average response within four seconds prior to the recall condition. The BOLD response of control subjects is depicted for comparison.

was often found to play a role in phonological processing and forming words (Dietz et al., 2005), as well as in word recognition (Cabeza and Nyberg, 2000). One step of the proposed model in **Figure 3** involves silently speaking. However, we did not find specific activation in, e.g., Broca's area. One explanation is that the control subjects also used inner speech to encode as many digits as possible. Moreover, because the SMs in the group were highly experienced in the encoding process, some of the sub-processes involving covert speech may have required less mental effort.

When connecting the mentally visualized object to the selected landmark, the visualized object becomes navigationally relevant. This may explain why the pHi is activated (Wegman and Janzen, 2011). Moreover, the repetition of the processes depicted in (**Figure 1**) automatically results in mental navigation that is known to involve not only the pHi but also BA 7 and 30 (Ghaem et al., 1997; Maguire et al., 1998, 2003; Kondo et al., 2005; Epstein, 2008; Auger et al., 2012). With the left-hemispheric activation of BA 7 and BA 39 during encoding, we found two areas that have been also found activated by other groups during recollection (Vilberg and Rugg, 2007; Spaniol et al., 2009). Those findings imply that the encoding process of SMs is basically a mixture of encoding and retrieval, as suggested by the scheme of **Figure 1**, where it is shown that items already learned may be used to memorize other new items.

During recall none of the encoding-related areas was specifically activated and the overall number of activated voxels was much smaller than in the encoding condition. This was surprising, since a comparison of **Figures 1** and **2** leads one to expect to find at least the same areas activated for mental navigation as for encoding. This result seems to stand in some contradiction to the transfer-appropriate process theory, which proposes that successful retrieval is accompanied by neuronal processes similar to those occurring during encoding (Rugg et al., 2008). However such a conclusion must be considered very carefully since due to our conjunction analyses we only analyzed very specific activated brain regions.

During recall, the activation in the motor area may be explained by the fact that SMs had to recite significantly more digits. This is in part supported by the BOLD response (**Figure 9**) which in control subjects falls off to baseline much earlier than in SMs. The anterior temporal lobe has been shown to be involved in the retrieval of proper names (Damasio et al., 2004; Bethmann et al., 2012), which are composed of more semantic features than common objects. The left anterior temporal lobe has also been shown to be involved in syntactic processing, independent of syntactic complexity (Friederici, 2002; Dronkers et al., 2004). Hence, it has been suggested that the left anterior temporal lobe contributes to the composition of sentence meaning (Vandenberghe et al., 2002; Humphries et al., 2006). Beyond that, the anterior temporal region has been proposed to be involved in the concatenation of sentences or propositions into stories (Mar, 2004).

Taking these suggestions together, we interpret the current recall-specific activation in the anterior STG to be related to the reconstruction of the exact sequence of objects along the mental route of landmarks. Because SMs make up vivid stories of how the visual objects, which represent the encoded information, are related to the landmarks of their routes, recalling the sequence of encoded information may be interpreted as reviewing a story rather than as mental navigation along a route. Such a difference in strategy may also explain the unexpected difference in the recruitment of brain areas during the encoding compared with the recall phase. Although we think that the described process in **Figure 2** is very accurate, it has to be considered that some modifications would be appropriate. Our results show that the process of mental movement from one location to the next seems to be much less intense than during encoding. Thus it may be reasonable to link directly from the last step of the process, "recall digits related to keyword", to the step "previously created story pops up". We have shown that with the dashed arrow in **Figure 2**.

Although many aspects of memory-related processes have been investigated, little is known about persons who have

trained their capabilities to memorize abstract and emotionally neutral data using the MoL (Maguire et al., 2003; Kondo et al., 2005; Konrad, 2014). The regions activated during encoding are consistent with those found by Maguire et al. (2003), except that we found activation in the pHi instead of in the hippocampus. The former was also found to be strongly activated in several other encoding studies (Spaniol et al., 2009). One explanation for the higher activation in SMs could be the fact that they encode additional information (e.g., the connection between landmark and object), since the pHi is also suggested by other studies (Epstein, 2008) to be involved in the encoding of contextual information (Davachi, 2006; Charan Ranganath, 2012) and navigationally relevant object information (Epstein, 2008; Wegman and Janzen, 2011). In another study naïve subjects were trained to use the MoL prior to the fMRI experiment (Kondo et al., 2005), which resulted in activation of the left fusiform and lingual gyrus during both encoding and recall. However, it should be noted that the subjects in this study were trained only briefly and thus were by no means SMs.

According to the hemispheric encoding/retrieval asymmetry model (HERA; Tulving et al., 1994) the left PFC should be more involved during encoding and the right PFC more involved during retrieval. We found a strong left lateralization for a few areas (**Table 1**) specifically activated by SMs during encoding but no specific activation in the PFC during encoding or during retrieval. The overall left dominance could indicate language-related and sequential processing while applying the MoL combined with a phonetic system.

In this study we aimed to interpret the fMRI data of SMs who used the MoL combined with a phonetic system to memorize long lists of digits. To understand which neuronal processes may potentially be involved, a scheme was developed of the different sub-processes that are employed when SMs fulfill this task. Although our data show some support for the proposition that language-related systems are involved in addition to the brain parts already known to play a role in encoding, this interpretation of the data is limited at the moment by the fact that the single sub-processes of the scheme cannot be separated within our experimental setup. Furthermore the overt behavior during the different conditions was different, which may cause some background noise in the data and some lack of control over all potential sub-processes. Thus there is a need for future studies focusing on partial aspects of the overall process. This could be achieved by scanning the SMs solely while they "walk" through their mental route without creating mental images or while they translate numbers into images without connecting them to specific locations.

A limitation of the fixed effects analysis is that it does not allow for inferences to the population. Nevertheless, to demonstrate the significance of the effects, we used very conservative criteria, as described in the Section "Materials and Methods". Furthermore, we show the BOLD response over time for each activated brain area that reveals how large the difference between SMs and controls is in terms of BOLD response amplitude. It also has to be stated that the potential heterogeneity of strategies of the naïve volunteers may limit the generalization of the results, which makes more specific research necessary.

In sum, we found that SMs use different networks for encoding and recall, with the latter process generating much less brain activation. This is in accordance with the everyday challenges of learning a language, playing a piece of music, or learning movement patterns. Once a pattern is learned it can be recalled and executed very quickly and with little conscious effort. However, learning strategies are seldom taught in schools or other institutions. One may speculate to which degree learning would gain efficiency when applying optimized strategies instead of trying to memorize data by endless repetition. At least our behavioral results show that the strategies of the superior memorizer are very efficient for learning digits. It would be interesting to investigate the efficiency of their strategies on general knowledge. Such a study could have an important influence on how knowledge is imparted in schools or universities.

Author Contributions

JM, ABr, and JB wrote the main manuscript text. JM also prepared **Figures 1–4**. ABr prepared **Figures 5–9**, was responsible for methods and data analysis. ABr, ABe, and ML contributed to the discussion. All authors reviewed the manuscript.

References

Auger, S. D., Mullally, S. L., and Maguire, E. A. (2012). Retrosplenial cortex codes for permanent landmarks. *PLoS ONE* 7:e43620. doi: 10.1371/journal.pone.0043620

Bethmann, A., Scheich, H., and Brechmann, A. (2012). The temporal lobes differentiate between the voices of famous and unknown people: an event-related fMRI study on speaker recognition. *PLoS ONE* 7:e47626. doi: 10.1371/journal.pone.0047626

Cabeza, R., and Nyberg, L. (2000). Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J. Cogn. Neurosci.* 12, 1–47. doi: 10.1162/08989290051137585

Charan Ranganath, M. R. (2012). Two cortical systems for memory-guided behaviour. *Nat. Rev. Neurosci.* 13, 713–726. doi: 10.1038/nrn3338

Damasio, H., Tranel, D., Grabowski, T., Adolphs, R., and Damasio, A. (2004). Neural systems behind word and concept retrieval. *Cognition* 92, 179–229. doi: 10.1016/j.cognition.2002.07.001

Davachi, L. (2006). Item, context and relational episodic encoding in humans. *Curr. Opin. Neurobiol.* 16, 693–700. doi: 10.1016/j.conb.2006.10.012

Dietz, N. A. E., Jones, K. M., Gareau, L., Zeffiro, T. A., and Eden, G. F. (2005). Phonological decoding involves left posterior fusiform gyrus. 26, 81–93. doi: 10.1002/hbm.20122

Dronkers, N. F., Wilkins, D. P., Van Valin, R. D., Redfern, B. B., and Jaeger, J. J. (2004). Lesion analysis of the brain areas involved in language comprehension. *Cognition* 92, 145–177. doi: 10.1016/j.cognition.2003.11.002

Epstein, R. A. (2008). Parahippocampal and retrosplenial contributions to human spatial navigation. *Trends Cogn. Sci.* 12, 388–396. doi: 10.1016/j.tics.2008.07.004

Friederici, A. D. (2002). Towards a neural basis of auditory sentence processing. *Trends Cogn. Sci.* 6, 78–84. doi: 10.1016/S1364-6613(00)01839-8

Ghaem, O., Mellet, E., Crivello, F., Tzourio, N., Mazoyer, B., Berthoz, A., et al. (1997). Mental navigation along memorized routes activates the hippocampus, precuneus, and insula. *Neuroreport* 8, 739–744. doi: 10.1097/00001756-199702100-00032

Guida, A., Gobet, F., Tardieu, H., and Nicolas, S. (2012). How chunks, long-term working memory and templates offer a cognitive explanation for neuroimaging data on expertise acquisition: a two-stage framework. *Brain Cogn.* 79, 221–244. doi: 10.1016/j.bandc.2012.01.010

Hu, Y., Ericsson, K. A., Yang, D., and Lu, C. (2009). Superior self-paced memorization of digits in spite of a normal digit span: the structure of a memorist's skill. *J. Exp. Psychol. Learn. Mem. Cogn.* 35, 1426–1442. doi: 10.1037/a0017395

Humphries, C., Binder, J. R., Medler, D. A., and Liebenthal, E. (2006). Syntactic and semantic modulation of neural activity during auditory sentence comprehension. *J. Cogn. Neurosci.* 18, 665–679. doi: 10.1162/jocn.2006.18.4.665

Johnson, M. R., and Johnson, M. K. (2014). Decoding individual natural scene representations during perception and imagery. *Front. Hum. Neurosci.* 8:59. doi: 10.3389/fnhum.2014.00059

Kim, H. (2011). Neural activity that predicts subsequent memory and forgetting: a meta-analysis of 74 fMRI studies. *Neuroimage* 54, 2446–2461. doi: 10.1016/j.neuroimage.2010.09.045

Kondo, Y., Suzuki, M., Mugikura, S., Abe, N., Takahashi, S., Iijima, T., et al. (2005). Changes in brain activation associated with use of a memory strategy: a functional MRI study. *Neuroimage* 24, 1154–1163. doi: 10.1016/j.neuroimage.2004.10.033

Konrad, B. N. (2014). *Characteristics and Neuronal Correlates of Superior Memory Performance*. Available at: http://edoc.ub.uni-muenchen.de/16636/ [Accessed May 20, 2014]

Maguire, E. A., Burgess, N., Donnett, J. G., Frackowiak, R. S., Frith, C. D., and O'Keefe, J. (1998). Knowing where and getting there: a human navigation network. *Science* 280, 921–924. doi: 10.1126/science.280.5365.921

Maguire, E. A., Valentine, E. R., Wilding, J. M., and Kapur, N. (2003). Routes to remembering: the brains behind superior memory. *Nat. Neurosci.* 6, 90–95. doi: 10.1038/nn988

Mar, R. A. (2004). The neuropsychology of narrative: story comprehension, story production and their interrelation. *Neuropsychologia* 42, 1414–1434. doi: 10.1016/j.neuropsychologia.2003.12.016

Raz, A., Packard, M. G., Alexander, G. M., Buhle, J. T., Zhu, H., Yu, S., et al. (2009). A slice of pi: an exploratory neuroimaging study of digit encoding and retrieval in a superior memorist. *Neurocase* 15, 361–372. doi: 10.1080/13554790902776896

Richman, H. B., Staszewski, J. J., Simon, H. A., Richman, H. B., Staszewski, J. J., and Simon, H. A. (1995). Simulation of expert memory using EPAM IV. *Psychol. Rev.* 102, 305–330. doi: 10.1037/0033-295X.102.2.305

Rugg, M. D., Johnson, J. D., Park, H., and Uncapher, M. R. (2008). Encoding-retrieval overlap in human episodic memory: a functional neuroimaging perspective. *Prog. Brain Res* 169, 339–352. doi: 10.1016/S0079-6123(07)00021-0

Scoville, W. B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* 20, 11–21. doi: 10.1136/jnnp.20.1.11

Spaniol, J., Davidson, P. S. R., Kim, A. S. N., Han, H., Moscovitch, M., and Grady, C. L. (2009). Event-related fMRI studies of episodic encoding and retrieval: meta-analyses using activation likelihood estimation. *Neuropsychologia* 47, 1765–1779. doi: 10.1016/j.neuropsychologia.2009.02.028

Squire, L. R., and Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science* 253, 1380–1386. doi: 10.1126/science.1896849

Tulving, E., Kapur, S., Craik, F. I., Moscovitch, M., and Houle, S. (1994). Hemispheric encoding/retrieval asymmetry in episodic memory: positron emission tomography findings. *Proc. Natl. Acad. Sci. U.S.A.* 91, 2016–2020. doi: 10.1073/pnas.91.6.2016

Vandenberghe, R., Nobre, A. C., and Price, C. J. (2002). The response of left temporal cortex to sentences. *J. Cogn. Neurosci.* 14, 550–560. doi: 10.1162/08989290260045800

Vilberg, K. L., and Rugg, M. D. (2007). Dissociation of the neural correlates of recognition memory according to familiarity, recollection, and amount of recollected information. *Neuropsychologia* 45, 2216–2225. doi: 10.1016/j.neuropsychologia.2007.02.027

Wegman, J., and Janzen, G. (2011). Neural encoding of objects relevant for navigation and resting state correlations with navigational ability. *J. Cogn. Neurosci.* 23, 3841–3854. doi: 10.1162/jocn_a_00081

Worthen, J. B. (2010). *Mnemonology: Mnemonics for the 21st Century*, 1st Edn. New York, NY: Psychology Press.

Yates, F. A. (1966). *The Art of Memory*. London: University of Chicago Press.

Yin, L.-J., Lou, Y.-T., Fan, M.-X., Wang, Z.-X., and Hu, Y. (2015). Neural evidence for the use of digit-image mnemonic in a superior memorist: an fMRI study. *Front. Hum. Neurosci.* 9:109. doi: 10.3389/fnhum.2015.00109

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 16 October 2014
doi: 10.3389/fnsys.2014.00200

Working memory training improves emotional states of healthy individuals

Hikaru Takeuchi[1]*, Yasuyuki Taki[1,2,3], Rui Nouchi[4], Hiroshi Hashizume[1], Atsushi Sekiguchi[4], Yuka Kotozaki[4], Seishu Nakagawa[5], Carlos Makoto Miyauchi[5], Yuko Sassa[1] and Ryuta Kawashima[1,2,4]

[1] Division of Developmental Cognitive Neuroscience, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[2] Division of Medical Image Analysis, Department of Community Medical Supports, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan
[3] Department of Radiology and Nuclear Medicine, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[4] Department of Advanced Brain Science, Smart Ageing International Research Center, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[5] Department of Functional Brain Imaging, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

Edited by:
Manuel Casanova, University of Louisville, USA

Reviewed by:
Hasan Ayaz, Drexel University, USA
Ariana Tart-Zelvin, Idaho State University, USA

****Correspondence:***
Hikaru Takeuchi, Smart Ageing International Research Center, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-cho, Aoba-ku, Sendai 980-8575, Japan
e-mail: takehi@idac.tohoku.ac.jp

Working memory (WM) capacity is associated with various emotional aspects, including states of depression and stress, reactions to emotional stimuli, and regulatory behaviors. We have previously investigated the effects of WM training (WMT) on cognitive functions and brain structures. However, the effects of WMT on emotional states and related neural mechanisms among healthy young adults remain unknown. In the present study, we investigated these effects in young adults who underwent WMT or received no intervention for 4 weeks. Before and after the intervention, subjects completed self-report questionnaires related to their emotional states and underwent scanning sessions in which brain activities related to negative emotions were measured. Compared with controls, subjects who underwent WMT showed reduced anger, fatigue, and depression. Furthermore, WMT reduced activity in the left posterior insula during tasks evoking negative emotion, which was related to anger. It also reduced activity in the left frontoparietal area. These findings show that WMT can reduce negative mood and provide new insight into the clinical applications of WMT, at least among subjects with preclinical-level conditions.

Keywords: working memory, training, plasticity, emotion, mood, anger, fMRI

INTRODUCTION

Working memory (WM) is the limited capacity storage system involved in the maintenance and manipulation of information over a short time period (Baddeley, 2003). It is a functionally important system that underlies a wide range of higher-order cognitive activities such as general intelligence, reasoning and problem solving, language comprehension, learning, and response inhibition (Osaka and Nishizaki, 2000; Baddeley, 2003). Reduced WM capacity (WMC) is also associated with a wide variety of emotional aspects, including mood disorders, anxiety, stress, greater emotional responses, and fewer regulatory behaviors (Sorg and Whitney, 1992; Klein and Boals, 2001; Weiland-Fiedler et al., 2004; Schmeichel et al., 2008).

The dorsolateral prefrontal cortex (DLPFC) plays a key role in the central executive system of WM (Baddeley, 2003). The premotor cortex is closely related to DLPFC, both anatomically, and functionally (Fuster, 2006; Takeuchi et al., 2012), and it plays important roles in some executive functions of WM (Wager et al., 2008). Both areas are consistently active during the execution of WM (Reuter-Lorenz et al., 2000). They also play important roles in emotional aspects, and an essential link exists between DLPFC to premotor areas and mood as described below. Functional deficits of DLPFC to dorsal premotor areas are associated with depression (Siegle et al., 2007). DLPFC to premotor areas and other widespread lateral prefrontal and medial prefrontal regions are activated to control negative emotions (Phan et al., 2005; Belden et al., 2014). Rapid-rate transcranial magnetic stimulation (rTMS) over DLPFC as well as premotor areas mitigates depression (Pascual-Leone et al., 1996; Johnson et al., 2013). Activity changes in DLPFC to dorsal premotor areas and the precentral gyrus have been associated with depression (Koenigs and Grafman, 2009; Stuhrmann et al., 2011). DLPFC lesions have been shown to cause apathy and greater vulnerability to fatigue and depression compared with other lesions (Fuster, 2006; Koenigs and Grafman, 2009), and structural abnormalities in DLPFC to premotor areas are associated with fatigue (Chaudhuri and Behan, 2004).

Physical exercise can affect the volume of DLPFC to premotor areas (Colcombe et al., 2004, 2006; Flöel et al., 2010) and improve the cognitive functions that are related to DLPFC to premotor areas (Smith et al., 2010); further, it can robustly improve mood (Arent and Landers, 2000). Other studies have shown that WM training (WMT) can affect the outcomes of psychological measures and neural systems (Uchida and Kawashima, 2008; Klingberg, 2010; Takeuchi et al., 2010b). However, whether WMT can improve cognitive performance is still a matter of debate because several studies have reported different conclusions (Jaeggi et al., 2008; Redick et al., 2013). A recent meta-analysis

indicated that WMT has robust effects on WMC and measures of inhibition and attention (Melby-Lervåg and Hulme, 2012). In addition, WMT has been clinically shown to improve fatigue in patients with multiple sclerosis (Takeuchi et al., 2010b) as well as the symptoms of attention deficit hyperactivity disorder (Klingberg et al., 2005), which can affect mood (e.g., impulsivity can lead to a failure in emotional regulation) (Apter et al., 1990). Structural and functional studies that have used magnetic resonance imaging (MRI), positron emission tomography (PET), and near infrared spectroscopy (NIRS), have linked WMT to changes in brain activity during WM and other related tasks, changes in gray and white matter structures, and dopamine D1 receptor density in prefrontal (including DLPFC and premoror) and posterior parietal areas (McNab et al., 2009; Klingberg, 2010; Takeuchi et al., 2010b, 2013b, 2014a; Buschkuehl et al., 2012; McKendrick et al., 2014). In summary, as described, TMS applied to DLPFC to premotor areas improves mood, whereas DLPFC lesions can deteriorate mood. Similarly, deteriorated emotional states have been associated with hypoactivity in DLPFC to premotor areas. Other interventions such as aerobic exercise can improve the structure and functioning of DLPFC to premotor areas and subsequently improve mood (Lawlor and Hopker, 2001). WMT leads to changes in several mechanisms of DLPFC to premotor areas and cognitive functions related to the areas (Takeuchi et al., 2010b, 2013b). Further, emotional states can be changed through a wide range of interventions in both clinical and nonclinical samples (McNair et al., 1992).

These previous findings have led us to question whether WMT can improve emotional states in individuals without apparent cognitive deficits. We investigated this question in the present study in young adults who underwent WMT. The subjects completed self-report questionnaires related to their emotional states before and after WMT and were examined with a functional MRI (fMRI) task to detect negative emotion-related brain activity as they identified faces with negative emotions (angry or afraid) (Hariri et al., 2002). Previously, this task has been used to examine whether individuals at risk for emotional problems (e.g., subjects with a genetic risk for impulsivity and violence) show different activity in emotion-related areas such as the anterior insula (Meyer-Lindenberg et al., 2006). Our primary aim was to investigate the effects of WMT on mood and its underlying neural activity. We believe that there is no evidence that indicates that improvements in emotional states are solely produced by increased emotional regulation. Considering that our emotional states contribute to our everyday well-being, it is important to investigate the extent of their plasticity and develop new methods to improve them, which could lead to the clinical application of WMT. We hypothesized that WMT improves an emotional state and alters neural activity related to negative emotions, particularly in DLPFC to premotor areas and areas related to emotional states. The increase or decrease in task-induced activity after training or interventions depends on many factors. Even when training leads to adaptations in performance or neural mechanisms, a training-related increase in the efficiency of the involved areas (hence, an activity decrease) or a training-related increase in recruitment of the critical areas (hence, an activity increase) may underlie these changes (Erickson et al., 2007). Thus, we did not predict the direction (increase or decrease) of changes.

MATERIALS AND METHODS

SUBJECTS

Subjects characteristics in each group and experiment

We recruited 97 students and graduates to participate in experiments in our laboratory. Of these 97 subjects, 81 were included in the longitudinal intervention experiment, which involved two intervention studies and three groups (a WMT group, another intervention group for another longitudinal study, and a non-intervention control group, **Figure 1**). The remaining

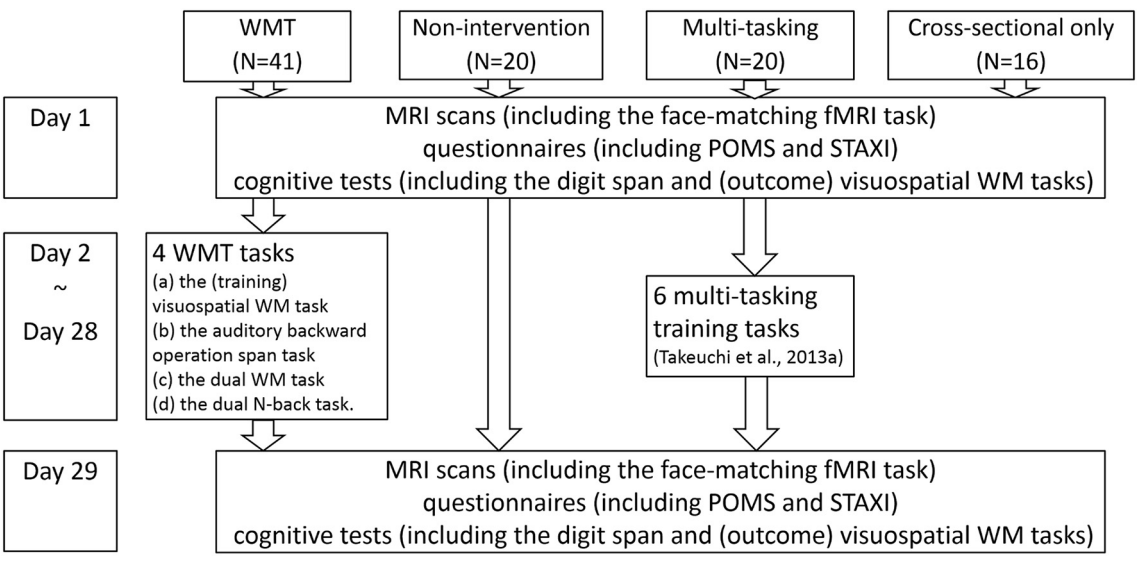

FIGURE 1 | The schema of this study's groups, tasks, and experimental design. A figure illustrating subject flow can be seen in our previous report (Takeuchi et al., 2014b). The measurements for day 1 and day 29 are in no particular order.

16 subjects were included in a cross-sectional experiment that examined brain activity during a face-matching task and completed only all other preintervention procedures [16 women; mean age = 21.6 years; standard deviation (SD) = 1.9; age range = 19–27]. Among the 81 subjects included in the longitudinal intervention experiment, 61 were assigned to the WMT or non-intervention control group. The WMT group consisted of 41 subjects (27 men, 14 women) with a mean age of 20.9 years (SD = 1.6; age range = 18–24). The non-intervention group consisted of 20 subjects (15 men, 5 women) with a mean age of 21.4 years (SD = 2.2; age range = 18–26). Among the 81 subjects included in the longitudinal intervention experiment, the remaining 20 subjects were assigned to a second intervention group (multitasking training group) which is irrelevant to the purpose of this study. Details of this intervention have been described elsewhere (Takeuchi et al., 2014b).

The WMT and control groups were initially similar ($P > 0.1$, two-tailed t-tests) in terms of age, sex, general intelligence scores, WM measures (visuospatial WM and digit span tasks) that were used as outcome measures (Takeuchi et al., 2013b), or mood measures used in this study. Subjects who could not be followed throughout the study because of illness (one subject in the control group) or who failed to submit the required data [one subject in the WMT group did not submit the postintervention Profile of Mood States (POMS) data] were excluded from the relevant analyses. For the description of the subjects flow in the present longitudinal experiment of 81 subjects, please see the figure from our previous report (Takeuchi et al., 2014b).

Subjects' inclusion and exclusion criteria

The study participants were college students at Tohoku University or graduates from the university in the previous year, who were recruited with advertisements posted on bulletin boards or via email, both of which specified the inclusion and exclusion criteria for participation in the study. The exclusion criteria included left-handedness, implanted metal devices, metal around the body, claustrophobia, the use of certain drugs, a history of psychiatric or neurological diseases, not having a laptop personal computer running Windows (Microsoft, USA), and previous participation in related experiments. We provided questionnaires to all potential participants to assess for psychiatric illnesses and to request disclosure of recent drug use. None of the patients reported a history of neurological or psychiatric illness. These questionnaires were administered during the pre-experiment explanation and based on voluntary self-report. Handedness was evaluated using the Edinburgh Handedness Inventory (Oldfield, 1971). All the subjects had normal vision.

Group assignment of participants and experimental periods

Group assignments were performed in a non-arbitrary manner. As described in our previous study (Takeuchi et al., 2013b), groups of participants completed the preintervention- and postinterevention-MRI studies and psychological experiments during different predetermined experimental periods (for example, one group participated in the periods starting 4 weeks from November 4th, another group participated in the periods starting 4 weeks from November 10th, and so on). Participants in one predetermined experimental period were randomly assigned to one of the two groups (e.g., the WMT group or the non-intervention control group). The two groups assigned were different for each predetermined experimental period. This means, for example, that participants in the 4-week periods starting from November 4th and November 25th were assigned to the WMT group or the non-intervention control group, but participants in the 4-week periods starting from November 10th and September 2nd were assigned to the MT training group or the non-intervention control group, yet participants in the 4-week periods starting from November 16th and September 10th were assigned to the WMT group or the MT training group. The participants selected the period they wished to participate in and were not notified that there were two intervention groups before the experiment. Which periods have which two groups are ordered in non-specific manner. Thus, basically, subjects were supposed to be assigned to one of three groups without any bias in a non-arbitrary manner.

The purpose of this study and those of other studies using the data of WMT groups in this study

In this experiment, we aimed to investigate a wide range of distinct research topics, including the effects of polymorphism on cognitive training. We previously investigated the effects of WMT on resting-state FC, regional cerebral blood flow at rest (resting-rCBF), and gray matter structure as well as performance on cognitive tests (Takeuchi et al., 2013b), none of which overlap the measures investigated in the present study. Given these diverse aims and the possible infinite sources of variation, we focused on the effect of WMT on emotional states. More subjects were included in the WMT group to facilitate analysis of polymorphism in WMT, as described above.

Ethical issues

Written informed consent was obtained from each individual for the projects in which they participated. The Ethics Committee of Tohoku University approved all procedures.

PROCEDURE

General procedures of WMT

The WMT task included four computerized tasks that were Borland C++ programs developed in-house. Subjects trained in the task 20–60 min a day for 27 days. We chose a 4-week intervention period because the duration of WMT programs is often 4–5 weeks (Takeuchi et al., 2010b). However, the total training time depended on the level and the time between trials. The length of each training period varied because we did not limit training by time to prevent subjects from finishing the training without completing the tasks properly. Instead, task completion was based on the number of correctly completed trials (in other words, we controlled the length of the tasks based on how many trials each subject completed the tasks correctly). Thus, subjects could not finish the tasks without completing them correctly no matter how much time they spent on them. This means that if the subject's condition was poor, they took longer to complete the training of the day. However, heterogeneity in the amount of overall training among subjects was unlikely because when the subjects recovered

the following day they would be able to finish the tasks more quickly (because the level of the tasks would have dropped when the subject's condition was poor). Subjects performed the tasks on their personal computers and were advised to perform the WMT task daily, with two training sessions a week conducted in the laboratory. A log that recorded task performance and the time when subjects completed each trial was used to determine the date and duration of training sessions. Further details can be found in our previous study (Takeuchi et al., 2013b). For the reasons why we used the non-intervention group as a control group, and possible consideration of placebo effects, please see Supplemental Discussion.

Monetary reward

Subjects including those of the control group, received a monetary reward based on the extent to which they participated in the experiments. For training period, to strictly control the amount of training and to motivate subjects to undertake regular training, (a) the subjects received a monetary reward for the training part of the experiment on the basis of the number of sessions they completed; however, (b) they were not given a monetary reward for excessive training (>27 sessions), and (c) the maximum amount of monetary reward was received when one training session was completed each day, even if 27 training sessions were completed. If multiple training sessions were performed in a day, the monetary reward for any second and subsequent training sessions was reduced. For the consideration effects of monetary rewards on moods, see Supplemental Discussion. Basically we failed to find such effects as monetary reward inducement of mood change after the intervention period in this kind of experiment.

TRAINING TASKS

The WMT task is described in our previous report (Takeuchi et al., 2013b). In brief, four WMT tasks were presented during a single training session. In each task, the difficulty (number of items to be remembered) level was modulated on the basis of the subject's performance. Four training tasks were used because increasing the task variability, stimuli, and training situations leads to a more successful transfer (Sweller et al., 1998; Yamnill and McLean, 2001; Green and Bavelier, 2008). The four WMT tasks were as follows: (a) a visuospatial WM task, (b) an auditory backward operation span task, (c) a dual WM task, and (d) a dual N-back task. Additional details are presented in Supplemental Methods. Note that the visuospatial WM task for WMT was different from that used as the outcome measure (Takeuchi et al., 2013b).

We quantified the subjects' performance on all trained WM tasks to analyze their training-related changes in the WM tasks (please see our previous study for details; Takeuchi et al., 2013b). The difference between the composite scores for the first three sessions and the last three sessions was represented as performance changes in the WMT tasks. This method was used in previous studies (Takeuchi et al., 2010a, 2013b, 2014a,b) because it provides a stable performance measure for multiple tasks across three training days.

PSYCHOLOGICAL OUTCOME MEASURES

Neuropsychological tests and questionnaires were administered for preintervention and postintervention evaluation.

POMS

The shortened Japanese version (Yokoyama, 2005) of the POMS (McNair et al., 1992) questionnaire was used to measure a participant's mood on the day of the experiment as well as in the preceding week. The POMS questionnaire consists of six individual subscales: tension/anxiety, depression/dejection, anger/hostility, vigor/activity, fatigue/inertia, and confusion/bewilderment. We used the POMS subscale score for the preceding week to investigate whether WMT improved emotional states. Since its release, POMS has proven to be an excellent measure of fluctuations in affective mood states across a broad population, including psychiatric outpatients, medical patients, and sports subjects (McNair et al., 1992). POMS identifies and assesses transient, fluctuating affective mood states (McNair et al., 1992). It can be administered on a weekly basis, which is a sufficient period for detecting the respondent's mood responses to his or her current life situation but still short enough to assess acute treatment effects (McNair et al., 1992).

State anger scale of the state–trait anger expression inventory

The State Anger scale of the State–Trait Anger Expression Inventory (Spielberger et al., 1999) was used to measure anger. The State Anger scale measures the subjects' mood at the time of the test. These questionnaires can be used to measure the mood of healthy subjects.

Other outcome measures

Several questionnaires designed to assess traits or states related to longer time periods were collected but are not described here. For other performance-type psychological cognitive tests used in this experiment, please see our previous study (Takeuchi et al., 2013b).

FACE-MATCHING TASK (fMRI TASK)

Task procedures

Subjects in the WMT and control groups participated in the fMRI session before and after completing the intervention period. During the fMRI session, subjects performed five blocks of the face-matching task that were alternated with six blocks of a control-matching task involving simple geometric shapes (there was no rest period between blocks). The face-matching task was used to map training-induced changes in negative emotion-related brain activity (Hariri et al., 2002). In each trial, an image of a face portraying anger or fear was presented at the top of a computer screen and was presented at the bottom left and right sides of the screen simultaneously with two additional images of the same face. One of the two bottom images was a face portraying anger, whereas the other face portrayed fear. Subjects were instructed to select one of the faces presented at the bottom of the screen that was identical to the target face (top). Images were presented sequentially per block. Images represented the target affect (angry or fear) and were derived from a standard set of pictures showing facial effects (Kamachi, 2001). In the control-matching task, the faces were replaced with simple geometric shapes, and

the subject was instructed to identify the two matching shapes. The subject had to push a button with the first or second finger if he/she selected the bottom left or right shape, respectively.

Four sets of stimuli, each with a duration of 4.0625 s, were presented in a block, with each block lasting 16.25 s Behavioral performance was recorded as the subject's accuracy and reaction time. For a schematic of the task, please refer to the study by Hariri et al. (2002).

Development of stimuli in the previous study

The technical details of how the standard set of pictures of facial effects (Kamachi, 2001) were developed are presented in Kamachi (2001) and Tamamiya and Hiraki (2013). As described in a previous study (Tamamiya and Hiraki, 2013), the database contains 4 females and 6 males and 3 pictures of each model displaying each facial expression. The database also contained the results of a preliminary experiment in which 27 young adults evaluated the intensity of facial expressions and classified the expressions into the following seven categories: happiness, sadness, surprise, anger, disgust, fear, and contempt. The results from that study were used to validate each facial expression.

Rationale for fMRI task paradigm

The behavioral task used in this study has been widely used to detect brain activity related to negative emotions, and it has become the gold standard for tasks that have this purpose. Increased activation in the relevant brain areas when viewing angry or fearful faces indicates the induction of negative emotions during the task (Hariri et al., 2002; Meyer-Lindenberg et al., 2006). These changes simply occurred when subjects viewed the faces without having to identify the emotions they portrayed; thus, this is an implicit task. The emotion face task differs from the present control task in the complexity of the stimuli; however, there are no reasons to assume that such differences contribute to brain activity. The reason for using geometrical shapes in the control task was not provided in its original description. We speculate that the aim of this task is to detect brain activity in emotion-related areas; however, how the activity was induced and what factors induced the activity may not be relevant. The same principle applies to the present study.

Other types of negative emotions include sadness or disgust, and faces showing these emotions could be used as stimuli. We only focused on anger and fear because of time limitations, and we chose to use the same procedures consistently among studies (Hariri et al., 2002; Meyer-Lindenberg et al., 2006). For further explanations of why we used this task and not a task for assessing emotional regulation, please see Supplemental Discussion.

IMAGE ACQUISITION AND ANALYSIS

MRI data acquisition was conducted using a 3-T Philips Achieva scanner. Forty-two transaxial gradient-echo images ($TE = 30$ ms, $FA = 90°$, slice thickness = 3 mm, $FOV = 192$ mm, matrix $= 64 \times 64$) covering the entire brain were acquired at a repetition time of 2.5 s using an echo planar sequence. For the session for negative emotion-related brain activities, 73 functional volumes were obtained. Diffusion-weighted data were acquired using a spin-echo EPI sequence ($TR = 10293$ ms, $TE = 55$ ms, $FOV = 22.4$ cm, $2 \times 2 \times 2$ mm^3 voxels, 60 slices, SENSE reduction factor = 2, number of acquisitions = 1). The diffusion weighting was isotropically distributed along 32 directions (b-value = 1,000 s/mm^2). Using a spin-echo EPI sequence ($TR = 10293$ ms, $TE = 55$ ms, $FOV = 22.4$ cm, $2 \times 2 \times 2$ mm^3 voxels, 60 slices), three images with no diffusion weighting (b-value = 0 s/mm^2) ($b = 0$ images) were acquired from 52 subjects and one $b = 0$ image was acquired from nine subjects. From the collected images, FA and ADC maps were calculated (Takeuchi et al., 2011c). These calculated FA and ADC images were used for preprocessing. Other imaging data included scans obtained with fMRI during WM, scans for resting state functional connectivity (FC), arterial spin labeling, and T1-weighted structural images, all of which were used in our previous study of WMT (Takeuchi et al., 2013b).

PREPROCESSING OF FUNCTIONAL ACTIVATION DATA

Preprocessing and analysis of functional activation data were performed using SPM8 implemented in Matlab. Before their analysis, BOLD images from the preintervention and postintervention scans were realigned and resliced to the mean image of the BOLD images from the preintervention scans. For each subject, the skull and skin appearing in the mean BOLD images and $b = 0$ images were removed through intensity thresholding of the spatially smoothed images, as described previously (Takeuchi et al., 2011b). All BOLD images of each subject were coregistered to a skull/skin-stripped $b = 0$ image using the skull/skin-stripped mean BOLD image. Because the $b = 0$ image was aligned with the FA image and ADC map, the BOLD image, $b = 0$ image, FA image, and ADC map were all aligned.

All images were subsequently normalized using a previously validated two-step new segmentation algorithm of diffusion images and the previously validated diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL)-based registration process (Takeuchi et al., 2013a). The voxel size of normalized BOLD images was $3 \times 3 \times 3$ mm^3.

For additional details on these normalization procedures and their validity, please refer to our previous study (Takeuchi et al., 2013a). In brief, we used these normalization procedures instead of coregistering BOLD and T1-weighted structural images (followed by normalization of the T1-weighted structural image) to ensure precise normalization. Because of the distortion caused by 3-T MRI, the brain's shape in BOLD and T1-weighted structural images can differ, preventing a precise normalization.

INDIVIDUAL-LEVEL STATISTICAL ANALYSIS OF FUNCTIONAL IMAGING DATA

A design matrix was fitted to each participant with one regressor for each of the face-matching task conditions (when compared with controlled task conditions) in the preintervention and postintervention scans using the standard hemodynamic response function (HRF). The design matrix weighted each raw image according to its overall variability to reduce the impact of movement artifacts (Diedrichsen and Shadmehr, 2005). Six parameters obtained by rigid body correction of head motion were regressed out using these variances with the regressor. We removed low-frequency fluctuations with a high-pass filter using

a cut-off value of 128 s. The individual-level statistical analyses were performed using a general linear model. After estimation, beta images were smoothed (6-mm full-width half-maximum) and taken to the second level or subjected to random-effect analysis.

In individual analyses, we examined changes in activation related to negative emotion (the face-matching task vs. the control-matching task) before and after a 4-week intervention period. The resulting maps for each participant represented changes in brain activity during the face-matching task condition between the preintervention and postintervention periods as well as the preintervention brain activity during the corresponding condition. The resulting data were forwarded to group analysis.

The present fMRI tasks did not include resting states. Thus, we were not able to determine whether neural activity was affected by interactions among training effects (preintervention, postintervention), task (emotional task, control task), or group (WMT, control group). Even if we included the resting state, fMRI paradigms for comparing individual differences, group differences, and training effects have resting-state differences in neural activity and suffer from the same limitations. Therefore, we used the gold standard procedure for this fMRI task and removed the resting states (Hariri et al., 2002). Furthermore, because repeated exposure to the same tasks alone greatly alters fMRI responses (Luauté et al., 2009), changes in the control group should be considered as a baseline in this type of experiment.

STATISTICAL GROUP-COMPARISON ANALYSIS OF PSYCHOLOGICAL DATA

Behavioral data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL). Because training-related improvements were our primary interest and the basis of our hypothesis, we compared test–retest changes in the WMT group with those in the control group using one-tailed One-Way analyses of covariance (ANCOVA), which is the analysis method used in previous studies of WMT (Klingberg et al., 2002, 2005; Takeuchi et al., 2011d, 2013b). The difference between preintervention and postintervention measures was used as the dependent variable, preintervention scores were the independent variable, and group (WMT or control) was the fixed factor ($P < 0.05$). We used ANCOVAs instead of repeated-measure ANOVAs to control for the effects of preintervention test scores. Statistical experts strongly recommend using ANCOVA instead of repeated-measure ANOVA in this type of study design (Dimitrov et al., 2003). With randomized designs, ANCOVA can reduce error variance, whereas with nonrandomized designs (or with analyses involving substantial preexisting group differences), ANCOVA can adjust the postintervention test means for preintervention test differences among groups (Dimitrov et al., 2003). One may recommend using postintervention test scores instead of differences between preintervention test and postintervention test measures. However, when the preintervention test scores are included as covariates, the two analyses return the same statistical value. Using two-tailed tests was not appropriate in this context because statistical tests should be performed against the hypotheses tested. In this study, the hypotheses did not involve a training-related reduction in mood states.

The behavioral data (accuracy and reaction time) recorded in the face-matching and control-matching tasks were analyzed with the same design but with two-tailed analyses because we did not expect changes in these measures (see Results for details).

STATISTICAL GROUP-LEVEL ANALYSIS OF IMAGING DATA

In the group-level imaging analysis, we tested for group-wise differences in functional activity changes across the entire brain during the emotional face-matching task (compared with the control-matching task). We performed voxel-wise ANCOVAs with the difference in each measure between preintervention scans and postintervention scans at each voxel as the dependent variable and the preintervention scan value at each voxel as the independent variable. Biological Parametrical Mapping (BPM) (Casanova et al., 2007) implemented in SPM5 made it possible for us to use these voxel-wise ANCOVAs by including images representing regional values as covariates. We used SPM5 because BPM was not designed and it has not been thoroughly tested against SPM8. Analysis was performed using SPM5 and images representing preintervention to postintervention changes in functional activity during the emotional face-matching task and functional activity during the emotional face-matching task in the preintervention scan. One may think that the difference in the number of subjects in each group may lead to significant differences in measures of preintervention to postintevention activation changes. However, because statistical analyses tested for differences in changes between groups and not within groups (followed by assertions that significant differences were observed in only one group but not in the other group), this was not possible.

Regions with significance were inferred using family-wise error-based cluster-level statistics (Friston et al., 1996). Only clusters with $P < 0.05$ after correction for multiple comparisons at cluster size with a voxel-level cluster-determining threshold of $P < 0.005$ (uncorrected) were considered statistically significant. This voxel-level cluster-determining threshold has been used in previous studies (Takeuchi et al., 2010a, 2011b), and the validation study (Hayasaka and Nichols, 2003) showed that this threshold does not cause anticonservativeness. If anything, it appears to lead to more conservative results compared with more stringent voxel-level cluster-determining thresholds.

We also performed ANCOVA (two-tailed) to compare group differences between the mean beta estimates of preintervention to postintervention changes in functional activity with the mean beta estimates of preintervention functional activity in clusters with significant WMT-related changes in whole brain analysis, preintervention difference of behavioral data (reaction time, accuracy) between the face emotion task and the control-matching task, as well as pre to post changes in difference of behavioral data (reaction time, accuracy) between the face emotion task and the control-matching task.

CORRELATIONS BETWEEN BEHAVIORAL DATA AND FUNCTIONAL ACTIVITY IN SIGNIFICANT CLUSTERS

Furthermore, to reveal the nature of functional activity in areas with significant WMT-related changes, we investigated the

association between functional activity and psychological variables using the preintervention data and multiple regression analyses. The dependent variables were the mean beta estimates of functional activity in the clusters with significant WMT-related changes identified in whole brain analysis described above. Independent variables were age, sex, and each psychological variable (scores of measures of emotional states). Data from 95 subjects were included in this analysis. Among the initial 97 subjects considered for the study (see Subjects subsection), data for one subject were not available because of a metal-related problem (Takeuchi et al., 2014b). The data from another subject were removed because the fMRI data were unsuitable. This reduced the number of subjects to 95. We added the data from the additional 16 subjects who only participated in the cross-sectional experiment because they had completed the same protocols for the preintervention period as the subjects in the present study, which included fMRI scans for negative emotion. Excluding these subjects was statistically harmful because it would have reduced the statistical power. For cross-sectional analysis of the brain images, the number of subjects analyzed to determine the effects of WMT (approximately 60 subjects: 41 subjects in the WMT group and 20 subjects in the control group) was quite small; therefore, additional subjects were included in this analysis.

Finally, we investigated whether training-related variables (changes in the composite score of the four WMT tasks), preintervention to postintervention emotional state changes, and preintervention to postintervention functional activity changes in the clusters with significant WMT-related changes identified above were related.

RESULTS

TRAINING DATA

As described in our previous study, to investigate the effect of WMT on resting-state FC, resting-rCBF, and regional gray matter volume using data from the same subjects (Takeuchi et al., 2013b), subjects in the WMT group completed on average 25.87 sessions ($SD = 2.18$) and at least 17 sessions during the 27-day intervention period. The performance on all four trained WM tasks during the last three training sessions was significantly improved compared with that during the first three training sessions (paired t-test, $P < 0.001$). Details of the training data and training-related changes in performance scores on cognitive tests (such as WM tasks) have been described previously (Takeuchi et al., 2013b). WMT task performance significantly improved from the first three training days to the last three training days for all tasks (Takeuchi et al., 2013b). The training-related performance change presented in the previous study is illustrated in Supplemental Table 1. Please note that these behavioral data apply to the training tasks performed during the training period, and no data are available for the control group. Compared with the control group, performance on the untrained verbal and visual WM tasks conducted on the day of the MRI sessions in the WMT significantly improved from preintervention to postintervention, as described previously (Takeuchi et al., 2013b).

THE EFFECT OF WMT ON EMOTIONAL STATES (MAIN PSYCHOLOGICAL ANALYSES OF THIS STUDY)

Compared with the control group, the WMT group showed significantly greater preintervention to postintervention reductions in POMS subscale scores for anger/hostility, depression/dejection, and fatigue/inertia but not for the tension/anxiety, vigor/activity, and confusion/bewilderment subscales. Compared with the control group, the WMT group also showed significantly greater preintervention to postintervention reductions for the STAXI score. The results for the psychological scales are shown in **Table 1**. In these analyses, the statistical values were not corrected for multiple comparisons, which was the case in some previous studies (Klingberg et al., 2002, 2005). Nevertheless, even when these values were corrected using the false discovery rate (Benjamini and Hochberg, 1995), the effects of WMT on the POMS anger/hostility subscale and the STAXI State Anger scale remained significant ($P < 0.05$, corrected). There were also tendencies toward WMT effects ($P < 0.1$) on the

Table 1 | Pretest and posttest scores for psychological measures (mean ± s.e.m.).

	WMT		Control (non-intervention)		Planned contrast	*P*-value[c]
	Pre	Post	Pre	Post		
POMS[a] –tension–anxiety	6.15 ± 4.66	6.55 ± 5.47	6.00 ± 4.03	6.95 ± 3.99	WMT < control	0.322
POMS—depression–dejection	4.50 ± 4.77	3.63 ± 4.39	3.47 ± 3.89	4.95 ± 4.03	WMT < control	0.048*
POMS—anger–hostility	3.73 ± 4.26	3.35 ± 3.85	2.63 ± 2.67	4.79 ± 4.08	WMT < control	0.009**
POMS—vigor–activity	8.28 ± 3.78	7.8 ± 4.54	9.42 ± 3.01	9.11 ± 3.05	WMT > control	0.740
POMS—fatigue–inertia	6.63 ± 4.66	6.18 ± 4.71	7.63 ± 4.34	8.53 ± 4.14	WMT < control	0.050*
POMS—confusion–bewilderment	4.85 ± 3.71	4.93 ± 3.66	4.37 ± 3.52	5.42 ± 3.85	WMT < control	0.219
STAXI[b]—state–anger	11.8 ± 3.7	11.1 ± 3.1	11.3 ± 3.3	12.9 ± 3.2	WMT < control	0.007**

[a] *Profile of Mood States.*

[b] *State–Trait Anger Expression Inventory.*

[c] *One-Way analyses of covariance with test–retest differences in psychological measures as dependent variables and pretest scores of the psychological measures as covariates.*

*$P < 0.05$.

**$P < 0.01$.

POMS depression/dejection and fatigue/inertia subscales, which is congruent with our hypothesis.

Comparisons with a control group are crucial in this type of intervention study. In the present study, average changes in mood scores were observed in the control group. In particular, the score of the POMS anger/hostility subscale significantly increased in the control group ($P = 0.044$, two-tailed paired-t). However, previous studies have shown that mood worsens in the season in which this experiment was performed. Therefore, the changes observed in the control groups may be expected without any intervention or experimental effects. For more details related to this issue, please see Discussion.

THE EFFECT OF WMT ON NEGATIVE EMOTION-RELATED BRAIN ACTIVITIES (MAIN NEUROIMAGING ANALYSES OF THIS STUDY)

We compared changes in negative emotion-related brain activity in the WMT and control groups. This analysis revealed a statistically significant decrease from preintervention measures to postintervention measures in negative emotion-related activities in an anatomical cluster spread around the left posterior insula (**Table 2**; **Figure 2**) and in the anatomical cluster extending from the inferior parietal lobule to the premotor area (**Table 2**; **Figure 3A**). The latter cluster slightly extended into Brodmann's area 8, which includes a small portion of DLPFC. The significance of these results was not affected when any preexisting or postintervention group differences in behavioral performance of fMRI tasks were accounted for.

To determine whether the training-related changes in activity were lateralized to the left hemisphere, we investigated the results using a lenient threshold (uncorrected $P < 0.05$). Please note that we are not making any conclusions about significant results for the right hemisphere using this second threshold; we are merely showing that laterality of the results is unlikely and that no such tendency was observed. This analysis revealed widespread clusters in the frontoparietal area bilaterally and a posterior insula cluster that included the abovementioned significant areas (**Figure 3B**). These three clusters were significant at $P < 0.05$ after correcting for multiple comparisons at the cluster level with a cluster-determining threshold of $P < 0.05$, uncorrected. Thus, the results may not necessarily be lateralized to the left side.

The *P*-values for comparisons between groups with ANCOVA (two-tailed) for the mean beta estimates for changes in functional activity from preintervention to postintervention with the mean beta estimates of preintervention functional activity in the significant clusters presented in **Figures 2**, **3A** were 0.0001 and 0.00003, respectively. We next performed ANCOVA (two-tailed) that added the following variables (a–d) as covariates using the mean values of each cluster with significant WMT-related changes: (a) accuracy of preintervention face-matching task—accuracy of preintervention control-matching task; (b) [accuracy of postintervention face-matching task—accuracy of postintervention control-matching task]—[accuracy of preintervention face-matching task—accuracy of preintervention control-matching task]; (c) reaction time of preintervention face-matching task—reaction time of preintervention control-matching task; and (d) [reaction time of postintervention face-matching task—reaction time of postintervention control-matching task]—[reaction time of preintervention face-matching task—reaction time of preintervention control-matching task]. *P*-values from ANCOVAs for the clusters presented in **Figures 2**, **3A** were 0.0001 and 0.00002, respectively. These ANCOVA comparisons showed that preexisting group differences in reaction

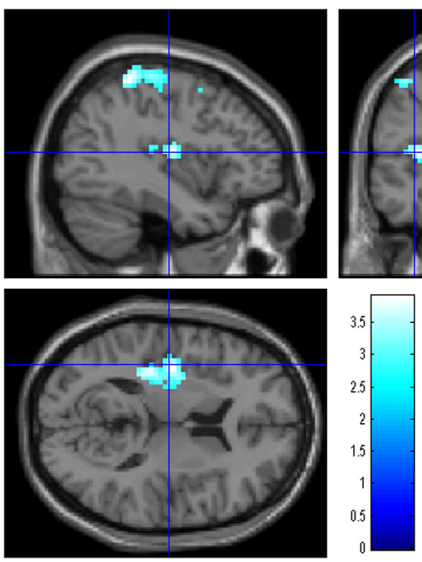

FIGURE 2 | The effect of WMT on negative emotion-related brain activity in the left posterior insula. Results are shown with the threshold of $P < 0.05$, corrected for multiple comparisons at the cluster-level with an underlying voxel-level of $P < 0.005$. Findings were overlaid on a "single-subject T1" SPM5 image. Blue represents the T score. Compared with the control intervention (non-intervention), WMT resulted in a significant decrease in functional activity in a cluster spread around the left posterior insula.

Table 2 | Activity related to WMT and negative emotion decreases compared with the control group.

Area		MNI coordinates of the peak value			T score of the peak value	Corrected *P*-value (cluster)
		x	*y*	*z*		
Posterior insula	L	−39	−15	12	3.91	0.031
Inferior parietal lobule/premotor area/precentral gyrus/postcentral gyrus	L	−39	−42	60	3.79	<0.001

time and accuracy as well as in changes to these behavioral measures did not affect group differences in preintervention to postintervention changes in functional activity for these clusters. We performed these comparisons because covariates (a–d) could not be added to the voxel-by-voxel whole brain analyses because of technical errors in the procedures.

BEHAVIORAL DATA FROM THE fMRI TASKS

The fMRI tasks used in the present study were not cognitively demanding. Therefore, these values were not expected to change, and there is no prior knowledge or theory that suggests individual differences of behavioral performance of this task are even remotely associated with emotion-related cognition. However, preintervetion and postintervention behavioral data for the fMRI tasks are provided in **Table 3**. ANCOVAs with preintervention to postintervention performance changes in each behavioral data set were used as dependent variables that corresponded to the preintervention performance data as covariates. The group difference as a fixed factor revealed no significant WMT-related effects on either accuracy or reaction time during the tasks.

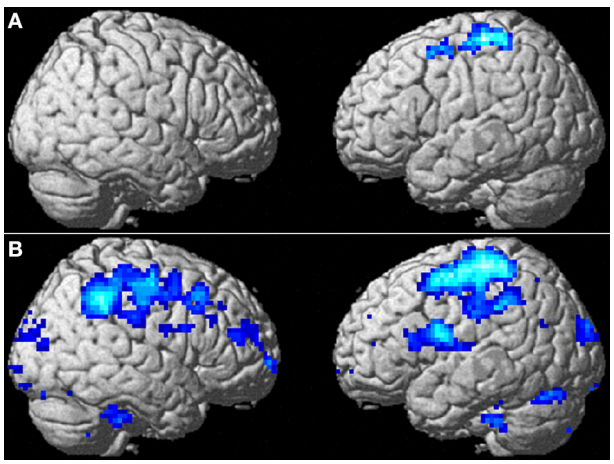

FIGURE 3 | The effect of WMT on negative emotion-related brain activity in the lateral part of the brain. (A) Significant results are $P < 0.05$, corrected for multiple comparisons at the cluster level with an underlying voxel level of $P < 0.005$. Blue represents the T score. Compared with the control intervention (non-intervention), WMT resulted in a significant decrease in functional activity in a cluster spread around the left frontoparietal area. **(B)** Tendencies are $P < 0.05$, uncorrected. Compared with the control intervention (non-intervention), WMT resulted in a tendency toward a decrease in functional activity in a cluster spread bilaterally around the extensive frontoparietal areas. Please note that we are not making any conclusions about significant results in the right hemisphere using this second threshold; we are merely showing that laterality of results is unlikely and that no such tendency was observed.

CORRELATION BETWEEN RELATED PSYCHOLOGICAL VARIABLES AND FUNCTIONAL ACTIVITY IN SIGNIFICANT CLUSTERS BEFORE THE INTERVENTION

To reveal the nature of the functional activity in areas with significant WMT-related changes, we used multiple regression analyses and region of interest (ROI) analysis to investigate the associations between functional activity and psychological variables using data from the preintervention session. In these analyses, the dependent variables were the mean beta estimates of functional activity within the significant clusters presented in **Figures 2**, **3A**, while the independent variables were age, sex, and the individual psychological variables. In particular, we tested whether activity in the posterior insula was associated with the state of anger. We examined data from the 95 subjects (see Subjects subsection and correlations between behavioral data and functional activity in significant clusters subsection of Methods).

As expected, activity in the left posterior insula cluster was significantly and positively correlated with the POMS anger/hostility subscale score ($P = 0.019$, $t = 2.38$; effect size $r = 0.228$, **Figure 4**); however, no significant associations were observed with the STAXI State Anger scale score; there was no evidence of outliers in the scatterplot. This correlation remained significant after excluding the 16 abovementioned subjects who only took part in the cross-sectional experiment ($P = 0.039$). The difference could be due to the higher sensitivity for POMS. No other psychological variables, including each POMS subscale score, showed significant correlations with activity. In addition, no significant correlations were observed when the dependent variable was activity in the left frontoparietal cluster.

The present analyses may appear to be slightly circular in nature because the mean values of clusters with significant WMT effects were used (please note that the same problems occur

Table 3 | Preintervention and postintervention behavioral data for the fMRI tasks (mean ± s.e.m.).

	WMT		Control (non-intervention)		Planned contrast	*P*-value[c]
	Pre	Post	Pre	Post		
Control task RT[a] (ms)	7767 ± 1294	7872 ± 1337	8299 ± 884	7905 ± 675	Two-tailed[b]	0.087
Emotional face task RT (ms)	12366 ± 2595	12779 ± 2927	12406 ± 1991	12632 ± 1518	Two-tailed[b]	0.754
Control task accuracy (%)	96.5 ± 3.9	96.8 ± 3.0	98.7 ± 2.0	97.6 ± 3.2	Two-tailed[b]	0.640
Emotional face task accuracy (%)	95.1 ± 6.7	94.3 ± 5.7	96.6 ± 3.7	95.8 ± 5.1	Two-tailed[b]	0.471

[a] *Reaction time.*

[b] *These tasks are not something one has to perform as much as he or she can; therefore, no training-related improvements in these behavioral data have been assumed.*

[c] *One-Way analyses of covariance with test–retest differences in psychological measures as dependent variables and preinterveniton scores of the psychological measures as covariates.*

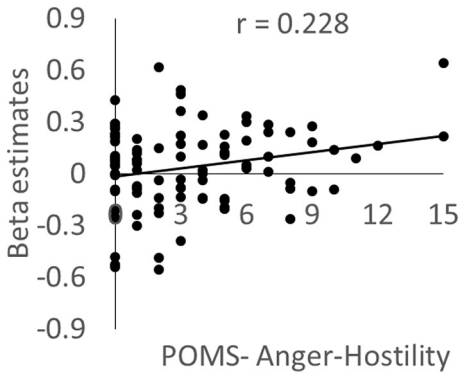

FIGURE 4 | The association between brain activity related to negative emotions and anger mood state. Scatterplot showing the relationship between the POMS Anger–Hostility subscale score and the mean value for brain activity during the preintervention period in a cluster in the left posterior insula in which a significant WMT effect was observed.

even when the peak values of clusters with WMT effects are used). However, even when the effects of group assignments were regressed out of our multiple regression analyses by creating and including a covariate of group assignment (WMT group $= -1$, control group $= +1$, other subjects $= 0$), the significance of the correlation between activity in the cluster in the left posterior insula and the POMS anger/hostility subscale score did not change ($P = 0.030$, $t = 2.20$). This shows that the circular nature of the present analysis does not undermine the finding of an association between anger and brain activity in the anterior insula. These results indicate that the increased activity observed during the face-matching task in fMRI was associated with the state of anger.

CORRELATIONS BETWEEN CHANGES IN RELATED PSYCHOLOGICAL VARIABLES AND CHANGES IN FUNCTIONAL ACTIVITY IN SIGNIFICANT CLUSTERS

Following this, we investigated whether training-related variables (changes in the composite score for the four WMT tasks), emotional state changes, and functional activity changes in two of the significant clusters identified above were related. For this analysis, we performed simple correlational analyses between (a) each functional activity change in the two significant clusters and changes in each score of emotional state measures; (b) each functional activity change in the two significant clusters and changes in the composite score for the WMT tasks; and (c) changes in each score of emotional state measures and changes in the composite score for the WMT tasks among subjects in the WMT group. We found no significant correlations; however, the changes in functional activity in the two significant clusters were highly and positively correlated ($P < 0.001$).

There could be a number of reasons for this lack of correlation. One possible reason is insufficient statistical power because we could only analyze data from subjects in the WMT group in this analysis. On the other hand, large individual differences were observed in the control group for emotional state changes, indicating that much of the variance in emotional state changes in WMT group was also unrelated to WMT. In addition, this experiment was not designed for this type of correlation analysis. Although the amount of training is correlated with training-related changes in neural mechanisms (Takeuchi et al., 2010a), we strictly controlled the amount of training performed by subjects in the WMT group (for the actual SD of the number of training sessions, please see Training data subsection of Results). Therefore, little variance was observed among subjects in this respect. To perform these types of correlation analyses successfully, we should not have controlled the amount of training so strictly. Finally, although WMT is effective in decreasing some negative emotional states, WMT is apparently a cognitively demanding and tiring task. Thus, moderate WMT may be effective for improving mood; however, subjects who exert more effort during training may experience an increase in negative feelings during training and consequently have higher scores for negative moods. This may complicate the association between training and mood changes and negate the correlations among WMT-related changes.

DISCUSSION

In the present study, we investigated whether WMT affected the emotional states and corresponding brain activity in healthy young adults. We observed that individuals who underwent WMT showed decreases in anger, depression, and fatigue as well as decreases in negative emotion-related brain activity in the left posterior insula and in an anatomical cluster near the left frontoparietal area. As discussed below, WMT may reduce anger by increasing the ability to cognitively manage emotional stimuli. WMT may also reduce depressive and fatigue states through previously reported training-induced increases in DLPFC and premotor cortex functioning. These results cannot be entirely explained by preexisting differences between groups because ANCOVA corrected for any preintervention differences. These effects are unlikely to be explained by unknown effects for the non-intervention group, even though the moods in the non-intervention group tended to worsen from the preintervention to postintervention period, as discussed below. The present findings provide new insight into the broader applications of WMT for psychiatric problems, at least among subjects with preclinical-level conditions.

WMT may lead to reduced anger by facilitating cognitive control of situations arousing emotion. Although WMC is not directly related to anger (Hofmann et al., 2008), mental health problems often occur with impaired WMC. For example, substance abuse leads to depression, decreased anger control, suicidal tendencies, and WM deficits (Hoffman et al., 2006). Subjects with a high-functioning WMC are less likely to respond automatically in many situations, including those that provoke emotion (Hofmann et al., 2008). Moreover, they are more likely to correct misapprehensions that lead to anger (Cummins, 2005) and appraise emotional stimuli in an unemotional manner. Thus, they are able to experience and express less emotion in response to such stimuli (Schmeichel et al., 2008). Training-related increases in WMC may lead to decreased anger expression through these mechanisms. Anger and WMC were not correlated despite these previous studies as well as the present study, which may be

because anger can lead to arousal and improve performance (Novaco, 1976). Thus, anger and WMC may not reinforce each other through positive feedback.

WMT-related decreases in activity in the frontoparietal region may reflect a decreased cognitive load, which may subsequently allow cognitive management of emotion-inducing stimuli that affect activity in the posterior insula. Numerous studies have shown the posterior insula is associated with pain, anger, and disgust (Calder et al., 2000; Williams et al., 2005; Schultheiss et al., 2008; Paulus et al., 2010). For example, injury to the posterior insula seems to lead to impaired recognition of disgust (Calder et al., 2000). In the present study, subjects performed a face-matching task that involved faces expressing negative emotions, and as they performed the task, activity in the posterior insula was positively correlated with anger/hostility. Thus, the WMT-related decrease in activity in the left posterior insula may reflect a decrease in emotional responses such as anger or other emotions evoked with negative mood-inducing stimuli. On the other hand, the left superior frontal and parietal regions are active during externally directed attention-demanding cognitive tasks (Fox et al., 2005), and this increased activity may generally reflect cognitive load (Takeuchi et al., 2012). Any changes in task-induced activity depends on several factors; and we did not predict the direction of such changes because training-related increases in efficiency (hence an activity decrease) or increase of recruitment of involved areas depend on multiple factors (Erickson et al., 2007). The face-matching task used in the present study appears to have been cognitively demanding because it required additional reaction time. Therefore, the WMT-related decrease in activity in this region may have been related to a decrease in cognitive load during the task. Considering that an increased cognitive capacity allows cognitive management of emotional stimuli, the decreased cognitive load reflected in the frontoparietal area may facilitate cognitive management of emotion-inducing signals that affect posterior insular activity. Alternatively, DLPFC to premotor areas, which are partially included in the frontoparietal area, have been associated with automatic emotion regulation (Mauss et al., 2007). Thus, reduced activity in this area may reflect the load associated with suppression of negative emotion. However, in the present task, the subjects were not required to suppress emotions. Therefore, the nature of task-related activity changes in this area during tasks that induce negative emotion as well as the mechanisms underlying this activity during explicit suppression of emotion remain to be investigated.

It is not clear why after WMT, the frontoparietal area showed activity changes when the extensive DLPFC area that is not overlapped with this area did not. One possibility is a lack of statistical power, considering the right DLPFC showed the same pattern of decreased training-related activity (**Figure 3B**). Further, training-related structural changes of the bilateral DLPFC (Takeuchi et al., 2013b) have been shown, and DLPFC is involved in the voluntary suppression of emotion (Lévesque et al., 2003).

WMT, like other interventions that stimulate DLPFC, may increase the baseline activity of DLPFC to premotor areas and lead to a concomitant reduction in depressive mood. Patients with depression are characterized by hypometabolism in the cortex, particularly in PFC (Fuster, 2006). Loss of initiative and failure to accurately construct thoughts and conversation are characteristics of depression and are underlain by DLPFC dysfunction (Fuster, 2006). TMS applied to DLPFC to premotor areas can decrease depression and increase resting-state activity in DLPFC to premotor areas (Pascual-Leone et al., 1996; Catafau et al., 2001; Knoch et al., 2006; Johnson et al., 2013). Training on complex divided attention speed tasks, which may well recruit DLPFC to premotor areas because divided attention and speed of complex cognitive tasks are both strongly associated with activity of DLPFC to premotor areas (Thomsen et al., 2004; Takeuchi et al., 2012), also prevents depression (Takeuchi and Kawashima, 2012). These findings suggest that strong cortical stimulation, particularly for DLPFC to premotor areas, leads to increased baseline activity in DLPFC to premotor areas and a concomitant reduction in depressive mood, through TMS or cognitive training. However, the possibility of WMT on clinically depressed subjects remains to be clarified. In the case of abovementioned divided attention speed tasks, the training prevents depression, but the effects of the training on subjects who had already developed depression were less clear (Takeuchi and Kawashima, 2012).

WMT may lead to a reduction in fatigue through training-related activity in DLPFC and its related circuits. It was previously shown that WMT can reduce fatigue in patients with multiple sclerosis (Vogt, 2005). Chronic fatigue syndrome is associated with reduced regional gray matter in the bilateral DLPFC (Okada et al., 2004). The fatigue seems to be related to dysfunction in the frontal–subcortical circuits (Okada et al., 2004). Consistent with this notion, lateral PFC lesions lead to apathy (Fuster, 2006). The present results extend the previous finding of WMT-related reductions in fatigue in patients to healthy young adults, and they show that training-related reductions in fatigue can occur without substantial fatigue and cognitive impairments (Takeuchi et al., 2010b). This change may be caused by the previously identified WMT-induced functional and structural changes in DLPFC (Takeuchi et al., 2010b).

Although the mood states in the control group tended to worsen, it is unlikely that the differences between groups in the present study were caused by effects specific to the control group. The group differences in emotional state changes appeared to be mediated by the average aggregation of emotional states in the control group. However, the present experiments were performed in autumn and winter, and a robust increase in negative mood states, as measured by POMS, has been reported during this period (Harris and Dawson-Hughes, 1993). It is also possible that such changes were strengthened because of distressing events at the university that occurred during winter (such as examinations). Even the patterns of mood changes in this study were similar to those observed in the previous study. Although negative moods increased from autumn to winter in both studies, the increases in depression–dejection and anger–hostility were the most robust, and the reduction in positive mood (vigor) was not as clear. Furthermore, we used the non-intervention group as the control, and because of this simple effect of time-related mood changes, this study design was a strength of our study. If certain meaningful active controls were applied, such as videogame playing, we may not have been able to eliminate the possibility of active control group effects on mood states. Because WMT

was limited to less than an hour, it is unlikely that reductions in everyday activities replaced by WMT would have strong effects. Thus, based on the use of a control group in intervention studies and the fact that we used a non-intervention control, it is inappropriate to attribute the group differences in mood change to any specific effect associated with the control group.

There are a few potential pitfalls or limitations to this study. While the state of anger reduction was statistically robust and was confirmed by two different measures, the reduction in fatigue and depression was only marginally significant. These WMT-related reductions in mood are congruent with those observed in previous clinical studies examining WMTs or stimulation applied to DLPFC to premotor areas during cognitive training or TMS; however, these findings warrant further replication.

With regard to the negative findings, no significant WMT-related changes in mood were found for anxiety or other POMS subscales. These findings may be attributed to factors such as a lack of statistical power and the inclusion of outliers. However, anxiety differs from apathy and depression (Fuster, 2006). Psychiatric problems such as anxiety and obsessive–compulsive disorder are associated with hyperactivity in the anterior brain regions (Fuster, 2006; Milad and Rauch, 2007), whereas excessive anxiety may be associated with reduced resting-state brain activity (Gur et al., 1987). In the case of anxiety, these anterior regions include parts of the WM network, such as the dorsal part of the anterior cingulate cortex (Milad et al., 2007). Surgical interventions affecting the dorsal part of the anterior cingulate can attenuate these psychiatric problems, including anxiety (Fuster, 2006; Milad et al., 2007). Functional enhancements in certain parts of the WM network, such as the dorsal part of the anterior cingulate cortex, may not reduce anxiety despite other beneficial effects, e.g., an increased cognitive capacity.

ACKNOWLEDGMENTS

We thank Yuki Yamada for operating the MRI scanner, Haruka Nouchi for conducting the psychological tests, the subjects, and all our other colleagues in IDAC, Tohoku University for their support. This study was supported by JST/RISTEX, JST/CREST, a Grant-in-Aid for Young Scientists (B) (KAKENHI 23700306) and a Grant-in-Aid for Young Scientists (A) (KAKENHI 25700012) from the Ministry of Education, Culture, Sports, Science, and Technology. The authors would like to thank Enago (www.enago.jp) for the English language review.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnsys.2014.00200/abstract

REFERENCES

Apter, A., Van Praag, H., Plutchik, R., Sevy, S., Korn, M., and Brown, S. (1990). Interrelationships among anxiety, aggression, impulsivity, and mood: a serotonergically linked cluster? *Psychiatry Res.* 32, 191–199. doi: 10.1016/0165-1781(90)90086-K

Arent, S., and Landers, M. (2000). The effects of exercise on mood in older adults: a meta-analytic. *J. Ageing Phys. Act.* 8, 407–430.

Bäckman, L., Nyberg, L., Soveri, A., Johansson, J., Andersson, M., Dahlin, E., et al. (2011). Effects of working-memory training on striatal dopamine release. *Science* 333, 718–718. doi: 10.1126/science.1204978

Baddeley, A. (2003). Working memory: looking back and looking forward. *Nat. Rev. Neurosci.* 4, 829–839. doi: 10.1038/nrn1201

Belden, A. C., Luby, J. L., Pagliaccio, D., and Barch, D. M. (2014). Neural activation associated with the cognitive emotion regulation of sadness in healthy children. *Dev. Cogn. Neurosci.* 9, 136–147. doi: 10.1016/j.dcn.2014.02.003

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B (Methodol.)* 57, 289–300.

Brown, A. K., Liu-Ambrose, T., Tate, R., and Lord, S. R. (2009). The effect of group-based exercise on cognitive performance and mood in seniors residing in intermediate care and self-care retirement facilities: a randomised controlled trial. *Br. J. Sports Med.* 43, 608–614. doi: 10.1136/bjsm.2008.049882

Buschkuehl, M., Jaeggi, S. M., and Jonides, J. (2012). Neuronal effects following working memory training. *Dev. Cogn. Neurosci.* 2, S167–S179. doi: 10.1016/j.dcn.2011.10.001

Calder, A. J., Keane, J., Manes, F., Antoun, N., and Young, A. W. (2000). Impaired recognition and experience of disgust following brain injury. *Nat. Neurosci.* 3, 1077–1078. doi: 10.1038/80586

Casanova, R., Srikanth, R., Baer, A., Laurienti, P. J., Burdette, J. H., Hayasaka, S., et al. (2007). Biological parametric mapping: a statistical toolbox for multimodality brain image analysis. *Neuroimage* 34, 137–143. doi: 10.1016/j.neuroimage.2006.09.011

Catafau, A. M., Perez, V., Gironell, A., Martin, J. C., Kulisevsky, J., Estorch, M., et al. (2001). SPECT mapping of cerebral activity changes induced by repetitive transcranial magnetic stimulation in depressed patients. A pilot study. *Psychiatry Res.* 106, 151–160. doi: 10.1016/S0925-4927(01)00079-8

Chaudhuri, A., and Behan, P. O. (2004). Fatigue in neurological disorders. *Lancet* 363, 978–988. doi: 10.1016/S0140-6736(04)15794-2

Clark, F., Azen, S., Zemke, R., Jackson, J., Carlson, M., Mandel, D., et al. (1997). Occupational therapy for independent-living older adults. A randomized controlled trial. *JAMA* 278, 1321–1326. doi: 10.1001/jama.1997.03550160041036

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 1166–1170. doi: 10.1093/gerona/61.11.1166

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., et al. (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3316–3321. doi: 10.1073/pnas.0400266101

Cummins, P. (2005). "The experience of anger," in *Personal Construct Psychotherapy*, eds D. Winter and L. Viney (London: Whurr), 239–255. doi: 10.1002/9780470713686.ch19

Dahlin, E., Neely, A. S., Larsson, A., Backman, L., and Nyberg, L. (2008). Transfer of learning after updating training mediated by the striatum. *Science* 320, 1510–1512. doi: 10.1126/science.1155466

Dash, D., McNab, F., and Klingberg, T. (2010). "Effect of dual working memory training on a reasoning task- an fMRI study," in *16th Annual Meeting of the Organization for Human Brain Mapping* (Barcelona).

Diedrichsen, J., and Shadmehr, R. (2005). Detecting and adjusting for artifacts in fMRI time series data. *Neuroimage* 27, 624–634. doi: 10.1016/j.neuroimage.2005.04.039

Dimitrov, D. M., Rumrill, J., and Phillip, D. (2003). Pretest-posttest designs and measurement of change. *Work* 20, 159–165.

Erickson, K. I., Colcombe, S. J., Wadhwa, R., Bherer, L., Peterson, M. S., Scalf, P. E., et al. (2007). Training-induced functional activation changes in dual-task processing: an FMRI study. *Cereb. Cortex* 17, 192–204. doi: 10.1093/cercor/bhj137

Flöel, A., Ruscheweyh, R., Krüger, K., Willemer, C., Winter, B., Völker, K., et al. (2010). Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link? *Neuroimage* 49, 2756–2763. doi: 10.1016/j.neuroimage.2009.10.043

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9673–9678. doi: 10.1073/pnas.0504136102

Friston, K. J., Holmes, A., Poline, J. B., Price, C. J., and Frith, C. D. (1996). Detecting activations in PET and fMRI: levels of inference and power. *Neuroimage* 4, 223–235. doi: 10.1006/nimg.1996.0074

Fuster, J. M. (2006). *The Prefrontal Cortex: Anatomy, Physiology, and Neuropsychology of the Frontal Lobe.* New York, NY: Raven Press.

Green, C. S., and Bavelier, D. (2008). Exercising your brain: a review of human brain plasticity and training-induced learning. *Psychol. Aging* 23, 692–701. doi: 10.1037/a0014345

Gur, R. C., Gur, R. E., Resnick, S. M., Skolnick, B. E., Alavi, A., and Reivich, M. (1987). The effect of anxiety on cortical cerebral blood flow and metabolism. *J. Cereb. Blood Flow Metab.* 7, 173–177. doi: 10.1038/jcbfm.1987.40

Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., and Weinberger, D. R. (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage* 17, 317–323. doi: 10.1006/nimg.2002.1179

Harris, S., and Dawson-Hughes, B. (1993). Seasonal mood changes in 250 normal women. *Psychiatry Res.* 49, 77–87. doi: 10.1016/0165-1781(93)90031-B

Hayasaka, S., and Nichols, T. E. (2003). Validating cluster size inference: random field and permutation methods. *Neuroimage* 20, 2343–2356. doi: 10.1016/j.neuroimage.2003.08.003

Hoffman, W. F., Moore, M., Templin, R., McFarland, B., Hitzemann, R. J., and Mitchell, S. H. (2006). Neuropsychological function and delay discounting in methamphetamine-dependent individuals. *Psychopharmacology* 188, 162–170. doi: 10.1007/s00213-006-0494-0

Hofmann, W., Gschwendner, T., Friese, M., Wiers, R. W., and Schmitt, M. (2008). Working memory capacity and self-regulatory behavior: toward an individual differences perspective on behavior determination by automatic versus controlled processes. *J. Pers. Soc. Psychol.* 95, 962–977. doi: 10.1037/a0012705

Hróbjartsson, A., and Gøtzsche, P. C. (2001). Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. *N. Engl. J. Med.* 344, 1594–1602. doi: 10.1056/NEJM200105243442106

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Johnson, K. A., Baig, M., Ramsey, D., Lisanby, S. H., Avery, D., McDonald, W. M., et al. (2013). Prefrontal rTMS for treating depression: location and intensity results from the OPT-TMS multi-site clinical trial. *Brain Stimul.* 6, 108–117. doi: 10.1016/j.brs.2012.02.003

Kamachi, M. (2001). *ATR Facial Affect Database (DB99) Schema. ATR Technical Report.* Kyoto: ATR-Promotions.

Klein, K., and Boals, A. (2001). The relationship of life event stress and working memory capacity. *Appl. Cogn. Psychol.* 15, 565–579. doi: 10.1002/acp.727

Klingberg, T. (2010). Training and plasticity of working memory. *Trends Cogn. Sci.* 14, 317–324. doi: 10.1016/j.tics.2010.05.002

Klingberg, T., Fernell, E., Olesen, P. J., Johnson, M., Gustafsson, P., Dahlström, K., et al. (2005). Computerized training of working memory in children with ADHD-a randomized, controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry* 44, 177–186. doi: 10.1097/00004583-200502000-00010

Klingberg, T., Forssberg, H., and Westerberg, H. (2002). Training of working memory in children with ADHD. *J. Clin. Exp. Neuropsychol.* 24, 781–791. doi: 10.1076/jcen.24.6.781.8395

Knoch, D., Treyer, V., Regard, M., Müri, R., Buck, A., and Weber, B. (2006). Lateralized and frequency-dependent effects of prefrontal rTMS on regional cerebral blood flow. *Neuroimage* 31, 641–648. doi: 10.1016/j.neuroimage.2005.12.025

Koenigs, M., and Grafman, J. (2009). The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav. Brain Res.* 201, 239–243. doi: 10.1016/j.bbr.2009.03.004

Lawlor, D. A., and Hopker, S. W. (2001). The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials. *BMJ* 322, 763–767. doi: 10.1136/bmj.322.7289.763

Lévesque, J., Eugène, F., Joanette, Y., Paquette, V., Mensour, B., Beaudoin, G., et al. (2003). Neural circuitry underlying voluntary suppression of sadness. *Biol. Psychiatry* 53, 502–510. doi: 10.1016/S0006-3223(02)01817-6

Luauté, J., Schwartz, S., Rossetti, Y., Spiridon, M., Rode, G., Boisson, D., et al. (2009). Dynamic changes in brain activity during prism adaptation. *J. Neurosci.* 29, 169–178. doi: 10.1523/JNEUROSCI.3054-08.2009

Mahncke, H. W., Connor, B. B., Appelman, J., Ahsanuddin, O. N., Hardy, J. L., Wood, R. A., et al. (2006). Memory enhancement in healthy older adults using a brain plasticity-based training program: a randomized, controlled study. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12523–12528. doi: 10.1073/pnas.0605194103

Mauss, I. B., Bunge, S. A., and Gross, J. J. (2007). Automatic emotion regulation. *Soc. Personal. Psychol. Compass* 1, 146–167. doi: 10.1111/j.1751-9004.2007.00005.x

McKendrick, R., Ayaz, H., Olmstead, R., and Parasuraman, R. (2014). Enhancing dual-task performance with verbal and spatial working memory training: continuous monitoring of cerebral hemodynamics with NIRS. *Neuroimage* 85, 1014–1026. doi: 10.1016/j.neuroimage.2013.05.103

McNab, F., Varrone, A., Farde, L., Jucaite, A., Bystritsky, P., Forssberg, H., et al. (2009). Changes in cortical dopamine D1 receptor binding associated with cognitive training. *Science* 323, 800–802. doi: 10.1126/science.1166102

McNair, D. M., Lorr, M., and Droppleman, L. F. (1992). *Profile of Mood States.* San Diego, CA: Educational and Industrial Testing Service.

Melby-Lervåg, M., and Hulme, C. (2012). Is working memory training effective? A meta-analytic review. *Dev. Psychol.* 49, 270–291. doi: 10.1037/a0028228

Meyer-Lindenberg, A., Buckholtz, J. W., Kolachana, B., Hariri, A. R., Pezawas, L., Blasi, G., et al. (2006). Neural mechanisms of genetic risk for impulsivity and violence in humans. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6269–6274. doi: 10.1073/pnas.0511311103

Milad, M. R., Quirk, G. J., Pitman, R. K., Orr, S. P., Fischl, B., and Rauch, S. L. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biol. Psychiatry* 62, 1191–1194. doi: 10.1016/j.biopsych.2007.04.032

Milad, M. R., and Rauch, S. L. (2007). The role of the orbitofrontal cortex in anxiety disorders. *Ann. N.Y. Acad. Sci.* 1121, 546–561. doi: 10.1196/annals.1401.006

Novaco, R. W. (1976). The functions and regulation of the arousal of anger. *Am. J. Psychiatry* 133, 1124–1128.

Okada, T., Tanaka, M., Kuratsune, H., Watanabe, Y., and Sadato, N. (2004). Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. *BMC Neurol.* 4:14. doi: 10.1186/1471-2377-4-14

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Olesen, P. J., Westerberg, H., and Klingberg, T. (2004). Increased prefrontal and parietal activity after training of working memory. *Nat. Neurosci.* 7, 75–79. doi: 10.1038/nn1165

Osaka, M., and Nishizaki, Y. (2000). *Brain and Working Memory (in Japanese).* Kyoto: Kyoto University Press.

Pascual-Leone, A., Rubio, B., Pallardó, F., and Catalá, M. D. (1996). Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. *Lancet* 348, 233–237. doi: 10.1016/S0140-6736(96)01219-6

Paulus, M. P., Simmons, A. N., Fitzpatrick, S. N., Potterat, E. G., Van Orden, K. F., Bauman, J., et al. (2010). Differential brain activation to angry faces by elite warfighters: neural processing evidence for enhanced threat detection. *PLoS ONE* 5:e10096. doi: 10.1371/journal.pone.0010096

Phan, K. L., Fitzgerald, D. A., Nathan, P. J., Moore, G. J., Uhde, T. W., and Tancer, M. E. (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biol. Psychiatry* 57, 210–219. doi: 10.1016/j.biopsych.2004.10.030

Price, D., Finniss, D., and Benedetti, F. (2008). A comprehensive review of the placebo effect: recent advances and current thought. *Annu. Rev. Psychol.* 59, 565–590. doi: 10.1146/annurev.psych.59.113006.095941

Redick, T. S., Shipstead, Z., Harrison, T. L., Hicks, K. L., Fried, D. E., Hambrick, D. Z., et al. (2013). No evidence of intelligence improvement after working memory training: a randomized, placebo-controlled study. *J. Exp. Psychol. Gen.* 142, 359–379. doi: 10.1037/a0029082

Reuter-Lorenz, P. A., Jonides, J., Smith, E. E., Hartley, A., Miller, A., Marshuetz, C., et al. (2000). Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. *J. Cogn. Neurosci.* 12, 174–187. doi: 10.1162/089892900561814

Schmeichel, B. J., Volokhov, R. N., and Demaree, H. A. (2008). Working memory capacity and the self-regulation of emotional expression and experience. *J. Pers. Soc. Psychol.* 95, 1526–1540. doi: 10.1037/a0013345

Schultheiss, O. C., Wirth, M. M., Waugh, C. E., Stanton, S. J., Meier, E. A., and Reuter-Lorenz, P. (2008). Exploring the motivational brain: effects of implicit power motivation on brain activation in response to facial expressions of emotion. *Soc. Cogn. Affect. Neurosci.* 3, 333–343. doi: 10.1093/scan/nsn030

Siegle, G. J., Thompson, W., Carter, C. S., Steinhauer, S. R., and Thase, M. E. (2007). Increased amygdala and decreased dorsolateral prefrontal BOLD responses in unipolar depression: related and independent features. *Biol. Psychiatry* 61, 198–209. doi: 10.1016/j.biopsych.2006.05.048

Smith, P. J., Blumenthal, J. A., Hoffman, B. M., Cooper, H., Strauman, T. A., Welsh-Bohmer, K., et al. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosom. Med.* 72, 239–252. doi: 10.1097/PSY.0b013e3181d14633

Sorg, B. A., and Whitney, P. (1992). The effect of trait anxiety and situational stress on working memory capacity. *J. Res. Pers.* 26, 235–241. doi: 10.1016/0092-6566(92)90041-2

Spielberger, C. D., Sydeman, S. J., Owen, A. E., and Marsh, B. J. (1999). "Measuring anxiety and anger with the State-Trait Anxiety Inventory (STAI) and the State-Trait Anger Expression Inventory (STAXI)," in *The Use of Psychological Testing for Treatment Planning and Outcomes Assessment, 2nd Edn.,* ed M. Maruish (Mahwah, NJ: Lawrence Erlbaum Associates Publishers), 993–1021.

Stuhrmann, A., Suslow, T., and Dannlowski, U. (2011). Facial emotion processing in major depression: a systematic review of neuroimaging findings. *Biol. Mood Anxiety Disord.* 1, 1–17. doi: 10.1186/2045-5380-1-10

Sweller, J., Van Merrienboer, J. J. G., and Paas, F. (1998). Cognitive architecture and instructional design. *Educ. Psychol. Rev.* 10, 251–296. doi: 10.1023/A:1022193728205

Takeuchi, H., and Kawashima, R. (2012). Effects of processing speed training on cognitive functions and neural systems. *Rev. Neurosci.* 23, 289–301. doi: 10.1515/revneuro-2012-0035

Takeuchi, H., Sekiguchi, A., Taki, Y., Yokoyama, S., Yomogida, Y., Komuro, N., et al. (2010a). Training of working memory impacts structural connectivity. *J. Neurosci.* 30, 3297–3303. doi: 10.1523/JNEUROSCI.4611-09.2010

Takeuchi, H., Sugiura, M., Sassa, Y., Sekiguchi, A., Yomogida, Y., Taki, Y., et al. (2012). Neural correlates of the difference between working memory speed and simple sensorimotor speed: an fMRI study. *PLoS ONE* 7:e30579. doi: 10.1371/journal.pone.0030579

Takeuchi, H., Taki, Y., Hashizume, H., Sassa, Y., Nagase, T., Nouchi, R., et al. (2011a). Effects of training of processing speed on neural systems. *J. Neurosci.* 31, 12139–12148. doi: 10.1523/JNEUROSCI.2948-11.2011

Takeuchi, H., Taki, Y., Hashizume, H., Sassa, Y., Nagase, T., Nouchi, R., et al. (2011b). Failing to deactivate: the association between brain activity during a working memory task and creativity. *Neuroimage* 55, 681–687. doi: 10.1016/j.neuroimage.2010.11.052

Takeuchi, H., Taki, Y., and Kawashima, R. (2010b). Effects of working memory training on cognitive functions and neural systems. *Rev. Neurosci.* 21, 427–450. doi: 10.1515/REVNEURO.2010.21.6.427

Takeuchi, H., Taki, Y., Nouchi, R., Hashizume, H., Sekiguchi, A., Kotozaki, Y., et al. (2013b). Effects of working memory-training on functional connectivity and cerebral blood flow during rest. *Cortex* 49, 2106–2125. doi: 10.1016/j.cortex.2012.09.007

Takeuchi, H., Taki, Y., Nouchi, R., Hashizume, H., Sekiguchi, A., Kotozaki, Y., et al. (2014a). Working memory training impacts the mean diffusivity in the dopaminergic system. *Brain Struct. Funct*. doi: 10.1007/s00429-014-0845-2. [Epub ahead of print].

Takeuchi, H., Taki, Y., Nouchi, R., Hashizume, H., Sekiguchi, A., Kotozaki, Y., et al. (2014b). Effects of multitasking-training on gray matter structure and resting state neural mechanisms. *Hum. Brain Mapp.* 35, 3646–3660. doi: 10.1002/hbm.22427

Takeuchi, H., Taki, Y., Sassa, Y., Hashizume, H., Sekiguchi, A., Fukushima, A., et al. (2011c). Verbal working memory performance correlates with regional white matter structures in the fronto-parietal regions. *Neuropsychologia* 49, 3466–3473 doi: 10.1016/j.neuropsychologia.2011.08.022

Takeuchi, H., Taki, Y., Sassa, Y., Hashizume, H., Sekiguchi, A., Fukushima, A., et al. (2011d). Working memory training using mental calculation impactsregional gray matter of the frontal and parietal regions. *PLoS ONE* 6:e23175. doi: 10.1371/journal.pone.0023175

Takeuchi, H., Taki, Y., Sassa, Y., Hashizume, H., Sekiguchi, A., Nagase, T., et al. (2013a). White matter structures associated with empathizing and systemizing in young adults. *Neuroimage* 77, 222–236. doi: 10.1016/j.neuroimage.2013.04.004

Tamamiya, Y., and Hiraki, K. (2013). Individual differences in the recognition of facial expressions: an event-related potentials study. *PLoS ONE* 8:e57325. doi: 10.1371/journal.pone.0057325

Thomsen, T., Rimol, L. M., Ersland, L., and Hugdahl, K. (2004). Dichotic listening reveals functional specificity in prefrontal cortex: an fMRI study. *Neuroimage* 21, 211–218. doi: 10.1016/j.neuroimage.2003.08.039

Uchida, S., and Kawashima, R. (2008). Reading and solving arithmetic problems improves cognitive functions of normal aged people: a randomized controlled study. *Age* 30, 21–29. doi: 10.1007/s11357-007-9044-x

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nat. Rev. Neurosci.* 6, 533–544. doi: 10.1038/nrn1704

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., and Ochsner, K. N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron* 59, 1037–1050. doi: 10.1016/j.neuron.2008.09.006

Weiland-Fiedler, P., Erickson, K., Waldeck, T., Luckenbaugh, D. A., Pike, D., Bonne, O., et al. (2004). Evidence for continuing neuropsychological impairments in depression. *J. Affect. Disord.* 82, 253–258. doi: 10.1016/j.jad.2003.10.009

Westerberg, H., Hirvikoski, T., Forssberg, H., and Klingberg, T. (2004). Visuo-spatial working memory span: a sensitive measure of cognitive deficits in children with ADHD. *Child Neuropsychol.* 10, 155–161. doi: 10.1080/09297040409609806

Williams, L. M., Das, P., Liddell, B., Olivieri, G., Peduto, A., Brammer, M. J., et al. (2005). BOLD, sweat and fears: fMRI and skin conductance distinguish facial fear signals. *Neuroreport* 16, 49–52. doi: 10.1097/00001756-200501190-00012

Yamnill, S., and McLean, G. N. (2001). Theories supporting transfer of training. *Hum. Res. Dev. Q.* 12, 195–208. doi: 10.1002/hrdq.7

Yokoyama, K. (2005). *POMS Shortened Version (in Japanese)*. Tokyo: Kanekoshobo.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 August 2014; accepted: 23 September 2014; published online: 16 October 2014.

Citation: Takeuchi H, Taki Y, Nouchi R, Hashizume H, Sekiguchi A, Kotozaki Y, Nakagawa S, Miyauchi CM, Sassa Y and Kawashima R (2014) Working memory training improves emotional states of healthy individuals. Front. Syst. Neurosci. ***8****:200. doi: 10.3389/fnsys.2014.00200*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

ORIGINAL RESEARCH
published: 28 April 2015
doi: 10.3389/fnsys.2015.00062

Adaptation to elastic loads and BMI robot controls during rat locomotion examined with point-process GLMs

Weiguo Song[1], *Iahn Cajigas*[2], *Emery N. Brown*[2,3,4] *and Simon F. Giszter*[1,5]*

[1] *Department of Neurobiology and Anatomy, Drexel University College of Medicine, Drexel University, Philadelphia, PA, USA,* [2] *Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA,* [3] *Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, USA,* [4] *Department of Anesthesia, Critical Care and Pain Medicine, Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA,* [5] *School of Biomedical Engineering, Drexel University, Philadelphia, PA, USA*

Currently little is known about how a mechanically coupled BMI system's actions are integrated into ongoing body dynamics. We tested a locomotor task augmented with a BMI system driving a robot mechanically interacting with a rat under three conditions: control locomotion (BL), "simple elastic load" (E) and "BMI with elastic load" (BMI/E). The effect of the BMI was to allow compensation of the elastic load as a function of the neural drive. Neurons recorded here were close to one another in cortex, all within a 200 micron diameter horizontal distance of one another. The interactions of these close assemblies of neurons may differ from those among neurons at longer distances in BMI tasks and thus are important to explore. A point process generalized linear model (GLM), was used to examine connectivity at two different binning timescales (1 ms vs. 10 ms). We used GLM models to fit non-Poisson neural dynamics solely using other neurons' prior neural activity as covariates. Models at different timescales were compared based on Kolmogorov-Smirnov (KS) goodness-of-fit and parsimony. About 15% of cells with non-Poisson firing were well fitted with the neuron-to-neuron models alone. More such cells were fitted at the 1 ms binning than 10 ms. Positive connection parameters ("excitation" ∼70%) exceeded negative parameters ("inhibition" ∼30%). Significant connectivity changes in the GLM determined networks of well-fitted neurons occurred between the conditions. However, a common core of connections comprising at least ∼15% of connections persisted between any two of the three conditions. Significantly almost twice as many connections were in common between the two load conditions (∼27%), compared to between either load condition and the baseline. This local point process GLM identified neural correlation structure and the changes seen across task conditions in the rats in this neural subset may be intrinsic to cortex or due to feedback and input reorganization in adaptation.

Keywords: augmenting BMI, elastic field, point-process general linear model, exoskeleton robot model system, motor adaptation

OPEN ACCESS

Edited by:
Mikhail Lebedev,
Duke University, USA

Reviewed by:
Monzurul Alam,
University of California, USA
Dean Robert Freestone,
The University of Melbourne, Australia
Matthew D. Golub,
Carnegie Mellon University, USA

***Correspondence:**
Simon F. Giszter,
Department of Neurobiology and Anatomy, Drexel University College of Medicine, Drexel University, 2900 Queen Lane, Philadelphia, PA, USA
sgiszter@drexelmed.edu

Received: *01 January 2015*
Accepted: *01 April 2015*
Published: *28 April 2015*

Citation:
Song W, Cajigas I, Brown EN and Giszter SF (2015) Adaptation to elastic loads and BMI robot controls during rat locomotion examined with point-process GLMs. Front. Syst. Neurosci. 9:62. doi: 10.3389/fnsys.2015.00062

Introduction

Brain Machine Interfaces (BMIs) hold the potential to provide functions to spinal-injured patients (Hochberg et al., 2006) or to augment normal motor functions in novel ways. The operation of BMIs depends on reliable neural decoding during ongoing behaviors and on a subject's adaptation of activity to achieve function across differing ongoing tasks. To date, most efforts in BMI have worked in mechanically isolated contexts and concentrated on highly-trained manipulation tasks of the forelimb (Chapin et al., 1999; Hochberg et al., 2006; Moritz et al., 2008; Velliste et al., 2008). They have largely neglected locomotion or its augmentation using invasive single unit methods, with notable exceptions (Fitzsimmons et al., 2009; Song et al., 2009; Manohar et al., 2012; Alam et al., 2014). Further, a future BMI application of lower limb control would likely require the user to manage on-line motor adaptations and integrate the lower limb BMI control with their whole body control.

The effect on neural modulation of BMI integration into the body scheme has not been thoroughly investigated. The stability of cortical neural responses remains controversial. Some data showed that cells can be functionally stable and BMI systems maintain performance across days with the same decoder (Serruya et al., 2003; Greenberg and Wilson, 2004; Chestek et al., 2007). However, significant neural plasticity was also reported in non-BMI force-field adaptation experiments and in BMI systems exposed to a given decoder across days (Li et al., 2001; Carmena et al., 2005; Jackson et al., 2006; Rokni et al., 2007; Zacksenhouse et al., 2007; Jarosiewicz et al., 2008; Ganguly and Carmena, 2009; Song and Giszter, 2011). Even when responses become fairly stable, and switching among BMI controls are achieved easily, for example after over-training, issues will remain in integrating and adapting the BMI controls to new tasks and mechanical contexts in activities of daily living. Conceivably, highly-trained BMI designs with complicated decoding algorithms may impede the use of the intrinsic plasticity of the brain to adapt and improve the BMI control in dynamic environments. Current efforts to develop adaptive decoders must balance their potential advantages against the potential for conflict with the user's own adaptation. An alternative strategy may be to provide simpler interfaces (Moritz et al., 2008), and allow the brain's plasticity and processing power to adapt and incorporate the novel actions.

Using a relatively simple interface strategy to generate a neural driven force from the robot, like Fetz and colleagues (Moritz et al., 2008), we examined the use of the BMI in this dynamic environment. Rats were exposed to three conditions during treadmill locomotion:(a) control, (b) simple elastic loading (E), and (c) BMI with elastic load (BMI/E) in which BMI lifting control was made available in parallel with the elastic load used in (b). Previously, we found that cells could modulate their firing patterns differently during each adaptation in different experimental conditions (Song and Giszter, 2011).

To understand the function of neuronal circuits and systems, it is essential to characterize the connections between individual neurons (Brown et al., 2004; Shigeyoshi, 2008; Stevenson and Körding, 2010) and their dynamics across tasks or condition changes. Correlations of cells relate to network connectivity and common inputs and processing. The cross-correlation, coherence and joint peri-stimulus time histogram (JPSTH) methods have been used for pairs of cells (Gerstein and Perkel, 1969; Jarvis and Mitra, 2001; Schneidman et al., 2006; Shigeyoshi, 2008). To characterize the strengths and any dynamics of the connections between neurons, variants of information based methods have been used in computational neuroscience for analyzing spiking neural systems (Strong et al., 1998). However, in many brain areas, each neuron receives input from a large population, and the generalized linear model (GLM) provides another framework to examine connectivity based on the point process representation of the spike trains (Brown et al., 2002; Truccolo et al., 2005; Acharya et al., 2008; Stevenson et al., 2008; Héliot et al., 2010). The GLM attempts to predict a neuron's firing patterns based on its own spikes and the spikes of other neurons, and on external inputs. By combining a KS statistical analysis for the goodness of fit in each model, it provides a powerful tool for neural network functional connectivity analysis (Brown et al., 2002; Truccolo et al., 2005; Acharya et al., 2008; Kositsky et al., 2009; Gerhard et al., 2011). By utilizing a point process framework and GLM, we examined the neural dynamics at the functional network level.

We examined "functional connectivity" which we here define as an analysis and estimation of how firing of each neuron appears to influence each other neuron in a given data set, given the data collected and included in the analysis, with such influences expressed in the most parsimonious way possible. This represents a compact description of firing pattern correlations and apparent influences on one another in the observed data. Because the neural activity is incompletely observed, it is necessarily limited in relation to anatomy and actual circuit structure. Results showed significant differences in network organization as estimated by the GLM analysis of functional connectivity under different conditions, with some fraction of "core connectivity" in the GLM models, and the observed firing pattern features, preserved across the trials.

Methods and Materials

Surgical Procedure and Neural Recording

Six adult Sprague-Dawley rats, weighing 250 $\sim$ 300 g, were used in these experiments. Care and treatment of the animals conformed to protocols approved by the University Laboratory Animal Resources and Institutional Animal Care and Use Committee of the College of Medicine of Drexel University. Methods are published in Song and Giszter (2011). The detailed procedures for pelvic and cortical implantations have also been described previously (Song and Giszter, 2011). For short, after anesthesia using KXA (63 mg/Kg Ketamine, 6 mg/Kg Xylazine, 0.05 mg/Kg Acepromazine), rats were implanted with a pelvic orthosis, which allowed a PHANTOM robot (Sensable Devices) to apply forces directly to the skeleton. Tetrode arrays, which consisted of 6 tetrodes positioned around the neck of an etched spear-shaped fine tungsten rod, were implanted stereotactically in the hindlimb / trunk area of the motor cortex (2.0 mm lateral of the midline, 1.6 mm caudal to bregma and 1.0–1.5 mm in depth). Recording sites of all electrodes in a rat were within from 50

to 200 microns of one another in the cortex using our probe design. Intracortical microstimulation of rat M1 in this recorded region generates hindlimb/trunk movements in all rats tested. At the end of the final recording session, strong electrolytic lesions were made and brain slices were cut in 40 μm sections to validate electrode placement. Neural data were recorded using a Cerebus system (Cyberkinetics, Inc. / Blackrock systems) after animals recovered from surgery. Neural signals were band-pass filtered (300–7.5 KHz) and digitized at a sampling frequency of 30 kHz. Spikes were detected online using thresholding. The detected spikes could be automatically classified on-line after setting the templates of each waveform. The on-line sorted spikes were used in a real time "BMI with elastic load" control. The controller and real-time OS code were custom laboratory written code (available on request). They implemented the algorithms in Equation (1) below, with the parameters noted there. The detected spike trains, as well as all the thresholded spike waveforms, were also saved for off-line analysis. Multiple single units were isolated off-line using Off-line Sorter (Plexon Neurotech Com.) after noise removal by cluster cutting method (Shoham et al., 2003). This analysis uses the offline sorted data. Up to 24 channels of neural activity at a time in a single array could be recorded, and 1 or 2 individual cells from most wires of the tetrodes could be recorded; around 15–38 well isolated single units were used in each trial.

Robot Controls and Training Protocols

The robotic system employed and the training protocol used have been described in detail previously (Song and Giszter, 2011). Briefly, different force fields (linear, non-linear, isotropic, anisotropic, piecewise discontinuities etc.) could be applied to the pelvis of the rat using our custom control software. This was achieved through an implanted orthosis, directly interacting with the skeleton using the orthosis attached robot. Robot control was updated at a frequency of 1 KHz, and these controls were synchronized and coupled with neural recordings. Neural data recorded could be used in the robot control scheme after a delay of under 3 ms from spike occurence. In elastic load trials (*abbrevation* ***E***), unidirectional (vertical forces only, i.e., radially anisotropic) elastic fields were used. Elastic loading (downward) fields F_e were applied in condition E by setting an equilibrium point on the horizontal equilibrium plane of the elastic field around 12.5 mm under the rat's normal pelvic height with a field stiffness of 44 N/m, which gives around 15% of the body weight. In trials termed here "BMI with elastic load" (abbrev. BMI/E), a neural driven lifting elastic force F_n was combined with the above simple elastic force F_e, and both forces were combined and applied simultaneously. The neural driven elastic force was calculated according to the equation

$$F_n = K_n \times N_{af} \times (X - X_{n0}), \tag{1}$$

where N_{af} was aggregate firing rate in the prior 100 ms window, $K_n = 80\ N/m$ was the stiffness, $X_{n0} = 55\,mm$ was the equilibrium point, and X the current vertical position of the pelvis with respect to the horizontal equilibrium plane. This gave the rat in BMI/E trials a means to offset the elastic load F_e using neural activity N_{af} to modulate an opposing force F_n. Experiments except where noted comprised one or more iterations of 3 conditions: 2 min of baseline (BL) with no loading, followed by 2 min of elastic load (E) in which the rat experienced F_e, and then 2 min of BMI + load (BMI/E) in which the rat had access to the BMI driven field F_n and could potentially offset F_e.

Rats were trained to walk on a treadmill at a speed ranging from 0.1 to 0.15 m/s depending on the normal preferred walking speed of individual rats. One daily experimental session used in analysis below was divided into three trials as described above: (i) the BL (baseline) trial, in which rats walked on treadmill without any force; (ii) the E (simple elastic load) trial; (iii) the BMI/E (BMI with elastic load) trial. Each session thus consisted of three 2-min trials of treadmill walking (∼120 steps) with 5 min intervals of rest occurred between each trial.

Neural Network Functional Connectivity Analysis by Using GLM Model

We follow Stevenson et al. (2009) and Okatan et al. (2005) in defining functional connectivity: we seek to characterize how each neuron appears to influence each other neuron in the data sets in the most parsimonious way possible. The neural modulation of single cells that was observed during BMI usage and motor adaptation observed in our experimental paradigm was previously reported (Song and Giszter, 2011).

The procedure for functional connectivity analysis was as shown in **Figure 1**. Briefly, neural spiking activity is modeled as a point process which is completely characterized by its conditional intensity function (Daley and Vere-Jones, 2003) defined as

$$\lambda\left(t \mid H_t\right) = \lim_{\Delta \to 0} \frac{P(N\left(t+\Delta\right) - N\left(t\right) = 1 \mid H_t)}{\Delta} \tag{2}$$

where H_t is all the history information from 0 up to time t, and $N(t)$ is a counting process containing the sum of all the spikes up to time t. The conditional intensity function can be considered a generalization of the "rate" for a homogenous Poisson process. Under the restriction that $\lambda\left(t|H_t\right)\Delta$ follows a distribution in the exponential family, the GLM framework can be used to fit statistical models for the conditional intensity function (McCullagh and Nelder, 1989). Goodness of fit can be assessed by Kolmogorov-Smirnov test (KS-test) after the time rescaling theorem (Brown et al., 2002).

The conditional intensity function of the ith spiking neuron was modeled with the spiking history of neighbor cells either at a short timescale (1 ms, $L = 1$) or long timescale (10 ms, $L = 10$) as Equation (3),

$$\lambda_i\left(t_k \mid H_t\right) = \exp\left(\mu_i + \sum\nolimits_{\substack{j=1 \\ j \neq i}}^{J} \beta_{ij} \cdot \Delta N_j\left(t_k\right)\right) \tag{3}$$

where J is the total number of neurons recorded in the current trial; μ_i is the baseline level of firing rate; $\Delta N_j\left(t_k\right) = N_j\left(t_k\right) - N_j\left(t_{k-L}\right)$ is the number of spikes of the jth neuron between time t_{k-L} and t_k, $L = 1$ or 10 for the short and long timescale model respectively, and β_{ij} is the regression coefficient to be estimated representing the strength of effect between the firing of the jth

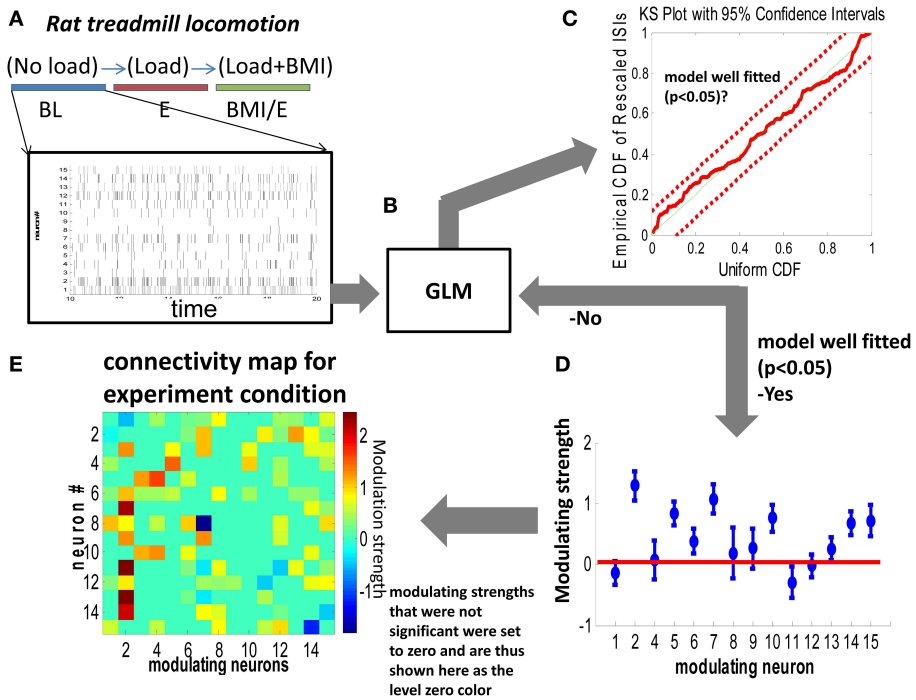

FIGURE 1 | Generalized linear model (GLM) based network connectivity analysis. (A,B) The conditional intensity function of each neuron was modeled with GLM by using a single step spiking history of the whole population of neurons over two different timescales (1 ms vs. 10 ms). **(C)** A Kolmogorov Smirnov (KS) criteria was used to test the goodness of fit of the model. According to the time rescaling theorem for point processes, the rescaled spike times under the true conditional intensity function would be independent, identically distributed, exponential random variables with unit rate. **(D)** For neurons which showed a significant nonzero contribution in the GLM model, the nonzero modeling strength was used to construct an element in the connectivity matrix (map) in **(E)**. Parameters whose confidence interval crossed zero (the horizontal red line) in **(D)** were deemed not significantly different from zero and were set to zero in the connectivity maps in **(E)**.

neuron in the short or long timescale and the current firing rate of the ith neuron. We term the β_{ij} as the functional connectivity coefficient of neuron j to neuron i. Note that with this definition, $\beta_{ij} \neq \beta_{ji}$ in general and both that both short and long timescale models have the same number of parameters. We define the functional connectivity map, $\boldsymbol{\beta}$, as the matrix containing β_{ij} in the ith row and jth column. The structure of $\boldsymbol{\beta}$ is one measure of the connections between neurons or a representation of the functional network between the neurons.

The activity of each neuron was modeled by its neighboring neurons' immediately preceding history within a 10 ms window (type A in Equation 3) and in a small number of test models by its own history (type B, self-history included - i.e., remove the $j \neq i$ constraint in Equation 3). The Type B model (with neural self history) was evaluated in 2 rats. Models with the self history inclusion on the timescales tested here differed little in their network neighbor interactions. They showed significant overlap of network connections with the similar networks identified and estimated without self history. However, they generally showed some reduction of external network connections (22% in BL, 11% in E, and 15% in BMI, when using 1 ms time bins) with increasing history parameters but also a decreasing quality metric (see below). Here we present data from the network models without self-history, consistent with our quality metric used (see below).

Statistical Methods

After GLM fitting (glmfit, MATLAB) for each neuron, a Kolmogorov-Smirnov (KS) test was applied to test the goodness of fitting. If the fitting was within the 95% confidence interval, we classified the neuron as well fitted. Then, the significant parameters that were nonzero ($p < 0.05$) were used to construct the functional connectivity map as in **Figure 1**.

In order to test the functional connectivity map changes between two conditions, and their significance, the functional connectivity map identified and estimated in each was converted to a binary functional connectivity matrix by setting the *significant* nonzero elements to one and the others to zero as in **Figure 4**. Connection density was defined as the percentage of significant connected links, in relation to the total possible links and used as a criterion for analysis and comparison of model structures and conditions. In a first pass analysis we modeled all recorded neurons as stationary Poisson processes to test for non-Poisson behavior. In a second pass analysis (type II models), we modeled those neurons whose firing was not fitted by a mean rate parameter, and thus non-Poisson by our test criterion used for model fit assessment (time rescaling theorem/Kolmogorov Smirnov tests with $p < 0.05$). Such cells could not be well-predicted simply by using a mean rate parameter (type I model) and were thus firing based on locomotion variables or one another. The number of such non-Poisson cells found differed

in the data sets, varying based on binning. We found such "non-Poisson patterned" cells using both 1 ms and 10 ms binning. In total 573 neurons were analyzed. Cell numbers that were non-Poisson were as follows: 1 ms binning: 287 (BL), 328 (E), 303(BMI/E); 10 ms: 348 (BL), 368 (E), 361 (BMI/E). The first pass analysis was equivalent to an assumption of the possible presence of neither locomotion task information nor neural interaction information in the cell firing. Cells not fitted in this way would need a more complex fitting, which could further improve conditional intensity function fit and KS statistic if modeled correctly, using neural or locomotion variable effects. The second pass analysis we used examined neural interactions alone. Effectively, we assumed that the first pass Poisson fitted cells were unrelated to the motor tasks, and were simply noise sources in the cortical network, though potentially important predictors of other neural firing. We ignored the external covariate effects, focusing on neural correlation alone. We compared GLM model structures to capture the non-Poisson population of neurons at a short timescale using 1 ms binning and at a longer timescale using 10 ms binning based on the parsimonious selection of GLM fitting. We sought models that captured the most neurons with the best levels of individual neuron parameter parsimony. To assess this model quality we calculated a model parameter Q,

$$Q = n/N \tag{4}$$

where n is the percentage of neurons with non-Poisson firing that were well fitted using the model, N is the square root of the significant connection density (number of possible connections growing as the square of neurons recorded). High Q values indicate good parsimonious fitting of the (non-Poisson) activity in the network, and the sparsest possible connectivity description of firing. If two models had the same numbers of well-predicted neurons total in a data set but one fitting had higher Q (i.e., fewer connections/ parameters required) it was considered a better model form. If two models fitted different numbers of neurons in a data set, but the model fitting of larger number of neurons had a much lower Q this would indicate a more complex individual neural functional connectivity was necessary in the model to capture the greater number of neurons and achieve fitting. When the activity of each neuron was modeled from its neighboring simultaneous firing neurons, but not using self history and recursive self connections, the overall parsimony and Q value obtained was significantly lower, so we only present data using the history effects of neighbor neurons not self history (Equation 3).

From the binary functional connectivity matrix of significant parameters found using models with the best Q, we could then test the significance of functional connectivity maps changes. We did by using a binomial distribution test. That is, by assuming the probability of keeping or changing the original link had the same chance, then if the probability of the pattern of change observed was less than 0.05, then a significant change had occurred. In practice this statistical test was likely unnecessary, since we found that only about 15–27% of model connections persisted across different conditions in an animal. Nonparametric signed rank tests (signrank, MATLAB) were used to compare the significance of such individual changes and differences across sessions and rats. All the tests were set to a significance level of 0.05. All data analysis was performed using custom software written in MATLAB. A suite of refined and related MATLAB tools including these is freely available (Cajigas et al., 2012).

Results

In total 21 robot and BMI interaction sessions were collected in 6 normal rats that were implanted with multi-electrode recording probes. Rats performed treadmill locomotion in three conditions: (1) unloaded baseline (BL), (2) a robot driven elastic load condition (E) in which rats experienced a vertical elastic load, and (3) a robot driven elastic load and BMI condition (BMI/E), see **Figure 1A**. In the last condition the rat could in principle offset elastic load using neural activity. In BMI/E the recorded neural activity of the rat regulated the stiffness of a second "BMI elastic field." This could potentially be employed to offset the elastic load, as was described in Song and Giszter (2011). We have reported previously that rats do quite quickly begin to adjust and to offset the load (Song and Giszter, 2011). We sought to assess changes in functional connectivity as rats proceeded from BL to E to BMI/E conditions. In order to do this we compared functional connections in GLM fitted networks for the data (**Figures 1A–E**) in each successive test condition. We thus separately fitted each condition. 21 fitted networks were here obtained and compared for each condition (BL, E, and BMI/E) using GLM models. We tested fits of the GLM models at two different binning timescales: 1 ms and 10 ms. In total 573 neurons were analyzed in the 21 sessions. Cell numbers that were non-Poisson and supported network connection modeling were as follows: 1 ms binning: 287 (BL), 328 (E), 303 (BMI/E); 10 ms: 348 (BL), 368 (E), 361 (BMI/E). In addition to these sessions, in control experiments we tested repetitions of the baseline, and repetitions of the full sequence of tests in a rat, to assess the connection and network drift (gradual network functional connection changes within a condition), relative to the measures of change we saw between conditions.

Network Fitting Procedures and Network Characteristics

We first modeled each neuron in each task condition (BL, E, and BMI/E) by using a 1 step spiking history of the ensemble neurons using either short timescale (1 ms bin) or longer timescale (10 ms bin) steps. Type I models ignored interactions, simply fitting cells independently as point processes to discover non-Poisson pattern cells. Type II models used cell interactions. We found that about 60% of recorded cells were well fitted at the short timescale. However, the number of well-predicted non-Poisson firing cells was not high. About 15% of such non-Poisson pattern cells were well-predicted using the short timescale. Significantly less cells were well-predicted in the long timescale, matching the total cell fitting patterns. The numbers of total and non-Poisson firing pattern cells that were well fitted at each timescale were not significantly different between the three different adaptation conditions. We used a measure Q of fitting complexity to compare the models and binnings (see Methods). The numbers of cells that were well-predicted and the Q statistic in both the shorter timescale (1 ms) and longer timescale (10 ms) analyses are

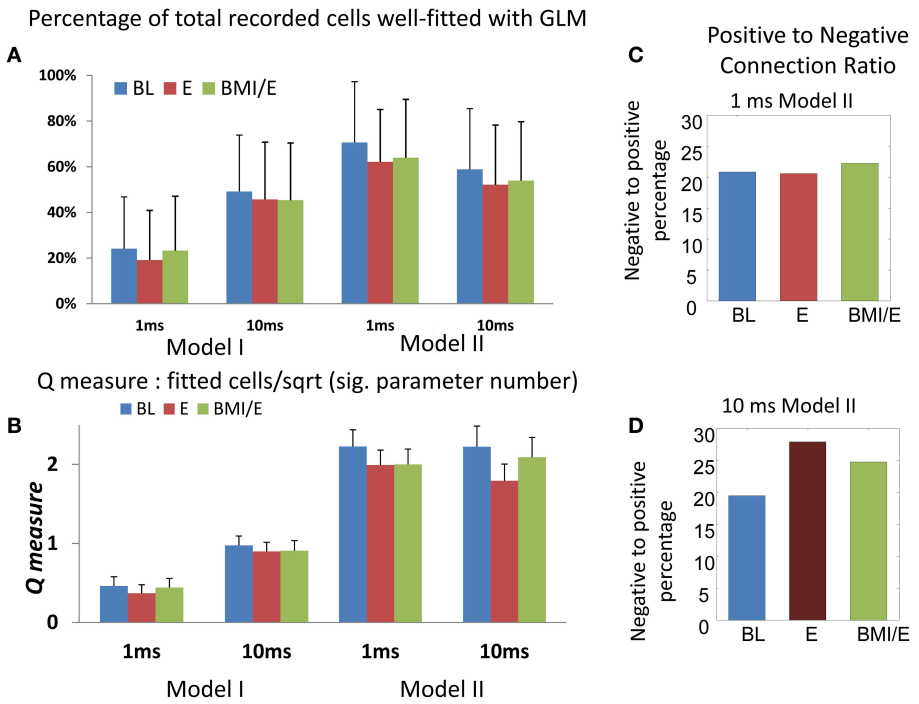

FIGURE 2 | Numbers of cells fitted with GLM model and Q-measure. (**A**) Percentage of total cells from the whole population ($n = 573$) fitted in each model and adaptation condition. The model II with 1 ms timescale had the highest capture of cells and best Q (indicated with the red dotted box). Note that despite good overall fits, only about 15% of neurons with firing interval statistics not captured by a mean rate parameter (non-Poisson firing) were well-predicted in the GLM networks here. More total and non-Poisson cells were fitted in the E condition than in the other two conditions (BL or BMI/E) at both short timescale (1 ms binning) and long timescale (10 ms binning). **(B)** The Q-measure. This was defined as the ratio of the percentage of neurons with non-Poisson firing that were well-predicted using the model, to the square root of the significant connection density required. Coupled with **(A)** the Q-measure analysis favors the type II models at the 1 ms binning scale. The error bars indicates mean ± S.E. **(C)** The percentage of significant negative parameters as a portion of significant positive modulation parameters in the 1 ms bin model II is shown in each task condition. **(D)** The percentage of significant negative parameters as a portion of significant positive modulation parameters in the 10 ms bin model II is shown in each task condition. The number of significant negative interactions was lower than positive ones in each network, while there was no significant difference between the different adaptation conditions for each model. Overall, in the 10 ms binned model II fits, the number of negatively modulating interactions increased. Abbrev. BL baseline, E elastic load, BMI/E brain machine interface and elastic load.

shown in **Figure 2**. The 10 ms binned fits were almost as good as the 1 ms model fits. Although a simple 1 step history linear filter is presented here, we also tested type I and type II models with basis function filters similar to Truccolo (Truccolo et al., 2010), and tested models with a 10 ms (five basis function) history in two rats. There was no significant improvement in the number of cells which could be well-predicted using KS-criteria, when compared to using the 1 ms binning used here. Thus, we kept the 1 step history window in our subsequent modeling. We present the analyses only for the simpler 1 ms bin and 10 ms history type II model. In the 1 ms bin short timescale, the GLM models captured the binary spike train behaviors, where temporal coding might feature, while the 10 ms bin long timescale GLM model corresponds more to rate coding. More cells could be well fitted using the short timescale models than with the long timescale models. This suggests that with 1 ms binning, the individual cells predicted (or "influenced") the short term information of the neighboring cells better (more) than was seen on the long timescale. Thus, the better-fitted functional connectivity network structures examined here using 1 ms binning likely represent short timescale processes.

We also examined the fitted networks' significant parameters to see if numbers of excitatory or inhibitory types of connections were dominant, and the average strengths of each type of connection. In each network, we found that the number of significant negative interactions ("inhibition") was lower than the number of positive ones ("excitation") constituting about on fifth on average (**Figures 2C,D**). When we compared these connection numbers for the models between experimental conditions, we found that there was no significant difference in the positive/negative parameter balances in models among the different task conditions. However, the number of negatively modulating interactions in the models using 10 ms binning and timescale were increased overall compared to 1 ms binning and timescale (**Figures 2C,D**).

Network Stability in Continuously Repeated Trials of a Condition, and Repetitions of Conditions after Delays and Condition Switches

In control trials, we found that in successive repetitions of the baseline conditions, the fitted networks showed some connection variability but that overall more than 30% of

connections were preserved over the entire series of repetitions, and around 40% or more similarity was found between tests of networks adjacent in time (**Figure 3A**). Similarly, we found that networks fitted to repetitions of each condition after other interleaved conditions showed more connection similarity with each other (**Figure 3B**) than with the networks fitted to different conditions that were adjacent in time, and this difference was significant under an assumption of random variations (e.g., for data in **Figure 3B**, one-tailed t-test $p < 0.05$).

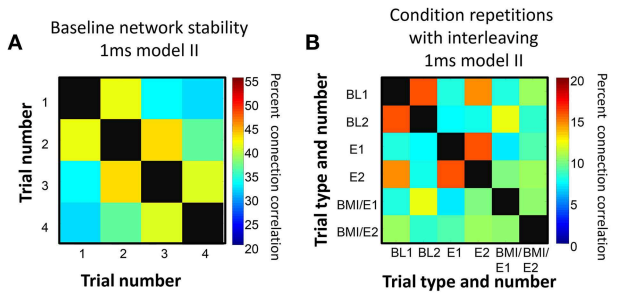

FIGURE 3 | Connection persistence within condition. (A) Repeated baseline recordings with 5 min pauses between them showed more than 30% of connections were preserved from the first to last recording. However, over the repetitions there was a slow drift in connection pattern. The matrix of percentage of common connections, and the color map scale are shown. On the diagonal, the black squares indicate 100% correlation (self to self). **(B)** Repetitions of the basic experiment used, with successive trials of BL, E, BMI/E in a rat. The matrix of common connection percentages is organized with BL1 and BL2 grouped, E1 and E2 grouped, and BMI/E1 and BMI/E2 grouped but these were separated in time in the experiment. The repetitions of each condition are generally more similar to one another than to other conditions. The level of within condition similarity is not consistent with random assortment of the matrix elements and their variance. On the diagonal, the black squares indicate 100% correlation (self to self). Note also that the color scale range in **A** and **B** differ.

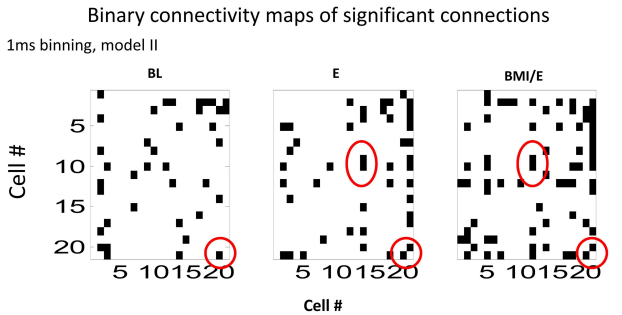

FIGURE 4 | Binary connectivity maps from the GLM fits in a typical session (from the same session as is shown in Figure 5). Significant connections are shown in black. The networks showed sparse connectivity. The connection density was higher for the 1 ms bin time scale that is shown (1 ms binning). Examples of a few connections found in common in the GLM fits in each condition, are circled in red. These show consistency in modeling of some connections across all the conditions. Abbrevations: BL, baseline; E, elastic load; BMI/E, brain machine interface and elastic load.

Network Changes across Different Adaptation Conditions

We next analyzed the model connectivity changes occuring between the three adaptation conditions when they followed in close succession in a complete experiment series. Our rationale was that although the numbers of cells, which could be well-predicted by either type I or type II GLM models were not significantly different among each adaptation task condition, the network structure might change or reorganize in these different contexts. Thus, we looked in detail at how the fitted network statistics changed in the transitions between the different task conditions, compared across conditions, and at the different binning timescales. Both the binary connection maps (**Figure 4**) and the functional connectivity strengths (**Figures 5**, **6**) showed significant differences at the two different model timescales (1 ms, and 10 ms). These also differed among the three adaptation conditions tested (BL, E, and BMI/E). Some fraction of the connections persisted across different task conditions (**Figure 4**, circled examples). Connections that persisted in common between the three adaptation conditions remained correlated in strengths (**Figure 5** red points in each plot), however other patterns of functional connectivity often changed significantly. The binary functional connectivity maps showed distinct patterns depending on timescales analyzed, as seen in **Figure 4**. Generally, we found that in the longer 10 ms binned data most connections were unique and many fewer were shared with the other adaptation conditions (not shown). We therefore focused accordingly on the short time scale 1 ms binned models (1 ms Type II Models) see **Figures 4**, **5**. We showed previously

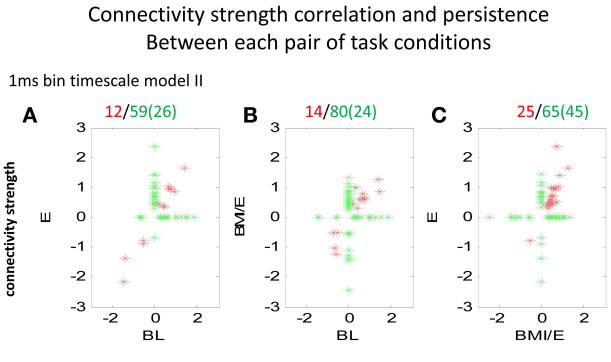

FIGURE 5 | Comparison of connectivity strengths between two conditions modeled at the 1 ms binned timescale. Each point marker in the figure denotes a significant parameter and connectivity strength from a pair of neurons, related between two conditions (**A** BL vs. E, **B** BL vs. BMI/E, and **C** E vs. BMI/E). The red markers denote that the connection between the neurons was significantly different from zero under both the two tested conditions, while the green marker denotes the connectivity of the neurons was only significantly different from zero under one condition (BL, E or BMI/E). The numbers in parentheses indicate number of cells on abscissa. Some of the network connectivity estimated with the GLM models shifted drastically, and much connectivity was unique to a given condition. However, there were also connections that persisted across conditions (red). These persistent connection strengths clearly also correlated. There were more connections in common between E and BMI/E in **C**, than in either **A** or in **B** when these were correlated to BL. Abbrevations: BL, baseline; E, elastic load; BMI/E, brain machine interface and elastic load.

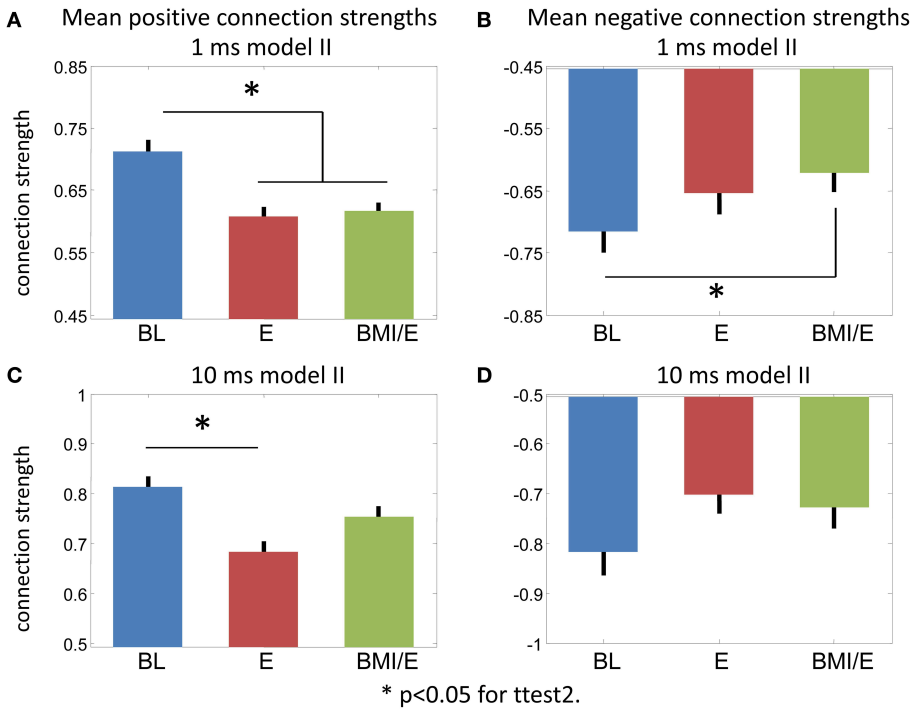

FIGURE 6 | Connection strength changes. (A,B) Model II 1 ms bin fitting. **(A)** Positive connections. **(B)** Negative connections. **(C,D)** Model II 10 ms bin fitting. (**C**) Positive connections. **(D)** Negative connections. * Significant difference in a two-tailed *t*-test, $p < 0.05$ (MATLAB ttest2).

that rats in BMI/E offset load while preserving more normal pelvic height compared to load alone (Song and Giszter, 2011). When rats experiencing applied loads in BMI/E were able to significantly offset these with the BMI, the network increased the number of connections during simple elastic load and BMI with elastic load conditions compared to baseline (e.g., see **Figures 4**, **5**). Further, significantly more of (almost double the number of) the connections persisted in the transition from Elastic load (E) to BMI (BMI/E) (∼27% in **Figure 5C**) compared to those that seen were in common between Baseline and Elastic load (∼16% in **Figure 5A**) or Baseline and BMI (∼15% in **Figure 5B**). This statistically significant difference in common or shared functional connectivity was biased to positive connections between the two load conditions (**Figure 5C**, one red negative connection in common out of 25 total common), but instead included higher fractions of negative parameter connections when examining connections that were in common with baseline patterns of firing (**Figure 5A**, 4/12 negative, and **Figure 5B**, 4/14).

While the numbers of significant connections increased in the E and BMI/E conditions, the mean parameter strengths also altered. **Figure 6** shows the average positive and negative connection strengths tended in 1 ms and 10 ms models. In keeping with the other results the 10 ms binning results had weaker significance, only differing in positive connection strength between BL and BMI/E. In the 1 ms models, all connection strengths tended to decrease in the loading conditions compared to baseline. These changes were significant for the positive connections in both load conditions in the 1 ms models, and for negative connections compared to BMI/E.

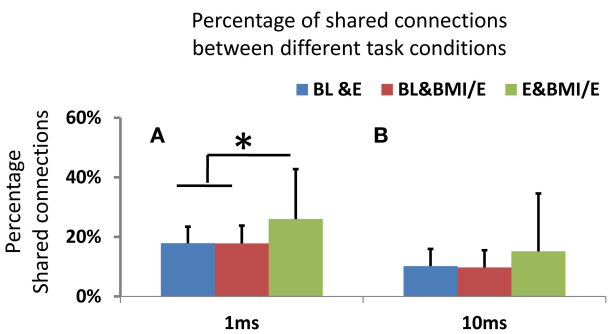

FIGURE 7 | (A) Shared connections between networks obtained in two conditions. On either 1 ms or 10 ms binned timescales, the networks showed significant differences in connectivity between two conditions (BL vs. E, BL vs. BMI/E and E vs. BMI/E) using binomial tests. However, with 1 ms binning there was a significantly higher percentage of shared connection between E and BMI/E than that between any other two conditions (BL vs. E and BL vs. BMI/E, indicated by *, two-tailed *t*-test, $p < 0.05$, MATLAB ttest2). **(B)** For the 10 ms binning there was no significant difference in the percentage of shared connection between any paired conditions, and percentages of shared connections were significantly lower than in the 1 ms binned GLM models.

By using functional connection density measures and the shared significant parameters as functional connectivity links or edges in a network graph, we compared the network structure changes. We found the number of common connections was significantly decreased in 10 ms timescale binned models compared to 1 ms binned (signed rank test, $p < 0.05$, MATLAB signrank), as in **Figure 7**. These differences held across baseline

condition and elastic load condition as well as BMI with elastic load. All the networks built from the GLM models showed low numbers of shared connections (15–27%) and significant differences (assessed by signed rank tests, $p < 0.05$) between any two conditions. However, there were significantly higher percentages of shared connections between the two load adaptation conditions of E and BMI/E than between either of the load with baseline condition shared connections (BL vs. E and BL vs. BMI/E) (**Figure 7A**). The percentage of shared connections between any two compared conditions was also higher in 1 ms binning than in 10 ms binning **Figure 7B**. These differences among conditions could not be explained by drift over time in BL e.g., in **Figure 3A**. This suggests that the active cortical circuitry may in part share a similar common structure when rats adapt under simple elastic load and under BMI with elastic load, and may also indicate that apart from rate encoding (analyzing in long timescale), short term temporal coding (binary spike timing) might capture some information regarding these changes on a short timescale.

In conclusion, the fitted GLM model networks of cells here showed significantly different connection maps between each task condition potentially indicating significant changes in network dynamics with task conditions. A core of between 15 and 27% of network connections were in common between the individual conditions and over time, but this common core differed in extent between different task condition pairs, with baseline having less in common with the other two (loaded) conditions.

Discussion

Using the methods here we previously demonstrated that rats could adapt their locomotion under a simple elastic load and use a BMI to offset elastic load effectively (Song and Giszter, 2011). GLM methods allow us to move beyond our prior analyses of coarse firing rate in this paradigm, and to add point process assessments of functional connectivity. The differences in BMI adaptation compared to the simple load in this paradigm involve alterations in firing patterns and rate based correlation relationships among neurons, but not average firing rates compared with elastic loading (Song and Giszter, 2011). We previously also observed changes in the number of force-related neurons, their peak firing and burst activity timing in the step cycle (Song and Giszter, 2011). Here we focus on neuron-neuron interactions at the point process level among neurons all collected within a range of from 30 to 200 microns of each other in each rats, with the recording electrode sites around layer V of motor cortex. These spatially close neurons may potentially have different connectivity from spatially further separated neurons. As we have noted above the functional connectivity we uncovered here represents the most parsimonious account of apparent neural influences among one another based on the observed neurons and firing. Because not all neurons are observed the account is necessarily incomplete and may infer Granger type causal links where none exist in the complete biology. However, subject to these limitations the functional connectivity may give descriptive insights into aspects of network operation. By using a GLM approach here, our data analysis has shown some features of cortical network dynamics occuring at different timescales during these adaptation processes. The neural network in hindlimb/trunk cortex of rats as assessed here in a subset of well-predicted neurons was extensively reorganized in terms of GLM parameters during adaptation to different elastic load force fields applied in locomotion. The networks shared more common connections between elastic load and BMI with elastic load condition than between any other condition pairs. More of the functional network connections identified were preserved during transitions from elastic load (E) to BMI with elastic load (BMI/E) conditions, than from baseline (BL) to elastic load (E). However, only about 15% of the total population of non-Poisson firing cells were well-predicted by our models here, which were built without reference to the external covariates, and relied only on strong neural firing pattern interactions. Internal network functional connectivity and the network based firing predictions that are presented here thus represent an account of a small fraction of the patterned activity. The models thus also indicate a high degree of independence of much of the non-Poisson pattern firing cells from one another from the GLM modeling perspective, consistent with classical rate coding models and other frameworks (e.g., Sanger, 2010, 2011, 2014). Likely the strongest predictive relationships of the firing of the 85% of unfitted non-Poisson cells will be with external covariates and tuning to feedback or motor drive. Nonetheless, the internal relationships revealed here in well-predicted cells provide some insights into the adaptation processes and dynamics in augmenting BMIs (see Omar et al., 2011). Unfortunately, low firing rates for the GLM point process models preclude a confident analysis of the activity changes within a condition as was possible with other methods in Song and Giszter (2011). The relations of the local activity relations here to overall function of the network remain to be determined.

It should be noted that caution should be taken when interpreting the results from model dynamics and differences here. As noted, the model did not consider external covariates, which might have made a strong contribution to the models. This was especially likely during elastic load conditions, and some if not most of the non-Poisson firing pattern cells that were not well fitted with neural interactions alone can be expected to be captured with inclusion of these. Whether and how the sparse connections identified here are correlated with the overall network states across tasks still needed to be tested. On the other hand, as the model here used spiking history of the ensemble of recorded cells, any effects of the potential external covariates on the well-predicted subset of cells analyzed here might be captured by its being filtered and incorporated into the history effects of connected neighboring neurons in the ensemble. The lagged parameters of GLM models of cells here generally had a more significant effect on a current cells' firing than any immediate (1 ms) synchronization to other firing cells consistent with causal interactions but no proof of such. Similiarly, it should be made clear that the connections or functional connectivity maps constructed here were derived only from "apparent pattern" among neural spikes. There might be no anatomical connectivity representing the apparent connections in the cortical network.

The differences between open loop BMIs, BMIs with feedback, physically connected BMIs and differences between BMIs for proximal locomotion vs. distal reaching are not well understood (Acharya et al., 2008; Héliot et al., 2010). However, these different effects may become crucial as various feedback based BMIs are introduced and the range and scope of available BMI prosthetic devices increases (Moritz et al., 2008; Velliste et al., 2008; Fitzsimmons et al., 2009; Héliot et al., 2010; Song and Giszter, 2011). Recent studies showed that cells in the primary motor cortex demonstrated learning related plasticity during force field adaptation (Li et al., 2001). A stable relation between neural activity and behavior can clearly be established in well trained animals (Serruya et al., 2003; Greenberg and Wilson, 2004; Chestek et al., 2007). Moritz et al. demonstrated that individual cells can be rate modulated to control Functional Electrical Stimulation (Moritz et al., 2008). Variations of neural firing patterns in such differing adaptation processes are not well understood at this point. For example, patterns might differ between novel voluntary tasks and more vegetative "built-in" or evolutionarily older tasks, such as locomotion. The rats adapted to load (E) and adapted differently to load with BMI (BMI/E). The identified *functional* cortical network parameters, as identified from firing observed here, also clearly adapted, and reorganized differently between conditions. Previously we found the distribution of average firing rate from the whole population was not significantly different among conditions (Song and Giszter, 2011). The numbers of significantly connected cells here were not significantly different across conditions in these models either. However, the numbers of common connections over time were higher in BMI with elastic load than baseline, and the network connection density even decreased during elastic loading alone when the rat adapted from the first half trial to the second half trial. Zacksenhouse et al. showed that there are firing rate changes in the early sessions with other BMI experiments (Zacksenhouse et al., 2007), and we found here that 70–85% of the sparse network connections of well-predicted cells reorganized depending on the context. For elastic load locomotion, the connection density actually decreased here, while in the BMI with elastic load locomotion condition, the network as modeled kept the same connection density. Similar results were obtained examining more standard long timescale correlations of rate in our earlier study (Song and Giszter, 2011). These data might indicate that rats need a longer time to achieve a stable and perhaps more sparse network state during BMI/E adaptation and conditioning when they were tested in our experimental framework, compared to the simpler elastic loaded locomotion. This evolving relationship between cortical firing effects, network changes, and feedback and relationships with external covariates is an area of active investigation using this framework.

When animals adapt their locomotion to new environments, initially cortex becomes more activated and engaged. Engaging a BMI in this period appears to alter and at least briefly stabilize the changes involved in the adaptation of neural network structure. Despite the small role of cortex in the normal locomotion of rats, cortex can be engaged in a BMI augmenting locomotion during this adaptation. Our data thus supports the idea that activity normally involved in monitoring, or simply correlated with and not normally driving a behavior in an animal, can nonetheless be engaged through motor adaptation processes to drive behavior in a BMI. Neuron-neuron correlation metrics estimated here provide one set of measures on the changes in neural dynamics on short adaptation time scales that can be extended to high practice use of BMIs and load adaptation over time, to examine transitions from naive user of an augmenting BMI to an expert practiced user. Understanding the patterns of these adaptive changes better may be essential in order to provide prostheses that can integrate seamlessly with normal motor adaptations and feedback effects across the many contexts encountered in activities of daily living.

Acknowledgments

This work was supported by NIH NS54894 to SG, NIH 1F31NS058275-01A2 to IC, NIH DP1 OD003646 to EB and R01 GM104948 to EB.

References

Acharya, S., Tenore, F., Aggarwal, V., Etienne-Cummings, R., Schieber, M. H., and Thakor, N. V. (2008). Decoding individuated finger movements using volume-constrained neuronal ensembles in the M1 hand area. *IEEE Trans. Neural Syst. Rehabil. Eng.* 16, 15–23. doi: 10.1109/TNSRE.2007.916269

Alam, M., Chen, X., Zhang, Z., Li, Y., and He, J. (2014). A brain-machine-muscle interface for restoring hindlimb locomotion after complete spinal transection in rats. *PLoS ONE* 9:e103764. doi: 10.1371/journal.pone.0103764

Brown, E. N., Barbieri, R., Ventura, V., Kass, R. E., and Frank, L. M. (2002). The time-rescaling theorem and its application to neural spike train data analysis. *Neural Comput.* 14, 325–346. doi: 10.1162/08997660252741149

Brown, E. N., Kass, R. E., and Mitra, P. P. (2004). Multiple neural spike train data analysis: state-of-the-art and future challenges. *Nat. Neurosci.* 7, 456–461. doi: 10.1038/nn1228

Cajigas, I., Malik, W. Q., and Brown, E. N. (2012). nSTAT: open-source neural spike train analysis toolbox for Matlab. *J. Neurosci. Methods* 211, 245–264. doi: 10.1016/j.jneumeth.2012.08.009

Carmena, J. M., Lebedev, M. A., Henriquez, C. S., and Nicolelis, M. A. L. (2005). Stable ensemble performance with single-neuron variability during reaching movements in primates. *J. Neurosci.* 25, 10172–10176. doi: 10.1523/JNEUROSCI.2772-05.2005

Chapin, J. K., Markowitz, R. A., Moxon, K. A., and Nicolelis, M. A. L. (1999). Direct real-time control of a robot arm using signals derived from neuronal population recordings in motor cortex. *Nat. Neurosci.* 2, 664–670. doi: 10.1038/10223

Chestek, C. A., Batista, A. P., Santhanam, G., Yu, B. M., Afshar, A., Cunningham, J. P., et al. (2007). Single-neuron stability during repeated reaching in macaque premotor cortex. *J. Neurosci.* 27, 10742–10750. doi: 10.1523/JNEUROSCI.0959-07.2007

Daley, D., and Vere-Jones, D. (2003). *An Introduction to the Theory of Point Processes.* New York, NY: Springer-Verlag.

Fitzsimmons, N. A., Lebedev, M. A., Peikon, I. D., and Nicolelis, M. A. L. (2009). Extracting kinematic parameters for monkey bipedal walking from cortical neuronal ensemble activity. *Front. Integr. Neurosci.* 3, 1–19. doi: 10.3389/neuro.07.003.2009

Ganguly, K., and Carmena, J. M. (2009). Emergence of a stable cortical map for neuroprosthetic control. *PLoS Biol.* 7:e1000153. doi: 10.1371/journal.pbio.1000153

Gerhard, F., Pipa, G., Lima, B., Neuenschwander, S., and Gerstner, W. (2011). Extraction of network topology from multi-electrode recordings: is there a small-world effect? *Front. Comput. Neurosci.* 5:4. doi: 10.3389/fncom.2011.00004

Gerstein, G. L., and Perkel, D. H. (1969). Simultaneously recorded trains of action potentials: analysis and functional interpretation. *Science* 164, 828–830. doi: 10.1126/science.164.3881.828

Greenberg, P. A., and Wilson, F. A. W. (2004). Functional stability of dorsolateral prefrontal neurons. *J. Neurophysiol.* 92, 1042–1055. doi: 10.1152/jn.00062.2004

Héliot, R., Ganguly, K., Jimenez, J., and Carmena, J. M. (2010). Learning in closed-loop brain-machine interfaces: modeling and experimental validation. *IEEE Trans. Syst. Man Cybern. B Cybern.* 99, 1–11. doi: 10.1109/TSMCB.2009.2036931

Hochberg, L. R., Serruya, M. D., Friehs, G. M., Mukand, J. A., Saleh, M., Caplan, A. H., et al. (2006). Neuronal ensemble control of prosthetic devices by a human with tetraplegia. *Nature* 442, 164–171. doi: 10.1038/nature04970

Jackson, A., Mavoori, J., and Fetz, E. E. (2006). Long-term motor cortex plasticity induced by an electronic neural implant. *Nature* 444, 56–60. doi: 10.1038/nature05226

Jarosiewicz, B., Chase, S. M., Fraser, G. W., Velliste, M., Kass, R. E., and Schwartz, A. B. (2008). Functional network reorganization during learning in a brain-computer interface paradigm. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19486–19491. doi: 10.1073/pnas.0808113105

Jarvis, M. R., and Mitra, P. P. (2001). Sampling properties of the spectrum and coherency in sequences of action potentials. *Neural Comput.* 13, 717–749. doi: 10.1162/089976601300014312

Kositsky, M., Chiappalone, M., Alford, S. T., and Mussa-Ivaldi, F. A. (2009). Brain-machine interactions for assessing the dynamics of neural systems. *Front. Neurorobot.* 3:1. doi: 10.3389/neuro.12.001.2009

Li, C. S., Padoa-Schioppa, C., and Bizzi, E. (2001). Neuronal correlates of motor performance and motor learning in the primary motor cortex of monkeys adapting to an external force field. *Neuron* 30, 594–607. doi: 10.1016/S0896-6273(01)00301-4

Manohar, A., Flint, R. D., Knudsen, E., and Moxon, K. A. (2012). Decoding hindlimb movement for a brain machine interface after a complete spinal transection. *PLoS ONE* 7:e52173. doi: 10.1371/journal.pone.0052173

McCullagh, P., and Nelder, J. (1989). *Generalized Linear Models, 2nd Edn*. Boca Raton, FL: Chapman and Hall/CRC. doi: 10.1007/978-1-4899-3242-6

Moritz, C. T., Perlmutter, S. I., and Fetz, E. E. (2008). Direct control of paralyzed muscles by cortical neurons. *Nature* 456, 639–642. doi: 10.1038/nature07418

Okatan, M., Wilson, M. A., and Brown, E. N. (2005). Analyzing functional connectivity using a network likelihood model of ensemble neural spiking activity. *Neural Comput.* 17, 1927–1961. doi: 10.1162/0899766054322973

Omar, C., Akce, A., Johnson, M., Bretl, T., Ma, R., Maclin, E., et al. (2011). A feedback information-theoretic approach to the design of brain-computer interfaces. *Int. J. Hum. Comput. Interact.* 27, 5–23. doi: 10.1080/10447318.2011.535749

Rokni, U., Richardson, A. G., Bizzi, E., and Seung, H. S. (2007). Motor learning with unstable neural representations. *Neuron* 54, 653–666. doi: 10.1016/j.neuron.2007.04.030

Sanger, T. D. (2010). Neuro-mechanical control using differential stochastic operators. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2010, 4494–4497. doi: 10.1109/IEMBS.2010.5626029

Sanger, T. D. (2011). Distributed control of uncertain systems using superpositions of linear operators. *Neural Comput.* 23, 1911–1934. doi: 10.1162/NECO_a_00151

Sanger, T. D. (2014). Risk-Aware Control. *Neural Comput.* 26, 2669–2691. doi: 10.1162/NECO_a_00662

Schneidman, E., Berry, M. J. II, Segev, R., and Bialek, W. (2006). Weak pairwise correlations imply strongly correlated network states in a neural population. *Nature* 440, 1007–1012. doi: 10.1038/nature04701

Serruya, M., Hatsopoulos, N., Fellows, M., Paninshki, L., and Donoghue, J. P. (2003). Robustness of neuroprosthetic decoding algorithms. *Biol. Cybern.* 88, 219–228. doi: 10.1007/s00422-002-0374-6

Shigeyoshi, F. (2008). behavior-dependent short term assembly dynamics in the medial prefrontal cortex. *Nat. Neurosci.* 11, 823–833. doi: 10.1038/nn.2134

Shoham, S., Fellows, M. R., and Normann, R. A. (2003). Robust, automatic spike sorting using mixtures of multivariate t-distributions. *J. Neurosci. Methods* 127, 111–122. doi: 10.1016/S0165-0270(03)00120-1

Song, W. G., and Giszter, S. F. (2011). Adaptation to a cortex controlled robot attached at the pelvis and engaged during locomotion in rats. *J. Neurosci.* 31, 3110–3128. doi: 10.1523/JNEUROSCI.2335-10.2011

Song, W. G., Ramakrishnan, A., Udoekwere, U. I., and Giszter, S. F. (2009). Multiple types of movement-related information encoded in hindlimb/trunk cortex in rats and potentially available for brain–machine interface controls. *IEEE Trans. Biomed. Eng.* 56, 2712–2716. doi: 10.1109/TBME.2009.2026284

Stevenson, I. H., and Körding,. K. P. (2010). On the similarity of functional connectivity between neurons estimated across timescales *PLoS ONE* 5:e9206. doi: 10.1371/journal.pone.0009206

Stevenson, I. H., Rebesco, J. M., Hatsopoulos, N. G., Haga, Z., Miller, L. E., and Körding, K. P. (2009). Bayesian inference of functional connectivity and network structure from spikes. *IEEE Trans. Neural Syst. Rehabil. Eng.* 17, 203–213. doi: 10.1109/TNSRE.2008.2010471

Stevenson, I. H., Rebesco, J. M., Miller, L. E., and Körding, K. P. (2008). Inferring functional connections between neurons. *Curr. Opin. Neurobiol.* 18, 582–588. doi: 10.1016/j.conb.2008.11.005

Strong, S. P., Koberle, R., de Ruyter van Steveninck, R. R., and Bialek, W. (1998). Entropy and information in neural spike trains. *Phys. Rev. Lett.* 80, 197–200. doi: 10.1103/PhysRevLett.80.197

Truccolo, W., Eden, U. T., Fellows, M. R., Donoghue, J. P., and Brown, E. N. (2005). A point process framework for relating neural spiking activity to spiking history, neural ensemble, and extrinsic covariate effects. *J. Neurophysiol.* 93, 1074–1089. doi: 10.1152/jn.00697.2004

Truccolo, W., Hochberg, L. R., and Donoghue, J. P. (2010). Collective dynamics in human and monkey sensorimotor cortex: predicting single neuron spikes. *Nat. Neurosci.* 13, 105–111. doi: 10.1038/nn.2455

Velliste, M., Perel, S., Spalding, M. C., Whitford, A. S., and Schwartz, A. B. (2008). Cortical control of a prosthetic arm for self-feeding. *Nature* 453, 1098–1101. doi: 10.1038/nature06996

Zacksenhouse, M., Lebedev, M. A., Carmena, J. M., O'Doherty, J. E., Henriquez, C., and Necolelis, M. A. L. (2007). Cortical modulations increase in early sessions with brain-machine interface. *PLoS ONE* 7, 1–10. doi: 10.1371/journal.pone.0000619

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 20 May 2014
doi: 10.3389/fnsys.2014.00090

Pharmacological enhancement of memory or cognition in normal subjects

Gary Lynch[1,2]*, Conor D. Cox[2] and Christine M. Gall[2]

[1] Department of Psychiatry and Human Behavior, University of California, Irvine, CA, USA
[2] Department of Anatomy and Neurobiology, University of California, Irvine, CA, USA

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Ioan Opris, Wake Forest University, USA
Rafael Roesler, Federal University of Rio Grande do Sul, Brazil
Sam Deadwyler, Wake Forest University Health Sciences, USA
Maryam Farahmandfar, Tehran University of Medical Sciences, Iran

***Correspondence:**
Gary Lynch, Department of Psychiatry and Human Behavior, Gillespie Neuroscience Research Facility, University of California, 837 Health Science Road, Irvine, CA, 92697-1275, USA
e-mail: glynch@uci.edu

The possibility of expanding memory or cognitive capabilities above the levels in high functioning individuals is a topic of intense discussion among scientists and in society at large. The majority of animal studies use behavioral endpoint measures; this has produced valuable information but limited predictability for human outcomes. Accordingly, several groups are pursuing a complementary strategy with treatments targeting synaptic events associated with memory encoding or forebrain network operations. Transcription and translation figure prominently in substrate work directed at enhancement. Notably, the question of why new proteins would be needed for a now-forming memory given that learning-driven synthesis presumably occurred throughout the immediate past has been largely ignored. Despite this conceptual problem, and some controversy, recent studies have reinvigorated the idea that selective gene manipulation is a plausible route to enhancement. Efforts to improve memory by facilitating synaptic encoding of information have also progressed, in part due of breakthroughs on mechanisms that stabilize learning-related, long-term potentiation (LTP). These advances point to a reductionistic hypothesis for a diversity of experimental results on enhancement, and identify under-explored possibilities. Cognitive enhancement remains an elusive goal, in part due to the difficulty of defining the target. The popular view of cognition as a collection of definable computations seems to miss the fluid, integrative process experienced by high functioning individuals. The neurobiological approach obviates these psychological issues to directly test the consequences of improving throughput in networks underlying higher order behaviors. The few relevant studies testing drugs that selectively promote excitatory transmission indicate that it is possible to expand cortical networks engaged by complex tasks and that this is accompanied by capabilities not found in normal animals.

Keywords: cognitive enhancement, learning, long term potentiation, ampakine, synaptic plasticity, BDNF, F-actin, positive AMPA receptor modulators

INTRODUCTION

The present review concerns three topics, two of which involve terms—enhancement and cognition—that are not sharply defined. Usage of the former seems straightforward when applied to memory, although it is often unclear whether accelerated acquisition or an increase in encoding strength is intended. But applied to cognition, claims for enhancement face the great problem of how to quantify something for which there is no consensus measurement system. The difficulty can be reduced by focusing on cognitive activities of a type that can be described in computational terms. This, however, raises questions about the extent to which the sampled process is representative, or a major component, of cognition as the term is typically used. In response, it could reasonably be argued that cognition is a collection of semi-independent operations (e.g., categorization, value assignment) (Sugrue et al., 2005; Tsunada and Sawaguchi, 2012) but this seems unsatisfactory because the phenomenon is experienced as being, if not unitary, then at least strongly coherent. Electrophysiological and brain imaging results showing coordinated activity across broad stretches of neocortex provide some support for the idea of a system that, while capable of periodically focusing on specific tasks, usually works by integrating a vast amount of disparate material into a product accessible to consciousness. A true cognitive enhancer might therefore take the form of a treatment that increases the speed or capacity of this assembly process.

Memory enhancement, as suggested, appears to be a much more tractable problem. Retention is easily measured as is the amount of training needed to produce a given score in a test subsequent to learning. But a curious problem emerges here: few of the many pharmacological agents that produce robust enhancement of memory in animals are found to have positive effects in humans. This observation has become the subject of intense public discussion, perhaps with growing skepticism about the utility of animal studies on memory enhancement. Some neuroscientists have argued that the "failure to predict" problem reflects the widespread use of paradigms that have little relevance to human learning. These workers have devised ingenious protocols that can be used in rodents and with minor modifications in humans (e.g., Bari et al., 2008; Demeter et al., 2008; Eichenbaum and Robitsek, 2009; Zeeb et al., 2009; Demeter and Sarter, 2013). There is every

reason to assume that these efforts will ultimately narrow the gap in cross-species comparisons. But there is a more fundamental issue from comparative biology that could underlie the failure-to-predict problem: humans are enormously encephalized animals and rodents aren't (neocortex makes up at least 77% of brain volume in human and just 31% in rat; Stephan et al., 1981; Swanson, 1995). Encephalization is hypothesized to result in a shift of functions from lower brain to cortex; from this perspective, humans may be using networks of a very different kind than those employed by rodents to solve similar problems.

An alternative to behaviorally based approaches to developing enhancers would be to focus on the neurobiological substrates of memory and cognition. This seems feasible in the case of memory because of the tremendous progress that has been made in identifying synaptic mechanisms that encode information. There is no good reason to think that these processes differ significantly between mammalian species and indeed comparative studies suggest that certain essential elements are evolutionarily ancient (Crystal and Glanzman, 2013). It follows from this that treatments acting on memory substrates in rodents are likely to have similar actions in human brain. Cognition again represents a much more challenging problem. However, the universally held assumption that cognitive operations arise from the transient formation of telencephalic networks points to a relatively simple idea for enhancement. Communication within and between cortical regions is mediated by glutamatergic transmission; if so, then agents that augment the release of glutamate, or the post-synaptic response to it, should facilitate the formation of cognition's substrates.

The following sections consider attempts to develop enhancers via actions on (i) different aspects of the complex machinery underlying learning-related synaptic modifications, or (ii) communication within and between cortical networks.

MEMORY ENHANCEMENT

Most research on memory enhancement deals with psychological events that precede the actual encoding of information. There is for example a very large literature describing attempts, typically using chemical agents, to increase the speed of learning by modulating arousal and attention (Lynch et al., 2011). It has become common to refer to resultant improvements as cognitive enhancement, presumably because key elements of cognition are being manipulated, but there are reasons to question this assumption (see below). There is a smaller, but rapidly growing, body of work directed at the machinery responsible for converting patterns of afferent activity into the long lasting increases in synaptic strength assumed to encode specific information. This section evaluates the latter material.

GENE EXPRESSION AND PROTEIN SYNTHESIS

Work in this area begins with the hypothesis that learning triggers the transcription or local translation of proteins that serve to consolidate the newly acquired memories, something that can take anywhere from many minutes to hours. Compounds that facilitate production of the pertinent RNAs or proteins could accordingly increase the likelihood that recent learning will lead to stable memory, and there are many reports of such effects (Guzowski et al., 2001; Plath et al., 2006; Katche et al., 2010, 2012). However, the basic idea that new protein synthesis is critical to memory formation has been controversial since its introduction more than 50 years ago (Abraham and Williams, 2008; Gold, 2008). Much of the dispute revolves around the necessary prediction that protein synthesis inhibitors will selectively block recently acquired memory; most papers report this result but others do not, or argue that observed disruptions to encoding are due to factors unrelated to synthesis (Routtenberg, 2008; Gold and Wrenn, 2012).

Beyond this, the protein synthesis argument faces certain conceptual problems. Learning is a continuous process in humans, and likely other mammals, with new encoding occurring many times a minute, as is evident with episodic memory. People recognize or recall a remarkable number of serial events when queried after a 90 min movie. Unless we make the very unlikely assumption that each item of information is encoded on a different neuron, it is difficult to see why, after hours of producing proteins needed for consolidation, a given cell would need further synthesis to stabilize a now forming memory. Along this line, it has been argued that animals exposed to an enriched environment which would entail constitutively elevated basal activity, and thus activity-driven protein synthesis, may not require additional synthesis to support LTP (Abraham and Williams, 2008) and the related encoding of hippocampus-dependent memories. There is, however, a special case in which transcription and/or broadly distributed translation could be required to securely encode a specific memory; namely, a circumstance in which continuous learning of similar material does *not* precede the new instance. Under these conditions, consolidation could depend upon proteins generated by the isolated learning episode. Note that this scenario loosely describes the great majority of animal studies testing for the contributions of protein synthesis. Certain of these arguments make relatively straightforward, readily tested predictions. For example, animals with a well-developed learning set could be given protein synthesis inhibitors after learning a single problem with or without having dealt with many such problems in the preceding hours. Such a paradigm can be achieved for rats using two-odor discriminations. If continual learning obviates the need for problem-specific synthesis, then the blockers should have no effect in a group given many trials prior to being introduced to the new test items.

There is a variant of the translation hypothesis that addresses the problem of why prior synthesis doesn't provide a sufficient supply of proteins for current learning. This involves the ample evidence for dendritic (local) translation from already in place mRNAs. One could posit a set of conditions in which new synthesis, even after recent experience, needs to occur post-acquisition for transfer into long-term storage; e.g., (1) translation occurs within very small dendritic compartments; (2) such active regions are only found in the immediate vicinity of recently modified synapses; and (3) newly formed proteins do not diffuse to any great degree. These circumstances would reduce the probability that proteins from earlier learning would be present at the large majority of current sites. But "synaptic tagging" experiments, conducted for instances where LTP in hippocampal slices is blocked by protein synthesis inhibitors, describe results that are not consistent with these postulates. Specifically, LTP induction at

one input protects subsequently induced potentiation at a second input to the same region from the effects of the inhibitor (Frey and Morris, 1997; Shires et al., 2012). Given the small number of synapses that generate EPSPs of conventional amplitudes, it is extremely likely that connections from the two inputs are, for the most part, located on different dendritic segments. It follows then that proteins from the first episode must have been synthesized, or traveled, throughout much of the dendritic arborization, a point that is reinforced by evidence for tagging in the apical dendrites after stimulation of basal afferents (Alarcon et al., 2006). It will be noted that these findings align with the broad idea that continual learning maintains relevant proteins at levels sufficient for LTP-related plasticity, obviating the need for synthesis after individual learning events.

The above discussion concerns interpretative issues rather than the likelihood of achieving enhancement using the transcription / translation strategy. It may well be the case that increasing within-cell levels of proteins that support consolidation reduces the requirements for encoding persistent memories and/or increases their stability. Signaling from synapses to the nucleus or to local protein synthesis machinery involves many steps and so is likely to be a variable and somewhat uncertain process. It would not be surprising, then, if the ongoing production of memory-related elements operates at a less than optimal rate even in high performing, normal subjects. In line with this, there are multiple demonstrations that treatment with compounds that inhibit particular histone deacetylases, leading to increased transcription of select gene families, can markedly enhance memory after single training sessions (Stefanko et al., 2009; McQuown et al., 2011). Also of interest are the numerous studies showing that selective phosphodiesterase-4 inhibitors have potent enhancing effects on memory. Inhibitors of this class (e.g., Rolipram), drive the protein kinase A—CREB transcription pathway implicated in learning in a broad array of animals (including invertebrates), and so is argued to be a very ancient, evolutionarily conserved memory substrate (Tully et al., 2003; Normann and Berger, 2008). Evidence that the same results obtain after extensive experience with similar problems in the recent past, and presumably a great deal of learning-driven transcription, would constitute support for there being less than optimal production of proteins needed for encoding under normal circumstances. This would certainly encourage the idea that enhanced protein synthesis is a viable route to augmented memory.

SYNAPTIC PLASTICITY AND MEMORY ENHANCEMENT

Most mechanism-based efforts directed at improving memory have focused on synaptic plasticity and in particular the long term potentiation (LTP) effect. Researchers since the late 19th century have argued that the enormous capacity of memory is best explained by assuming that physical encoding of new information occurs at small numbers of connections between neurons. The discovery of LTP demonstrated that individual synapses in the cortical telencephalon do in fact possess the properties expected for a memory substrate (Bliss and Collingridge, 1993; Lynch, 1998, 2004b; Morris, 2003). The increase in transmission strength (magnitude of EPSCs) develops quickly, persists for a remarkable period (weeks at least) (Staubli and Lynch, 1987; Abraham, 2003), and does not disturb already potentiated contacts as likely required for a high capacity memory system. A very large body of experimental work has confirmed the tight connection between LTP and diverse instances of memory (e.g., Roman et al., 1987; Rioult-Pedotti et al., 2000; Whitlock et al., 2006). Moreover, LTP is intimately related to the theta rhythm, an oscillation long associated with learning (Buzsaki, 2005; Vertes, 2005; Snider et al., 2013); i.e., five brief (30 ms) bursts of high frequency stimulation pulses (a pattern that mimics "theta bursting" during learning) prove to be near optimal for inducing extremely stable LTP but only when separated by the period of the theta wave (Larson et al., 1986; Capocchi et al., 1992). The reasons for this have been identified (**Figure 1**).

These observations suggest the possibility of enhancing learning with drugs that promote theta activity and correlated bursts of high frequency discharges. Agents such as physostigmine, that facilitate central cholinergic transmission, promote the theta rhythm (Olpe et al., 1987; Hasselmo, 2006) and are reported to improve learning scores in certain experimental situations. Notably, drugs of this type are among the few treatments approved for Alzheimer's Disease (Clarke and Francis, 2005; Noetzli and Eap, 2013). However, cholinergic systems perform varied functions in brain, some of which are homeostatic in nature. This likely explains why drugs targeting cholinergic mechanisms have not gained widespread acceptance as plausible enhancers. Another approach based on theta activity involves the large hyperpolarizing potentials triggered within target neurons by the short train of theta bursts used to induce LTP. These after-hyperpolarizing potentials (AHPs), set in motion by cell discharges, persist throughout the duration of the theta train and serve to counteract the depolarization needed to unblock the voltage dependent, synaptic NMDA receptors. Influx of calcium through these receptors, followed by release of the cation from intracellular stores, triggers the chain of events leading to potentiation (**Figure 1**). AHPs are mediated by a set of voltage- and calcium-sensitive potassium channels, prominent among which is the SK3 channel (Hosseini et al., 2001). The bee toxin apamin blocks this channel with some selectivity and, as predicted, augments post-synaptic responses to theta burst trains; this results in a striking increase in the magnitude of LTP (Kramar et al., 2004). While a number of studies have found substantial improvements in rodent learning with apamin treatment (Ikonen and Riekkinen, 1999; Brennan et al., 2008; Vick et al., 2010), this is not a likely enhancer because of toxicology issues. But given increasing interest in applications of channel blockers for diverse clinical problems, the apamin results suggest an intriguing mechanistic target for the development of enhancers. It is of note in this regard that Brain Derived Neurotrophic Factor (BDNF), which appears to be released from terminals by theta bursts (Balkowiec and Katz, 2000; Chen et al., 2010b), also reduces AHPs at least in rats (Kramar et al., 2004). Elevating endogenous levels of this neurotrophin, which can be achieved by pharmacological manipulations described later, thus provides another avenue for enhancement.

Identification of the initial triggers for LTP, as schematized in **Figure 1**, pointed to NMDA receptor-mediated calcium influxes as a logical target for enhancement. The existence of multiple

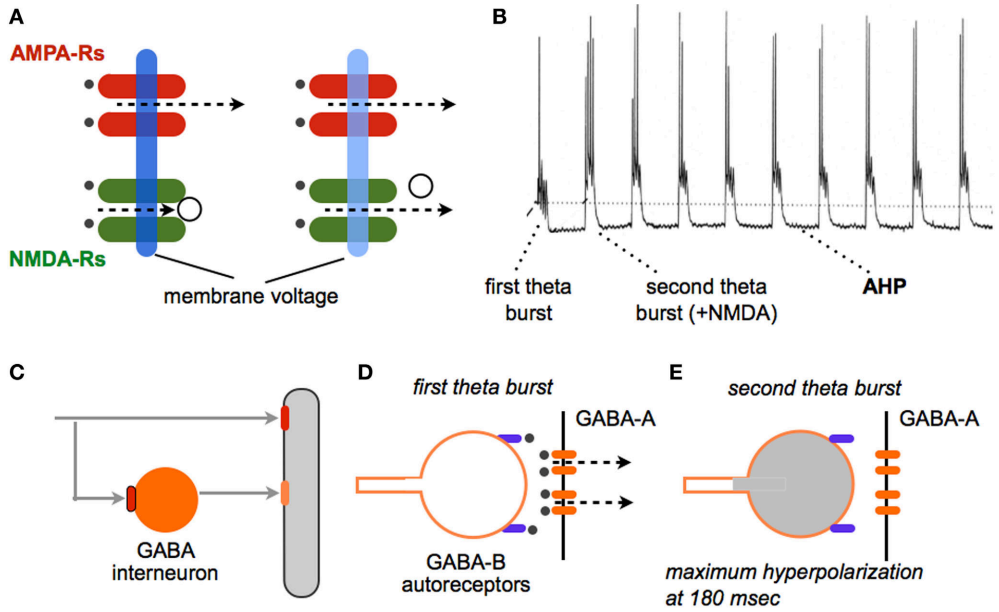

FIGURE 1 | Why theta burst stimulation (TBS) is so effective at inducing LTP. TBS (Larson et al., 1986) mimics a firing pattern found in cortical neurons during learning (Otto et al., 1991) and elicits a robust, non-decremental LTP that persists for weeks (at least). **(A)** (left side). A single stimulation pulse releases glutamate (black dots) and a partial membrane depolarization via current flux through AMPA receptors (dotted line). NMDA receptors do not open because of voltage dependent block of the ion channel (open circle). (right side) Trains of high frequency stimulation cause a greater depolarization (light blue) that removes the channel block and thereby allows current flow through calcium permeant NMDA receptors. Calcium is the initial trigger for LTP. **(B)** Intracellular recording shows that the first theta burst (four pulses at 100 Hz) in a train causes a relatively modest depolarization accompanied by a single spike; NMDA receptors make a very small contribution to this response. A second burst administered after a delay corresponding to the period of the theta wave produces a more profound depolarization with multiple spikes; this burst response contains a large NMDA receptor mediated component. Note that each theta burst in the train is followed by a large after-hyperpolarization (AHP). The AHP, which is largely mediated by calcium and voltage dependent potassium channels, tends to counteract the depolarization produced by the burst, thus capping the magnitude of NMDA receptor responses. **(C–E)** How the second and subsequent bursts generate large depolarization and unblock NMDA receptors. **(C)** A glutamatergic axon innervates a pyramidal cell dendrite (gray) and a feedforward, GABAergic interneuron (orange); note that *both* contacts use AMPA receptors (red). **(D)** A first theta burst triggers GABA release from the interneuron onto the pyramidal neuron thereby producing a di-synaptic (slightly delayed) IPSC via post-synaptic GABA-A receptors (orange ellipses); this shunts the EPSCs produced at neighboring glutamatergic synapses. The released GABA also binds to pre-synaptic, metabotrophic GABA-B auto-receptors on the releasing interneuron terminal (purple). **(E)** The auto-receptors hyperpolarize the GABAergic terminal and block release, an effect that reaches its maximum at the period of the theta wave. A theta burst arriving at this time point generates an excitatory response that is only weakly counteracted by the opening of post-synaptic GABA-A receptors **(**see **B)**.

modulatory sites (e.g., for glycine and polyamine) on the receptors suggested a plausible route for building positive allosteric drugs (Monaghan et al., 2012). Most of this effort has been directed toward treatments for neuropathology and psychiatric disorders, most notably schizophrenia and depression (Labrie and Roder, 2010; Dang et al., 2014), rather than memory enhancement. Perhaps the most widely studied agent of this type is D-cycloserine, a compound that targets the glycine binding pocket on the receptor and facilitates channel opening (Sheinin et al., 2001; Dravid et al., 2010). It has been known for some time that the site is important for induction of LTP (Oliver et al., 1990) and, as expected from this, D-cycloserine enhances various forms of memory in animals (Flood et al., 1992; Baxter et al., 1994; Tsai et al., 1999; Normann and Berger, 2008; Peters and De Vries, 2013). There is also evidence that the endogenous neurosteroid pregnenolone sulfate (Wu et al., 1991), and other steroid-like substances (Madau et al., 2009), promote the opening of NMDA receptors and facilitate both LTP and memory. Also of note, recent work led to discovery of a naturally occurring cholesterol metabolite that facilitates NMDA receptor currents through a novel oxysterol modulatory site and markedly increases the magnitude of LTP (Paul et al., 2013). The development of positive NMDA receptor modulators is clearly a promising area with regard to enhancement.

Increasing current flux through AMPA receptors results in greater post-synaptic depolarization and thereby promotes removal of the voltage block on NMDA receptors. This suggests that increasing AMPA receptor currents should facilitate the induction of LTP. Tests of this became possible with the invention of AMPA receptor modulators that freely enter the brain and increase fast glutamatergic transmission (Lynch, 2004a). The initial positive modulators were small benzamide compounds but subsequent work from many laboratories resulted in diverse families of compounds that slow deactivation or desensitization (or both) of ligand bound AMPA receptors. Here we will refer to all agents of this type by the term, "ampakines," used for the original compounds. Through a series of electrophysiological and X-ray crystallography studies, the mechanism of ampakine action

is now fairly well understood. As illustrated in **Figure 2**, each subunit of the tetrameric AMPA receptor has two large extracellular domains that form a "clamshell" that closes upon glutamate binding (Sun et al., 2002). Relaxation to the resting state, and transmitter release, terminates current flow; this process is referred to as "deactivation." The four subunits form two dimers, an arrangement that can be disrupted by ligand binding; under these conditions the channel closes but the transmitter is retained. This interesting, high affinity (slow dissociation constant) state constitutes the desensitized condition of the receptor (Hall et al., 1993). It was originally thought that desensitization is the normal route for terminating the EPSC but it now appears that deactivation is responsible for the decay rate of the synaptic response. The ampakine binding pocket is located at the dimer interface near the hinge of the clamshell (Jin et al., 2005); this strategic position explains how ampakines can affect both deactivation and desensitization (Arai et al., 1996) (**Figure 2**). Apparently, the orientation of the compounds within the pocket determines which of the two processes is most affected. There is overlap between AMPA and NMDA receptor pharmacology: compounds widely used to block the former also exhibit high affinity antagonism of the glycine modulatory site on the latter (Kessler et al., 1989). However, the ampakine pocket is distant to the extracellular domain of AMPA receptor antagonist binding and there is no evidence that these drugs affect NMDA receptor-gated currents.

Early work established that ampakines enhance both LTP and memory (Granger et al., 1993; Staubli et al., 1994), results that have been multiply replicated by different groups (Lynch, 2004a; Lynch and Gall, 2013). Versions of the drugs that simply slow deactivation lower the threshold for inducing LTP whereas those that affect both deactivation and desensitization also raise the ceiling on the degree of potentiation produced by theta bursts (Arai et al., 2002). By changing rate constants for both receptor inactivation processes, the latter compounds lead to much longer EPSCs and thus prolonged NMDA receptor-mediated calcium influxes. This presumably explains their greater potency. Surprisingly, there appear to have been no studies testing for

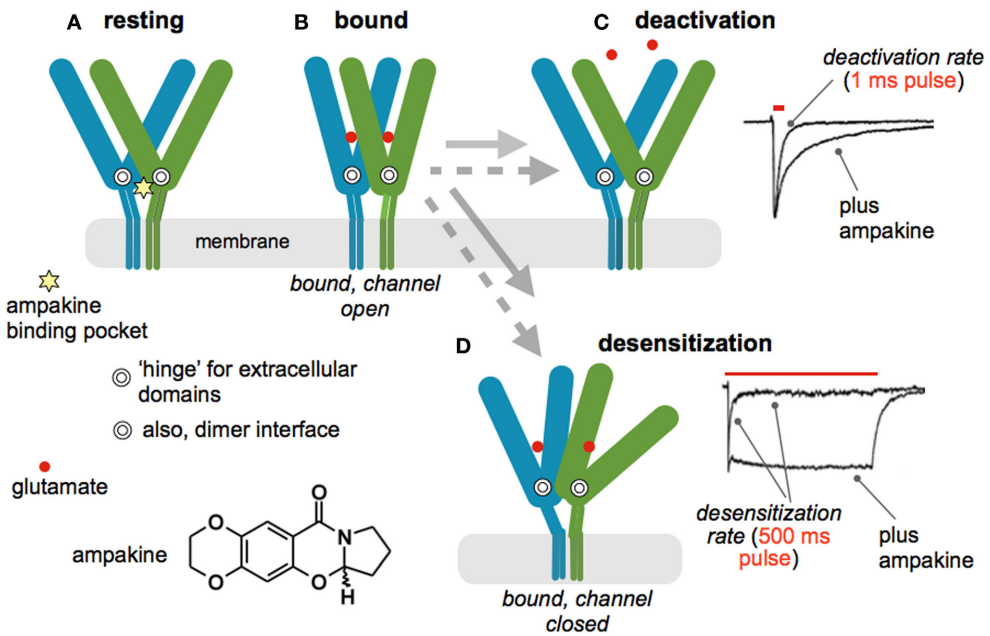

FIGURE 2 | Mode of action for positive allosteric modulators of AMPA receptors (ampakines). (A) Schematic shows two of the four subunits that comprise the AMPA receptor tetramer in the resting state; the C-tails are not included. Each subunit has two large extracellular domains that form a "clamshell" containing the glutamate binding site. The hinge of the structure is indicated by the double circle. The subunits dimerize at a zone close to the hinge. The ampakine pocket (star) is strategically located at the dimer interface adjacent to the two hinges. There are thus four neurotransmitter, and two ampakine, sites on the full AMPA receptor. **(B)** Glutamate binding is accompanied by a closing of each subunits' clamshell, resulting in opening of the ion channel and inward flux of current. The receptor then shifts into one of two configurations; the gray arrows denote the time required for the transitions in the presence (dotted) and absence (solid line) of an ampakine. **(C)** Normally, single transmission events are followed by opening of the extracellular domains and release of the transmitter, a process referred to as "deactivation." The upper trace to the right describes deactivation after a one ms pulse of glutamate to an excised patch: delivery of the ligand causes a sharp influx of current that decays after rapid washout. Bound ampakines slow reopening, resulting in a significant retardation of deactivation (bottom trace). **(D)** Prolonged stimulation of the receptor can disrupt the dimer configuration, leading to a condition in which transmitter remains bound but the ion channel returns to the closed state (desensitization). The upper trace to the right describes an instance of this in which glutamate was applied for 500 msec. An initial influx of current was followed by decay, despite continuing presence of the transmitter, to a steady state value about 1/10 of the peak flux. Ampakines stabilize the dimer configuration and, as predicted, greatly slow desensitization—current flow continues throughout the 500 ms application of glutamate. The receptor structural dynamics, including interactions with an ampakine, illustrated here are based on X-ray crystallography studies (Sun et al., 2002; Jin et al., 2005); physiological data are from patches taken from hippocampal slices (Arai et al., 1996, 2002; Arai and Lynch, 1998).

differential actions of the two functional classes of ampakine on learning.

Notably, the positive influence of acutely administered ampakines on memory are reported for aged and young animals (Granger et al., 1993, 1996; Shors et al., 1995) as well as for a broad array of species and learning tasks (Lynch, 2004a; Bernard et al., 2010). Very few effects in human have been published although one study using a short half-life, deactivation-only drug obtained evidence for memory enhancement in different tasks including ones involving complex processing (Ingvar et al., 1997).

LEARNING-RELATED SYNAPTIC MODIFICATIONS AS A TARGET FOR ENHANCEMENT STRATEGIES

The discovery of LTP (Bliss and Lomo, 1973) greatly simplified what had already been an extended search for the substrates of memory. An early and critical clue came with electron microscopic evidence that stable potentiation is accompanied by changes in the morphology of dendritic spines (Lee et al., 1979, 1980; Chang and Greenough, 1984), an observation recently and convincingly confirmed by live imaging experiments (Matsuzaki et al., 2004; Harvey and Svoboda, 2007; Kramar et al., 2012b). The initial studies also described results suggestive of an increase in synapse size and there are now data pointing to a similar effect after LTP (Chen et al., 2007) and learning (Fedulov et al., 2007). The observed anatomical restructuring implied that induction events for LTP or memory result in substantial alterations to the actin cytoskeleton. Tests of this, using a newly developed *in situ* method for labeling F-actin in hippocampal slices, found that theta bursts cause a dramatic increase in the number of spines with high concentrations of polymerized actin (Lin et al., 2005; Kramar et al., 2006). The newly formed filaments proved to be unstable for a period of 5–10 min, after which they were unaffected by depolymerizing agents (Rex et al., 2009, 2010). The experimental question then became one of how the very brief AMPA and NMDA receptor events that induce LTP lead to the formation of new actin cytoskeleton. Work using Fluorescence Deconvolution Tomography for assessing concentrations of activated signaling proteins at individual synapses, along with the use of selective inhibitors, identified multiple, GTPase-initiated signaling pathways involved in the assembly and stabilization of actin filament networks following theta burst stimulation (Kramar et al., 2009; Rex et al., 2009, 2010; Seese et al., 2012). Particularly relevant to the present topic, these studies also described membrane receptors that modulate the activity of cascades leading to the cytoskeletal reorganization required for consolidation of LTP (**Figure 3**).

Brief treatments with BDNF partially activate at least two of the signaling pathways shown in **Figure 3** and potently facilitate

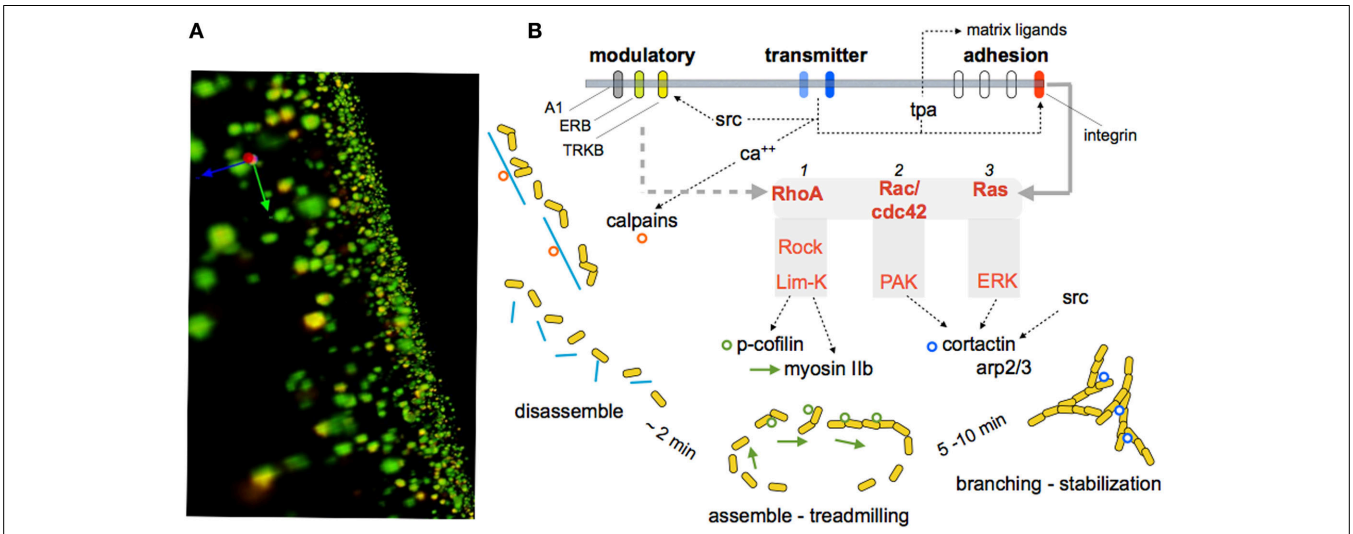

FIGURE 3 | Signaling events responsible for reorganizing the synaptic cytoskeleton and consolidating LTP. The substrate map for LTP stabilization is largely based on work using hippocampal slices, although some of the steps have been observed in learning studies. **(A)** Immunolabeled synapses surrounding the LTP site as reconstructed using Fluorescence Deconvolution Tomography (Seese et al., 2013): The green elements reflect immunostaining for PSD95, a protein that is evenly distributed within post-synaptic densities at excitatory (glutamatergic) synapses. Phosphorylated (inactivated) cofilin was immunolabeled with red fluorescence. Co-localization (p-Cofilin/PSD95) results in yellow labeling. The technique supports counts and size-measures for about 40,000 synapses per image z-stack and 160,000 synapses per slice, and calculates the number of these synaptic elements that are co-localized with the signaling protein of interest (p-Cofilin in this instance). These values are then compared for slices that did or did not receive theta burst simulation. **(B)** Schematic shows signaling pathways activated at excitatory synapses by theta burst stimulation. Transmitter receptors i) increase calcium which stimulates calpain, a spine protease (Perlmutter et al., 1988) that cleaves cross-linking proteins (blue lines) for the subsynaptic cytoskeleton, and ii) activates synaptic adhesion receptors belonging to the integrin family (Babayan et al., 2012). Integrins then engage at least two Rho family GTPases that promote the assembly of dynamic actin filaments (RhoA to cofilin and myosin) (Rex et al., 2009) and, over a period of several minutes, branching and stabilization of the reorganized cytoskeleton. The latter processes involve Rac/Cdc42 signaling to cortactin and ARP2/3. The synaptic membrane also contains receptors for the releasable factors adenosine, estrogen, and BDNF (A1, ERB, TrkB, respectively). These receptors positively and negatively (A1) influence the signaling pathways, probably at the level of the GTPases. Studies using neutralizing antisera, genetic manipulations, toxins, and enzyme blockers confirm certain key links in the model and show that disrupting these specific actin regulatory pathways blocks the consolidation, but not initial expression, of LTP.

both theta burst-driven actin polymerization and LTP (Chen et al., 2006; Rex et al., 2007). It seems likely that the LTP effects reflect both direct actions on the actin regulatory pathways and the above noted influence on AHPs generated during the theta stimulation trains (see above). Notably, scavenging extracellular BDNF blocks the stabilization of LTP produced by theta burst stimulation (Kovalchuk et al., 2002; Rex et al., 2007) as well as the associated activation of actin regulatory signaling and increases in spine F-actin (**Figure 4**); activity-induced release of the neurotrophic factor thus emerges as a key ingredient in the normal production of learning-related synaptic changes. In all, increases in BDNF signaling appear to be a biologically plausible means for enhancing memory. Peripheral administration of the protein is unlikely to have robust central effects but brain permeant agonists for its synaptic TrkB receptor have been developed and shown to improve function in varied conditions of impairment (Andero et al., 2012; Schmid et al., 2012; Ding et al., 2013; Jiang et al., 2013). Reports on how these compounds affect memory in normals have only begun to appear but initial studies indicate that acute systemic treatment can improve object recognition, object location and fear memory when given just before training (Andero et al., 2011; Bollen et al., 2013); for object location memory administration 3 h after training was also effective. These results encourage the expectation that acute systemic treatment with a TrkB agonist can facilitate both initial encoding and mechanisms of consolidation for at least some forms of memory. Further work is needed to determine the range of learning and cognitive functions that respond to this strategy and if this occurs without deleterious side effects.

Another route for utilizing BDNF in memory studies is suggested by the observation that transcription of the factor is positively regulated by neuronal activity (Isackson et al., 1991; Gall, 1992). It follows from this that increases in excitatory drive to neurons, as for example produced by ampakines, should upregulate the neurotrophin. A sizable number of studies using individual or a series of daily injections of the positive modulators have confirmed this basic idea (Lauterborn et al., 2000; Rex et al., 2006; Simmons et al., 2009; Bernard et al., 2010; Haditsch et al., 2013). The treatments rescue theta burst-induced actin polymerization and LTP in a number of animal models of human conditions in which memory loss and/or intellectual disabilities are prominent, including those for normal aging, low estrogen

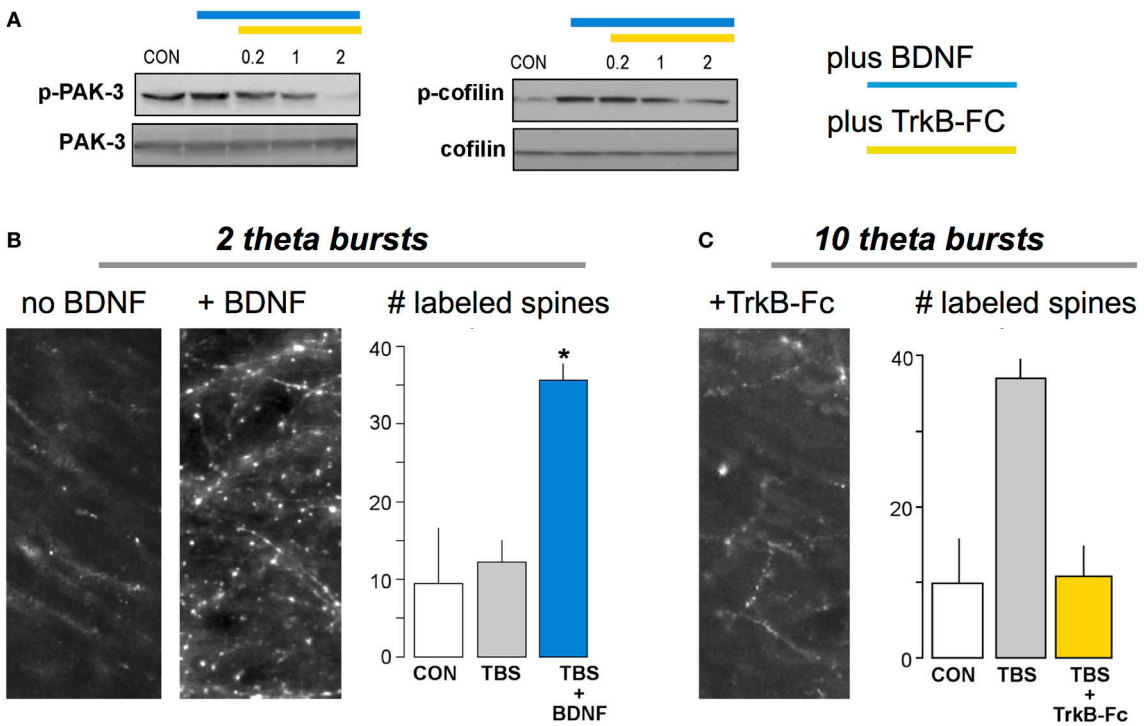

FIGURE 4 | BDNF enhances theta burst induced actin signaling and cytoskeletal assembly at hippocampal synapses. (A) Brief infusion of BDNF (60 ng/ml) into adult hippocampal slices increases phosphorylation of PAK and cofilin (immunoblots), two of the signaling proteins included in the schematic for LTP consolidation (**Figure 3**). Phosphorylation was blocked by addition of the extracellular BDNF scavenger TrkB-Fc (applied at 0.2–2 μg/ml). There were no evident effects of either treatment on levels of total PAK or cofilin. **(B)** Labeling of filamentous (F-) actin with fluorescence-tagged phalloidin applied to slices 10 min after delivery of two theta bursts (TBS), a number too small to generate LTP if applied alone. Pretreatment with BDNF (right image) caused a marked increase in TBS-induced labeling of dendritic spines relative to labeling in ACSF-bathed slices receiving similar stimulation. Graph: Summary of the number of spines with intense concentrations of F-actin, as assessed using automated counting for a fixed sampling field, confirmed these observations (CON, slices received low frequency stimulation only; $^{*}p < 0.01$ vs. CON). **(C)** Similar to **(B)** except that ten theta bursts were used. Pretreatment with TrkB-Fc completely blocked the otherwise robust increase in F-actin positive spines seen with ten burst TBS. Modified from Rex et al. (2007).

levels, early stage Huntington Disease, and Angelman syndrome (Rex et al., 2006; Simmons et al., 2009; Baudry et al., 2012; Kramar et al., 2012a). When tested, daily injections also reduced or eliminated memory impairments (Simmons et al., 2009; Baudry et al., 2012). Several weeks of daily ampakine treatment were shown to be well tolerated. They also markedly reduced pathology and improved motor functioning in a mouse model of early onset Huntington Disease (Simmons et al., 2011); subsequent work with systemic administration of a TrkB agonist obtained similar results (Simmons et al., 2013).

Although it is apparent that semi-chronic ampakine treatment increases BDNF protein levels, and has potent brain effects predicted from this, there appear to be no studies testing for influences of up-regulating BDNF on learning in normal, high functioning animals. This likely reflects an assignment of greater importance to treatment than to enhancement with regard to drug development. But the exciting results obtained with up-regulation and receptor agonists with regard to brain disorders make BDNF-based strategies one of the more promising mechanism-grounded approaches to achieving memory enhancement.

The substrate map for LTP consolidation includes estrogen receptor beta as a second membrane agent that exerts a powerful modulatory influence over the actin signaling leading to LTP consolidation. Thirty minute infusions of estrogen, at physiological concentrations, cause a modest increase in baseline transmission in hippocampus but a striking facilitation of LTP (Cordoba Montoya and Carrer, 1997; Foy et al., 1999; Bi et al., 2000; Kramar et al., 2009). Recent work showed that these effects are due to activation of one of the actin regulatory cascades initiated by theta bursts (i.e., RhoA>ROCK>LIMK>cofilin—see **Figure 3**) and the assembly of new filamentous actin in spine heads (Kramar et al., 2009). Unlike the case for BDNF, there are several reports that estrogen improves memory scores in high functioning subjects across tasks and species (Frye et al., 2007; Liu et al., 2008; Hara et al., 2014). Evidence for similar effects in humans appears to be lacking (Grodstein, 2013) although several studies describe a decline in verbal memory with surgical menopause and improvements with hormone replacement (Brinton, 2009). Beyond needing further evidence for effects in cognitively normal individuals, a primary barrier to development of an estrogen-based enhancement strategy lies in the fact that the steroid affects many fundamental cellular processes in brain and the periphery, and is known to facilitate certain types of cancer. More restricted actions can be had using agonists selective for the hormone's beta receptor which is, to a degree, concentrated in brain; such agonists are highly effective in LTP studies (Kramar et al., 2009). Evidence that estrogen is synthesized by hippocampal neurons and that hormone of local origin contributes significantly to hippocampal synaptic plasticity (Ooishi et al., 2012; Vierk et al., 2012) should also be noted here. Thus, it may be possible to find means to promote normal, likely activity-dependent, estrogen actions in a regionally restricted manner.

INTEGRATION: MANY PATHS TO THE SAME END

Brain scientists had proposed increases in the strength of connections between neurons as the substrate of memory before the introduction of the word "synapse" (Cajal, 1894). The idea is intuitively attractive since such increases would clearly alter the operation of cortical networks and thus behavior. In essence, it describes microscopic events that, when implemented at many sites, could be the physical instantiation of the macroscopic phenomenon of memory. From this perspective, the most direct route to memory enhancement would involve facilitating physiologically produced, long lasting increases in synaptic responses. Developing what is still only an outline of the machinery that induces, expresses, and consolidates LTP then shaped ideas about how to produce facilitation. To some extent, it also led to a unification that is perhaps under-appreciated: an unrelated array of enhancement candidates such as steroids, trophic factors, positive modulators of glutamate receptors, and channel blockers can now be seen to operate at specific levels within the same cell biological framework (Lynch et al., 2013). Optimistically, we may be approaching a reductionistic (simplifying) conceptual event with regard to enhancing encoding of specific pieces of information. Notably, something of this kind may also be going on for appreciating shared mechanistic impairments present in quite different disorders that interfere with learning: work with a sizable number of rodent models suggests that conditions with disparate etiologies result in a common endpoint failure in cytoskeletal reorganization (Lynch and Gall, 2013).

But there are warning signs with regard to the possibility that the current substrate model may be overly tailored to a specific instance of learning-related plasticity, and in particular to that found in a particular dendritic lamina (stratum radiatum) of a particular hippocampal subfield (CA1). Even within that subfield, there is good evidence that the basal dendritic field exhibits a different form of LTP (Arai et al., 1994; Kramar and Lynch, 2003). And it is now well established that the peculiar mossy fiber connections between dentate gyrus and field CA3 use a form of long lasting potentiation that bears little resemblance to that found in apical field CA1 (Staubli, 1992; Schmitz et al., 2003). It is not unreasonable to expect that additional plasticity variants will be discovered as parametric studies are carried out for other telencephalic connections; e.g., the corticostriatal glutamatergic synapses (Jia et al., 2010) or the olfactory and associational afferents to piriform cortex (Jung et al., 1990). While these observations greatly complicate predictions about the behavioral effects of putative enhancers, they also offer intriguing possibilities concerning specificity of action. That is, there are reasons to think that different forms of synaptic potentiation may underlie different types, or aspects, of memory. An explicit proposal of this type has been advanced for the basal and apical dendrites of field CA1 (Arai et al., 1994; Kramar and Lynch, 2003): The easily induced, readily erased LTP in the basal dendritic field seems well suited for transient encoding while the higher threshold and more rapidly stabilized form in the apical field is more appropriate for long term memory. An arrangement of this type would be useful in addressing the problem of how to accomplish, through repeated sampling, low noise extraction of constancies from a novel environment (apical dendrites) while at the same time transiently storing a great deal of information much of which can be discarded as being irrelevant (basal dendrites). In any event, testing experimental compounds on various

forms of plasticity could lead to agents that target particular forms of memory.

COGNITIVE ENHANCEMENT

DOES AUGMENTING MEMORY ENHANCE COGNITION?

Memory is such a prominent part of cognition that it seems obvious that enhancing the one will improve the other. However, there may be good computational reasons that cognitive benefit is gained when acquisition is less than optimal in terms of speed and strength. Animals faced with new and complex circumstances need to encode regular features without storing variable, low information elements. Otherwise, as noted earlier, the resultant memories will be noisy and less predictive of future encounters. The spaced trials effect—wherein, temporally separated training trials more efficiently support encoding than does a single "massed" session—can be seen as one adaptation toward better capture of regularities in the learning environment (Hintzman, 1976; Commins et al., 2003; Cepeda et al., 2006). That is, spacing ensures that only elements that are regularly present will be incorporated into memory while transient features will not. An enhancer could obviate the need for spacing by producing strong memory on an initial trial but would be expected to result in a noisy representation.

Tests of the above point are lacking but LTP experiments have produced what may be pertinent results. The original descriptions of links between theta burst afferent stimulation and LTP showed that, absent other manipulations, trains of ten bursts produced near maximal potentiation (Larson et al., 1986), a result that led to what has become a standard paradigm. Recently, however, it was found that a second theta train doubles the level of potentiation but only if it is delayed by about 60 min (**Figure 5**); a third stimulation train produces still more potentiation but only if it is applied at least 60 min after the second. Additional work suggested that this LTP "spaced trials effect" reflects the presence of a large population of synapses with high plasticity thresholds that are "primed" by the first theta episode and then shifted into the potentiated state by the second (Kramar et al., 2012b). These effects fit naturally within the above described substrate map for LTP: activation of synaptic integrins by a first theta burst train was followed by an approximately one hour period before these receptors could be re-engaged by additional stimulation (Babayan et al., 2012). They also set the stage for a first test of how a drug that enhances memory affects a physiological analogue of the spaced trials effect. The results were clear: infusion of an ampakine prior to theta train #1 produced the expected enhancement in the amplitude of LTP1 but also occluded further increases in the level of potentiation following a second, delayed theta train administered in the absence of the drug (Kramar et al., 2012b). Thus, the ampakine enhanced initial encoding (as multiply reported) but did so at the expense of effects of spaced stimulation, and presumably the computational advantages associated with spacing (Lynch and Gall, 2013 for a discussion).

The preceding example describes a situation in which pharmacologically augmenting memory would likely not result in enhanced cognition, at least in complex environments lacking explicit guidelines for effective performance. These are routine circumstances in which demands on cognition are high. But a great deal of cognition involves instances in which significant cues and appropriate responses are salient and predetermined, and irrelevant information is minimized. Under these conditions, enhanced encoding could be of great use in building or expanding

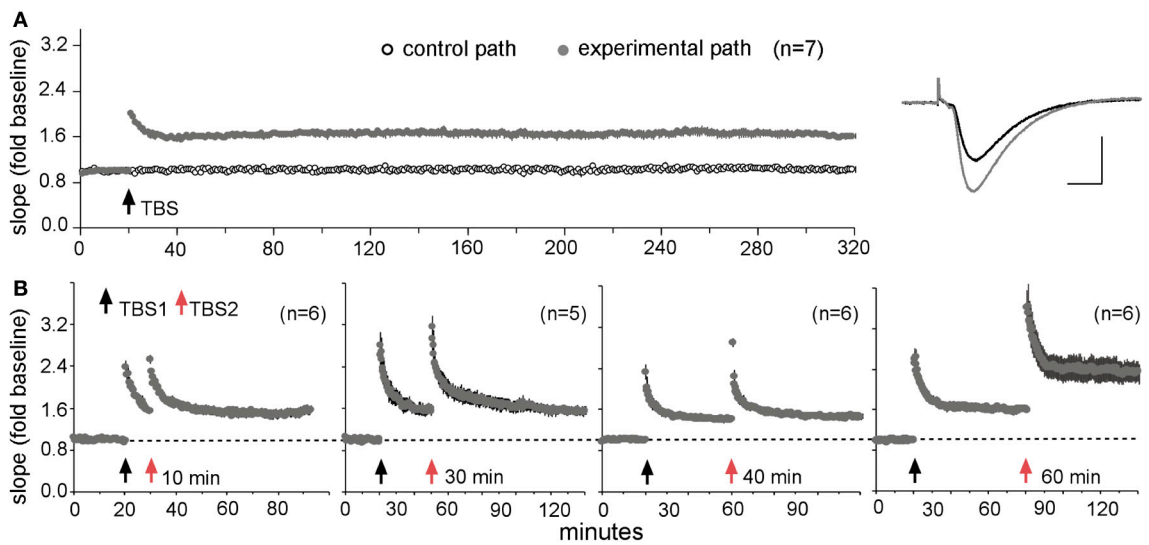

FIGURE 5 | A "spaced trials effect" for LTP. (A) Theta burst stimulation (TBS) produces stable LTP. A single train of ten bursts was delivered to one input to the CA1b recording field after 20 min of collecting baseline synaptic responses to 3/min stimulation pulses in adult rat hippocampal slices. A second input to the same dendritic region received 3/min pulses throughout the session. Note that the potentiation of the experimental input did not decay over 5 h of recording (means ± s.e.m.s for seven slices); traces at right show representative baseline (black) and potentiated (gray) responses. **(B)** Effect of a second theta train (TBS2) applied at various times after TBS1. TBS2 produced no further increases in the slopes of the responses when delayed by 10, 30, or 40 min after TBS1, but doubled the magnitude of LTP when applied after a 60 min interval. Modified from Kramar et al. (2012b).

cognitive structures. Thus, the effects of memory enhancement on cognition could prove to be situationally dependent with clear benefits in some cases and neutral or even negative influence in others.

NETWORKS AND COGNITIVE ENHANCEMENT

Discussions of neurobiological processes underlying cognition inevitably begin with the immensely complicated networks formed by cortical neurons, if for no other reason than a lack of realistic alternatives. This fundamental idea suggests two paths to *acute* enhancement. First, improving throughput within established networks should lead to faster computation and better utilization of cognitive time. Second, augmented synaptic communication could allow for the transient assembly of larger than normal networks (e.g., incorporation of additional cortical regions) to deal with a particular problem, and thus the opportunity to execute more complex or even entirely novel computations. In this sense better throughput would add capabilities, perhaps the surest measure of cognitive enhancement. Increased plasticity might add a third route to enhanced cognition by allowing for construction of functional networks that would not likely emerge under normal conditions; however, as noted in the preceding section, positive versions of such effects may be limited to particular circumstances.

There are multiple manipulations that should result in improved throughput. Communication between collections of neurons is greatly improved by synchronizing their activity, something that is accomplished in the cortical telencephalon by system-wide rhythms. These patterns are induced by diffuse ascending projections from the lower brain and drugs that affect these have predictable strong effects on rhythmic activity (Staubli and Xu, 1995; Kowalczyk et al., 2013). But, as mentioned in the discussion of memory, the diffuse systems influence a broad range of brain functions including ones that are vital to survival. And so, as in the case of memory, they do not represent a promising avenue toward enhancement in high functioning individuals. A more likely approach would be to increase transmitter release or post-synaptic responses to transmitter binding at the glutamatergic connections used for the great bulk of intra-cortical communication.

Adenosine, which depresses glutamate release via presynaptic A1 receptors (Dunwiddie and Haas, 1985), is increased in the extracellular environment during repetitive firing by two mechanisms: rapid release from post-synaptic neurons followed by slower release of ATP from glia which is then converted to adenosine by ecto-5′-nucleotidase, an enzyme located on glial membranes (Klyuch et al., 2012; Wall and Dale, 2013). These observations represent a significant part of the tripartite model (terminal bouton, spine, astrocyte) of fast, excitatory transmission (Araque et al., 1999). Selective antagonists of the A1 receptor increase glutamate release in slices and these compounds do indeed reverse impairments in LTP in slices of middle-aged rat hippocampus (Rex et al., 2005). However, despite evidence that the compounds enter the brain (Wall and Dale, 2013), there has been surprisingly little work on *in vivo* effects after peripheral administration. Perhaps the lack of interest with regard to network operations reflects understandable concern about the important roles played by adenosine in the periphery, including actions on the heart and lungs.

Nicotinic receptors for acetylcholine are also found on glutamatergic terminals where they promote release (Wonnacott, 1997) and there is evidence that this increases network throughput (Gioanni et al., 1999). Alpha7-containing and alpha4/beta2 subtypes of the receptors both appear to be effective in this regard (Dickinson et al., 2008). However, the situation is complicated by the likelihood that compounds targeting nicotinic receptors act on cholinergic and GABAergic neurons as well (Wonnacott, 1997; Alkondon and Albuquerque, 2001); moreover, it is not clear that these receptors are present throughout glutamatergic networks. In all, nicotinic receptor agonists and positive allosteric modulators can be assumed to affect portions of excitatory circuitry in the telencephalon while at the same time modifying local processing—via modulation of cholinergic input, interneurons, and glutamatergic collaterals—at individual relays. Net effects will be complex but there is good evidence that the compounds acting on frontal networks enhance "top-down" mechanisms for focusing attention (Sarter et al., 2009). Since pertinent drugs are already in clinical trials (Holmes et al., 2011; Demeter and Sarter, 2013), nicotinic compounds, and especially those targeting the alpha4/beta2 receptor subtype concentrated in brain, have to be seen as one of the most promising of current approaches to cognitive enhancement.

The ampakine compounds described in the earlier section on memory enhancement seem particularly appropriate for improving communication within and between cortical regions. Their mode of action has the virtue of relative simplicity: an extensive body of research from many laboratories has not uncovered any evidence for effects on targets other than AMPA receptors. And they produce the same facilitation of fast, excitatory transmission after peripheral administration as seen with infusions into brain slices. Indeed, ampakines appear to be the only agents so far shown to cause comparable *in vitro*/*in vivo* facilitation of EPSPs. These points lead to two critical experimental questions. First, does increasing monosynaptic transmission result in greater output from a polysynaptic network? This might seem to be a foregone conclusion but each step in a series of neuronal stations has local processing mechanisms (relays are not passive transferal points) dominated by an impressive collection of different types of inhibitory interneurons. These inhibitory elements respond both to inputs directly and to discharges from principle (glutamatergic) neurons; they also form complex local networks among themselves. It is therefore possible that strong inputs are dampened and normalized to a degree such that the second stage of a network may not pass on a larger than normal signal in the presence of an ampakine. Second, assuming augmentation of the signal does occur, what are the functional consequences of enhanced network throughput?

Brain slices provide for the simplest and most compelling tests for circuit behavior because anatomically precise stimulation and recording is possible and extrinsic modulatory (cholinergic, etc.) inputs (cholinergic, serotonergic, etc.) that might influence downstream responses are excluded. Work of this kind has established that weak facilitation of monosynaptic transmission with an ampakine results in a greatly amplified response from the

output stage of the trisynaptic intra-hippocampal circuit (Sirvio et al., 1996). These observations accord with the broad idea that facilitated transmission at one connection will lead to a greater number of cells transmitting to the next. Repeated across many stages, each responding to the ampakine, this will produce a multiplier effect for drug action. This argument points to the conclusion that ampakine-type drugs will exert much greater effects in the long chains of glutamatergic neurons that constitute cortical networks than in the much simpler circuits found at lower levels of the neuraxis. Why the multiplier effect doesn't ultimately result in abnormal discharges likely reflects the above mentioned inhibitory interneurons whose influence on projection neurons also grows with increasing glutamatergic drive, as seen in input / output measurements in conventional hippocampal slice experiments. Since inhibition generated by arrival of a glutamatergic input is di- (and multi-) synaptic, there is brief widow in which network facilitation is operative. Sophisticated multi-scale (biophysics, synapses, neurons, and connectivity) computational work indicates the manner in which enhanced throughput can produce useful effects in complex cortical circuits (Bouteiller et al., 2011). However, the effects of increased EPSPs on network responses to rhythmic or complex stimulation are a critical and as yet unstudied issue.

Evidence for enhanced throughput has also been obtained in studies using *in vivo* analyses of hippocampal projections to frontal cortex (Baumbarger et al., 2001) and chronic recordings from the output stage of hippocampus (Hampson et al., 1998b). The latter rat study showed that the number of cells discharging during key steps in performing a complex task was substantially increased by systemic treatment with an ampakine (**Figure 6**). Given that the recording site was the terminus of the primary intra-hippocampal circuit, one can reasonably assume that the observed results reflected an augmentation of drug action through a polysynaptic network similar to those described for ampakines infused into hippocampal slices (Sirvio et al., 1996). But the possibility that augmented excitatory drive on ascending biogenic amine systems, whether from the drugs or the behavioral activity they produce, results in generalized increases

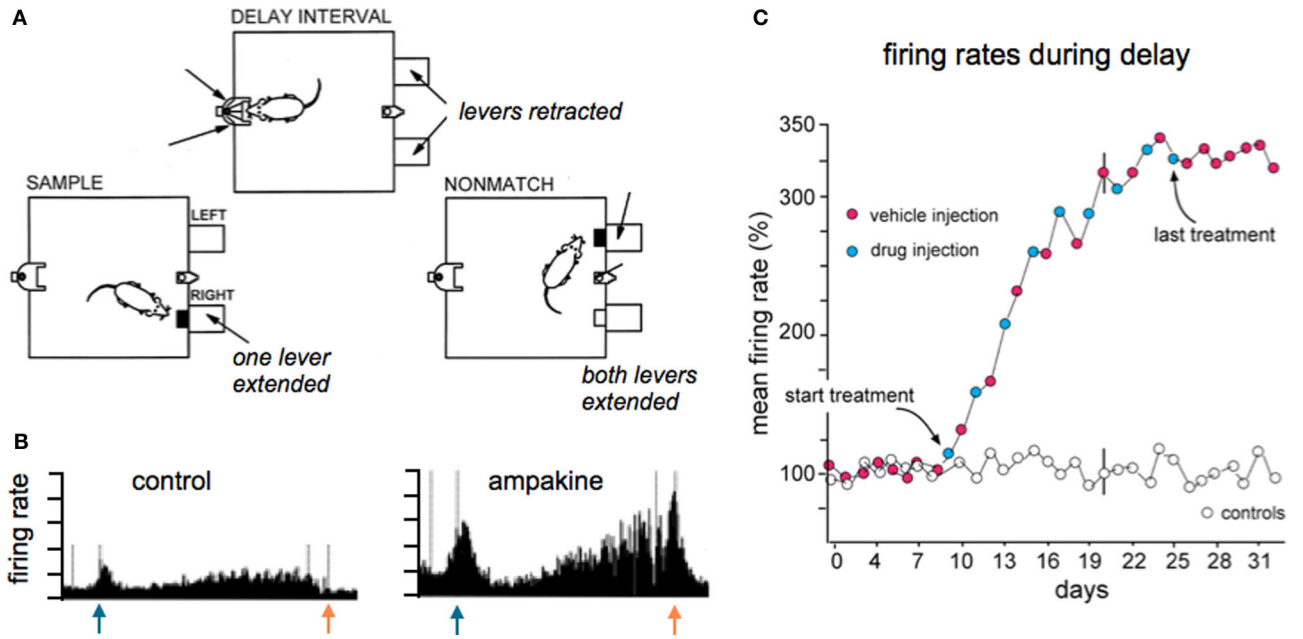

FIGURE 6 | Ampakine treatment increases neuronal activity in the terminal stage of the intra-hippocampal network and this is accompanied by supra-normal performance on a complex task. (A) Adult rats were extensively trained in the delayed non-match to sample (DNMS) task, illustrated here, wherein they were required to sequentially (i) press a bar to receive a reward (sample phase; left) at which time a light appeared on the opposite wall of the arena, (ii) move to the wall with the light during the "delay phase" (top) and perform a nose poke for random intervals until the light extinguished, and then (iii) return to the original side of the arena and press the bar that had not been originally selected (non-match phase; right). The level of success in executing the task reached asymptote after weeks of training, after which the animals were separated into vehicle or drug treated groups. Injections were given every other day. Animals given the ampakine prior to individual training sessions showed a progressive increase in correct responses over the next 2+ weeks of daily testing, ultimately reaching levels far higher than the asymptotic level maintained by vehicle-only rats (not shown; see Hampson et al., 1998b). **(B)** Chronic recordings from the same hippocampal neuron collected during task performance prior to and after the start of drug treatment. As shown, the ampakine dramatically increased the firing rate most particularly after the initial response to the sample (blue arrow) and in the delay phase leading up to the non-match choice (red arrow); this pattern and ampakine-related change was typical of field CA1 cells. **(C)** Plot shows the mean firing rate of all units recorded during the delay phase expressed as a percent of the baseline firing rate (prior to ampakine treatment): the progressive increase in firing rate in the experimental rats paralleled the increase in successful trials in the same rats. Rats given vehicle only (open circles) exhibited no change in cell firing or successful task execution over the full period of testing. Note that the increased firing in the drug group was still present on vehicle days (red dots), and indeed for the week following cessation of treatment, an effect that is interpreted as being due to facilitation of LTP-type plasticity on drug days. Adapted from Hampson et al. (1998b).

in neuronal excitability cannot be excluded in these *in vivo* studies.

FUNCTIONAL EFFECTS OF ENHANCED NETWORKS

There are relatively straightforward results showing the effects of increasing throughput in cortical networks in simple experimental paradigms. For example, unilateral lesions of the nigro-striatal projections result in a circling response to dopamine agonists; ampakines significantly expand activation of the motor cortex on the side of the lesion and this is associated with a suppression of rotations (Hess et al., 2003). Note that in this case circuits are selectively brought into play that are directly germane to the problem faced by the animal. The expectation of a more subtle version of this effect under conditions in which cortex is performing complex calculations constitutes one basis for hypothesizing that improved network throughput will result in acute enhancement of cognition. It should be noted here that the development of very fast algorithms for extracting core spatio-temporal activity patterns from multi-electrode recordings has made it possible to insert, via stimulation at many sites, information rich patterns to networks in behaving animals. These advances have opened the way to experimental testing of fundamental, long-standing assumptions about how cortical circuits process complex signals from the environment. One recent study of this kind that is particularly germane to the present discussion showed that delivery to CA1 of a "correct" pattern of activation predicted from CA3 recordings during the sample phase of a match to sample problem markedly enhanced performance of monkeys on the subsequent decision phase in difficult versions of the task (Hampson et al., 2013). These dramatic findings encourage the idea that facilitating partial or "weak" network patterns can lead to pronounced improvements in the ability of animals, including primates, to deal with complexity.

As mentioned earlier, enhancement could take the form of acceleration of cognitive activities, and thus allowing for more computations in the same time frame, or expansion of networks and potentially new types of operations. One route for testing the latter possibility would be to overtrain animals to the point at which optimal performance is fully established and then to determine if network facilitation through enhanced transmission allows the subject to go beyond normal limits. There is very little work of this kind for ampakines or any other putative enhancer but suggestive results have been described. The study noted above in which ampakines expanded the hippocampal response during learning (**Figure 6**) also found that overtrained rats significantly improved their learning scores under the influence of the drug. Remarkably, the animals then continued to perform at supranormal levels in the absence of the ampakine. Detailed analyses showed that the animals shifted response strategies in a manner that reduced proactive interference between trials (Hampson et al., 1998a). In essence, the drugs opened the way to expanded networks and the development of higher order rules that cannot otherwise be acquired even with weeks of training. Another example of going well beyond normal limits has been described for monkeys performing a challenging delayed match-to-sample problem (Porrino et al., 2005). The animals were trained to asymptote to identify, as indicated by movement of a computer cursor, a previously seen real world cue from a group of similar objects. Performance on the task was increased dramatically with ampakine pretreatment. Brain imaging studies then uncovered a remarkable result: the ampakine intensified activity in frontal and temporal cortices but also led to the engagement of a superior parietal region, the precuneus, which was inactive during vehicle trials. The precuneus is thought to be critical for envisioning future actions by humans. In any case, these results in a primate provide an example in which expansion of cortical networks is associated with a lifting of limits on performance in a cognitively demanding problem.

These few studies using overtrained animals, exciting though the findings may be, are hardly sufficient to establish the general point that increases in network throughput result in beyond normal performance on challenging problems. Experiments of this type are not common because they involve major investments in time and technology. And it will be noted that they focus on problems that are sharply defined with regard to cues and appropriate responses. One can fairly ask if these conditions capture the essence of cognition as a free flowing processing of the enormous complexities generated by the exterior and interior worlds of humans. This point is picked up in the following section.

FUTURE NEUROBIOLOGICAL STUDIES ON COGNITIVE ENHANCEMENT

A question running through this review, and alluded to immediately above, concerns the extent to which we can consider problem solving by animals as a fair descriptor of cognition. One can hardly question the proposition that the analyses of different computations performed by distinct frontal subfields in rats (e.g., credit assignment to particular serial actions, set shifting, focusing of attention; Turner et al., 2004; Sugrue et al., 2005; Demeter and Sarter, 2013) will provide deep insights into how humans resolve real world issues. But here we encounter the problem of how to define cognition and whether or not it can be understood in simple computational terms. To be specific, what might be needed are studies testing whether putative enhancers improve the performance of sophisticated (highly experienced) subjects dealing with novel circumstances of great complexity and without the benefit of external supervision. If nothing else, this would bring experiments closer to the human condition and thereby help explain why animal studies on cognition and memory have such a poor record in predicting human outcomes.

Much of the present discussion centered on the proposition that enhancing network throughput will have positive effects on cognition (**Figures 7A,B**). It will be recognized that most of the material presented in support of this idea dealt with specific neuronal circuits or opportunistic discoveries of network expansion. A more systematic, agnostic description of how experimental compounds affect the vast number of forebrain circuits is badly needed. This could be obtained using activity-regulated immediate early gene expression to provide an index of the recent history of neuronal firing. Such analyses would provide a picture of the networks assembled to deal with complex circumstances, surely an initial step toward a mechanism based theory of cognitive operations, and add an information rich step for the screening of experimental compounds. There is also the possibility that network maps of drug effects

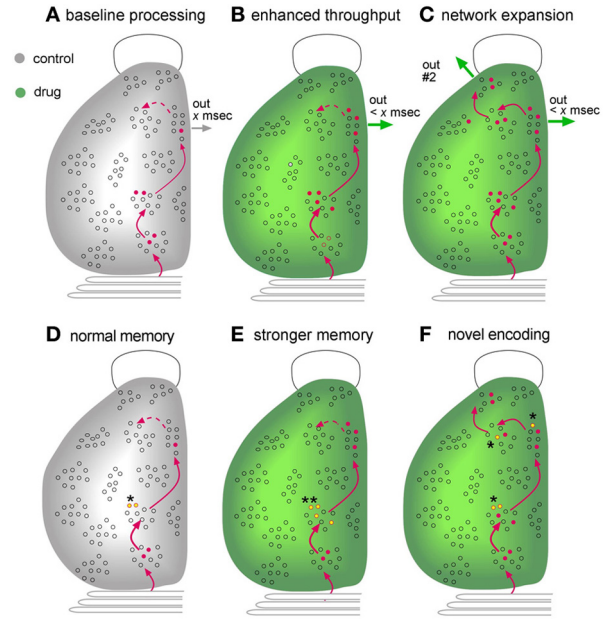

FIGURE 7 | Mapping as an endpoint measure for evaluating the effects of putative enhancers. Schematic illustration of a dorsal view of the left side of a rodent brain, with an open outline of the olfactory bulb at top and the cerebellum at bottom. Gray is for control subjects while green denotes results obtained in the presence of an experimental treatment. **(A–C)** Network Mapping. The figures describe the distribution of cells activated during learning of a novel environment, as could be determined from maps assembled from serial sections labeled for activation of Immediate-Early Genes (see text). Combining this information with neuroanatomy (red lines) would provide a picture of the extent to which known circuits were engaged during learning. **(A)** Interaction with the environment increases the number of cells firing (red dots) in three successive stages of a transcortical network, resulting in output from the third stage. A fourth region that that is weakly innervated by preceding network stages (dotted line) is not engaged. **(B)** A compound that facilitates excitatory synaptic transmission increases the number of neurons that respond to the input and thereby reduces circuit-throughput time (< *x* ms) and generates a more robust network output. **(C)** An additional possibility, supported by experimental work, is that improved transmission adds regions to the network, resulting in novel outputs ("out #2"). Potentially, the cortex in this instance would gain new capabilities for dealing with complex problems. **(D–F)** Mapping of encoding sites (engrams). Recent advances have made it possible to identify synapses that are modified by recent learning. Mapping studies for hippocampus strongly suggest that such effects are restricted to a small number of sites in a multiple stage network (see text). **(D)** Under control conditions, memory-related synaptic changes, such as LTP, are restricted to stage #2 (yellow dots, asterisk) of one network engaged during learning. **(E)** An enhancer could increase the number of neurons on which such synaptic changes occur in regions in which they are normally found (double asterisks). Such an effect is predicted to result in the formation of stronger memory (e.g., more resistant to extinction). **(F)** Improved transmission relating to an experimental treatment reduces the threshold for inducing memory-related synaptic alterations such as LTP. It follows from this that the manipulation could result in synaptic modifications in regions at which they do not normally occur (multiple asterisks). This is predicted to produce an elaborated, novel representation of the learned material.

would be predictive: measures of intensified activity within, or expansion of, behaviorally engaged circuits (**Figure 7C**) should lead to explicit hypotheses about the origins of psychological changes.

Another type of mapping may also prove useful in future searches for cognitive enhancers (**Figures 7D–F**). As noted, changes in the numbers of individual synapses associated with LTP-related actin regulatory proteins have been detected in hippocampus following learning. Notably, the labeled synapses were larger than their neighbors (Fedulov et al., 2007), an effect that was also seen with LTP (Chen et al., 2007). Continuing advances in the technology for identifying synapses engaged in plastic changes that underlie learning have made it possible to plot the distribution, across entire cross-sections of the hippocampus, of subfields that reliably contain many such contacts. A first study using the mapping method to plot synapses undergoing plasticity following learning of a new environment detected only three out of forty-two sampling zones within hippocampus that match this description; imposing a response contingency that interfered with free exploration eliminated the effect (Cox et al., 2014). These results cannot be taken as indicating that learning related synaptic adjustments only occur within these three sites; it is entirely possible that such effects are present in many regions but vary between subjects and/or are not numerically large enough to be detected with current procedures. But the results strongly suggest that encoding of one type of spatial information is not homogeneously distributed but instead occurs at high levels in a surprisingly small number of locations. In essence, they constitute a first, albeit crude memory map that covers one septo-temporal segment of the hippocampus.

Localizing memory has been a much discussed topic among brain scientists since the early days of the last century; subsequent attempts to map the distribution of encoding sites acquired an evocative title: "The search for the engram" (Thompson et al., 1976; Thompson and Krupa, 1994). Maps, or engrams, are of evident importance to the development of neurobiological theories of how memories are recalled but they are also of potential significance with regard to network events related to cognitive enhancement. The discussion to this point has stressed the effects of transiently facilitating network throughput but it is noteworthy that certain of the manipulations suggested for this purpose, such as increasing neurotrophic factor signaling, may also promote stable changes in connectivity. But would such changes simply increase the efficacy (e.g., throughput time) of extant circuits or would they allow for the emergence of new and persistent networks? This distinction returns to the earlier consideration of enhancement as reflecting faster processing or the introduction of new capabilities. The technology for mapping the location of learning-driven synaptic modifications may allow for neurobiological, as opposed to purely behavioral, tests of the question: does an experimental manipulation intensify the map (more synapses with LTP-related changes at normal sites) vs. creating additional regions with high numbers of modified connections (**Figures 7D–F**).

In all, a futuristic combination of network mapping with localization of synaptic changes could shift the evaluation of putative enhancers from exclusively behavioral endpoints to the presumed network substrates of cognitive operations. Such a step would likely lead to the many advantages in problem conceptualization historically associated with reductionism in science.

ACKNOWLEDGMENTS

Research described here was supported, in part, by grants NS045260 from NINDS, N0014-10-1-007 from ONR, and #1146708 from NSF, and an award to Gary Lynch from the UC Irvine Center for Autism Research and Translation. Conor D. Cox was supported by NSF fellowship DGE088392.

REFERENCES

Abraham, W. C. (2003). How long will long-term potentiation last? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 735–744. doi: 10.1098/rstb.2002.1222

Abraham, W. C., and Williams, J. M. (2008). LTP maintenance and its protein synthesis-dependence. *Neurobiol. Learn. Mem.* 89, 260–268. doi: 10.1016/j.nlm.2007.10.001

Alarcon, J. M., Barco, A., and Kandel, E. R. (2006). Capture of the late phase of long-term potentiation within and across the apical and basilar dendritic compartments of CA1 pyramidal neurons: synaptic tagging is compartment restricted. *J. Neurosci*. 26, 256–264. doi: 10.1523/JNEUROSCI.3196-05.2006

Alkondon, M., and Albuquerque, E. X. (2001). Nicotinic acetylcholine receptor alpha7 and alpha4beta2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *J. Neurophysiol.* 86, 3043–3055.

Andero, R., Daviu, N., Escorihuela, R. M., Nadal, R., and Armario, A. (2012). 7,8-dihydroxyflavone, a TrkB receptor agonist, blocks long-term spatial memory impairment caused by immobilization stress in rats. *Hippocampus*. 22, 399–408. doi: 10.1002/hipo.20906

Andero, R., Heldt, S. A., Ye, K., Liu, X., Armario, A., and Ressler, K. J. (2011). Effect of 7,8-dihydroxyflavone, a small-molecule TrkB agonist, on emotional learning. *Am. J. Psychiatry* 168, 163–172. doi: 10.1176/appi.ajp.2010.10030326

Arai, A., Black, J., and Lynch, G. (1994). Origins of the variations in long-term potentiation between synapses in the basal versus apical dendrites of hippocampal neurons. *Hippocampus* 4, 1–9. doi: 10.1002/hipo.450040103

Arai, A. C., Xia, Y. F., Rogers, G., Lynch, G., and Kessler, M. (2002). Benzamide-type AMPA receptor modulators form two subfamilies with distinct modes of action. *J. Pharmacol. Exp. Ther.* 303, 1075–1085. doi: 10.1124/jpet.102.040360

Arai, A., Kessler, M., Ambros-Ingerson, J., Quan, A., Yigiter, E., Rogers, G., et al. (1996). Effects of a centrally active benzoylpyrrolidine drug on AMPA receptor kinetics. *Neuroscience* 75, 573–585. doi: 10.1016/0306-4522(96)00263-1

Arai, A., and Lynch, G. (1998). The waveform of synaptic transmission at hippocampal synapses is not determined by AMPA receptor desensitization. *Brain Res.* 799, 230–234. doi: 10.1016/S0006-8993(98)00446-6

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215. doi: 10.1016/S0166-2236(98)01349-6

Babayan, A. H., Kramar, E. A., Barrett, R. M., Jafari, M., Haettig, J., Chen, L. Y., et al. (2012). Integrin dynamics produce a delayed stage of long-term potentiation and memory consolidation. *J. Neurosci.* 32, 12854–12861. doi: 10.1523/JNEUROSCI.2024-12.2012

Balkowiec, A., and Katz, D. M. (2000). Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA *in situ*. *J. Neurosci*. 20, 7417–7423.

Bari, A., Dalley, J. W., and Robbins, T. W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat. Protoc.* 3, 759–767. doi: 10.1038/nprot.2008.41

Baudry, M., Kramar, E., Xu, X., Zadran, H., Moreno, S., Lynch, G., et al. (2012). Ampakines promote spine actin polymerization, long-term potentiation, and learning in a mouse model of Angelman syndrome. *Neurobiol. Dis.* 47, 210–215. doi: 10.1016/j.nbd.2012.04.002

Baumbarger, P. J., Muhlhauser, M., Zhai, J., Yang, R., and Nisenbaum, E. S. (2001). Positive modulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in prefrontal cortical pyramidal neurons by a novel allosteric potentiator. *J. Pharmacol. Exp. Ther*. 298, 86–102.

Baxter, M. G., Lanthorn, T. H., Frick, K. M., Golski, S., Wan, R. Q., and Olton, D. S. (1994). C-cycloserine, a novel cognitive enhancer, improves spatial memory in aged rats. *Neurobiol. Aging*. 15, 207–213. doi: 10.1016/0197-4580(94)90114-7

Bernard, K., Danober, L., Thomas, J. Y., Lebrun, C., Munoz, C., Cordi, A., et al. (2010). Drug focus: S 18986: a positive allosteric modulator of AMPA-type glutamate receptors pharmacological profile of a novel cognitive enhancer. *CNS Neurosci. Ther.* 16, e193–212. doi: 10.1111/j.1755-5949.2009.00088.x

Bi, F., Broutman, G., Foy, M. R., Thompson, R. F., and Baudry, M. (2000). The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. *Proc. Natl. Acad. Sci. U.S.A*. 97, 3606–3607. doi: 10.1073/pnas.97.7.3602

Bliss, T. V., and Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39. doi: 10.1038/361031a0

Bliss, T. V. P., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol*. 232, 334–356.

Bollen, E., Vanmierlo, T., Akkerman, S., Wouters, C., Steinbusch, H. M., and Prickaerts, J. (2013). 7,8-dihydroxyflavone improves memory consolidation processes in rats and mice. *Behav. Brain Res.* 257, 8–12. doi: 10.1016/j.bbr.2013.09.029

Bouteiller, J. M., Allam, S. L., Hu, E. Y., Greget, R., Ambert, N., Keller, A. F., et al. (2011). Integrated multiscale modeling of the nervous system: predicting changes in hippocampal network activity by a positive AMPA receptor modulator. *IEEE Trans. Biomed. Eng.* 58, 3008–3011. doi: 10.1109/TBME.2011.2158605

Brennan, A. R., Dolinsky, B., Vu, M. A., Stanley, M., Yeckel, M. F., and Arnsten, A. F. (2008). Blockade of IP3-mediated SK channel signaling in the rat medial prefrontal cortex improves spatial working memory. *Learn. Mem.* 15, 93–96. doi: 10.1101/lm.767408

Brinton, R. D. (2009). Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol. Sci.* 30, 212–222. doi: 10.1016/j.tips.2008.12.006

Buzsaki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus* 15, 827–840. doi: 10.1002/hipo.20113

Cajal, S. R. (1894). La fine structure des centres nerveux. *Proc. R. Soc. Lond.* 55, 444–468. doi: 10.1098/rspl.1894.0063

Capocchi, G., Zampolini, M., and Larson, J. (1992). Theta burst stimulation is optimal for induction of LTP at both apical and basal dendritic synapses on hippocampal CA1 neurons. *Brain Res.* 591, 332–336. doi: 10.1016/0006-8993(92)91715-Q

Cepeda, N. J., Pashler, H., Vul, E., Wixted, J. T., and Rohrer, D. (2006). Distributed practice in verbal recall tasks: a review and quantitative synthesis. *Psychol. Bull.* 132, 354–380. doi: 10.1037/0033-2909.132.3.354

Chang, F.-L. F., and Greenough, W. T. (1984). Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. *Brain Res.* 309, 35–46. doi: 10.1016/0006-8993(84)91008-4

Chen, L. Y., Rex, C. S., Casale, M. S., Gall, C. M., and Lynch, G. (2007). Changes in synaptic morphology accompany actin signaling during LTP. *J. Neurosci.* 27, 5363–5372. doi: 10.1523/JNEUROSCI.0164-07.2007

Chen, L. Y., Rex, C. S., Sanaiha, Y., Lynch, G., and Gall, C. M. (2010b). Learning induces neurotrophin signaling at hippocampal synapses. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7030–7035. doi: 10.1073/pnas.0912973107

Chen, T. J., Gehler, S., Shaw, A. E., Bamburg, J. R., and Letourneau, P. C. (2006). Cdc42 participates in the regulation of ADF/cofilin and retinal growth cone filopodia by brain derived neurotrophic factor. *J. Neurobiol.* 66, 103–114. doi: 10.1002/neu.20204

Clarke, N. A., and Francis, P. T. (2005). Cholinergic and glutamatergic drugs in Alzheimer's disease therapy. *Expert Rev. Neurother.* 5, 671–682. doi: 10.1586/14737175.5.5.671

Commins, S., Cunningham, L., Harvey, D., and Walsh, D. (2003). Massed but not spaced training impairs spatial memory. *Behav. Brain Res.* 139, 215–223. doi: 10.1016/S0166-4328(02)00270-X

Cordoba Montoya, D. A., and Carrer, H. F. (1997). Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. *Brain Res*. 778, 430–438. doi: 10.1016/S0006-8993(97)01206-7

Cox, C. D., Rex, C. S., Palmer, L. C., Babayan, A. H., Pham, D. T., Corwin, S. D., et al. (2014). A map of LTP-related synaptic changes in dorsal hippocampus following unsupervised learning. *J. Neurosci.* 34, 3033–3041. doi: 10.1523/JNEUROSCI.4159-13.2014

Crystal, J. D., and Glanzman, D. L. (2013). A biological perspective on memory. *Curr. Biol.* 23, R728–731. doi: 10.1016/j.cub.2013.07.082

Dang, Y. H., Ma, X. C., Zhang, J. C., Ren, Q., Wu, J., Gao, C. G., et al. (2014). Targeting of NMDA receptors in the treatment of major depression. *Curr. Pharm. Des.* 19, 1–9. doi: 10.2174/13816128196661401101204 35

Demeter, E., and Sarter, M. (2013). Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology* 64, 294–304. doi: 10.1016/j.neuropharm.2012.06.060

Demeter, E., Sarter, M., and Lustig, C. (2008). Rats and humans paying attention: cross-species task development for translational research. *Neuropsychology* 22, 787–799. doi: 10.1037/a0013712

Dickinson, J. A., Kew, J. N., and Wonnacott, S. (2008). Presynaptic alpha 7- and beta 2-containing nicotinic acetylcholine receptors modulate excitatory amino acid release from rat prefrontal cortex nerve terminals via distinct cellular mechanisms. *Mol. Pharmacol.* 74, 348–359. doi: 10.1124/mol.108.046623

Ding, Z. B., Wu, P., Luo, Y. X., Shi, H. S., Shen, H. W., Wang, S. J., et al. (2013). Region-specific role of Rac in nucleus accumbens core and basolateral amygdala in consolidation and reconsolidation of cocaine-associated cue memory in rats. *Psychopharmacology (Berl)*. 228, 427–437. doi: 10.1007/s00213-013-3050-8

Dravid, S. M., Burger, P. B., Prakash, A., Gaballe, M. T., Yadav, R., Le, P., et al. (2010). Structural determinants of D-cycloserine efficacy at the NR1/NR2C NMDA receptors. *J. Neurosci.* 30, 2741–2754. doi: 10.1523/JNEUROSCI.5390-09.2010

Dunwiddie, T. V., and Haas, H. L. (1985). Adenosine increases synaptic facilitation in the *in vitro* rat hippocampus: evidence for a presynaptic site of action. *J. Physiol. (Lond).* 369, 365–377.

Eichenbaum, H., and Robitsek, R. J. (2009). Olfactory memory: a bridge between humans and animals in models of cognitive aging. *Ann. N.Y. Acad. Sci.* 1170, 658–663. doi: 10.1111/j.1749-6632.2009.04012.x

Fedulov, V., Rex, C. S., Simmons, D. A., Palmer, L., Gall, C. M., and Lynch, G. (2007). Evidence that long-term potentiation occurs within individual hippocampal synapses during learning. *J. Neurosci.* 27, 8031–8039. doi: 10.1523/JNEUROSCI.2003-07.2007

Flood, J. G., Morley, J. E., and Langhorn, T. H. (1992). Effect on memory processing by D-cycloserine, an agonist of the NMDA/glycine rceptor. *Eur. J. Pharmacol.* 221, 249–254. doi: 10.1016/0014-2999(92)90709-D

Foy, M. R., Xu, J., Xie, X., Brinton, R. D., Thompson, R. F., and Berger, T. W. (1999). 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J. Neurophysiol.* 81, 925–929.

Frey, U., and Morris, R. G. M. (1997). Synaptic tagging and long-term potentiation. *Nature* 385, 533–536. doi: 10.1038/385533a0

Frye, C. A., Duffy, C. K., and Walf, A. A. (2007). Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. *Neurobiol. Learn. Mem.* 88, 208–216. doi: 10.1016/j.nlm.2007.04.003

Gall, C. M. (1992). Regulation of brain neurotrophin expression by physiological activity. *Trends Pharmacol. Sci.* 13, 401–403. doi: 10.1016/0165-6147(92)90123-N

Gioanni, Y., Rougeot, C., Clarke, P. B., Lepouse, C., Thierry, A. M., and Vidal, C. (1999). Nicotinic receptors in the rat prefrontal cortex: increase in glutamate release and facilitation of mediodorsal thalamo-cortical transmission. *Eur. J. Neurosci.* 11, 18–30. doi: 10.1046/j.1460-9568.1999.00403.x

Gold, P. E. (2008). Protein synthesis and memory. Introduction. *Neurobiol. Learn. Mem.* 89, 199–200. doi: 10.1016/j.nlm.2007.12.005

Gold, P. E., and Wrenn, S. M. (2012). Cycloheximide impairs and enhances memory depending on dose and footshock intensity. *Behav. Brain Res.* 233, 293–297. doi: 10.1016/j.bbr.2012.05.010

Granger, R., Deadwyler, S., Davis, M., Moskovitz, B., Kessler, M., Rogers, G., et al. (1996). Facilitation of glutamate receptors reverses an age-associated memory impairment in rats. *Synapse* 22, 332–337. doi: 10.1002/(SICI)1098-2396(199604)22:4%3C332::AID-SYN4%3E3.0.CO;2-C

Granger, R., Staubli, U., Davis, M., Perez, Y., Nilsson, L., Rogers, G. A., et al. (1993). A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependent task. *Synapse* 15, 326–329. doi: 10.1002/syn.890150409

Grodstein, F. (2013). Hormone therapy in younger women and cognitive health. *JAMA Intern. Med.* 173, 1437–1438. doi: 10.1001/jamainternmed.2013.6827

Guzowski, J. F., Setlow, B., Wagner, E. K., and McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif268. *J. Neurosci.* 21, 5089–5098.

Haditsch, U., Anderson, M. P., Freewoman, J., Cord, B., Babu, H., Brakebusch, C., et al. (2013). Neuronal Rac1 is required for learning-evoked neurogenesis. *J. Neurosci.* 33, 12229–12241. doi: 10.1523/JNEUROSCI.2939-12.2013

Hall, R. A., Kessler, M., Quan, A., Ambros-Ingerson, J., and Lynch, G. (1993). Cyclothiazide decreases [3H]AMPA binding to rat brain membranes: evidence that AMPA receptor desensitization increases agonist affinity. *Brain Res.* 628, 345–348. doi: 10.1016/0006-8993(93)90978-V

Hampson, R. E., Song, D., Opris, L., Santos, L. M., Shin, D. C., Gerhardt, G. A., et al. (2013). Facilitation of memory encoding in primate hippocampus by a neuroprosthesis that promotes task-specific neural firing. *J. Neural Eng.* 10, 066013. doi: 10.1088/1741-2560/10/6/066013

Hampson, R., Rogers, G., Lynch, G., and Deadwyler, S. (1998a). Facilitative effects of the ampakine CX516 on short-term memory in rats: enhancement of delayed-nonmatch-to-sample performance. *J. Neurosci.* 18, 2740–2747.

Hampson, R., Rogers, G., Lynch, G., and Deadwyler, S. (1998b). Facilitative effects of the ampakine CX516 on short-term memory in rats: correlations with hippocampal neuronal activity. *J. Neurosci.* 18, 2748–2763.

Hara, Y., Yuk, F., Puri, R., Janssen, W. G., Rapp, P. R., and Morrison, J. H. (2014). Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. *Proc. Natl. Acad. Sci. U.S.A.* 111, 486–491. doi: 10.1073/pnas.1311310110

Harvey, C. D., and Svoboda, K. (2007). Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* 450, 1195–1200. doi: 10.1038/nature06416

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Curr. Opin. Neurobiol.* 16, 710–715. doi: 10.1016/j.conb.2006.09.002

Hess, U. S., Whalen, S. P., Sandoval, L. M., Lynch, G., and Gall, C. M. (2003). Ampakines reduce methamphetamine-driven rotation and activate neocortex in a regionally selective fashion. *Neuroscience* 121, 509–521. doi: 10.1016/S0306-4522(03)00423-8

Hintzman, D. L. (1976). "Repetition and memory," in *The Psychology of Learning and Motivation,* ed G. H. Bower (New York, NY: Academic Press; Elsevier), 47–91.

Holmes, A. D., Copland, D. A., Silburn, P. A., and Chenery, H. J. (2011). Acute nicotine enhances strategy-based semantic processing in Parkinson's disease. *Int. J. Neuropsychopharmacol.* 14, 877–885. doi: 10.1017/S1461145710001665

Hosseini, R., Benton, D. C., Dunn, P. M., Jenkinson, D. H., and Moss, G. W. (2001). SK3 is an important component of K(+) channels mediating the after-hyperpolarization in cultured rat SCG neurones. *J. Physiol.* 535, 323–334. doi: 10.1111/j.1469-7793.2001.00323.x

Ikonen, S., and Riekkinen, P. Jr. (1999). Effects of apamin on memory processing of hippocampal-lesioned mice. *Eur. J. Pharmacol.* 382, 151–156. doi: 10.1016/S0014-2999(99)00616-0

Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G. A., et al. (1997). Enhancement by an ampakine of memory encoding in humans. *Exp. Neurol.* 146, 553–559. doi: 10.1006/exnr.1997.6581

Isackson, P. J., Murray, K., Huntsman, M., and Gall, C. M. (1991). BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. *Neuron* 6, 937–948. doi: 10.1016/0896-6273(91)90234-Q

Jia, Y., Gall, C. M., and Lynch, G. (2010). Presynaptic BDNF promotes postsynaptic long-term potentiation in the dorsal striatum. *J. Neurosci.* 30, 14440–14445. doi: 10.1523/JNEUROSCI.3310-10.2010

Jiang, M., Peng, Q., Liu, X., Jin, J., Hou, Z., Zhang, J., et al. (2013). Small-molecule TrkB receptor agonists improve motor function and extend survival in a mouse model of Huntington's disease. *Hum. Mol. Genet.* 22, 2462–2470. doi: 10.1093/hmg/ddt098

Jin, R., Clark, S., Weeks, A. M., Dudman, J. T., Gouaux, E., and Partin, K. M. (2005). Mechanism of positive allosteric modulators acting on AMPA receptors. *J. Neurosci.* 25, 9027–9036. doi: 10.1523/JNEUROSCI.2567-05.2005

Jung, M. W., Larson, J., and Lynch, G. (1990). Long-term potentiation of monosynaptic EPSPs in rat piriform cortex *in vitro*. *Synapse* 6, 279–283. doi: 10.1002/syn.890060307

Katche, C., Bekinschtein, P., Slipczuk, L., Goldin, A., Izquierdo, I. A., Cammarota, M., et al. (2010). Delayed wave of c-Fos expression in the dorsal hippocampus involved specifically in persistence of long-term memory storage. *Proc. Natl. Acad. Sci. U.S.A.* 107, 349–354. doi: 10.1073/pnas.0912931107

Katche, C., Goldin, A., Gonzalez, C., Bekinschtein, P., and Medina, J. H. (2012). Maintenance of long-term memory storage is dependent on late posttraining Egr-1 expression. *Neurobiol. Learn. Mem.* 98, 220–227. doi: 10.1016/j.nlm.2012.08.001

Kessler, M., Baudry, M., and Lynch, G. (1989). Quinoxaline derivatives are high-affinity antagonists of the NMDA receptor-associated glycine sites. *Brain Res.* 489, 377–382. doi: 10.1016/0006-8993(89)90875-5

Klyuch, B. P., Dale, N., and Wall, M. J. (2012). Deletion of ecto-5′-nucleotidase (CD73) reveals direct action potential-dependent adenosine release. *J. Neurosci.* 32, 3842–3847. doi: 10.1523/JNEUROSCI.6052-11.2012

Kovalchuk, Y., Hanse, E., Kafitz, K. W., and Konnerth, A. (2002). Postsynaptic induction of BDNF-mediated long-term potentiation. *Science*. 295, 1729–1735. doi: 10.1126/science.1067766

Kowalczyk, T., Bocian, R., and Konopacki, J. (2013). The generation of theta rhythm in hippocampal formation maintained *in vitro*. *Eur. J. Neurosci.* 37, 679–699. doi: 10.1111/ejn.12091

Kramar, E. A., Babayan, A. H., Gavin, C. F., Cox, C. D., Jafari, M., Gall, C. M., et al. (2012b). Synaptic evidence for the efficacy of spaced learning. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5121–5126. doi: 10.1073/pnas.1120700109

Kramar, E. A., Chen, L. Y., Brandon, N. J., Rex, C. S., Liu, F., Gall, C. M., et al. (2009). Cytoskeletal changes underlie estrogen's acute effects on synaptic transmission and plasticity. *J. Neurosci.* 29, 12982–12993. doi: 10.1523/JNEUROSCI.3059-09.2009

Kramar, E. A., Chen, L. Y., Lauterborn, J. C., Simmons, D. A., Gall, C. M., and Lynch, G. (2012a). BDNF upregulation rescues synaptic plasticity in middle-aged ovariectomized rats. *Neurobiol. Aging* 33, 708–719. doi: 10.1016/j.neurobiolaging.2010.06.008

Kramar, E. A., Lin, B., Lin, C. Y., Arai, A. C., Gall, C. M., and Lynch, G. (2004). A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J. Neurosci.* 24, 5151–5161. doi: 10.1523/JNEUROSCI.0800-04.2004

Kramar, E. A., Lin, B., Rex, C. S., Gall, C. M., and Lynch, G. (2006). Integrin-driven actin polymerization consolidates long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5579–5584. doi: 10.1073/pnas.0601354103

Kramar, E. A., and Lynch, G. (2003). Developmental and regional differences in the consolidation of long-term potentiation. *Neuroscience* 118, 387–398. doi: 10.1016/S0306-4522(02)00916-8

Labrie, V., and Roder, J. C. (2010). The involvement of the NMDA receptor d-serine/glycine site in the pathophysiology and treatment of schizophrenia. *Neurosci. Biobehav. Rev.* 34, 351–372. doi: 10.1016/j.neubiorev.2009.08.002

Larson, J., Wong, D., and Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Res.* 368, 347–350. doi: 10.1016/0006-8993(86)90579-2

Lauterborn, J. C., Lynch, G., Vanderklish, P., Arai, A., and Gall, C. M. (2000). Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J. Neurosci.* 20, 8–21.

Lee, K., Oliver, M., Schottler, F., Creager, R., and Lynch, G. (1979). Ultrastructural effects of repetitive synaptic stimulation in the hippocampal slice preparation: a preliminary report. *Exp. Neurol.* 65, 478–480. doi: 10.1016/0014-4886(79)90114-6

Lee, K., Schottler, F., Oliver, M., and Lynch, G. (1980). Brief bursts of high-frequency stimulation produce two types of structural changes in rat hippocampus. *J. Neurophysiol.* 44, 247–258.

Lin, B., Kramar, E. A., Bi, X., Brucher, F. A., Gall, C. M., and Lynch, G. (2005). Theta stimulation polymerizes actin in dendritic spines of hippocampus. *J. Neurosci.* 25, 2062–2069. doi: 10.1523/JNEUROSCI.4283-04.2005

Liu, F., Day, M., Muniz, L. C., Bitran, D., Arias, R., Revilla-Sanchez, R., et al. (2008). Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. *Nat. Neurosci.* 11, 334–343. doi: 10.1038/nn2057

Lynch, G. (1998). Memory and the brain: unexpected chemistries and a new pharmacology. *Neurobiol. Learn. Mem.* 70, 82–100. doi: 10.1006/nlme.1998.3840

Lynch, G. (2004a). AMPA receptor modulators as cognitive enhancers. *Curr. Opin. Pharmacol.* 4, 4–11. doi: 10.1016/j.coph.2003.09.009

Lynch, G., and Gall, C. M. (2013). Mechanism based approaches for rescuing and enhancing cognition. *Front. Neurosci.* 7:143. doi: 10.3389/fnins.2013.00143

Lynch, G., Kramar, E. A., Babayan, A. H., Rumbaugh, G., and Gall, C. M. (2013). Differences between synaptic plasticity thresholds result in new timing rules for maximizing long-term potentiation. *Neuropharmacology* 64, 27–36. doi: 10.1016/j.neuropharm.2012.07.006

Lynch, G., Palmer, L. C., and Gall, C. M. (2011). The likelihood of cognitive enhancement. *Pharmacol. Biochem. Behav.* 99, 116–129. doi: 10.1016/j.pbb.2010.12.024

Lynch, M. A. (2004b). Long-term potentiation and memory. *Physiol. Rev.* 84, 87–136. doi: 10.1152/physrev.00014.2003

Madau, P., Clark, A. G., Neale, S. A., Smith, L., Hamilton, N., Thompson, F., et al. and Lambert, J. J. (2009). "Program no. 613.2. Org 1, Org 2, and Org 3 potentiate NMDA-receptor mediated responses *in vitro*," in *Society for Neuroscience Annual Meeting Planner* (Chicago, IL: Society for Neuroscience).

Matsuzaki, M., Honkura, N., Ellis-Davies, G. C., and Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature*. 429, 761–766. doi: 10.1038/nature02617

McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., Alenghat, T., et al. (2011). HDAC3 is a critical negative regulator of long-term memory formation. *J. Neurosci.* 31, 764–774. doi: 10.1523/JNEUROSCI.5052-10.2011

Monaghan, D. T., Irvine, M. W., Costa, B. M., Fang, G., and Jane, D. E. (2012). Pharmacological modulation of NMDA receptor activity and the advent of negative and positive allosteric modulators. *Neurochem. Int.* 61, 581–592. doi: 10.1016/j.neuint.2012.01.004

Morris, R. G. (2003). Long-term potentiation and memory. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 643–647. doi: 10.1098/rstb.2002.1230

Noetzli, M., and Eap, C. B. (2013). Pharmacodynamic, pharmacokinetic and pharmacogenetic aspects of drugs used in the treatment of Alzheimer's disease. *Clin. Pharmacokinet.* 52, 225–241. doi: 10.1007/s40262-013-0038-9

Normann, C., and Berger, M. (2008). Neuroenhancement: status quo and perspectives. *Eur. Arch. Psychiatry Clin. Neurosci.* 258(Suppl. 5), 110–114. doi: 10.1007/s00406-008-5022-2

Oliver, M. W., Kessler, M., Larson, J., Schottler, F., and Lynch, G. (1990). Glycine site associated with the NMDA receptor modulates long-term potentation. *Synapse* 5, 265–270. doi: 10.1002/syn.890050403

Olpe, H. R., Klebs, K., Kung, E., Campiche, P., Glatt, A., Ortmann, R., et al. (1987). Cholinomimetics induce theta rhythm and reduce hippocampal pyramidal cell excitability. *Eur. J. Pharmacol.* 142, 275–283. doi: 10.1016/0014-2999(87)90117-8

Ooishi, Y., Kawato, S., Hojo, Y., Hatanaka, Y., Higo, S., Murakami, G., et al. (2012). Modulation of synaptic plasticity in the hippocampus by hippocampus-derived estrogen and androgen. *J. Steroid Biochem. Mol. Biol.* 131, 37–51. doi: 10.1016/j.jsbmb.2011.10.004

Otto, T., Eichenbaum, H., Wiener, S. I., and Wible, C. G. (1991). Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation. *Hippocampus* 1, 181–192. doi: 10.1002/hipo.450010206

Paul, S. M., Doherty, J. J., Robichaud, A. J., Belfort, G. M., Chow, B. Y., Hammond, R. S., et al. (2013). The major brain cholesterol metabolite 24(s)-hydroxycholesterol is a potent allosteric modulator of n-methyl-d-aspartate receptors. *J. Neurosci.* 33, 17290–17300. doi: 10.1523/JNEUROSCI.2619-13.2013

Perlmutter, L. S., Siman, R., Gall, C., Seubert, P., Baudry, M., and Lynch, G. (1988). The ultrastructural localization of calcium-activated protease 'calpain' in rat brain. *Synapse* 2, 79–88. doi: 10.1002/syn.890020111

Peters, J., and De Vries, T. J. (2013). D-cycloserine administration directly to infralimbic medial prefrontal cortex enhances extinction memory in sucrose-seeking animals. *Neuroscience* 230, 24–30. doi: 10.1016/j.neuroscience.2012.11.004

Plath, N., Ohana, O., Dammermann, B., Errington, M. L., Schmitz, D., Gross, C., et al. (2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron* 52, 437–444. doi: 10.1016/j.neuron.2006.08.024

Porrino, L., Daunais, J., Rogers, G., Hampson, R., and Deadwyler, S. (2005). Facilitation of task performance and removal of the effects of sleep deprivation

by an ampakine (CX717) in nonhuman primates. *PLoS Biol.* 3:e299. doi: 10.1371/journal.pbio.0030299

Rex, C. S., Chen, L. Y., Sharma, A., Liu, J., Babayan, A. H., Gall, C. M., et al. (2009). Different Rho GTPase-dependent signaling pathways initiate sequential steps in the consolidation of long-term potentiation. *J. Cell Biol.* 186, 85–97. doi: 10.1083/jcb.200901084

Rex, C. S., Gavin, C. F., Rubio, M. D., Kramar, E. A., Chen, L. Y., Jia, Y., et al. (2010). Myosin IIb regulates actin dynamics during synaptic plasticity and memory formation. *Neuron*. 67, 603–617. doi: 10.1016/j.neuron.2010.07.016

Rex, C. S., Kramar, E. A., Colgin, L. L., Lin, B., Gall, C. M., and Lynch, G. (2005). Long-term potentiation is impaired in middle-aged rats: regional specificity and reversal by adenosine receptor antagonists. *J. Neurosci.* 25, 5956–5966. doi: 10.1523/JNEUROSCI.0880-05.2005

Rex, C. S., Lauterborn, J. C., Lin, C. Y., Kramar, E. A., Rogers, G. A., Gall, C. M., et al. (2006). Restoration of long-term potentiation in middle-aged hippocampus after induction of brain-derived neurotrophic factor. *J. Neurophysiol.* 96, 677–685. doi: 10.1152/jn.00336.2006

Rex, C. S., Lin, C. Y., Kramar, E. A., Chen, L. Y., Gall, C. M., and Lynch, G. (2007). Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. *J. Neurosci.* 27, 3017–3029. doi: 10.1523/JNEUROSCI.4037-06.2007

Rioult-Pedotti, M. S., Friedman, D., and Donoghue, J. P. (2000). Learning-induced LTP in neocortex. *Science* 290, 533–536. doi: 10.1126/science.290.5491.533

Roman, F., Staubli, U., and Lynch, G. (1987). Evidence for synaptic potentiation in a cortical network during learning. *Brain Res*. 418, 221–226. doi: 10.1016/0006-8993(87)90089-8

Routtenberg, A. (2008). The substrate for long-lasting memory: if not protein synthesis, then what? *Neurobiol. Learn. Mem.* 89, 225–233. doi: 10.1016/j.nlm.2007.10.012

Sarter, M., Parikh, V., and Howe, W. M. (2009). nAChR agonist-induced cognition enhancement: integration of cognitive and neuronal mechanisms. *Biochem. Pharmacol.* 78, 658–667. doi: 10.1016/j.bcp.2009.04.019

Schmid, D. A., Yang, T., Ogier, M., Adams, I., Mirakhur, Y., Wang, Q., et al. (2012). A TrkB small molecule partial agonist rescues TrkB phosphorylation deficits and improves respiratory function in a mouse model of Rett syndrome. *J. Neurosci.* 32, 1803–1810. doi: 10.1523/JNEUROSCI.0865-11.2012

Schmitz, D., Mellor, J., Breustedt, J., and Nicoll, R. A. (2003). Presynaptic kainate receptors impart an associative property to hippocampal mossy fiber long-term potentiation. *Nat. Neurosci.* 6, 1058–1063. doi: 10.1038/nn1116

Seese, R. R., Babayan, A. H., Katz, A. M., Cox, C. D., Lauterborn, J. C., Lynch, G., et al. (2012). LTP induction translocates cortactin at distant synapses in wild-type but not Fmr1 knock-out mice. *J. Neurosci.* 32, 7403–7413. doi: 10.1523/JNEUROSCI.0968-12.2012

Seese, R. R., Chen, L. Y., Cox, C. D., Schulz, D., Babayan, A. H., Bunney, W. E., et al. (2013). Synaptic abnormalities in the infralimbic cortex of a model of congenital depression. *J. Neurosci.* 33, 13441–13448. doi: 10.1523/JNEUROSCI.2434-13.2013

Sheinin, A., Shavit, S., and Benveniste, M. (2001). Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. *Neuropharmacology* 41, 151–158. doi: 10.1016/S0028-3908(01)00073-9

Shires, K. L., Da Silva, B. M., Hawthorne, J. P., Morris, R. G. M., and Martin, S. J. (2012). Synaptic tagging and capture in the living rat. *Nature Comm*. 3:1246. doi: 10.1038/ncomms2250

Shors, T. J., Servatius, R. J., Thompson, R. F., Rogers, G., and Lynch, G. (1995). Enhanced glutamatergic neurotransmission facilitates classical conditioning in the freely-moving rat. *Neurosci. Lett*. 186, 153–156. doi: 10.1016/0304-3940(95)11309-K

Simmons, D. A., Belichenko, N. P., Yang, T., Condon, C., Monbureau, M., Shamloo, M., et al. (2013). A small molecule TrkB ligand reduces motor impairment and neuropathology in R6/2 and BACHD mouse models of Huntington's disease. *J. Neurosci.* 33, 18712–18727. doi: 10.1523/JNEUROSCI.1310-13.2013

Simmons, D. A., Mehta, R. A., Lauterborn, J. C., Gall, C. M., and Lynch, G. (2011). Brief ampakine treatments slow the progression of Huntington's disease phenotypes in R6/2 mice. *Neurobiol. Dis.* 41, 436–444. doi: 10.1016/j.nbd.2010.10.015

Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M., et al. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4906–4911. doi: 10.1073/pnas.0811228106

Sirvio, J., Larson, J., Quach, C. N., Rogers, G. A., and Lynch, G. (1996). Effects of pharmacologically facilitating glutamatergic transmission in the trisynaptic intrahippocampal circuit. *Neuroscience* 74, 1025–1035. doi: 10.1016/0306-4522(96)00170-4

Snider, J., Plank, M., Lynch, G., Halgren, E., and Poizner, H. (2013). Human cortical theta during free exploration encodes space and predicts subsequent memory. *J. Neurosci.* 33, 15056–15068. doi: 10.1523/JNEUROSCI.0268-13.2013

Staubli, U. (1992). A peculiar form of potentiation in mossy fiber synapses. *Epilepsy Res. Suppl.* 7, 151–157.

Staubli, U., and Lynch, G. (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Res.* 435, 227–234. doi: 10.1016/0006-8993(87)91605-2

Staubli, U., Rogers, G., and Lynch, G. (1994). Facilitation of glutamate receptors enhances memory. *Proc. Natl. Acad. Sci. U.S.A*. 91, 777–781. doi: 10.1073/pnas.91.2.777

Staubli, U., and Xu, F. B. (1995). Effects of 5-HT3 receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. *J. Neurosci.* 15, 2445–2452.

Stefanko, D. P., Barrett, R. M., Ly, A. R., Reolon, G. K., and Wood, M. A. (2009). Modulation of long-term memory for object recognition via HDAC inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9447–9452. doi: 10.1073/pnas.0903964106

Stephan, H., Frahm, H., and Baron, G. (1981). New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* 35, 1–29. doi: 10.1159/000155963

Sugrue, L. P., Corrado, G. S., and Newsome, W. T. (2005). Choosing the greater of two goods: neural currencies for valuation and decision making. *Nat. Rev. Neurosci.* 6, 363–375. doi: 10.1038/nrn1666

Sun, Y., Olson, R., Horning, M., Armstrong, N., Mayer, M., and Gouaux, E. (2002). Mechanism of glutamate receptor desensitization. *Nature* 417, 245–253. doi: 10.1038/417245a

Swanson, L. W. (1995). Mapping the human brain: past, present, and future. *Trends Neurosci*. 18, 471–474. doi: 10.1016/0166-2236(95)92766-J

Thompson, R. F., Berger, T. W., Cegavske, C. F., Patterson, M. M., Roemer, R. A., Teyler, T. J., et al. (1976). The search for the engram. *Am. Psychol.* 31, 209–226. doi: 10.1037/0003-066X.31.3.209

Thompson, R. F., and Krupa, D. J. (1994). Organization of memory traces in the mammalian brain. *Ann. Rev. Neurosci*. 17, 519–549. doi: 10.1146/annurev.ne.17.030194.002511

Tsai, G. E., Falk, W. E., Gunther, J., and Coyle, J. T. (1999). Improved cognition in Alzheimer's disease with sort-term D-cycloserine treatment. *Am. J. Psychiatry* 156, 467–469.

Tsunada, J., and Sawaguchi, T. (2012). Neuronal categorization and discrimination of social behaviors in primate prefrontal cortex. *PLoS ONE* 7:e52610. doi: 10.1371/journal.pone.0052610

Tully, T., Bourtchouladze, R., Scott, R., and Tallman, J. (2003). Targeting the CREB pathway for memory enhancers. *Nat. Rev. Drug Discov*. 2, 267–277. doi: 10.1038/nrd1061

Turner, D. C., Clark, L., Pomarol-Clotet, E., McKenna, P., Robbins, T. W., and Sahakian, B. J. (2004). Modafinil improves cognition and attentional set shifting in patients with chronic schizophrenia. *Neuropsychopharmacology* 29, 1363–1373. doi: 10.1038/sj.npp.1300457

Vertes, R. P. (2005). Hippocampal theta rhythm: a tag for short-term memory. *Hippocampus*. 15, 923–935. doi: 10.1002/hipo.20118

Vick, K. A. T., Guidi, M., and Stackman, R. W. Jr. (2010). *In vivo* pharmacological manipulation of small conductance Ca(2+)-activated K(+) channels influences motor behavior, object memory and fear conditioning. *Neuropharmacology* 58, 650–659. doi: 10.1016/j.neuropharm.2009.11.008

Vierk, R., Glassmeier, G., Zhou, L., Brandt, N., Fester, L., Dudzinski, D., et al. (2012). Aromatase inhibition abolishes LTP generation in female but not in male mice. *J. Neurosci.* 32, 8116–8126. doi: 10.1523/JNEUROSCI.5319-11.2012

Wall, M. J., and Dale, N. (2013). Neuronal transporter and astrocytic ATP exocytosis underlie activity-dependent adenosine release in the hippocampus. *J. Physiol*. 591, 3853–3871. doi: 10.1113/jphysiol.2013.253450

Whitlock, J. R., Heynen, A. J., Shuler, M. G., and Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science* 313, 1093–1097. doi: 10.1126/science.1128134

Wonnacott, S. (1997). Presynaptic nicotinic ACh receptors. *Trends Neurosci*. 20, 92–98. doi: 10.1016/S0166-2236(96)10073-4

Wu, F. S., Gibbs, T. T., and Farb, D. H. (1991). Pregnenolone sulfate: a positive allosteric modulator at the n-methyl-d-aspartate receptor. *Mol. Pharmacol.* 40, 333–336.

Zeeb, F. D., Robbins, T. W., and Winstanley, C. A. (2009). Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. *Neuropsychopharmacology* 34, 2329–2343. doi: 10.1038/npp.2009.62

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 April 2014; accepted: 30 April 2014; published online: 20 May 2014.
Citation: Lynch G, Cox CD and Gall CM (2014) Pharmacological enhancement of memory or cognition in normal subjects. Front. Syst. Neurosci. ***8****:90. doi: 10.3389/fnsys.2014.00090*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 28 April 2014
doi: 10.3389/fnsys.2014.00069

Enhancement of cognitive and neural functions through complex reasoning training: evidence from normal and clinical populations

Sandra B. Chapman*[1] *and* ***Raksha A. Mudar*[2]**

[1] *Center for BrainHealth®, The University of Texas at Dallas, Dallas, TX, USA*
[2] *Department of Speech and Hearing Science, University of Illinois at Urbana-Champaign, Champaign, IL, USA*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Jitendra Sharma, Massachusetts Institute of Technology, USA
Aaron P. Blaisdell, University of California, Los Angeles, USA

****Correspondence:***
Sandra B. Chapman, Center for BrainHealth®, The University of Texas at Dallas, 2200 W Mockingbird Ln, Dallas, TX, 75235, USA
e-mail: schapman@utdallas.edu

Public awareness of cognitive health is fairly recent compared to physical health. Growing evidence suggests that cognitive training offers promise in augmenting cognitive brain performance in normal and clinical populations. Targeting higher-order cognitive functions, such as reasoning in particular, may promote generalized cognitive changes necessary for supporting the complexities of daily life. This data-driven perspective highlights cognitive and brain changes measured in randomized clinical trials that trained gist reasoning strategies in populations ranging from teenagers to healthy older adults, individuals with brain injury to those at-risk for Alzheimer's disease. The evidence presented across studies support the potential for Gist reasoning training to strengthen cognitive performance in trained and untrained domains and to engage more efficient communication across widespread neural networks that support higher-order cognition. The meaningful benefits of Gist training provide compelling motivation to examine optimal dose for sustained benefits as well as to explore additive benefits of meditation, physical exercise, and/or improved sleep in future studies.

Keywords: cognitive training, gist reasoning, cognition, neural, brain plasticity

INTRODUCTION

Five decades of research have shown that the brain is modifiable in the context of stimulating cognitive experiences and in response to cognitive training (e.g., Bennett et al., 1964; Schooler, 1984; Schooler et al., 1999). Cognitive training involves guided practice on tasks targeting specific cognitive functions (e.g., working memory, attention) or specific cognitive strategies (Clare and Woods, 2004; Martin et al., 2011). In general, evidence from most cognitive training studies reveal improvements in performance, especially in cognitive functions directly targeted by training (Nyberg et al., 2003; Dahlin et al., 2008; Jaeggi et al., 2008; Valenzuela et al., 2008; Carlson et al., 2009; Zelinski, 2009; Anguera et al., 2013; Chapman et al., 2013). However, much remains to be learned about the kinds of training programs that provide meaningful changes beyond the specific skills trained. From a public health perspective, cognitive training will be deemed useful if the training has generalized benefits and builds cognitive capacities to support performance in day-to-day tasks (Anand et al., 2011).

Concerted efforts are being directed toward exploring the realities of augmenting cognitive performance. Memory training has been a key focus of cognitive training programs motivated by extant evidence that declines in memory commonly occur with normal aging and brain diseases/injuries (e.g., Papp et al., 2009; Gates et al., 2011). Memory training mostly yields short-term improvements in memory; however, benefits do not generalize/transfer to other cognitive functions (Papp et al., 2009; Valenzuela and Sachdev, 2009; Martin et al., 2011; Tardif and Simard, 2011; Reijnders et al., 2012; Teixeira et al., 2012; Thompson et al., 2013), nor do they produce long-term benefits (Rebok et al., 2014). Emerging evidence suggests that reasoning training has widespread and lasting benefits that may guard against and restore cognitive losses in aging and/or disease (Willis et al., 2006; Anand et al., 2011; Chapman et al., 2013; Rebok et al., 2014). For instance, in one of the largest randomized cognitive training trials to date, i.e., the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) trial, participants who underwent reasoning training (i.e., focused on solving problems related to serial patterns and sequences) showed less decline in self-reported Independent Activities of Daily Living over a 10-year period compared to the memory training group (i.e., strategies to improve verbal episodic memory) (Rebok et al., 2014).

A growing body of evidence suggests that advanced reasoning engages gist-based processing (Reyna and Brainerd, 1995, 2011; Reyna and Lloyd, 2006). In the current paper, we present a perspective on a particular type of reasoning training that targets *gist-based reasoning* abilities. Gist reasoning is defined as the ability to synthesize and create abstract meanings from the literal content/information, a skill essential for academic, occupational, and functional competence. The primary objective of strategy-driven Gist reasoning training is to improve the ability to abstract generalized meanings from complex information and to incorporate these strategies into everyday tasks. Gist training is informed by cognitive theories of discourse meaning structure (Van Dijk et al., 1983) and information processing (Reyna and Brainerd,

1995), specifically van Dijk and Kintsch's macrostructure/global meanings and Reyna and Brainerd's gist representation. In this perspective paper, we provide a general framework of Gist reasoning training and highlight converging findings from Gist reasoning training studies across adolescent and adult populations. This novel strategy-based approach to cognitive training may provide insights and future directions to guide testing and development of training protocols that have ecological validity/real life application.

GENERAL OVERVIEW OF COGNITIVE TRAINING PROTOCOLS

In the distinct studies summarized below, we examined the potential for Gist reasoning training to improve cognitive performance as compared to control protocol/s (active control/wait-list control) (see **Table 1**) using a pseudo-randomized control design. We present data from studies on two groups of adolescents [i.e., typically developing eighth graders; youth with traumatic brain injury (TBI)] and three adult populations (i.e., adults with TBI, adults with early mild cognitive impairment, and cognitive healthy adults). Overall, the length of the training period across studies was short-term, ranging from 8 to 12 sessions delivered over one to two months in 45 to 60 min. duration, with each study protocol being identical for all participants within the trial. When an active control protocol was used (Memory strategy or New learning), it was comparable to the Gist reasoning training in length, complexity, and active group discussion/engagement.

GIST REASONING TRAINING

The Gist reasoning training (also referred to as gist training) is a strategy- rather than content-based program. The protocol entails three core strategies: *strategic attention, integrated reasoning*, and *innovation* summarized in **Table 1**, delineated in a training manual, and defined in more detail elsewhere (e.g., Gamino et al., 2010; Vas et al., 2011; Chapman et al., 2012; Chapman and Mudar, 2013). The strategies facilitate cognitive control and depth of encoding to facilitate knowledge acquisition and creation. The strategy instruction is hierarchical and dynamically interdependent, with each strategy building on previous strategies, and involves practice that encourages integrating all steps when tackling mental activities both inside and outside of training.

Table 1 | Brief description of experimental and control training protocols.

Training	Description
EXPERIMENTAL TRAINING	
Gist Reasoning Training (Gamino et al., 2010; Anand et al., 2011; Vas et al., 2011; Chapman et al., 2013; Mudar et al., 2013; Motes et al., 2014; Cook et al., under review)	Hierarchical Strategies Strategic Attention: Consciously blocking/inhibiting distractions and irrelevant/less relevant information Integrated Reasoning: Binding explicit facts with world knowledge to construct generalized/abstracted meanings Innovation: Deriving multiple interpretations and generalized applications beyond the concrete content reflecting fluency and fluidity of thinking
CONTROL TRAINING	
Memory Strategy Training (Gamino et al., 2010; Cook et al., under review)	Training of bottom-up memory strategies: focus on encoding, rehearsal, retrieval practice, cross modality associations and developing mnemonics
New Learning Training (Vas et al., 2011; Mudar et al., 2013) (Gamino et al., 2010)	New Learning about brain functions and influences on cognition Example topics covered: Brain Structures and Functions; The Neuron; Neuroplasticity and Neurogenesis; Memory and the Brain; Executive Functions of the Brain; Effects of Sleep and Stress on the Brain; Diet and Exercise and the Brain; Social Bonds and the Brain. This program was originally developed at the Rotman Institute, Canada and is referred to as Brain health workshop (Binder et al., 2008).
Wait-list controls (Chapman et al., 2013; Motes et al., 2014)	No contact group

EVIDENCE OF GAINS FROM GIST TRAINING vs. CONTROL CONDITIONS

COGNITIVE TRAINING IN YOUTH (GAMINO ET AL., 2010; MOTES ET AL., 2014; COOK ET AL., UNDER REVIEW)

Typically developing adolescents

In this study, middle-school students (8th graders) were randomly assigned to one of three training protocols, either the Gist reasoning, or one of two control groups, i.e., the Memory training or the New learning (Gamino et al., 2010). The trainings were delivered over 9 class periods lasting 45 min over four weeks. These students were from lower SES backgrounds (92% living in poverty). The three groups were comparable in age, gender, memory, and cognitive abilities at baseline. Outcomes comparing pre- to post- training performances were scored by researchers blinded to individuals and group identity.

Results revealed that gist-trained students improved ability to abstract novel meanings from lengthy classroom-type texts. Additionally, these gist-trained students showed significant improvement in memory for facts, a skill not targeted in training. We found a significant relation between gains in ability to abstract meanings and a real life school measure, the Texas Assessment of Knowledge and Skills (TAKS) reading testing "Applying Critical Thinking Skills." In contrast, students in the Memory training showed improvements only in memory for isolated facts with no gains in abstracting meanings. The New learning group did not show any significant gains. These findings suggest that gist reasoning strategies may have a broader impact on learning that improves deeper understanding of information encountered beyond shallow learning of isolated facts.

In a subsequent study Motes et al. (2014) examined the effects of Gist training on neural mechanisms related to inhibitory

control using electroencephalograph (EEG). Participants in the Gist training group vs. a Wait-list control group completed three visual go/no-go tasks that involved varying levels of semantic categorization (basic to more abstract superordinate categorization) both before and after training or a comparable duration in the case of the controls. The findings revealed that participants in the Gist group showed significant improvement in inhibition (i.e., ability to withhold behavioral responses on no-go trials) following training unlike the control group across basic and superordinate categorization tasks. Furthermore, those in the Gist trained group showed EEG changes suggestive of improved processing efficiency, as reflected by significant reduction in P3 no-go amplitude post-training compared to pre-training. No such differences were observed in the Wait-list controls. Overall, both the behavioral findings and the electrophysiological data across these two studies suggest that Gist training appears to enhance inhibitory responses both at the behavioral and neural level in typically developing middle school children. However, these findings have to be further validated using additional procedures (e.g., positive and negative patterning) in future studies.

Adolescents with TBI

The benefits of Gist training vs. Memory training in adolescents with TBI (ages 12–20 years) at chronic stages post injury (>6 months) was evaluated in a recent study (Cook et al., under review). Participants received one-on-one training delivered in eight sessions of 45 min duration over a four week period. The text materials for the Memory training were largely the same content as used in the Gist protocol. The findings revealed that the Gist-trained group significantly improved in their ability to abstract/synthesize meanings as compared to the Memory-trained group. The Gist-trained group also showed enhanced performance on cognitive measures for memory for facts, working memory (Digit Span backwards and Letter-Number Sequencing—WAIS III or WISC IV), and inhibition (Color Word Interference—D-KEFS). None of these latter cognitive skills were specifically targeted during training, suggesting spill-over effects of the Gist training to untrained cognitive domains. The Memory trained group's performance on memory for facts approached significance; however, results failed to show significant gains in domains of abstracting meaning, working memory, inhibition, or other cognitive areas.

Taken together, evidence from these three studies implicates the potential to enhance cognitive performance in areas of cognitive control in typically developing teens and teens with TBI, beyond the traditional treatment phase of 3 months post injury. Augmenting cognitive performance in normally developing populations and individuals at chronic stages post brain injury represents newer and promising areas of investigation.

ADULT COGNITIVE TRAINING TRIALS (ANAND ET AL., 2011; VAS ET AL., 2011; CHAPMAN ET AL., 2013)

Adults with TBI

In a randomized trial, the effects of Gist-reasoning training were compared to an active control training involving New learning in adults with TBI (ages 29–65 years) in chronic stages post-injury (>one year) (Vas et al., 2011). The Gist-trained group exhibited significant gains in abstracting meanings from complex information as compared to the New learning training group. Moreover, the Gist-trained group showed significant enhancement on measures of immediate memory (Digit Span Forward—WAIS-III), executive functions of working memory (Letter Number-Sequence—WAIS III and Daneman and Carpenter listening span) and cognitive switching (Color-Word Interference task—D-KEFS), and non-verbal reasoning (Matrix Reasoning—WAIS III). None of these latter cognitive processes were specifically targeted during training, adding further evidence of generalized benefits from Gist training. Furthermore, this group reported significant improvement in daily life skills (GOS-E, Functional Status Examination, Community Integration Questionnaire), such as increased socialization (e.g., initiating and planning activities with family and friends), higher levels of life productivity (e.g., active job seeking, setting up interviews, improved work efficiency and output), improved personal management (e.g., completing household responsibilities and house upkeep) and better sense of overall well-being. These reported real life gains from Gist training are not likely due to placebo effects since both groups believed they were receiving the experimental training. These training-related gains were maintained at 6-month follow-up. Despite comparable levels of active and engaged learning, the New Learning group failed to show significant gains on any of the performance measures.

Adults with pre-clinical Alzheimer's (Mild Cognitive Impairment)

This study compared immediate benefits of Gist training vs. New learning on cognition in individuals with Mild Cognitive Impairment (MCI) in a random assignment design (Mudar et al., 2013). Groups were comparable in age, Mini-Mental State Examination (MMSE) and episodic memory scores at baseline. Both groups received 8 h of training over a period of 4 weeks. We found differential cognitive gains between the two groups. Significant improvement was observed in the Gist-trained group on measures of abstract meaning, strategic attention, memory (immediate and delayed recall Logical Memory subtest-WMS III) and abstract verbal reasoning (Similarities-WAIS III). In contrast, participants in the New learning group showed significant improvements in remembering facts on an experimental text memory measure and on the Sorting test (D-KEFS). These findings suggest that both trainings offered some benefit for those with a progressive neurologic disease; however, Gist training offered broader benefits. Given the lack of pharmacological treatment options in slowing cognitive deterioration in pre-clinical stages of dementia, one current focus is on identifying non-pharmacological options (e.g., cognitive training) that can slow the rate of cognitive deterioration. Although maintenance of gains from Gist training needs to be studied in larger trials, even short-term increases in cognitive performance offer a glimmer of hope. Different doses and more frequent time intervals of cognitive training needs to be examined to harness optimal benefits in terms of maintaining cognitive capacity and slowing decline.

Cognitively healthy adults

The short-term effects of (i.e., 12 weeks of 1-h in-person training/week + 2 h/week homework) Gist training on cognitive and neural plasticity was examined in cognitively healthy

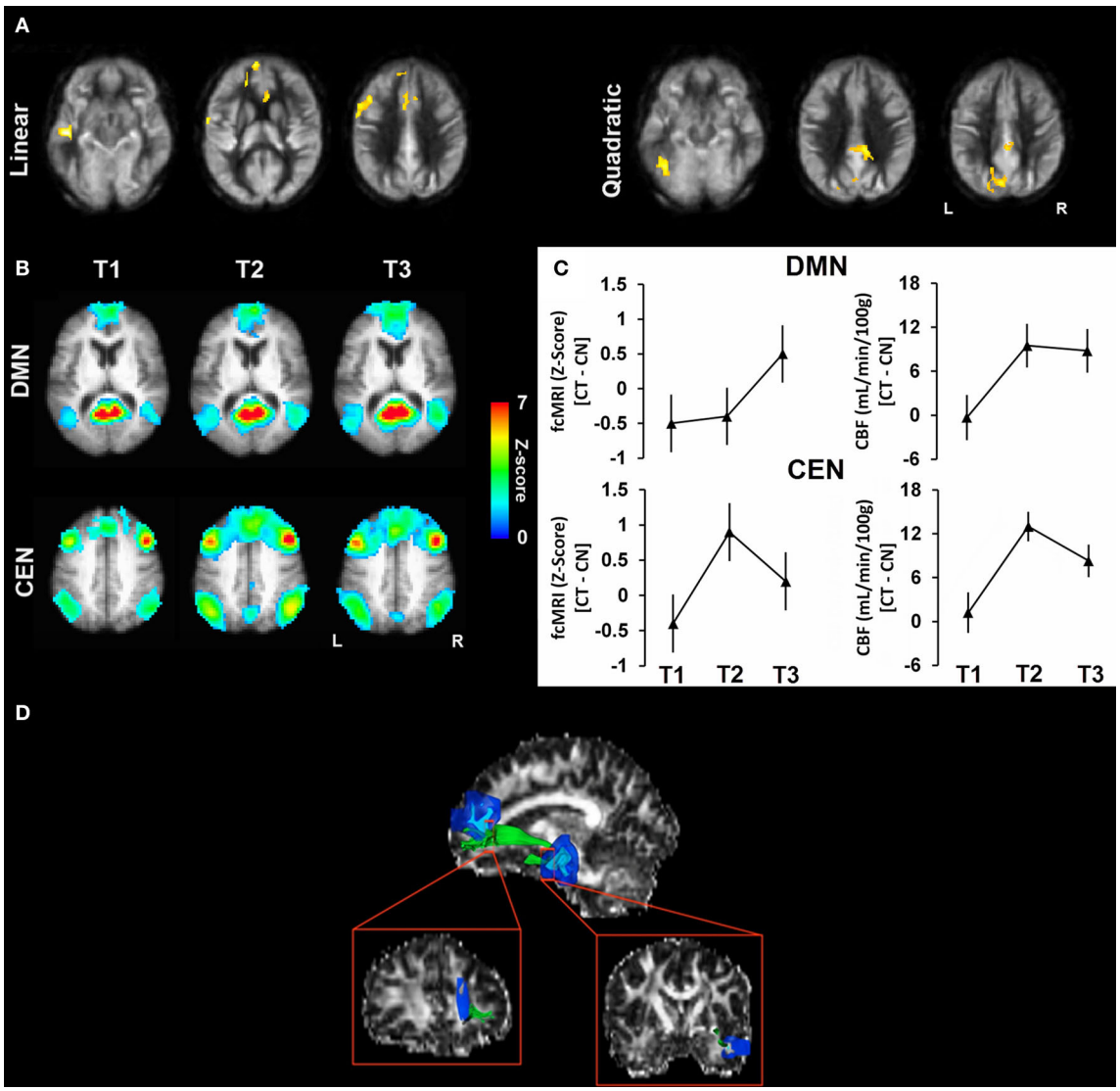

FIGURE 1 | This figure illustrates the convergence of neural plasticity findings in the cognitive training vs. control group across cerebral blood flow, functional connectivity, and structural DTI changes implicating functional brain changes more frequent and rapid than structural plasticity comparing changes at T2 and T3 to baseline T1 measures. (A) Results of CBF voxel-based comparison superimposed on an average CBF map of all participants for linear and quadratic interaction contrasts. **(B)** The average functional connectivity maps (i.e., DMN and CEN) of the cognitive training group are overlaid on their average T1-weighted image. **(C)** Mean increase in fcMRI z-scores (left column) and mean change in absolute CBF (right column) are shown for DMN and CEN across time periods. **(D)** A representative participant's uncinate fasciculus (green) is overlaid on his fractional anisotropy map from DTI (Chapman et al., 2013).

adults (55—75 years of age), well-screened to rule out early dementia, as compared to a Wait-list control group (Chapman et al., 2013). Individuals were assessed at baseline, at mid-point and immediately post-training. The Gist-trained group showed generalized cognitive gains consistent with an earlier pilot study in healthy older adults in which participants improved in cognitive switching (Trail Making Test Part B), concept abstraction (Similarities-WAIS III), and fluency (COWAT letter fluency) (Anand et al., 2011). We examined changes in large-scale brain networks in terms of brain blood flow (CBF) and functional and structural connectivity, in addition to the relationship between improved cognitive performance and brain changes. Results revealed significant increases in global CBF (total brain) as well as increased regional CBF measures as measured with Arterial Spin Labeling in two distinct and major brain networks tied to higher-order cognition, namely the Default Mode Network (DMN) and the Central Executive Network (CEN). Specifically, the DMN and CEN have been associated with top-down, cognitive control processes (Bressler and Menon, 2010). We also found corroborating patterns of increased functional connectivity in these very same major brain networks as the increased CBF (**Figure 1**). Additionally, we found significantly increased

structural connectivity as measured with diffusion tensor imaging (DTI) in the left uncinate fasciculus—the white matter tract that connects the middle temporal lobe to the superior medial frontal gyri after 12 weeks of training. The finding of increased white matter integrity in this select brain region perhaps suggests that some degree of atrophy at the level of white matter tracts in healthy aging may be reversible through Gist training. The expansion of the uncinate is intriguing and perhaps suggests that this pathway plays a role in synthesizing new learning—linking the memory center (left middle temporal region) to a brain region that is implicated in abstraction skills (e.g., the left superior medial frontal gyrus).

Whereas a handful of studies have reported cognitive gains and brain changes in response to cognitive training (Nyberg et al., 2003; Boyke et al., 2008; Belleville et al., 2011; Rosen et al., 2011), this study provided some of the first convergent findings across multiple brain imaging platforms (CBF, connectivity, white matter) and cognition supporting the promise of strategy-based top-down cognitive training to enhance brain integrity (Chapman et al., 2013). We acknowledge these findings are preliminary and should be interpreted with caution since the gist training group was compared to a wait-list control group. Nonetheless, the significant relation between brain changes and cognitive improvement provides impetus that the brain gains from gist-training may be possible and warrant further study with active controls.

CONCLUSIONS AND FUTURE OPPORTUNITIES

This synopsis of key findings across studies in normal and clinical populations indicates Gist reasoning training has the potential to improve cognitive performance beyond skills trained with the likelihood of enhancing underlying neural systems, as well as real life functional abilities. The significant improvements were achieved after relatively short-term training periods. The gains were documented in pseudo-randomly assigned trials comparing the experimental Gist reasoning training to control groups using objective measures.

We postulate that top-down strategy-based cognitive training may yield efficient and easily adoptable methods of mental practice to achieve broad-based benefits. To guide future cognitive training trials, we offer several explanations why Gist reasoning training may augment higher-order executive functions. First, gist reasoning takes advantage of the human brain's preferential bias toward understanding generalized/gist meanings (Bartlett, 1932; Reyna, 1996; Gabrieli, 2004). Extant evidence has demonstrated that while memory for details is lost fairly quickly, memory for global/gist meanings is preserved when delayed recall is examined, whether tested 30 min, one day or even a week later (Bransford and Franks, 1971; Bransford et al., 1972; Mandler and Rabinowitz, 1981; Reyna, 1996; Norman and Schacter, 1997; Kahana and Wingfield, 2000; Gabrieli, 2004). Second, gist reasoning requires an active process of meaning-abstraction where the incoming details are integrated within one's repository of world knowledge by a conscious, controlled manipulation of input into précised ideas (Johnson-Laird, 1983; Van Dijk et al., 1983; Frederksen and Donin, 1991; Zwaan and Radvansky, 1998; Chapman and Mudar, 2013). This integration of incoming data with prior knowledge necessitates activation of top-down processing with enhanced depth of encoding compared to simple representation of literal input. Third, gist reasoning is a practical skill that the majority of people from adolescence to old age can implement and practice throughout their daily normal mental activities (Lloyd and Reyna, 2009; Gamino et al., 2010; Vas et al., 2011; Motes et al., 2014). Examples of gist reasoning are meaning-creations illustrated by generating interpretations, themes, take-home messages, synopses, or generalized statements, to mention a few forms. Fourth, accruing evidence suggests that such a top-down approach to processing engages broad-based brain networks (Gazzaley et al., 2005; Chen et al., 2006, 2011; Chapman et al., 2013). We propose that when the neural activity of major brain networks is increased through complex and meaningful cognitive activities involving gist reasoning, the outcomes may be manifested at multiple levels of cognitive performance and neural health.

Immense potential exists to augment cognitive performance and enhance neural systems through top-down cognitive activity, such as gist reasoning. Future research opportunities should combine multiple approaches simultaneously from the growing armamentaria shown to enhance cognitive performance and brain functions to examine additive benefits. The current reported benefits from Gist reasoning training motivate future trials where the gist training protocol is combined with methods such as short-term meditation (Tang et al., 2007), sensory processing training (Mahncke et al., 2006), aerobic exercise (Kramer and Erickson, 2007; Chapman et al., 2013), or protocols to improve sleep (Nedergaard, 2013). Also critical is examining subjects' motivational level as a factor. Expanded efforts to identify combinatorial protocols that strengthen cognitive performance and recoup losses will be of major public health significance, with the ultimate goal to more fully harness the brain's capacity to be strengthened in health, disease, and injury.

FUNDING

This work was supported by grant from the National Institute of Health (RC1-AG035954, R01-NS067015, R01-AG033106, NICHD R21-HD062835), Department of Defense (W81XWH-11-2-0194) and by grants from the T. Boone Pickens Foundation, the RGK foundation, the Lyda Hill Foundation, Dallas Foundation, and Dee Wyly Distinguished University Endowment.

ACKNOWLEDGMENTS

The authors thank Jacquelyn Gamino, Lori Cook, Asha Vas, Mike Motes, Molly Keebler, Audette Rackley, Jennifer Zientz, Erin Venza, Hsueh-Sheng Chiang, Justin Eroh, Ali Perez, and the rest of the cognitive training team for their contribution to the research discussed in this perspective.

REFERENCES

Anand, R., Chapman, S. B., Rackley, A., Keebler, M., Zientz, J., and Hart, J. (2011). Gist reasoning training in cognitively normal seniors. *Int. J. Geriatr. Psychiatry* 26, 961–968. doi: 10.1002/gps.2633

Anguera, J. A., Boccanfuso, J., Rintoul, J. L., Al-Hashimi, O., Faraji, F., Janowich, J., et al. (2013). Video game training enhances cognitive control in older adults. *Nature* 501, 97–101. doi: 10.1038/nature12486

Bartlett, F. C. (1932). *Remembering: An Experimental and Social Study*. Cambridge: Cambridge University.

Belleville, S., Clément, F., Mellah, S., Gilbert, B., Fontaine, F., and Gauthier, S. (2011). Training-related brain plasticity in subjects at risk of developing Alzheimer's disease. *Brain* 134, 1623–1634. doi: 10.1093/brain/awr037

Bennett, E. L., Diamond, M. C., Krech, D., and Rosenzweig, M. R. (1964). Chemical and Anatomical Plasticity of the Brain. *Science* 610–619. doi: 10.1126/science.146.3644.610

Binder, D. T. G., O'Connor, C., Levine, B. (2008). *Brain HealthWorkshop*. Rotman Research Institute, Baycrest, Toronto: ON; University of California, Berkeley: CA.

Boyke, J., Driemeyer, J., Gaser, C., Büchel, C., and May, A. (2008). Training-induced brain structure changes in the elderly. *J. Neurosci.* 28, 7031–7035. doi: 10.1523/JNEUROSCI.0742-08.2008

Bransford, J. D., Barclay, J. R., and Franks, J. J. (1972). Sentence memory: a constructive versus interpretive approach. *Cogn. Psychol.* 3, 193–209. doi: 10.1016/0010-0285(72)90003-5

Bransford, J. D., and Franks, J. J. (1971). The abstraction of linguistic ideas. *Cogn. Psychol.* 2, 331–350. doi: 10.1016/0010-0285(71)90019-3

Bressler, S. L., and Menon, V. (2010). Large-scale brain networks in cognition: emerging methods and principles. *Trends Cogn. Sci.* 14, 277–290. doi: 10.1016/j.tics.2010.04.004

Carlson, M. C., Erickson, K. I., Kramer, A. F., Voss, M. W., Bolea, N., Mielke, M., et al. (2009). Evidence for neurocognitive plasticity in at-risk older adults: the experience corps program. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 1275–1282. doi: 10.1093/gerona/glp117

Chapman, S. B., Aslan, S., Spence, J. S., Hart, J. J., Bartz, E. K., Didehbani, N., et al. (2013). Neural mechanisms of brain plasticity with complex cognitive training in healthy seniors. *Cereb. Cortex*. doi: 10.1093/cercor/bht234. [Epub ahead of print].

Chapman, S. B., Gamino, J. F., and Mudar, R. A. (2012). "Higher-order strategic gist reasoning in adolescence," in *The Adolescent Brain: Learning, Reasoning, and Decison Making,* eds V. Reyna, S. B. Chapman, M. R. Dougherty, and J. Confrey, 123–151. doi: 10.1037/13493-005

Chapman, S. B., and Mudar, R. A. (2013). Discourse gist: a window into the brain's complex cognitive capacity. *Discourse Stud.* 15, 519–533. doi: 10.1177/1461445613501444

Chen, A. J., Nycum, T. J., Novakovic-Agopian, T., Song, S., Gary, T., Rome, S., et al. (2011). Training of goal-directed attention regulation enhances control over neural processing for individuals with brain injury. *Brain* 134, 1541–1554. doi: 10.1093/brain/awr067

Chen, A. J.-W., Abrams, G. M., and D'Esposito, M. (2006). Functional reintegration of prefrontal neural networks for enhancing recovery after brain injury. *J. Head Trauma Rehabil.* 21, 107–118. doi: 10.1097/00001199-200603000-00004

Clare, L., and Woods, R. T. (2004). Cognitive training and cognitive rehabilitation for people with early-stage Alzheimer's disease: a review. *Neuropsychol. Rehabil.* 14, 385–401. doi: 10.1080/09602010443000074

Dahlin, E., Neely, A. S., Larsson, A., Bäckman, L., and Nyberg, L. (2008). Transfer of learning after updating training mediated by the striatum. *Science* 320, 1510–1512. doi: 10.1126/science.1155466

Frederksen, C. H., and Donin, J. (1991). Constructing multiple semantic representations in comprehending and producing discourse. *Adv. Psychol.* 79, 19–44. doi: 10.1016/S0166-4115(08)61547-2

Gabrieli, J. (2004). Memory: pandora's hippocampus? *Cerebrum* 6, 39–48.

Gamino, J. F., Chapman, S. B., Hull, E. L., and Lyon, G. R. (2010). Effects of higher-order cognitive strategy training on gist-reasoning and fact-learning in adolescents. *Front. Psychol.* 1:188. doi: 10.3389/fpsyg.2010.00188

Gates, N. J., Sachdev, P. S., Fiatarone Singh, M. A., and Valenzuela, M. (2011). Cognitive and memory training in adults at risk of dementia: a systematic review. *BMC Geriatr.* 11:55. doi: 10.1186/1471-2318-11-55

Gazzaley, A., Cooney, J. W., McEvoy, K., Knight, R. T., and D'Esposito, M. (2005). Top-down enhancement and suppression of the magnitude and speed of neural activity. *J. Cogn. Neurosci.* 17, 507–517. doi: 10.1162/0898929053279522

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Johnson-Laird, P. N. (1983). *Mental Models*: *Towards* a *Cognitive Science* of *Language, Inference, and Consciousness*. Cambridge, MA: Harvard University Press.

Kahana, M. J., and Wingfield, A. (2000). A functional relation between learning and organization in free recall. *Psychon. Bull. Rev.* 7, 516–521. doi: 10.3758/BF03214365

Kramer, A. F., and Erickson, K. I. (2007). Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function. *Trends Cogn. Sci.* 11, 342–348. doi: 10.1016/j.tics.2007.06.009

Lloyd, F. J., and Reyna, V. F. (2009). Clinical gist and medical education. *JAMA* 302, 1332–1333. doi: 10.1001/jama.2009.1383

Mahncke, H. W., Connor, B. B., Appelman, J., Ahsanuddin, O. N., Hardy, J. L., Wood, R. A., et al. (2006). Memory enhancement in healthy older adults using a brain plasticity-based training program: a randomized, controlled study. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12523–12528. doi: 10.1073/pnas.0605194103

Mandler, G., and Rabinowitz, J. C. (1981). Appearance and reality: does a recognition test really improve subsequent recall and recognition? *J. Exp. Psychol. Hum. Learn. Mem.* 7, 79. doi: 10.1037/0278-7393.7.2.79

Martin, M., Clare, L., Altgassen, A. M., Cameron, M. H., and Zehnder, F. (2011). Cognition-based interventions for healthy older people and people with mild cognitive impairment. *Cochrane Database Syst. Rev.* CD006220. doi: 10.1002/14651858.CD006220.pub2

Motes, M. A., Gamino, J. F., Chapman, S. B., Rao, N. K., Maguire, M. J., Brier, M. R., et al. (2014). Inhibitory control gains from higher-order cognitive strategy training. *Brain Cogn.* 84, 44–62. doi: 10.1016/j.bandc.2013.10.007

Mudar, R. A., Chiang, H., Eroh, J. T., Rackley, A., Venza, E., Martin-Cook, K., et al. (2013). "Benefits of cognitive training in individuals with mild cognitive impairment," in *Poster Presentation - Cognitive Neuroscience Society Annual Meeting,* April 2013 (San Francisco, CA).

Nedergaard, M. (2013). Garbage truck of the brain. *Science* 340, 1529–1530. doi: 10.1126/science.1240514

Norman, K. A., and Schacter, D. L. (1997). False recognition in younger and older adults: exploring the characteristics of illusory memories. *Mem. Cognit.* 25, 838–848. doi: 10.3758/BF03211328

Nyberg, L., Sandblom, J., Jones, S., Neely, A. S., Petersson, K. M., Ingvar, M., et al. (2003). Neural correlates of training-related memory improvement in adulthood and aging. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13728–13733. doi: 10.1073/pnas.1735487100

Papp, K. V., Walsh, S. J., and Snyder, P. J. (2009). Immediate and delayed effects of cognitive interventions in healthy elderly: a review of current literature and future directions. *Alzheimers Dement.* 5, 50–60. doi: 10.1016/j.jalz.2008.10.008

Rebok, G. W., Ball, K., Guey, L. T., Jones, R. N., Kim, H. Y., King, J. W., et al. (2014). Ten year effects of the advanced cognitive training for independent and vital elderly cognitive training trial on cognition and everyday functioning in older adults. *J. Am. Geriatr. Soc.* 62, 16–24. doi: 10.1111/jgs.12607

Reijnders, J., Van Heugten, C., and Van Boxtel, M. (2012). Cognitive interventions in healthy older adults and people with mild cognitive impairment; a systematic review. *Ageing Res. Rev*. 12, 263–275. doi: 10.1016/j.arr.2012.07.003

Reyna, V. F. (1996). Conceptions of memory development with implications for reasoning and decision making. *Ann. Child Dev.* 12, 87–118.

Reyna, V. F., and Brainerd, C. J. (1995). Fuzzy-trace theory: an interim synthesis. *Learn. Individ. Differ.* 7, 1–75. doi: 10.1016/1041-6080(95)90031-4

Reyna, V. F., and Brainerd, C. J. (2011). Dual processes in decision making and developmental neuroscience: a Fuzzy-Trace model. *Dev. Rev.* 31, 180–206. doi: 10.1016/j.dr.2011.07.004

Reyna, V. F., and Lloyd, F. J. (2006). Physician decision making and cardiac risk: effects of knowledge, risk perception, risk tolerance, and fuzzy processing. *J. Exp. Psychol. Appl.* 12, 179–195. doi: 10.1037/1076-898X.12.3.179

Rosen, A. C., Sugiura, L., Kramer, J. H., Whitfield-Gabrieli, S., and Gabrieli, J. D. (2011). Cognitive training changes hippocampal function in mild cognitive impairment: a pilot study. *J. Alzheimers Dis.* 26, 349–357. doi: 10.3233/JAD-2011-0009

Schooler, C. (1984). Psychological effects of complex environments during the life span: a review and theory. *Intelligence* 8, 259–281. doi: 10.1016/0160-2896(84)90011-4

Schooler, C., Mulatu, M. S., and Oates, G. (1999). The continuing effects of substantively complex work on the intellectual functioning of older workers. *Psychol. Aging* 14, 483. doi: 10.1037/0882-7974.14.3.483

Tang, Y.-Y., Ma, Y., Wang, J., Fan, Y., Feng, S., Lu, Q., et al. (2007). Short-term meditation training improves attention and self-regulation. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17152–17156. doi: 10.1073/pnas.0707678104

Tardif, S., and Simard, M. (2011). Cognitive stimulation programs in healthy elderly: a review. *Int. J. Alzheimers Dis.* 2011:378934. doi: 10.4061/2011/378934

Teixeira, C. V. L., Gobbi, L. T. B., Corazza, D. I., Stella, F., Costa, J. L. R., and Gobbi, S. (2012). Non-pharmacological interventions on cognitive functions in older people with mild cognitive impairment (MCI). *Arch. Gerontol. Geriatr.* 54, 175–180. doi: 10.1016/j.archger.2011.02.014

Thompson, T. W., Waskom, M. L., Garel, K.-L. A., Cardenas-Iniguez, C., Reynolds, G. O., Winter, R., et al. (2013). Failure of working memory training to enhance cognition or intelligence. *PLoS ONE* 8:e63614. doi: 10.1371/journal.pone.0063614

Valenzuela, M. J., Sachdev, P., Wen, W., Chen, X., and Brodaty, H. (2008). Lifespan mental activity predicts diminished rate of hippocampal atrophy. *PLoS ONE* 3:e2598. doi: 10.1371/journal.pone.0002598

Valenzuela, M., and Sachdev, P. (2009). Can cognitive exercise prevent the onset of dementia? Systematic review of randomized clinical trials with longitudinal follow-up. *Am. J. Geriatr. Psych.* 17, 179–187. doi: 10.1097/JGP.0b013e3181953b57

Van Dijk, T. A., Kintsch, W., and Van Dijk, T. A. (1983). *Strategies of Discourse Comprehension.* New York, NY: Academic Press.

Vas, A. K., Chapman, S. B., Cook, L. G., Elliott, A. C., and Keebler, M. (2011). Higher-order reasoning training years after traumatic brain injury in adults. *J. Head Trauma Rehabil.* 26, 224–239. doi: 10.1097/HTR.0b013e318218dd3d

Willis, S. L., Tennstedt, S. L., Marsiske, M., Ball, K., Elias, J., Koepke, K. M., et al. (2006). Long-term effects of cognitive training on everyday functional outcomes in older adults. *JAMA* 296, 2805–2814. doi: 10.1001/jama.296.23.2805

Zelinski, E. M. (2009). Far transfer in cognitive training of older adults. *Restor. Neurol. Neurosci.* 27, 455–471. doi: 10.3233/RNN-2009-0495

Zwaan, R. A., and Radvansky, G. A. (1998). Situation models in language comprehension and memory. *Psychol. Bull.* 123:162. doi: 10.1037/0033-2909.123.2.162

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2014; accepted: 10 April 2014; published online: 28 April 2014.
Citation: Chapman SB and Mudar RA (2014) Enhancement of cognitive and neural functions through complex reasoning training: evidence from normal and clinical populations. Front. Syst. Neurosci. ***8****:69. doi: 10.3389/fnsys.2014.00069*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 09 May 2014
doi: 10.3389/fnsys.2014.00081

Changes in cerebellar activity and inter-hemispheric coherence accompany improved reading performance following Quadrato Motor Training

Tal Dotan Ben-Soussan[1,2*†], Keren Avirame[1,3†], Joseph Glicksohn[1,4], Abraham Goldstein[1,5], Yuval Harpaz[1] and Michal Ben-Shachar[1,6]

[1] The Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, Israel
[2] Research Institute for Neuroscience, Education and Didactics, Cognitive Neurophysiology Laboratory, Patrizio Paoletti Foundation, Assisi, Italy
[3] Department of Neurology, Charité - Universitätsmedizin, Berlin, Germany
[4] Department of Criminology, Bar-Ilan University, Ramat-Gan, Israel
[5] Department of Psychology, Bar-Ilan University, Ramat-Gan, Israel
[6] Department of English, Linguistics Division, Bar-Ilan University, Israel

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Froylán Gómez-lagunas, UNAM, Mexico
Panagiotis D. Bamidis, Aristotle University of Thessaloniki, Greece

***Correspondence:**
Tal Dotan Ben-Soussan, Research Institute for Neuroscience, Education and Didactics, Cognitive Neurophysiology Laboratory, Patrizio Paoletti Foundation, via Cristoforo Cecci 2, 06081 - Santa Maria degli Angeli, Assisi (PG), Italy
e-mail: research@fondazionepatriziopaoletti.org

† These authors have contributed equally to this work.

Dyslexia is a multifactorial reading deficit that involves multiple brain systems. Among other theories, it has been suggested that cerebellar dysfunction may be involved in dyslexia. This theory has been supported by findings from anatomical and functional imaging. A possible rationale for cerebellar involvement in dyslexia could lie in the cerebellum's role as an oscillator, producing synchronized activity within neuronal networks including sensorimotor networks critical for reading. If these findings are causally related to dyslexia, a training regimen that enhances cerebellar oscillatory activity should improve reading performance. We examined the cognitive and neural effects of Quadrato Motor Training (QMT), a structured sensorimotor training program that involves sequencing of motor responses based on verbal commands. Twenty-two adult Hebrew readers (12 dyslexics and 10 controls) were recruited for the study. Using Magnetoencephalography (MEG), we measured changes in alpha power and coherence following QMT in a within-subject design. Reading performance was assessed pre- and post-training using a comprehensive battery of behavioral tests. Our results demonstrate improved performance on a speeded reading task following one month of intensive QMT in both the dyslexic and control groups. Dyslexic participants, but not controls, showed significant increase in cerebellar oscillatory alpha power following training. In addition, across both time points, inter-hemispheric alpha coherence was higher in the dyslexic group compared to the control group. In conclusion, the current findings suggest that the combination of motor and language training embedded in QMT increases cerebellar oscillatory activity in dyslexics and improves reading performance. These results support the hypothesis that the cerebellum plays a role in skilled reading, and begin to unravel the underlying mechanisms that mediate cerebellar contribution in cognitive and neuronal augmentation.

Keywords: dyslexia, MEG, motor training, cerebellum, alpha power, coherence, reading

INTRODUCTION

Reading is a basic ability necessary in every-day life. Failure to acquire literacy early in the schooling years may have serious consequences for an individual's academic achievements, well-being and employment prospects. Dyslexia, which is characterized by difficulties with accurate and fluent word recognition, poor spelling and decoding abilities, is the most common learning disability, with a prevalence rate of about 10% in school-age children (Deffenbacher et al., 2004). Longitudinal studies further indicate that dyslexia is a chronic condition that persists into adulthood (Shaywitz et al., 2008).

Difficulties in learning to read are commonly thought to derive from impaired phonemic representations and phonological processing (Bradley and Bryant, 1983; Ramus, 2004). This phonological deficit has been associated with aberrant cortical responses and altered asymmetry of activity in frontal and temporal language- and reading-related areas (Ramus, 2004; Dufor et al., 2007; Gabrieli, 2009), as well as with structural and functional abnormalities of the cerebellum (Pernet et al., 2009b).

The involvement of the cerebellum in higher cognitive functions such as language was once a controversial issue (Leiner et al., 1993; Rao et al., 2001). However, much evidence has

been gathered in recent years to support this view. Initially, studies in patients with cerebellar disease reported significant deficits in verbal fluency (Akshoomoff et al., 1992; Appollonio et al., 1993). Later, cerebellar involvement was found in other aspects of language, such as phonological and semantic processing (for reviews see Stoodley and Schmahmann, 2009; Stoodley and Stein, 2011). In addition, structural imaging demonstrated that lower cerebellar declive volumes are associated with impaired reading abilities, suggesting that the cerebellum may be a biomarker of dyslexia (Pernet et al., 2009b). In fact, the cerebellar and frontal differences between dyslexics and controls are the most consistent (for reviews see Pernet et al., 2009a,b). For example, Eckert et al. (2003) found that the volume of the right anterior lobe of the cerebellum significantly distinguished dyslexic from control participants, and was correlated with reading measured by a single-word reading task.

According to broader theories, dyslexia is not limited to phonological difficulties but encompasses a wide range of neurodevelopmental deficits that can be traced back to the sensorimotor systems (Stein, 2001; Galaburda et al., 2006). It follows that difficulties in phonological processing related to dyslexia are secondary to impairments in basic sensory and motor processing. Some posit an impairment at an early stage in which fast incoming sensory information is processed in the magnocellular system (Stein and Walsh, 1997), while others have suggested a fundamental deficit in the integration of rapidly successive transient signals (Tallal et al., 1996) or in the detection of regularities in sound sequences (Oganian and Ahissar, 2012). These approaches all put forward the premise that sensorimotor alterations might be the source of the core reading impairments observed in dyslexia.

Although the role of the motor system in dyslexia is still controversial, it is by no means a novel proposal that dyslexia involves a motor component. Already in the 1930's, Orton observed abnormal clumsiness in dyslexic children. He suggested that clumsy children could have difficulties in learning complex body movements as well as movements which are necessary for speech and writing (Orton, 1937). Studies of dyslexic participants have found impaired motor performance in a variety of tasks such as speed of tapping, heel-toe placement, rapid successive finger opposition, accuracy in copying, learning and execution of motor sequence (Nicolson et al., 2001; De Kleine and Verwey, 2009). This body of evidence supports the claims regarding the functional interactions between motor control systems, language and reading (for reviews see Hickok et al., 2011; Buckner, 2013).

The importance of cerebellar oscillatory function in neuroplasticity (Swinnen, 2002; De Zeeuw et al., 2011) and its role in motor acquisition, such as bimanual skills (e.g., Andres et al., 1999), have long been acknowledged in studies related to motor learning. Since impaired motor skills were often observed in dyslexics, some researchers attributed dyslexics' cognitive and motor deficiencies to abnormal development and functioning of the cerebellum (Nicolson et al., 1999, 2001). These findings lead to the claim that the role of the cerebellum is not limited to regulating the rate, force, rhythm, and accuracy of movements, but also the speed, capacity, consistency and appropriateness of cognitive processes (Schmahmann, 2004; Hölzel et al., 2011; Buckner, 2013).

Consequently, several training studies aimed to improve reading through integrated sensory stimulation, incorporating visuomotor and vestibular home-based exercise program lasting 6 months (Reynolds et al., 2003; Reynolds and Nicolson, 2007). In their study, the intervention group improved in a range of motor skills, such as cerebellar/vestibular and eye movement tests, as well as in the Dyslexia Screening Test, more than the control group. Although the authors could only speculate about the neural mechanisms underlying these motor and cognitive improvements, they pointed to the involvement of cerebellar function in mediating these behavioral changes. In order to improve reading and spelling in dyslexia, other studies investigated the effect of normalizing oscillatory activity on reading and spelling using neurofeedback (Breteler et al., 2010). Following 10 weeks of neurofeedback training, the intervention group showed improved spelling in contrast to the control group; however, no improvement was found in reading performance in either group. In addition, a significant increase in alpha coherence was found, which was interpreted as an indication that attentional processes account for the observed improvement in spelling, while no correlation was found between the two measures. So far, the link between training-induced changes in cerebellar alpha oscillatory activity and reading skills remained unexplored.

In the current study, we explore the possible potential interactions between sensorimotor and reading systems, and the role of the cerebellum as a mediator between them. In a preliminary attempt to understand the causal relationship between these constructs and their role in dyslexia, we examined how reading skills change as a result of a highly-structured form of sensorimotor training. We applied *Quadrato Motor Training* (QMT), a new sensorimotor whole-body training that involves following a structured set of simple oral instructions, by stepping to the instructed corner in a square. Recently, we demonstrated that one session of QMT can improve cognitive function, including creativity and spatial cognition, in comparison to two alternative training regimens that did not combine motor and cognitive aspects (Ben-Soussan et al., 2013, 2014). In the current study, the QMT is applied for a period of one month, in order to test its efficacy in inducing plasticity.

We have chosen magneto-encephalography (MEG) as the main tool for assessing changes in brain activity, due to its excellent resolution in the temporal domain, as well as its superiority to EEG in terms of effective spatial resolution (Kanda et al., 2000; Genow et al., 2004). In fact, it has been explicitly argued that MEG could be an excellent tool for evaluating the neural correlates of training-induced changes in dyslexia because of its ability to localize the sources of the alpha activation in parallel to the examination of long-distance alpha coherence (Salmelin, 2007). Further theoretical motivation for this choice is provided by the temporal sampling framework (TSF), which has been recently proposed to connect the observed sensorimotor deficits in dyslexia to temporal alterations in neuronal oscillations (Goswami, 2011). We therefore set out to examine the effects of QMT in a group of adult dyslexics and matched controls using MEG alpha power and coherence as electrophysiological dependent measures, as well as reading performance and verbal fluency as cognitive measures.

We hypothesized that dyslexics would show reduced alpha power, altered alpha coherence and lower reading skills at baseline in comparison to controls. We further hypothesized that QMT would increase cerebellar alpha power due to the important role of cerebellar alpha power in voluntary action (Tesche and Karhu, 2000; Ivry et al., 2002). Increased cerebellar alpha power would then serve to normalize alpha coherence (Basar et al., 1997; Andres et al., 1999; Silberstein et al., 2003; Silberstein, 2006; Güntekin and Basar, 2010) and improve reading (Goswami, 2011). We therefore tested whether a 4-week period of daily QMT would: (a) Enhance alpha power and normalize alpha coherence in dyslexic adults; (b) Enhance reading performance. Finally, we tested whether changes in alpha power and inter-hemispheric alpha coherence would correlate with behavioral changes in reading.

METHODS

PARTICIPANTS

Twenty-two native Hebrew speakers participated in the study: 12 dyslexic participants (5 females and 7 males; mean age = 30 (±6); years of education = 15 (±1)) and 10 controls (7 females and 3 males; mean age = 27 (±5); years of education = 14(±2)). We recruited volunteers who had been previously diagnosed as dyslexic by a clinical or educational psychologist and had a documented history of reading and spelling difficulties. We excluded participants who were further diagnosed with comorbid disorders, including Attention Deficit Hyperactivity Disorder (ADHD), Attention Deficit Disorder (ADD) and developmental coordination disorder (Ramus et al., 2003). All participants provided written informed consent to take part in the study.

PROCEDURE

The study included three phases: pre-training assessment, QMT training, and post-training assessment (See **Figure 1**). The pre-training session (Day 1) included the following components in this fixed order: (a) Cognitive testing (about 30 min, see Section Cognitive Tasks below); (b) MEG measurements (about 15 min, see Section MEG Data Acquisition below); and (c) QMT training (about 7 min, see Section Quadrato Motor Training below). Post-training assessment took place at the lab on Day 29, and included the cognitive and MEG components as on Day 1 in the same order using matched versions of the cognitive tasks (see Section Cognitive Tasks). Due to technical problems, one dyslexic participant did not complete the behavioral tasks; additionally,

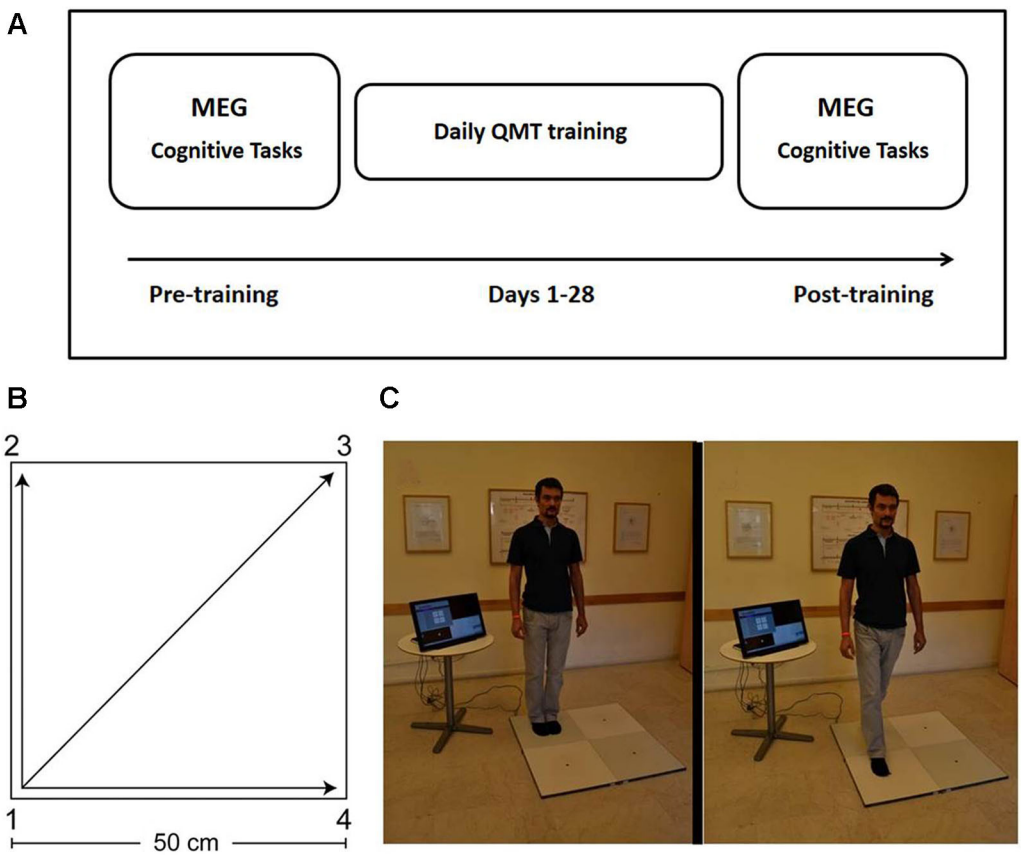

FIGURE 1 | Study design and Quadrato Motor Training. (A) Experimental protocol. MEG and behavioral measurements were conducted before and after 4 weeks of QMT training (see text for details). Two different versions of the cognitive tests were presented pre- and post-training in a counterbalanced order. **(B)** The spatial layout of the training space. **(C)** Practice setup. The trainee listens to recorded instructions and takes a step towards the target point. Figure adapted from Ben-Soussan et al. (2014). Quadrato Motor Training = QMT; Magnetoencephalography = MEG.

the behavioral data for the post-training verbal fluency tasks was incomplete for two dyslexic and one control participants. In those cases where participants failed to complete certain behavioral tests, their MEG measurements were still included in the analysis of the MEG data in order to increase statistical power given the small sample size.

QUADRATO MOTOR TRAINING

The participant stood in a quiet room at one corner of a 0.5 m × 0.5 m square and made movements in response to verbal instructions given by an audio tape recording. Participants were instructed to keep the eyes focused straight ahead and their hands loose at the side of the body. They were also told to immediately continue with the next instruction and not to stop due to mistakes. At each corner, there are three possible directions to move. The training thus consists of 12 possible movements (**Figure 1**). The daily training consisted of a sequence of 69 commands, lasting 7 min. Two variables that were addressed in other studies of motor learning are limb velocity and the decision regarding the responding limb (Criscimagna-Hemminger et al., 2002; Donchin et al., 2003). In order to control these parameters, we used a movement-sequence paced at a rate of an average of 0.5 Hz (similar to a slow walking rate), and we instructed the participants to begin all movements with the leg closest to the center of the square. Starting on day 2, daily QMT sessions were conducted by the participants at home. Home training lasted 27 consecutive days (from Day 2 to Day 28), and lasted 7 min each day.

COGNITIVE TASKS

The cognitive tasks were performed before the MEG measurement, and lasted for about 30 min. The order of tasks was fixed, starting with the reading test, category-based fluency and then letter-based fluency task. Each task had two different versions, and each of these versions was assigned to the pre- or post-training session in a counterbalanced manner across subjects.

Reading test

This test examines single-word reading speed and accuracy. A list of forty five written Hebrew words of increasing difficulty was presented and participants were asked to accurately read as many words as possible from the list in 1 min. The level of difficulty of the words was controlled in terms of word length and number of syllables. In order to minimize learning effects from the pre-test to the post-test, two non-overlapping word lists were created. Each list was presented either before or after training, in a counterbalanced manner. The two lists of 45 words were sampled from a database of rated Hebrew words (Levy-Drori and Henik, 2006), and were matched item-by-item for concreteness, availability of context, familiarity, number of letters and number of syllables. Since several participants from the control group finished the list of words in less than 1 min, the final score represents the number of words which were read correctly in the first 30 s.

Category-based fluency task

Participants were asked to say in 1 min as many words as possible belonging to a given semantic category. Two semantic categories were used alternately: (a) Animals; (b) Fruits and vegetables. One category was presented in the pre-training session (Day 1) and the other in the post-training session (Day 29), and the order of the categories was counterbalanced across subjects. Fruits and vegetables were treated as one category in order to avoid the ambiguity between botanical definitions and common usage (as in "avocado"). These categories were chosen because they have comparable norms, and in order to avoid test-retest influence by repeating the same category (Kavé, 2005).

Letter-based fluency task

Participants were asked to say in one minute as many words as possible that start with a given letter. We used two Hebrew letters: Bet (/b/) and Gimel (/g/). One letter served for pre-training and the other for post-training, and the order of the categories was counterbalanced across subjects. These letters were chosen because they have comparable norms, and in order to avoid test-retest influence by repeating the same letter (Kavé, 2005).

MEG DATA ACQUISITION

Power and coherence measures were collected using the MEG at the beginning and at the end of the month, after performing the cognitive tasks. MEG recordings were conducted with a whole-head 248-channel magnetometer array (4-D Neuroimaging, Magnes 3600 WH) in a magnetically shielded room. During the Rest condition, the participants were asked to refrain from moving and from falling asleep. In addition, the participants were asked to keep their eyes closed, in order to reduce ocular artifacts in the measured signals and to facilitate the localization of potential generator regions of the alpha resting–state oscillations (Goldman et al., 2002). Data acquisition took 15 min. We also collected MEG data using two active tasks which will be reported elsewhere. Before acquiring the data, the head-shape of each subject was digitized. Reference coils located approximately 30 cm above the head oriented by the *x*, *y* and *z* axes were used to record environmental noise. Three accelerometers, one for each axis, attached to the MEG gantry were used to record building vibrations in order to remove artifacts caused by them. The data were digitized at a sampling rate of 1017.25 Hz, and a 0.1 to 400 Hz band-pass filter was used online. The 50 Hz line power fluctuations were recorded directly from the power-line in order to remove the artifact on the MEG sensors.

PREPROCESSING MEG DATA

Power line, heartbeat, and vibration artifacts were removed automatically (Tal and Abeles, 2013). The data were then divided into 1 s epochs. Muscle artifact was estimated by examining the absolute value of all the MEG channels for every epoch, after applying a 20 Hz high-pass filter. For each epoch, the mean absolute value was computed. These values were then converted to z-scores, and epochs with *z*-scores greater than 3 standard deviations were rejected. Eye blinks were not considered as possible artifact for the alpha power processing, because the participants had their eyes closed and did not blink. Some eye movement artifact was still present in the data, but this was in a lower frequency range and was negligible in the alpha frequency range. Two of the 248

channels were noisy (one of the channels registered a constant zero value and the other exceeded 1 nanotesla); these channels were therefore excluded from all sensor and source level analysis. For left-right coherence computation, their homolog channels were omitted as well.

SOURCE LOCALIZATION

Source localization was applied for the alpha (7–13 Hz) frequency band. Synthetic Aperture Magnetometry (SAM) beamforming (Robinson and Vrba, 1999) was used with multiple spheres forward solution based on the digitized headshape. The neural activity was estimated for a grid of points covering the volume of the brain with 5 mm intervals. The power of activity was calculated for every grid point and for every epoch. Since raw beamforming results are biased toward deep sources it was necessary to normalize the images in order to keep the noise level equal throughout the whole volume of the brain. For this purpose, a pseudo-*z* score was calculated by averaging the power of every location, across epochs, divided by its noise estimate. The noise estimate was determined by the weights (the spatial filter). Deep sources generally have weights with higher values and are therefore noisier. Dividing the power of activity by the square of the weight norm can compensate for this bias (see Equation 3 in Sekihara et al., 2004). The absolute value of the weights of a particular location serves as a noise estimate. The resulting images represent the increase of alpha compared to noise, without being biased to deep sources. The pseudo-*z* value for each location was visualized as the color of voxels in the resulting functional images. The images were transformed to Talairach space by fitting a template MRI to the individual headshapes using SPM8 (Friston et al., 2007) and FieldTrip (Open Source Software for Advanced Analysis of MEG, Oostenveld et al., 2011) packages used with Matlab® R2010b. In order to control for multiple comparisons, a simulation was applied using an Analysis of Functional NeuroImages (AFNI) function (AlphaSim) which determines the probability to get significant clusters of different sizes at random. According to the simulation, at current parameters (given the template brain and spatial resolution used), clusters of voxels with a *p*-value smaller than 0.05 and exceeding one cubic cm (8 voxels) do not count as random noise. We decided to be even more conservative and to take only clusters containing more than 20 voxels at a threshold of $p < 0.005$ (Bunge et al., 2001).

COHERENCE

The coherence between left and right sensors was computed using FieldTrip. The data were first baseline-corrected by subtracting the mean of every epoch from the MEG traces. Eighteen channels located along the midline of the helmet were omitted from the coherence analysis, because these channels are likely to present with high coherence based on proximity, since spatially close sensors are likely to pick up very similar activities (Lehnertz et al., 2014).

Fourier transform was then computed using a spectral smoothing box of 1 Hz (meaning that the 10 Hz bin includes 9 to 11 Hz oscillations). The frequencies per time window were computed using a DPSS bell-shaped window. The resulting complex spectrum was used to assess coherence between each channel and its homolog. The coherence value of the 18 channels located along the mid-line of the helmet was set to one, representing perfect coherence between each channel and itself (the vertical red line in **Figure 4**). The coherence was projected onto a two-dimensional map of the sensor array, using the same left-right coherence value for the left as well as the right sensor, thus creating symmetrical maps of coherence. After the creation of the maps, channels *close* to the midline were excluded from the statistical analysis (in addition to the midline channels). This step was necessary because channels near the midline had high coherence values which did not represent cortical coherence, but simply the fact that the activity of one brain region was measured by two nearby channels. The channel pairs chosen were at least 11 cm apart in order to avoid "false coherence" resulting from close channels that pick up the same source and covered the lateral area of increased coherence (greater than 0.3; **Figure 4**). This procedure resulted in 59 channel pairs for which left-right coherence was statistically evaluated. Usually when studying inter-hemispheric differences, coherence is computed for one artifact-free channel for each region (e.g., from frontal, central, parietal, and temporal regions) and is computed for channels located in the corresponding regions of the two hemispheres (Osipova et al., 2003; Kikuchi et al., 2011). In fact, the number of chosen pairs is conventionally determined by *apriori* assumptions and therefore restricted to particular regions of interest. However, due to the exploratory nature of the current study, it was important to expand the search across multiple sensors.

STATISTICAL ANALYSIS

Mixed design ANOVA was used to test the effects of QMT on performance in the reading and verbal fluency tasks, with Training (pre-training, post-training) as a within-subject factor and Group (dyslexic, control) as a between-subjects factor. Statistical parametric maps were produced from MEG data using the AFNI package (Cox, 2012). Mixed design ANOVA was used to test the effects of QMT on alpha activity, i.e., alpha power and alpha coherence. Pearson correlation was used to test the association between behavioral and neuronal changes. The correlation threshold was $p < 0.05$. *Post hoc* comparisons were conducted using *t*-tests.

RESULTS

COGNITIVE RESULTS

Reading task

Performance on the speeded reading task was entered into a 2 (Group) by 2 (Training) ANOVA. First, a significant main effect for Group [$F_{(1,19)} = 7.80$, $p < 0.05$] was observed, indicating that, across both time points, the number of words correctly read by the controls ($M = 38.5$, SD = 6.3) was higher than the number of words correctly read by the dyslexic participants ($M = 27.6$, SD = 10.7). Secondly, the analysis yielded a main effect for Training [$F_{(1,19)} = 6.89$, $p < 0.05$], showing that QMT improved single-word reading performance across both groups (see **Figure 2**). Finally, the interaction between Training and Group was not significant ($p > 0.9$).

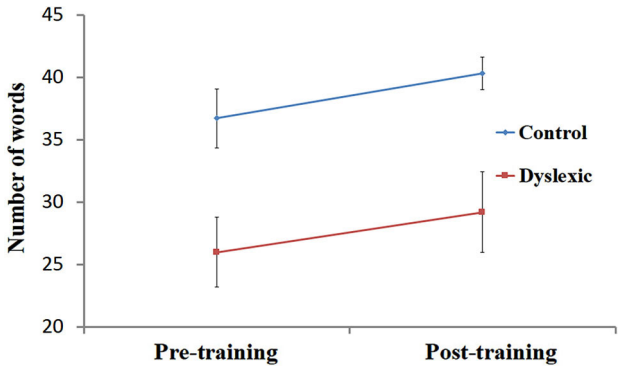

FIGURE 2 | Reading performance pre- and post-training. Number of words read correctly (mean ± SEM) in 30 s, pre- and post-training in the dyslexic (red) and control (blue) groups.

Verbal fluency

We conducted two separate analyses for the category-based and letter-based fluency tasks using a 2 (Group) by 2 (Training) ANOVA. No significant main effects or interactions were found, for either the category-based or the letter-based fluency task. The mean scores (i.e., number of words generated in 1 min) of the control participants for the category-based or letter-based fluency were similar to the norms (Kavé, 2005).

Based on previous studies demonstrating significant differences in phonological fluency between dyslexic and controls (Rack et al., 1992; Reid et al., 2007), we conducted a planned comparison between the groups for letter-based fluency, separately for pre- and post-QMT. While a marginally significant difference in phonological fluency was found pre-training between the dyslexic and control groups [$t_{(19)} = 2.08$, $p = 0.051$], no such difference was found following the training. In addition, no differences were found between the groups in semantic fluency, neither before nor after training. See **Table 1**.

Table 1 | Mean scores of letter-based and category-based fluency tasks as a function group and training.

	Letter				Category			
	Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dyslexic	8.7	2.4	9.0	1.9	23.9	5.2	24.9	4.3
Control	10.9	3.1	10.4	2.1	23.8	5.1	25.6	4.0

MEG RESULTS

Between-group differences in cerebellar alpha power

We first examined the effect of QMT on alpha power using a mixed design ANOVA, with Training as a within-subject factor and Group (Dyslexia, Control) as a between-subjects factor, for each voxel. The ANOVA revealed a significant Group × Training interaction in a cluster in the right cerebellum. The center of mass of this cluster was located in the right culmen (Talairach coordinates (in mm): 12, −37, −22; $F_{(1,20)} = 13.3$, $p < 0.0025$; See **Figure 3A**). Before training, cerebellar alpha power was significantly lower in the dyslexic group compared to the control group ($t_{(20)} = 3.88$, $p = 0.001$). Following 4 weeks of daily QMT, cerebellar alpha power significantly increased in the dyslexic group ($t_{(11)} = 3.08$, $p = 0.01$) in contrast to the control group which showed no significant change following training (see **Figure 3B**).

The ANOVA also revealed a significant Group × Training interaction for three frontal clusters, located in the right superior frontal gyrus (SFG) (Talairach coordinates in (mm): 23, 53, 17) [$F_{(1,20)} = 16.08$, $p < 0.001$], supplementary motor area (SMA) (Talairach coordinates in (mm): 13, 18, 42) [$F_{(1,20)} = 22.41$, $p < 0.001$] and the left middle frontal gyrus (Talairach coordinates in (mm): −27, 8, 57) [$F_{(1,20)} = 16.01$, $p < 0.001$]. While there were no significant differences between the groups in these areas prior to training, the control group showed a significant decrease in alpha power in the left medial frontal gyrus (MFG) ($t_{(9)} = 4.54$, $p < 0.005$), right SFG ($t_{(9)} = 3.73$, $p < 0.005$) and SMA ($t_{(9)} = 3.69$, $p < 0.005$) following 4 weeks of daily QMT. On the other hand, the opposite pattern was observed in the dyslexic group in which alpha power increased in the right SFG ($t_{(11)} = 2.66$, $p < 0.05$).

Coherence

Inter-hemispheric alpha coherence was tested using a mixed design 2-way ANOVA for each of the 59 channels, with Training as within-subject factor and Group as between-subjects factor. Across both time points, inter-hemispheric alpha coherence was significantly higher in the dyslexic group compared to the control group for five channel pairs ($F_{(1,21)} > 8.35$, $p < 0.01$, uncorrected; See **Figure 4**). No main effect for Training or interaction was found.

Neuro-cognitive correlations

In order to study the possible associations between change in alpha activity and change in reading performance, we calculated Pearson correlations between behavioral and neuronal change within each group. This analysis was motivated by previous studies relating reading, cerebellar activity and alpha coherence (Nicolson et al., 2001; Weiss and Mueller, 2003; Arns et al., 2007). Change in speeded reading was calculated as the difference between the number of words read correctly in 30 s before and after training. Change in cerebellar alpha power was calculated as the difference between pre- and post- training cerebellar alpha power of the cluster which was found to have the significant Group × Training interaction. Change in inter-hemispheric alpha coherence was calculated as the difference between pre- and post-training values of the bilateral temporal alpha coherence. No significant correlation was found between change in cerebellar alpha power and change in reading in the two groups. Yet, as can be seen in **Figure 5**, change in temporal alpha coherence was positively correlated with the change in reading score ($r = 0.58$, $p < 0.05$, $n = 11$; uncorrected) in the dyslexic group but not in the control group. Using the Fisher r-to-z transformation, we calculated the z value to assess the significance of the difference between two correlation coefficients. The results indicated a significant difference between the two correlation values ($z = 1.87$, $p < 0.05$).

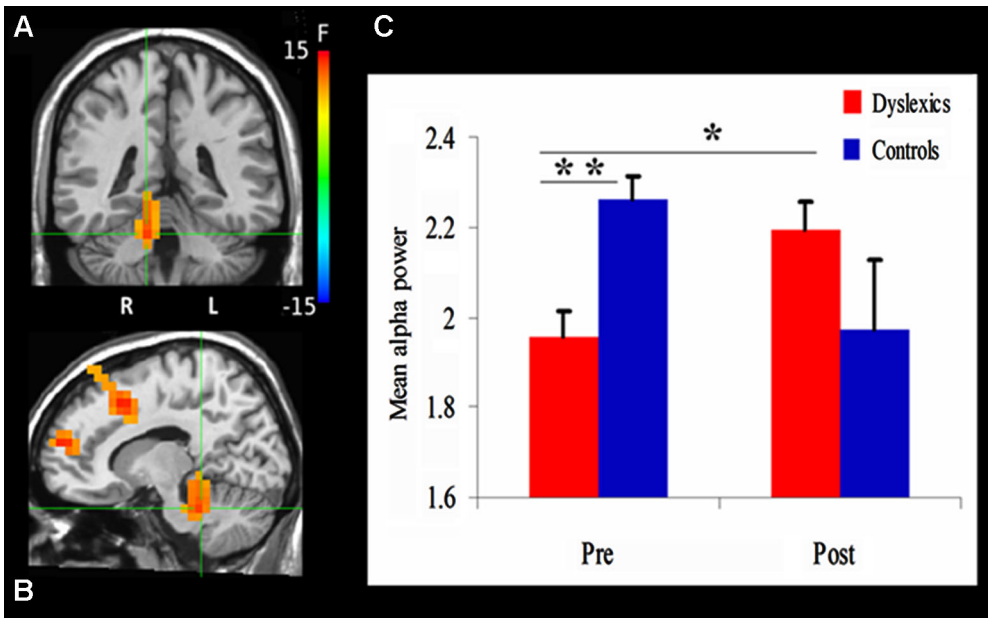

FIGURE 3 | Changes in alpha power. (A) and **(B)** demonstrate the significant clusters resulting from the Group (dyslexics, controls) by Training (pre-training, post-training) interaction. Voxels are colored by the *F* statistics, overlaid on coronal **(A)** and sagittal **(B)** views. The statistical map is thresholded at $p <$ 0.0025 in addition to a cluster size threshold of 20 voxels. The focus point (green cross) is positioned in the right culmen (Talairach coordinate: 12, −37, −22). **(C)** The bar graph shows alpha power as a function of Group and Training (mean + SEM). * $p = 0.01$; ** $p = 0.001$.

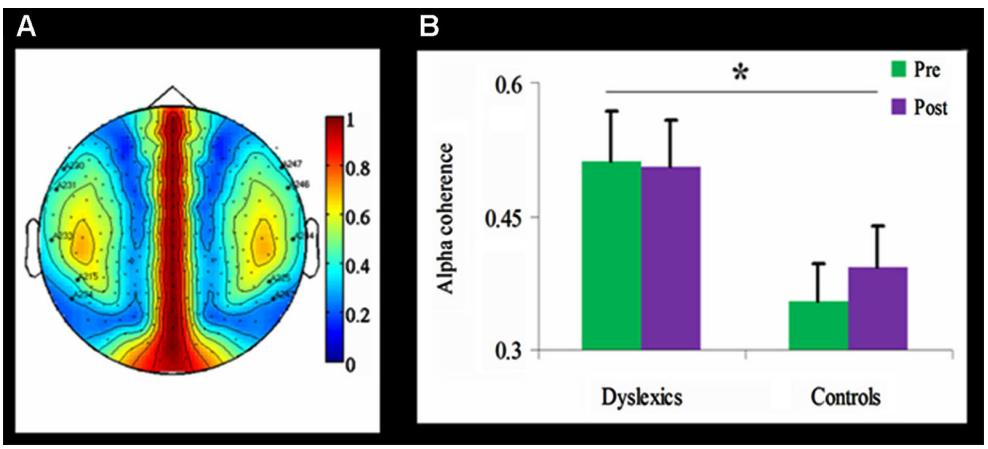

FIGURE 4 | Alpha coherence in the dyslexic and control groups. **(A)** Group differences in inter-hemispheric alpha coherence. The coherence was higher over temporal channels in the dyslexic group compared with the control group (* $p < 0.01$, uncorrected). **(B)** Temporal alpha coherence as a function of Group and Training (mean + SEM), demonstrating significant group differences between the dyslexic and control groups (* $p < 0.01$, uncorrected), as well as a null effect of the training in the dyslexic group and a trend toward an increase in alpha coherence in the control group.

DISCUSSION

Our results contribute two novel findings with regards to the cerebellar involvement in dyslexia: First, we show that cerebellar alpha activity prior to training is lower in dyslexics compared to controls. Second, a 4-week training program enhanced cerebellar alpha activity in dyslexics, but not in controls. Two other important findings are reported here for the first time: First, QMT over a period of 4 weeks improves reading speed in adults, and second, the improvement in reading performance is associated with increase in temporal alpha coherence in the dyslexic group. Below, we discuss these results in the context of different approaches to dyslexia and examine the possible role of the cerebellum in this neurodevelopmental disorder.

QMT ENHANCES PERFORMANCE IN A SPEEDED READING TASK

This study was inspired by the controversial body of research that examines the connection between reading and the sensorimotor

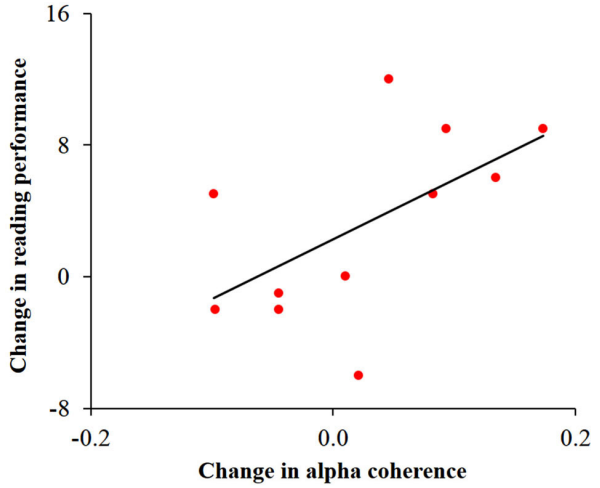

FIGURE 5 | Correlation between change in temporal alpha coherence and change in reading performance in the dyslexic group. Change in alpha coherence, calculated by the subtraction of pre- from post-training, was positively correlated with the change in number of words read correctly in 30 s ($r = 0.58$, $p < 0.05$).

systems (e.g., Flöel et al., 2003; Pulvermüller, 2005), in the context of novel discoveries about the benefit of daily sensorimotor practice for cognition (Ben-Soussan et al., 2013). We found improved reading skills in both groups as a result of 4-week QMT. Commonly, experimental studies attempt to enhance reading abilities and phonological functions by providing a training program for the target skill. Here we show that sensorimotor training might be beneficial for improving reading skills even though the practice relies on faculties that are not directly related to reading. Indeed, the QMT is based on a series of motor responses to verbal commands, which involve functions such as spatial cognition and response inhibition (Ben-Soussan et al., 2014). QMT-related improvement in the speeded reading task was found using different stimulus lists at each time point (pre- and post-training) and is thus considered to result from QMT and not from test-retest effects. This finding is also in agreement with previous results showing cognitive improvement in non-dyslexics adults even following short term QMT (Ben-Soussan et al., 2013, 2014).

In addition to group differences in reading score measured at baseline, a trend of a lower score in the phonological fluency task was observed pre-training in dyslexics compared to controls. This trend was not observed following 4 weeks of daily QMT, suggesting that QMT helped in normalizing the performance on this task. Again, the use of different categories at each time point (pre- and post-training) provided support to the interpretation that it is probably the QMT that normalized performance and not the repetition of the task. In addition, no differences were found between the groups in semantic fluency, neither before nor after training. This supports the view that dyslexia is more related to phonological than to semantic impairment (Leggio et al., 2000).

QMT ENHANCES CEREBELLAR ALPHA POWER IN DYSLEXIC ADULTS

In line with our hypothesis, cerebellar alpha power was significantly lower in the dyslexic group prior to training in comparison to the control group (see **Figure 3C**). Importantly, following 4 weeks of daily QMT, we found that cerebellar alpha significantly increased in the dyslexic group in the right culmen, a region which has been previously reported to be related to language processing (Luke et al., 2002; Pernet et al., 2009a,b; Rudner et al., 2013). This finding may reflect the role of the cerebellum as a general timing mechanism for both sensorimotor and cognitive processes (Ivry, 1997; Tesche and Karhu, 2000; Ivry et al., 2002; Tesche et al., 2007), such as the acquisition of sensorimotor skills and response readiness (Martin et al., 2006). It might also be linked to the critical involvement of the cerebellum in the coordination of smooth movements, maintenance of balance and posture, visually guided movements and motor learning (for review see Manto et al., 2012), which are inherent components of the QMT.

In addition to cerebellar changes, we found differences between dyslexics and controls in frontal alpha activity following 4 weeks of daily QMT. While alpha power in the left MFG and SMA significantly decreased in the control group, the dyslexic group showed increased alpha power in right SFG. These regions have been previously reported to be related to movement and language processing (Binder et al., 1997; Eckert et al., 2003; Neumann et al., 2004). Contrary to the dyslexics, the control group showed a trend towards a reduction in cerebellar alpha power; however, there was a notable increase of the control group's dispersion around the mean, which might account for the lack of statistical significance of this effect. In line with previous findings on motor practice, it is possible that the reduction of frontal and cerebellar activity indicates that practice became simpler as control of movement and coordination improve (Lacourse et al., 2004).

Previous work based on one session of training reported decreased frontal alpha activity in healthy young subjects (Ben-Soussan et al., 2013). This decreased frontal activity was mostly observed following simple motor training, indicating that changes in these regions might be related to motor learning as well as action observation and intention understanding (Exner et al., 2002; Dapretto et al., 2005). These results are compatible with Goldberg et al. (2006) who reported a complete segregation between self-related and sensorimotor activity in relevant cortical regions using functional neuroimaging. Their results showed that frontal regions were functionally inactive during sensorimotor tasks and active during self-engaged tasks. It is therefore possible that reduced activity of frontal regions at rest in controls signifies automaticity of sensorimotor components as a result of the repetition of the same sequence of QMT for 4 weeks.

ALPHA COHERENCE IN DYSLEXIC ADULTS COMPARED TO CONTROLS

Alpha coherence is important for cognitive and sensory processing (Weiss and Mueller, 2003; Ben-Soussan et al., 2013). Previously, EEG studies revealed increased coherence in dyslexic children, especially between temporal areas during rest (Shiota et al., 2000; Arns et al., 2007). Contrary to prior results showing increased inter-hemispheric alpha coherence following a single

session of QMT (Ben-Soussan et al., 2013), in the current study neither group showed a significant increase in alpha coherence following one month of daily QMT. It should be noted that the previous results were obtained using EEG and not MEG. In fact, calculating connectivity from sensor level recordings is not straightforward, as these recordings are highly dependent on the effects of field spread. In other words, coherence measured by MEG reflects fewer sources because the spatial scale of the MEG sensors is smaller resulting in inflated estimates. Moreover, EEG and MEG are different in their sensitivity to radial and tangential dipoles (Srinivasan et al., 2007). This points to the necessity to integrate different methods in the study of training-induced plasticity.

Importantly, in the current study coherence analysis confirmed increased inter-hemispheric alpha coherence in the dyslexic group compared to the control group across time points. The increased inter-hemispheric coherence, especially between the temporal areas, may reflect the connection between left and right superior temporal sulci, which are considered to be necessary for phonological processing (Hickok and Poeppel, 2004). These findings converge with independent data from diffusion imaging showing that children with lower phonological and reading skills have higher anisotropy in temporal-callosal fiber tracts (Ben-Shachar et al., 2007; Dougherty et al., 2007).

Consequently, we propose that the increased coherence found in the dyslexic group may reflect a compensation mechanism (Roberts and Kraft, 1989; Arns et al., 2007). This suggestion further accords with the view that both left and right posterior superior temporal cortices are required for phonological processing (Hickok and Poeppel, 2007). Indeed, earlier models of dyslexia promoted the premise that complex cognitive functions, such as the translation of graphic symbols into a phonemic code, depend on component processes from both cerebral hemispheres, and that at least some subtypes of dyslexia may be due to abnormal inter-hemispheric communication (Gazzaniga, 1973; Gladstone and Best, 1985; Wolff et al., 1990). The association between change in coherence and reading performance revealed a significant positive correlation only in the dyslexic group, suggesting that the underlying mechanisms of improved reading observed in this study are connected with increased inter-hemispheric communication in the alpha range. Due to the low power of the correlation analysis ($N = 11$) and the non-significant effect of training on alpha coherence, this finding should be treated as suggestive, and should be tested in future larger MEG studies of developmental dyslexia. Nonetheless, the positive correlation reveals that participants who showed higher improvement of speeded reading also demonstrated increased bilateral temporal alpha coherence, in addition to the general increase in coherence observed in dyslexia. Some researchers aimed at normalizing brain activity (and consequently ameliorating behavioral and cognitive deficits) in various developmental disorders by suppressing hyper-connectivity (Pineda et al., 2012). Similarly, in stroke rehabilitation, applying brain stimulation to inhibit inappropriate activity of non-specialized areas has been argued to offer an effective avenue of treatment (Naeser et al., 2005). However, ameliorating cognitive deficits in developmental disorders may not necessarily be achieved through suppressing abnormal connectivity, because the observed hyper-connectivity does not necessarily reflect a dysfunction (Arns et al., 2007). Indeed, findings from neurofeedback training show that, contrary to the expected effects, 6 months of training induced an increase in alpha coherence, which might be related to improved attention (Breteler et al., 2010).

TOWARDS A NEW APPROACH TO UNDERSTANDING AND TREATING DYSLEXIA

Existing methods of treating dyslexia usually rely on phonetic and reading materials which aim at dealing directly with the linguistic impairments. Nevertheless, dyslexia, as well as other developmental disorders, should not be interpreted as being impairments in a single cognitive process (Castles and Coltheart, 1993; Pernet et al., 2009a). These cognitive impairments should rather be regarded as the endpoint of an abnormal developmental process, reflecting the interactions of multiple potentially deficient processes as well as compensatory processes (Thomas and Karmiloff-Smith, 2002). The current study attempted to investigate dyslexia-related differences in specific regions, in inter-hemispheric coherence, and in response to intervention. In this way, the differences between groups in the training-induced electrophysiological effects may provide further insight into the deficient and compensatory processes that characterize dyslexia.

In line with our results, we suggest that both the deficient cerebellar alpha power and possibly compensatory alpha coherence may be connected to the cerebellum's role as a generator of alpha activity, and that sensorimotor training may lead to cerebellar plasticity which could eventually rebalance the system. In this respect, altered cerebellar oscillatory activity may be the source of the deficit in dyslexia since it could be viewed as a neural system that mediates cortical communication (Andres et al., 1999; Silberstein et al., 2003). Our preliminary findings also disclose that sensorimotor training can be a practical intervention in dyslexia because of its potential to facilitate cerebellar oscillatory activity. Exploring how neuronal oscillation and cerebellar function change as a result of training may have valuable implications for educational neuroscience.

LIMITATIONS

The current study is a preliminary attempt to examine empirically the question of system modulation, which is required for improving reading in dyslexia. The main limitations of the current study are the small sample size and the use of only one training paradigm. The choice of QMT was made based on previous studies in which it was demonstrated that cognitive changes, namely increased creativity and improved spatial cognition, are QMT specific, and are not observed in two control groups (Ben-Soussan et al., 2013, 2014). In the future, a study on a larger sample that includes several training regimes may extend the current results. In future research, it would be important to include a passive control group; in particular, a dyslexic passive control group would ensure that any test-retest effects that were controlled for with normal-readers and with the two different versions of the tasks are not different in participants with dyslexia.

So far, EEG studies have generally avoided studying cerebellar function because of the complex folding of the cerebellar cortex. As for MEG, signals can be obtained from the cerebellum especially within the alpha range (Ivry, 2000; Park et al., 2011). However, source localization makes it difficult to distinguish between signals arising in the cerebellar cortex and deep nuclei. Thus, our results should be interpreted keeping these limitations in mind. Regarding the coherence analysis, the statistical significance was not corrected for 59 comparisons and should be therefore evaluated with caution. However, since no previous study has shown similar left-right coherence effects it was impossible for us to focus on channels of interest and reduce the number of multiple comparisons. The results we report here can therefore be considered as exploratory, and should be confirmed in future studies. We expect that future studies will utilize independent imaging methods in order to examine the role of the cerebellum in reading and dyslexia, and the impact of QMT on cerebellar activity and connectivity.

CONCLUSIONS

The current MEG study is in line with previous studies suggesting that dyslexia may be related to cerebellar dysfunction. Four weeks of daily QMT enhances reading performance and cerebellar alpha oscillations in dyslexic participants. In addition, improved reading performance in dyslexics correlates with inter-hemispheric temporal alpha coherence. Our results suggest that cerebellar impairment in dyslexia can be modulated by sensorimotor training. Most importantly, the investigation of training-induced effects on reading performance provides a unique opportunity to gain insight into the relation between behavioral and neuronal changes. A better understanding of the functional coordination between cortical regions and the cerebellum would be necessary to pinpoint some of the underlying sources of dyslexia and to create training paradigms for clinical purposes. The current study provides an important step in bringing together different approaches to study the sources and treatment of dyslexia and the scientific value of sensorimotor training.

ACKNOWLEDGMENTS

We would like to thank the participants who let us examine their minds and brains. This study was supported by the Italy-Israel R&D Cooperation Program (Number 44186) awarded to Joseph Glicksohn and Tal Dotan Ben-Soussan and by the Israeli Center of Research Excellence (I-CORE) in Cognition (I-CORE Program 51/11).

REFERENCES

Akshoomoff, N. A., Courchesne, E., Press, G. A., and Iragui, V. (1992). Contribution of the cerebellum to neuropsychological functioning: evidence from a case of cerebellar degenerative disorder. *Neuropsychologia* 30, 315–328. doi: 10.1016/0028-3932(92)90105-u

Andres, F. G., Mima, T., Schulman, A. E., Dichgans, J., Hallett, M., and Gerloff, C. (1999). Functional coupling of human cortical sensorimotor areas during bimanual skill acquisition. *Brain* 122, 855–870. doi: 10.1093/brain/122.5.855

Appollonio, I. M., Grafman, J., Schwartz, V., Massaquoi, S., and Hallett, M. (1993). Memory in patients with cerebellar degeneration. *Neurology* 43, 1536–1536. doi: 10.1212/wnl.43.8.1536

Arns, M., Peters, S., Breteler, R., and Verhoeven, L. (2007). Different brain activation patterns in dyslexic children: evidence from EEG power and coherence patterns for the double-deficit theory of dyslexia. *J. Integr. Neurosci.* 6, 175–190. doi: 10.1142/s0219635207001404

Basar, E., Schurmann, M., Basar-Eroglu, C., and Karakas, S. (1997). Alpha oscillations in brain functioning: an integrative theory. *Int. J. Psychophysiol.* 26, 5–29. doi: 10.1016/s0167-8760(97)00753-8

Ben-Shachar, M., Dougherty, R. F., and Wandell, B. A. (2007). White matter pathways in reading. *Curr. Opin. Neurobiol.* 17, 258–270. doi: 10.1016/j.conb.2007.03.006

Ben-Soussan, T. D., Berkovich-Ohana, A., Glicksohn, J., and Goldstein, A. (2014). A suspended act: increased reflectivity and gender-dependent electrophysiological change following Quadrato Motor Training. *Front. Psychol.* 5:55. doi: 10.3389/fpsyg.2014.00055

Ben-Soussan, T. D., Glicksohn, J., Goldstein, A., Berkovich-Ohana, A., and Donchin, O. (2013). Into the square and out of the box: the effects of Quadrato Motor Training on creativity and alpha Coherence. *PLoS One* 8:e55023. doi: 10.1371/journal.pone.0055023

Binder, J. R., Frost, J. A., Hammeke, T. A., Cox, R. W., Rao, S. M., and Prieto, T. (1997). Human brain language areas identified by functional magnetic resonance imaging. *J. Neurosci.* 17, 353–362.

Bradley, L., and Bryant, P. E. (1983). Categorizing sounds and learning to read: a causal connection. *Nature* 301, 419–421. doi: 10.1038/301419a0

Breteler, M. H., Arns, M., Peters, S., Giepmans, I., and Verhoeven, L. (2010). Improvements in spelling after QEEG-based neurofeedback in dyslexia: a randomized controlled treatment study. *Appl. Psychophysiol. Biofeedback* 35, 5–11. doi: 10.1007/s10484-009-9105-2

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron* 80, 807–815. doi: 10.1016/j.neuron.2013.10.044

Bunge, S. A., Ochsner, K. N., Desmond, J. E., Glover, G. H., and Gabrieli, J. D. (2001). Prefrontal regions involved in keeping information in and out of mind. *Brain* 124, 2074–2086. doi: 10.1093/brain/124.10.2074

Castles, A., and Coltheart, M. (1993). Varieties of developmental dyslexia. *Cognition* 47, 149–180. doi: 10.1016/0010-0277(93)90003-e

Cox, R. W. (2012). AFNI: what a long strange trip it's been. *Neuroimage* 62, 743–747. doi: 10.1016/j.neuroimage.2011.08.056

Criscimagna-Hemminger, S. E., Donchin, O., Gazzaniga, M. S., and Shadmehr, R. (2002). Learning dynamics of reaching movements generalizes from dominant to non-dominant arm. *J. Neurophysiol.* 89, 168–176. doi: 10.1152/jn.00622.2002

Dapretto, M., Davies, M. S., Pfeifer, J. H., Scott, A. A., Sigman, M., Bookheimer, S. Y., et al. (2005). Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. *Nat. Neurosci.* 9, 28–30. doi: 10.1038/nn1611

De Kleine, E., and Verwey, W. B. (2009). Motor learning and chunking in dyslexia. *J. Mot. Behav.* 41, 331–338. doi: 10.3200/jmbr.41.4.331-338

De Zeeuw, C. I., Hoebeek, F. E., Bosman, L. W., Schonewille, M., Witter, L., and Koekkoek, S. K. (2011). Spatiotemporal firing patterns in the cerebellum. *Nat. Rev. Neurosci.* 12, 327–344. doi: 10.1038/nrn3011

Deffenbacher, K. E., Kenyon, J. B., Hoover, D. M., Olson, R. K., Pennington, B. F., DeFries, J. C., et al. (2004). Refinement of the 6p21. 3 quantitative trait locus influencing dyslexia: linkage and association analyses. *Hum. Genet.* 115, 128–138. doi: 10.1007/s00439-004-1126-6

Donchin, O., Francis, J. T., and Shadmehr, R. (2003). Quantifying generalization from trial-by-trial behavior of adaptive systems that learn with basis functions: theory and experiments in human motor control. *J. Neurosci.* 23, 9032–9045.

Dougherty, R. F., Ben-Shachar, M., Deutsch, G. K., Hernandez, A., Fox, G. R., and Wandell, B. A. (2007). Temporal-callosal pathway diffusivity predicts phonological skills in children. *Proc. Natl. Acad. Sci. U S A* 104, 8556–8561. doi: 10.1073/pnas.0608961104

Dufor, O., Serniclaes, W., Sprenger-Charolles, L., and Démonet, J. F. (2007). Top-down processes during auditory phoneme categorization in dyslexia: a PET study. *Neuroimage* 34, 1692–1707. doi: 10.1016/j.neuroimage.2006.10.034

Eckert, M. A., Leonard, C. M., Richards, T. L., Aylward, E. H., Thomson, J., and Berninger, V. W. (2003). Anatomical correlates of dyslexia: frontal and cerebellar findings. *Brain* 126, 482–494. doi: 10.1093/brain/awg026

Exner, C., Koschack, J., and Irle, E. (2002). The differential role of premotor frontal cortex and basal ganglia in motor sequence learning: evidence from focal basal ganglia lesions. *Learn. Mem.* 9, 376–386. doi: 10.1101/lm.48402

Flöel, A., Ellger, T., Breitenstein, C., and Knecht, S. (2003). Language perception activates the hand motor cortex: implications for motor theories of speech perception. *Eur. J. Neurosci.* 18, 704–708. doi: 10.1046/j.1460-9568.2003.02774.x

Friston, K. J., Ashburner, J. T., Kiebel, S. J., Nichols, T. E., and Penny, W. D. (2007). *Statistical Parametric Mapping: The Analysis of Functional Brain Images.* London: Academic Press.

Gabrieli, J. D. (2009). Dyslexia: a new synergy between education and cognitive neuroscience. *Science* 325, 280–283. doi: 10.1126/science.1171999

Galaburda, A. M., LoTurco, J., Ramus, F., Fitch, R. H., and Rosen, G. D. (2006). From genes to behavior in developmental dyslexia. *Nat. Neurosci.* 9, 1213–1217. doi: 10.1038/nn1772

Gazzaniga, M. S. (1973). Brain theory and minimal brain dysfunction. *Ann. N Y Acad. Sci.* 205, 89–92. doi: 10.1111/j.1749-6632.1973.tb43166.x

Genow, A., Hummel, C., Scheler, G., Hopfengärtner, R., Kaltenhäuser, M., Buchfelder, M., et al. (2004). Epilepsy surgery, resection volume and MSI localization in lesional frontal lobe epilepsy. *Neuroimage* 21, 444–449. doi: 10.1016/j.neuroimage.2003.08.029

Gladstone, M., and Best, C. T. (1985). "Developmental dyslexia: the potential role of interhemispheric collaboration in reading acquisition," in *Hemispheric Function Collaboration in the Child*, ed C. T. Best (San Francisco: Academic Press), 87–118.

Goldberg, I. I., Harel, M., and Malach, R. (2006). When the brain loses its self: prefrontal inactivation during sensorimotor processing. *Neuron* 50, 329–339. doi: 10.1016/j.neuron.2006.03.015

Goldman, R. I., Stern, J. M., Engel, J. Jr., and Cohen, M. S. (2002). Simultaneous EEG and fMRI of the alpha rhythm. *Neuroreport* 13, 2487–2492. doi: 10.1097/00001756-200212200-00022

Goswami, U. (2011). A temporal sampling framework for developmental dyslexia. *Trends Cogn. Sci.* 15, 3–10. doi: 10.1016/j.tics.2010.10.001

Güntekin, B., and Basar, E. (2010). A new interpretation of P300 responses upon analysis of coherences. *Cogn. Neurodynm.* 4, 107–118. doi: 10.1007/s11571-010-9106-0

Hickok, G., and Poeppel, D. (2004). Dorsal and ventral streams: a framework for understanding aspects of the functional anatomy of language. *Cognition* 92, 67–99. doi: 10.1016/j.cognition.2003.10.011

Hickok, G., and Poeppel, D. (2007). The cortical organization of speech processing. *Nat. Rev. Neurosci.* 8, 393–402. doi: 10.1038/nrn2113

Hickok, G., Houde, J., and Rong, F. (2011). Sensorimotor integration in speech processing: computational basis and neural organization. *Neuron* 69, 407–422. doi: 10.1016/j.neuron.2011.01.019

Hölzel, B. K., Carmody, J., Vangel, M., Congleton, C., Yerramsetti, S. M., Gard, T., et al. (2011). Mindfulness practice leads to increases in regional brain gray matter density. *Psychiatry Res.* 191, 36–43. doi: 10.1016/j.pscychresns.2010.08.006

Ivry, R. (1997). Cerebellar timing systems. *Int. Rev. Neurobiol.* 41, 555–573. doi: 10.1016/s0074-7742(08)60370-0

Ivry, R. (2000). Exploring the role of the cerebellum in sensory anticipation and timing: commentary on Tesche and Karhu. *Hum. Brain Mapp.* 9, 115–118. doi: 10.1002/(sici)1097-0193(200003)9:3<115::aid-hbm1>3.3.co;2-x

Ivry, R. B., Spencer, R. M., Zelaznik, H. N., and Diedrichsen, J. (2002). The cerebellum and event timing. *Ann. N Y Acad. Sci.* 978, 302–317. doi: 10.1111/j.1749-6632.2002.tb07576.x

Kanda, M., Nagamine, T., Ikeda, A., Ohara, S., Kunieda, T., Fujiwara, N., et al. (2000). Primary somatosensory cortex is actively involved in pain processing in human. *Brain Res.* 853, 282–289. doi: 10.1016/s0006-8993(99)02274-x

Kavé, G. (2005). Phonemic fluency, semantic fluency and difference scores: normative data for adult Hebrew speakers. *J. Clin. Exp. Neuropsychol.* 27, 690–699. doi: 10.1080/13803390490918499

Kikuchi, M., Shitamichi, K., Yoshimura, Y., Ueno, S., Remijn, G. B., Hirosawa, T., et al. (2011). Lateralized theta wave connectivity and language performance in 2-to 5-year-old children. *J. Neurosci.* 31, 14984–14988. doi: 10.1523/jneurosci.2785-11.2011

Lacourse, M. G., Turner, J. A., Randolph-Orr, E., Schandler, S. L., and Cohen, M. J. (2004). Cerebral and cerebellar sensorimotor plasticity following motor imagery-based mental practice of a sequential movement. *J. Rehabil. Res. Dev.* 41, 505–524. doi: 10.1682/jrrd.2004.04.0505

Leggio, M. G., Silveri, M. C., Petrosini, L., and Molinari, M. (2000). Phonological grouping is specifically affected in cerebellar patients: a verbal fluency study. *J. Neurol. Neurosurg. Psychiatry* 69, 102–106. doi: 10.1136/jnnp.69.1.102

Lehnertz, K., Ansmann, G., Bialonski, S., Dickten, H., Geier, C., and Porz, S. (2014). Evolving networks in the human epileptic brain. *Physica D* 267, 7–15. doi: 10.1016/j.physd.2013.06.009

Leiner, H. C., Leiner, A. L., and Dow, R. S. (1993). Cognitive and language functions of the human cerebellum. *Trends Neurosci.* 16, 444–447. doi: 10.1016/0166-2236(93)90072-t

Levy-Drori, S., and Henik, A. (2006). Concreteness and context availability in lexical decision tasks. *Am. J. Psychol.* 119, 45–65. doi: 10.2307/20445318

Luke, K. K., Liu, H. L., Wai, Y. Y., Wan, Y. L., and Tan, L. H. (2002). Functional anatomy of syntactic and semantic processing in language comprehension. *Hum. Brain Mapp.* 16, 133–145. doi: 10.1002/hbm.10029

Manto, M., Bower, J. M., Conforto, A. B., Delgado-García, J. M., da Guarda, S. N. F., Gerwig, M., et al. (2012). Consensus paper: roles of the cerebellum in motor control—the diversity of ideas on cerebellar involvement in movement. *Cerebellum* 11, 457–487. doi: 10.1007/s12311-011-0331-9

Martin, T., Houck, J. M., Bish, J. P., Kičić, D., Woodruff, C. C., Moses, S. N., et al. (2006). MEG reveals different contributions of somatomotor cortex and cerebellum to simple reaction time after temporally structured cues. *Hum. Brain Mapp.* 27, 552–561. doi: 10.1002/hbm.20200

Naeser, M. A., Martin, P. I., Nicholas, M., Baker, E. H., Seekins, H., Kobayashi, M., et al. (2005). Improved picture naming in chronic aphasia after TMS to part of right Broca's area: an open-protocol study. *Brain Lang.* 93, 95–105. doi: 10.1016/j.bandl.2004.08.004

Neumann, K., Euler, H. A., Gudenberg, A. W. V., Giraud, A. L., Lanfermann, H., Gall, V., et al. (2004). The nature and treatment of stuttering as revealed by fMRI: a within-and between-group comparison. *J. Fluency Disord.* 28, 381–410. doi: 10.1016/j.jfludis.2003.07.003

Nicolson, R. I., Fawcett, A. J., and Dean, P. (2001). Developmental dyslexia: the cerebellar deficit hypothesis. *Trends Neurosci.* 24, 508–511. doi: 10.1016/s0166-2236(00)01896-8

Nicolson, R. I., Fawcett, A. J., Berry, E. L., Jenkins, I. H., Dean, P., and Brooks, D. J. (1999). Association of abnormal cerebellar activation with motor learning difficulties in dyslexic adults. *Lancet* 353, 1662–1667. doi: 10.1016/s0140-6736(98)09165-x

Oganian, Y., and Ahissar, M. (2012). Poor anchoring limits dyslexics' perceptual, memory and reading skills. *Neuropsychologia* 50, 1895–1905. doi: 10.1016/j.neuropsychologia.2012.04.014

Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J. M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG and invasive electrophysiological data. *Comput. Intell. Neurosci.* 2011:156869. doi: 10.1155/2011/156869

Orton, S. T. (1937). *Reading, Writing and Speech Problems in Children.* New York: Norton.

Osipova, D., Ahveninen, J., Kaakkola, S., Jääskeläinen, I. P., Huttunen, J., and Pekkonen, E. (2003). Effects of scopolamine on MEG spectral power and coherence in elderly subjects. *Clin. Neurophysiol.* 114, 1902–1907. doi: 10.1016/s1388-2457(03)00165-2

Park, H., Kang, E., Kang, H., Kim, J. S., Jensen, O., Chung, C. K., et al. (2011). Cross-frequency power correlations reveal the right superior temporal gyrus as a hub region during working memory maintenance. *Brain Connect.* 1, 460–472. doi: 10.1089/brain.2011.0046

Pernet, C., Andersson, J., Paulesu, E., and Demonet, J. F. (2009a). When all hypotheses are right: a multifocal account of dyslexia. *Hum. Brain Mapp.* 30, 2278–2292. doi: 10.1002/hbm.20670

Pernet, C., Poline, J., Demonet, J., and Rousselet, G. (2009b). Brain classification reveals the right cerebellum as the best biomarker of dyslexia. *BMC Neurosci.* 10:67. doi: 10.1186/1471-2202-10-67

Pineda, J. A., Juavinett, A., and Datko, M. (2012). Self-regulation of brain oscillations as a treatment for aberrant brain connections in children with autism. *Med. Hypotheses* 79, 790–798. doi: 10.1016/j.mehy.2012.08.031

Pulvermüller, F. (2005). Brain mechanisms linking language and action. *Nat. Rev. Neurosci.* 6, 576–582. doi: 10.1038/nrn1706

Rack, J. P., Snowling, M. J., and Olson, R. K. (1992). The nonword reading deficit in developmental dyslexia: a review. *Read. Res. Q.* 27, 29–53.

Ramus, F. (2004). Neurobiology of dyslexia: a reinterpretation of the data. *Trends Neurosci.* 27, 720–726. doi: 10.1016/j.tins.2004.10.004

Ramus, F., Pidgeon, E., and Frith, U. (2003). The relationship between motor control and phonology in dyslexic children. *J. Child Psychol. Psychiatry* 44, 712–722. doi: 10.1111/1469-7610.00157

Rao, S. M., Mayer, A. R., and Harrington, D. L. (2001). The evolution of brain activation during temporal processing. *Nat. Neurosci.* 4, 317–323. doi: 10.1038/85191

Reid, A. A., Szczerbinski, M., Iskierka-Kasperek, E., and Hansen, P. (2007). Cognitive profiles of adult developmental dyslexics: theoretical implications. *Dyslexia* 13, 1–24. doi: 10.1002/dys.321

Reynolds, D., and Nicolson, R. I. (2007). Follow-up of an exercise-based treatment for children with reading difficulties. *Dyslexia* 13, 78–96. doi: 10.1002/dys.331

Reynolds, D., Nicolson, R. I., and Hambly, H. (2003). Evaluation of an exercise-based treatment for children with reading difficulties. *Dyslexia* 9, 48–71. doi: 10.1002/dys.235

Roberts, T. A., and Kraft, R. H. (1989). Developmental differences in the relationship between reading comprehension and hemispheric alpha patterns: an EEG study. *J. Educ. Psychol.* 81, 322–328. doi: 10.1037/0022-0663.81.3.322

Robinson, S. E., and Vrba, J. (1999). "Functional neuroimaging by synthetic aperture magnetometry (SAM)," in *Recent Advances in Biomagnetism*, eds T. Yoshimoto, M. Kotani, S. Kuriki, H. Karibe and N. Nakasato (Sendai: Tohuku University Press), 302–305.

Rudner, M., Karlsson, T., Gunnarsson, J., and Rönnberg, J. (2013). Levels of processing and language modality specificity in working memory. *Neuropsychologia* 51, 656–666. doi: 10.1016/j.neuropsychologia.2012.12.011

Salmelin, R. (2007). Clinical neurophysiology of language: the MEG approach. *Clin. Neurophysiol.* 118, 237–254. doi: 10.1016/j.clinph.2006.07.316

Schmahmann, J. D. (2004). Disorders of the cerebellum: ataxia, dysmetria of thought and the cerebellar cognitive affective syndrome. *J. Neuropsychiatry Clin. Neurosci.* 16, 367–378. doi: 10.1176/appi.neuropsych.16.3.367

Sekihara, K., Nagarajan, S. S., Poeppel, D., and Marantz, A. (2004). Asymptotic SNR of scalar and vector minimum-variance beamformers for neuromagnetic source reconstruction. *IEEE Trans. Biomed. Eng.* 51, 1726–1734. doi: 10.1109/tbme.2004.827926

Shaywitz, S. E., Morris, R., and Shaywitz, B. A. (2008). The education of dyslexic children from childhood to young adulthood. *Annu. Rev. Psychol.* 59, 451–475. doi: 10.1146/annurev.psych.59.103006.093633

Shiota, M., Koeda, T., and Takeshita, K. (2000). Cognitive and neurophysiological evaluation of Japanese dyslexia. *Brain Dev.* 22, 421–426. doi: 10.1016/s0387-7604(00)00167-4

Silberstein, R. B. (2006). Dynamic sculpting of brain functional connectivity and mental rotation aptitude. *Prog. Brain Res.* 159, 63–76. doi: 10.1016/s0079-6123(06)59005-3

Silberstein, R. B., Danieli, F., and Nunez, P. L. (2003). Fronto-parietal evoked potential synchronization is increased during mental rotation. *Neuroreport* 14, 67–71. doi: 10.1097/00001756-200301200-00013

Srinivasan, R., Winter, W. R., Ding, J., and Nunez, P. L. (2007). EEG and MEG coherence: measures of functional connectivity at distinct spatial scales of neocortical dynamics. *J. Neurosci. Methods* 166, 41–52. doi: 10.1016/j.jneumeth.2007.06.026

Stein, J. (2001). The magnocellular theory of developmental dyslexia. *Dyslexia* 7, 12–36. doi: 10.1002/dys.186

Stein, J., and Walsh, V. (1997). To see but not to read; the magnocellular theory of dyslexia. *Trends Neurosci.* 20, 147–152. doi: 10.1016/s0166-2236(96)01005-3

Stoodley, C. J., and Schmahmann, J. D. (2009). Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *Neuroimage* 44, 489–501. doi: 10.1016/j.neuroimage.2008.08.039

Stoodley, C. J., and Stein, J. F. (2011). The cerebellum and dyslexia. *Cortex* 47, 101–116. doi: 10.1016/j.cortex.2009.10.005

Swinnen, S. P. (2002). Intermanual coordination: from behavioural principles to neural-network interactions. *Nat. Rev. Neurosci.* 3, 348–359. doi: 10.1038/nrn807

Tal, I., and Abeles, M. (2013). Cleaning MEG artifacts by external cues. *J. Neurosci. Methods* 217, 31–38. doi: 10.1016/j.jneumeth.2013.04.002

Tallal, P., Miller, S. L., Bedi, G., Byma, G., Wang, X., Nagarajan, S. S., et al. (1996). Language comprehension in language-learning impaired children improved with acoustically modified speech. *Science* 271, 81–84. doi: 10.1126/science.271.5245.81

Tesche, C. D., Moses, S. N., Houck, J. M., Martin, T., Hanlon, F. M., Jackson, E., et al. (2007). Dynamics of frontal and cerebellar activation during aversive conditioning: an MEG study. *Int. Congr. Ser.* 1300, 437–440. doi: 10.1016/j.ics.2007.02.057

Tesche, C. D., and Karhu, J. J. (2000). Anticipatory cerebellar responses during somatosensory omission in man. *Hum. Brain Mapp.* 9, 119–142. doi: 10.1002/(sici)1097-0193(200003)9:3<119::aid-hbm2>3.3.co;2-i

Thomas, M., and Karmiloff-Smith, A. (2002). Are developmental disorders like cases of adult brain damage? Implications from connectionist modelling. *Behav. Brain Sci.* 25, 727–750. doi: 10.1017/s0140525x02000134

Weiss, S., and Mueller, H. M. (2003). The contribution of EEG coherence to the investigation of language. *Brain Lang.* 85, 325–343. doi: 10.1016/s0093-934x(03)00067-1

Wolff, P. H., Michel, G. F., Ovrut, M., and Drake, C. (1990). Rate and timing precision of motor coordination in developmental dyslexia. *Dev. Psychol.* 26, 349–359. doi: 10.1037/0012-1649.26.3.349

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2014; accepted: 18 April 2014; published online: 09 May 2014.
*Citation: Ben-Soussan TD, Avirame K, Glicksohn J, Goldstein A, Harpaz Y and Ben-Shachar M (2014) Changes in cerebellar activity and inter-hemispheric coherence accompany improved reading performance following Quadrato Motor Training. Front. Syst. Neurosci. **8**:81. doi: 10.3389/fnsys.2014.00081*
This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

OPINION ARTICLE
published: 12 March 2014
doi: 10.3389/fnsys.2014.00034

Increased intelligence is a myth (so far)

Richard J. Haier*

Emeritus, Pediatrics, School of Medicine, University of California, Irvine, CA, USA
**Correspondence: rjhaier@uci.edu*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
James M. Broadway, University of California Santa Barbara, USA
Michael Linderman, Norconnect Inc, USA

Keywords: intelligence, *g*-factor, brain imaging, cognitive training, ratio scales, IQ testing

On one hand, intelligence testing is one of the great successes of psychology (Hunt, 2011). Intelligence test scores predict many real world phenomena and have many well-validated practical uses (Gottfredson, 1997; Deary et al., 2010). Intelligence test scores also correlate to structural and functional brain parameters assessed with neuroimaging (Haier et al., 1988; Jung and Haier, 2007; Deary et al., 2010; Penke et al., 2012; Colom et al., 2013a) and to genes (Posthuma et al., 2002; Hulshoff Pol et al., 2006; Chiang et al., 2009, 2012; Stein et al., 2012). On the other hand, intelligence test scores are often misunderstood and can be misused. This paper focuses on a basic misunderstanding that permeates many of the recent reports of increased intelligence following short-term cognitive training. Several of these reports have been published in prominent journals and received wide public attention (Jaeggi et al., 2008, 2011; Mackey et al., 2011).

The basic misunderstanding is assuming that intelligence test scores are units of measurement like inches or liters or grams. They are not. Inches, liters and grams are ratio scales where zero means zero and 100 units are twice 50 units. Intelligence test scores estimate a construct using interval scales and have meaning only relative to other people of the same age and sex. People with high scores generally do better on a broad range of mental ability tests, but someone with an IQ score of 130 is not 30% smarter then someone with an IQ score of 100. A score of 130 puts the person in the highest 2% of the population whereas a score of 100 is at the 50th percentile. A change from an IQ score from 100 to 103 is not the same as a change from 133 to 136. This makes simple interpretation of intelligence test score changes impossible.

Most recent studies that have claimed increases in intelligence after a cognitive training intervention rely on comparing an intelligence test score before the intervention to a second score after the intervention. If there is an average change score increase for the training group that is statistically significant (using a dependent t-test or similar statistical test), this is treated as evidence that intelligence has increased. This reasoning is correct if one is measuring ratio scales like inches, liters or grams before and after some intervention (assuming suitable and reliable instruments like rulers to avoid erroneous Cold Fusion-like conclusions that apparently were based on faulty heat measurement); it is not correct for intelligence test scores on interval scales that only estimate a relative rank order rather than measure the construct of intelligence. Even though the estimate has considerable predictive value and correlates to brain and genetic measures, it is not a measurement in the same way we measure distance, liquid, or weight even if individual change scores are used in a pre-post design.

SAT scores, for example, are highly correlated to intelligence test scores (Frey and Detterman, 2004). Imagine a student takes the SATs when quite ill. The scores likely are a bad estimate of the student's ability. If the student retakes the test sometime later when well, does an increase in score mean the student's intelligence has increased, or that the newer score is now just a better estimate? The same is true for score changes following SAT preparatory courses. Many colleges and universities allow applicants to submit multiple SAT scores and the highest score typically carries the most weight; there are many spurious reasons for low scores but far fewer for high scores. Change scores from lowest to highest carry little if any weight. By contrast, change in a person's weight after some intervention is unambiguous.

In studies of the effect of cognitive training on intelligence, it is also important to understand that all intelligence test scores include a certain amount of imprecision or error. This is called the standard error of measurement and can be quantified as an estimate of a "true" score based on observed scores. The standard error of measuring inches or liters is usually zero assuming you have perfectly reliable, standard measurement devices. Intelligence tests generally show high test-retest reliability but they also have a standard error, and the standard error is often larger for higher scores than for lower scores. Any intelligence test score change after an intervention needs to be considered relative to the standard error of the test. Studies that use a single test to estimate intelligence before and after an intervention are using less reliable and more variable scores (bigger standard errors) than studies that combine scores from a battery of tests.

Change scores are never easy to interpret and require sophisticated statistical methods and research designs with appropriate control groups. If you try a training intervention in individuals all of whom have pre-intervention scores below the population mean, for example, re-testing with or without any intervention, may result in higher scores due to the statistical phenomenon of regression to the mean, or due to simple test practice, especially if equivalent alternative forms of

the test are not used. Quasi-experimental designs like post-test only with large samples and random assignment do not have all the same interpretation difficulties as pre-post designs. They have promise but most reviewers are more inclined to value pre-post changes. Latent variable techniques also avoid many of the difficulties of pre-post interval scale changes and they have promise in large samples (Ferrer and McArdle, 2010).

When change scores are used, it is important to identify individual differences even within a group where the average change score statistically increases after an intervention. Imagine a group of 100 students received cognitive training and 100 others received some control intervention. The mean change score in the training group may statistically show a greater increase than the controls. How many of the 100 individuals who received the training actually show an increase? Do they differ in any way from the individuals in the same group who do not show an increase? Does item analysis show whether increased scores are due more to easy test items or hard ones? What about any individuals in the control group that show change score increases as large as shown in the training group? If all 200 participants ultimately get the same training, will the rank order of individuals based on the post-training score be any different than the rank order based on the pre-training scores? If not, what has been accomplished? Most studies do not report such analyses, although newer training studies are addressing issues of multiple measure assessment of intelligence and individual differences (Colom et al., 2013b; Jaeggi et al., 2013). Burgaleta et al provide a good example of showing IQ changes subject-by-subject (Burgaleta et al., 2014).

Nonetheless, the main point is that to make the most compelling argument that intelligence increases after an intervention, a ratio scale of intelligence is required. None yet exists and meaningful progress may require a new way of defining intelligence based on measureable brain or information processing variables. For example, gray and white matter density in specific brain regions assessed by imaging and expressed as a profile of standard scores based on a normative group might substitute for intelligence test scores (Haier, 2009). Work by Engle and colleagues suggests that working memory capacity and perceptual speed are possible ways to assess fluid intelligence (Broadway and Engle, 2010; Redick et al., 2012) based on a large body of research that shows faster mental processing speed and increased memory capacity are related to higher intelligence.

Jensen has written extensively about an evolution from psychometrics to mental "chronometrics"—the use of response time in milliseconds to measure information processing in a standard way (Jensen, 2006). He argued that the construct of intelligence could be replaced in favor of ratio scale measures of speed of information processing assessed during standardized cognitive tasks like the Hick paradigm. Such measures, for example, would help advance research about the underlying neurophysiology of mental speed and might lead to a more advanced definition of intelligence. Jensen concluded his book on chronometry with this call to action: "... chronometry provides the behavioral and brain sciences with a universal absolute scale for obtaining highly sensitive and frequently repeatable measurements of an individual's performance on specially devised cognitive tasks. Its time has come. Let's get to work!" (p. 246).

This is a formidable challenge and a major priority for intelligence researchers. Collaboration among psychometricians and cognitive psychologists will be key. There are now a number of studies that fail to replicate the claims of increased intelligence after short-term memory training and various reasons are proposed (Colom et al., 2013b; Harrison et al., 2013). Given our narrow focus here, we note one failure to replicate also assessed working memory capacity and perceptual speed; no transfer effects were found (Redick et al., 2013) and there is reason to suggest that other positive transfer studies may be erroneous (Tidwell et al., 2013). For now, cognitive training results are more inconsistent than not, especially for putative intelligence increases. Nonetheless, it is encouraging that cognitive researchers are working on these issues despite a pervasive indifference or negativity to intelligence research in Psychology in general and for many funding agencies.

In the broader context, intelligence includes more than one component. However, the construct of interest usually is defined by psychometric methods as a general factor common to all mental abilities called the *g*-factor (Jensen, 1998). Fluid intelligence, the focus of several cognitive training studies, is one of several broad intelligence factors and it is highly correlated to *g*. The *g*-factor is estimated by intelligence tests but it is not synonymous with IQ or any other test score; some tests are more *g*-loaded than others. As noted, a score on an intelligence test has little meaning without comparing it to the scores of other people. That is why all intelligence tests require normative groups for comparison and why norm groups need to be updated periodically, as demonstrated by the Flynn Effect of gradual generational increases in intelligence test scores; although whether *g* shows the Flynn effect is still unsettled (te Nijenhuis and van der Flier, 2013). Psychometric estimations of *g* and other intelligence factors have generated strong empirical findings about the nature of intelligence and individual differences, mostly based on correlation studies. These interval assessments, however, are not sufficient to take research to the next step of experimental interventions to increase intelligence.

Speaking about science, Carl Sagan observed that extraordinary claims require extraordinary evidence. So far, we do not have it for claims about increasing intelligence after cognitive training or, for that matter, any other manipulation or treatment, including early childhood education. Small statistically significant changes in test scores may be important observations about attention or memory or some other elemental cognitive variable or a specific mental ability assessed with a ratio scale like milliseconds, but they are not sufficient proof that general intelligence has changed. As in all branches of science, progress depends on ever more sophisticated measurement that drives more precise definitions—think about the evolution of definition for a "gene" or an "atom". Even with sophisticated interval-based assessment techniques (Ferrer and McArdle, 2010), until we have better measures, especially ratio scales, we need to acknowledge the basic measurement problem and exercise abundant restraint when

reporting putative intelligence increases or decreases.

In the future, there may be strong empirical rationales for spending large sums of money on cognitive training or other interventions aimed at improving specific mental abilities or school achievement (in addition to the compelling moral arguments to do so), but increasing general intelligence is quite difficult to demonstrate with current tests. Increasing intelligence, however, is a worthy goal that might be achieved by interventions based on sophisticated neuroscience advances in DNA analysis, neuroimaging, psychopharmacology, and even direct brain stimulation (Haier, 2009, 2013; Lozano and Lipsman, 2013; Santarnecchi et al., 2013; Legon et al., 2014). Developing equally sophisticated ratio measurement of intelligence must go hand-in-hand with developing promising interventions.

ACKNOWLEDGMENTS

A version of this paper was presented at the annual meeting of the International Society for Intelligence Research, San Antonio, Texas, December 15, 2012 in a symposium on Improving IQ (chaired by S. Jaeggi and R. Colom).

REFERENCES

Broadway, J. M., and Engle, R. W. (2010). Validating running memory span: measurement of working memory capacity and links with fluid intelligence. *Behav. Res. Methods* 42, 563–570. doi: 10.3758/BRM.42.2.563

Burgaleta, M., Johnson, W., Waber, D. P., Colom, R., and Karama, S. (2014). Cognitive ability changes and dynamics of cortical thickness development in healthy children and adolescents. *Neuroimage* 84, 810–819. doi: 10.1016/j.neuroimage.2013.09.038

Chiang, M. C., Barysheva, M., McMahon, K. L., de Zubicaray, G. I., Johnson, K., Montgomery, G. W., et al. (2012). Gene network effects on brain microstructure and intellectual performance identified in 472 twins. *J. Neurosci.* 32, 8732–8745. doi: 10.1523/JNEUROSCI.5993-11.2012

Chiang, M. C., Barysheva, M., Shattuck, D. W., Lee, A. D., Madsen, S. K., Avedissian, C., et al. (2009). Genetics of brain fiber architecture and intellectual performance. *J. Neurosci.* 29, 2212–2224. doi: 10.1523/JNEUROSCI.4184-08.2009

Colom, R., Burgaleta, M., Roman, F. J., Karama, S., Alvarez-Linera, J., Abad, F. J., et al. (2013a). Neuroanatomic overlap between intelligence and cognitive factors: morphometry methods provide support for the key role of the frontal lobes. *Neuroimage* 72, 143–152. doi: 10.1016/j.neuroimage.2013.01.032

Colom, R., Roman, F. J., Abad, F. J., Shih, P. C., Privado, J., Froufe, M., et al. (2013b). Adaptive n-back training does not improve fluid intelligence at the construct level: gains on individual tests suggest that training may enhance visuospatial processing. *Intelligence* 41, 712–727. doi: 10.1016/j.intell.2013.09.002

Deary, I. J., Penke, L., and Johnson, W. (2010). The neuroscience of human intelligence differences. *Nat. Rev. Neurosci.* 11, 201–211. doi: 10.1038/nrn2793

Ferrer, E., and McArdle, J. J. (2010). Longitudinal modeling of developmental changes in psychological research. *Curr. Dir. Psychol. Sci.* 19, 149–154. doi: 10.1177/0963721410370300

Frey, M. C., and Detterman, D. K. (2004). Scholastic assessment or g? The relationship between the scholastic assessment test and general cognitive ability (vol 15, pg 373, 2004). *Psychol. Sci.* 15, 641. doi: 10.1111/j.0956-7976.2004.00687.x

Gottfredson, L. S. (1997). Why g matters: the complexity of everyday life. *Intelligence* 24, 79–132. doi: 10.1016/S0160-2896(97)90014-3

Haier, R. J. (2009). Neuro-intelligence, neuro-metrics and the next phase of brain imaging studies. *Intelligence* 37, 121–123. doi: 10.1016/j.intell.2008.12.006

Haier, R. J. (2013). *The Intelligent Brain*. Chantilly, VA: The Great Courses Company. Available online at: http://www.thegreatcourses.com/tgc/courses/course_detail.aspx?cid=1642

Haier, R. J., Siegel, B. V., Nuechterlein, K. H., Hazlett, E., Wu, J. C., Paek, J., et al. (1988). Cortical Glucose metabolic-rate correlates of abstract reasoning and attention studied with positron emission tomography. *Intelligence* 12, 199–217. doi: 10.1016/0160-2896(88)90016-5

Harrison, T. L., Shipstead, Z., Hicks, K. L., Hambrick, D. Z., Redick, T. S., and Engle, R. W. (2013). Working memory training may increase working memory capacity but not fluid intelligence. *Psychol. Sci.* 24, 2409–2419. doi: 10.1177/0956797613492984

Hulshoff Pol, H. E., Schnack, H. G., Posthuma, D., Mandl, R. C. W., Baare, W. F., van Oel, C., et al. (2006). Genetic contributions to human brain morphology and intelligence. *J. Neurosci.* 26, 10235–10242. doi: 10.1523/JNEUROSCI.1312-06.2006

Hunt, E. B. (2011). *Human Intelligence*. Cambridge; NY: Cambridge University Press.

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Shah, P. (2011). Short- and long-term benefits of cognitive training. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10081–10086. doi: 10.1073/pnas.1103228108

Jaeggi, S. M., Buschkuehl, M., Shah, P., and Jonides, J. (2013). The role of individual differences in cognitive training and transfer. *Mem. Cognit.* doi: 10.3758/s13421-013-0364-z. [Epub ahead of print].

Jensen, A. R. (1998). *The g Factor: The Science of Mental Ability*. Westport, CT: Praeger.

Jensen, A. R. (2006). *Clocking the Mind: Mental Chronometry and Individual Differences*. New York, NY: Elsevier.

Jung, R. E., and Haier, R. J. (2007). The Parieto-Frontal Integration Theory (P-FIT) of intelligence: converging neuroimaging evidence. *Behav. Brain Sci.* 30, 135–154. doi: 10.1017/S0140525X07001185

Legon, W., Sato, T. F., Opitz, A., Mueller, J., Barbour, A., Williams, A., et al. (2014). Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. *Nat. Neurosci.* 17, 322–329. doi: 10.1038/nn.3620

Lozano, A. M., and Lipsman, N. (2013). Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron* 77, 406–424. doi: 10.1016/j.neuron.2013.01.020

Mackey, A. P., Hill, S. S., Stone, S. I., and Bunge, S. A. (2011). Differential effects of reasoning and speed training in children. *Dev. Sci.* 14, 582–590. doi: 10.1111/j.1467-7687.2010.01005.x

Penke, L., Maniega, S. M., Bastin, M. E., Hernandez, M. C. V., Murray, C., Royle, N. A., et al. (2012). Brain white matter tract integrity as a neural foundation for general intelligence. *Mol. Psychiatry* 17, 1026–1030. doi: 10.1038/mp.2012.66

Posthuma, D., De Geus, E. J., Baare, W. F., Hulshoff Pol, H. E., Kahn, R. S., and Boomsma, D. I. (2002). The association between brain volume and intelligence is of genetic origin. *Nat. Neurosci.* 5, 83–84. doi: 10.1038/nn0202-83

Redick, T. S., Shipstead, Z., Harrison, T. L., Hicks, K. L., Fried, D. E., Hambrick, D. Z., et al. (2013). No evidence of intelligence improvement after working memory training: a randomized, placebo-controlled study. *J. Exp. Psychol. Gen.* 142, 359–379. doi: 10.1037/a0029082

Redick, T. S., Unsworth, N., Kelly, A. J., and Engle, R. W. (2012). Faster, smarter? Working memory capacity and perceptual speed in relation to fluid intelligence. *J. Cogn. Psychol.* 24, 844–854. doi: 10.1080/20445911.2012.704359

Santarnecchi, E., Polizzotto, N. R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., et al. (2013). Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr. Biol.* 23, 1449–1453. doi: 10.1016/j.cub.2013.06.022

Stein, J. L., Medland, S. E., Vasquez, A. A., Hibar, D. P., Senstad, R. E., Winkler, A. M., et al. (2012). Identification of common variants associated with human hippocampal and intracranial volumes. *Nat. Genet.* 44, 552–561. doi: 10.1038/ng.2250

te Nijenhuis, J., and van der Flier, H. (2013). Is the Flynn effect on g?: A meta-analysis. *Intelligence* 41, 802–807. doi: 10.1016/j.intell.2013.03.001

Tidwell, J. W., Dougherty, M. R., Chrabaszcz, J. R., Thomas, R. P., and Mendoza, J. L. (2013). What counts as evidence for working memory training? Problems with correlated gains and dichotomization. *Psychon. Bull. Rev.* doi: 10.3758/s13423-013-0560-7. [Epub ahead of print].

Received: 29 January 2014; accepted: 23 February 2014; published online: 12 March 2014.

Citation: Haier RJ (2014) Increased intelligence is a myth (so far). Front. Syst. Neurosci. ***8****:34. doi: 10.3389/fnsys.2014.00034*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in Systems Neuroscience

ORIGINAL RESEARCH
published: 02 June 2015
doi: 10.3389/fnsys.2015.00086

Transfer of training from one working memory task to another: behavioural and neural evidence

Erin L. Beatty[1], Marie-Eve Jobidon[1], Fethi Bouak[1], Ann Nakashima[1], Ingrid Smith[1], Quan Lam[1], Kristen Blackler[1], Bob Cheung[1] and Oshin Vartanian[1,2]*

[1] Defence Research and Development Canada – Toronto Research Centre, Toronto, ON, Canada, [2] Department of Psychology, University of Toronto Scarborough, Toronto, ON, Canada

N-back working memory (WM) tasks necessitate the maintenance and updating of dynamic rehearsal sets during performance. The delayed matching-to-sample (dMTS) task is another WM task, which in turn involves the encoding, maintenance, and retrieval of stimulus representations in sequential order. Because both n-back and dMTS engage WM function, we hypothesized that compared to a control task not taxing WM, training on the n-back task would be associated with better performance on dMTS by virtue of training a shared mental capacity. We tested this hypothesis by randomly assigning subjects ($N = 43$) to train on either the n-back (including 2-back and 3-back levels) or an active control task. Following training, dMTS was administered in the fMRI scanner. The n-back group performed marginally better than the active control group on dMTS. In addition, although the n-back group improved more on the less difficult 2-back level than the more difficult 3-back level across training sessions, it was improvement on the 3-back level that accounted for 21% of the variance in dMTS performance. For the control group, improvement in training across sessions was unrelated to dMTS performance. At the neural level, greater activation in the left inferior frontal gyrus, right posterior parietal cortex, and the cerebellum distinguished the n-back group from the control group in the maintenance phase of dMTS. Degree of improvement on the 3-back level across training sessions was correlated with activation in right lateral prefrontal and motor cortices in the maintenance phase of dMTS. Our results suggest that although n-back training is more likely to improve performance in easier blocks, it is improvement in more difficult blocks that is predictive of performance on a target task drawing on WM. In addition, the extent to which training on a task can transfer to another task is likely due to the engagement of shared cognitive capacities and underlying neural substrates—in this case WM.

Keywords: working memory, n-back, cognitive training, delayed matching-to-sample, prefrontal cortex

OPEN ACCESS

Edited by:
Mikhail Lebedev,
Duke University, USA

Reviewed by:
Erika K. Hussey,
University of Illinois, USA
Tilo Strobach,
Medical School Hamburg, Germany
Mary M. Torregrossa,
University of Pittsburgh, USA

****Correspondence:***
Erin L. Beatty,
Defence Research and Development Canada – Toronto Research Centre,
1133 Sheppard Avenue West,
Toronto, ON M3K 2C9, Canada
erin.beatty@drdc-rddc.gc.ca

Received: *25 March 2015*
Accepted: *20 May 2015*
Published: *02 June 2015*

Citation:
Beatty EL, Jobidon M-E, Bouak F, Nakashima A, Smith I, Lam Q, Blackler K, Cheung B and Vartanian O (2015) Transfer of training from one working memory task to another: behavioural and neural evidence. Front. Syst. Neurosci. 9:86. doi: 10.3389/fnsys.2015.00086

Introduction

Working memory (WM) can be defined as "a multicomponent system for active maintenance of information in the face of ongoing processing and/or distraction" (Conway et al., 2005, p. 770). Recently, there has been great theoretical and applied interest in the prospects of WM training for improving cognition. This interest stems from the possibility that improvements in WM

performance as a function of training might be transferable to other mental activities similarly drawing on WM capacity (Klingberg, 2010; Morrison and Chein, 2011; Buschkuehl et al., 2012). Although there is evidence to show that WM training can produce improvements in verbal as well as visuospatial WM, reliable evidence regarding far transfer to untrained tasks is presently lacking (for review see Melby-Lervåg and Hulme, 2013).

An important factor that might affect transfer is the goodness-of-fit between the specific capacity enhanced during training and the cognitive requirements of the untrained activity. For example, Harrison et al. (2013) showed that training on simple and complex WM span tasks led to improved performance on similar tasks (i.e., reading span and rotation span), despite the use of material with different surface features. Thus, structural and functional similarities between the trained and untrained tasks (e.g., both necessitate the suppression of distractors) appear to increase likelihood of transfer. The same conclusion can be drawn from the study conducted by Dahlin et al. (2008) who demonstrated transfer to a test of WM (i.e., letter memory) after 5 weeks of training in a specific aspect of WM—updating. The control group did not receive any training or specific activity. Importantly, using functional magnetic resonance imaging (fMRI), the researchers were also able to determine that the transfer effect was based on a joint training-related increase in brain activation in the trained and target tasks in the striatum. No transfer was observed to the Stroop task—a task that does not involve updating, and does not typically engage the striatum. Dahlin et al.'s (2008) results suggest that to obtain transfer, it is necessary to train specific aspects of WM (e.g., updating) that are functionally shared by the trained and target tasks. In turn, likelihood of transfer is increased to the extent that training-related changes in neural function occur in the same brain region recruited in relation to the trained process (e.g., updating) in both tasks.

Consistent with these process-specific findings, Salminen et al. (2012) examined transfer effects from WM training to executive functions. Importantly, they mapped particular cognitive processes engaged by their WM training task (i.e., dual n-back) to four aspects of executive functions, and measured transfer effects separately for each of those four processes: updating, coordination of concurrent performance, task switching, and attention. Their results demonstrated transfer from WM training to all aspects of executive function except coordination of concurrent performance, which the authors attributed to a "lack of commonalities" between the trained and target tasks (e.g., differences in the extent to which speeded processing was necessary for optimal performance). Salminen et al.'s (2012) results reinforce the notion that transfer effects depend on specific cognitive processes shared by the WM training and target tasks (see also Persson et al., 2007; Karbach and Kray, 2009; Sprenger et al., 2013; Salminen et al., 2015).

Building on the idea that shared capacities increase the likelihood of transfer of training, we conducted a study to test the hypothesis that training on one WM task would be more strongly associated with better performance on another WM task than training on a task that does not tax WM function. Our training task consisted of the n-back task—one of the most commonly used tasks to assess WM performance in the cognitive neuroscience literature (Kane and Engle, 2002). The n-back task requires that participants decide, on a trial-by-trial basis, whether a stimulus presented in the current trial matches a target stimulus presented a specific number of trials earlier in the sequence. The letter *n* denotes the specific number of trials that separate the current trial from the target trial. This task necessitates both maintenance and updating of dynamic rehearsal sets during performance (Kane et al., 2007). In contrast, participants in the active control group completed the 4-choice reaction time (RT) task (Dollins et al., 1993), which consists of pressing one of four buttons as quickly as possible when one of four target locations on a screen is highlighted (each target being matched to a given button). This task is not hypothesized to tax WM function.

Our target WM task consisted of the delayed matching-to-sample (dMTS) task, a classic measure of short-term visual WM from the animal learning and WM literatures (Miller et al., 1996). dMTS involves the encoding, maintenance, and retrieval of stimulus representations in sequential order (see **Figure 1**). Specifically, during *encoding* participants memorize the stimulus, during *maintenance* they maintain the stimulus in WM, and during *retrieval* they press the button corresponding to the stimulus that matches the stimulus presented during *encoding*. Importantly, both n-back and dMTS are considered to be WM tasks (Rottschy et al., 2012), although as noted above they include different sub-processes. An analysis of n-back and dMTS demonstrates that both engage the maintenance function of WM. Specifically, the n-back task necessitates that stimuli be maintained in WM across presentations so that decisions (match vs. no match) can be made. In turn, in dMTS a stimulus must be maintained for specific delay durations in WM to enable subsequent recognition among the available candidates. We therefore hypothesized that training the maintenance function of WM during n-back would confer an advantage to dMTS performance by virtue of influencing its maintenance phase, because that phase necessitates the maintenance of visual representations in WM.

Importantly, n-back training could also impact the encoding and/or retrieval phases of dMTS because both tasks likely share those sub-processes beyond maintenance alone. For example, although there are explicit and compartmentalized encoding and maintenance phases within dMTS, the updating function inherent in the n-back very likely requires the encoding of memory representations as well as their retrieval for making matching decisions.

In order to pinpoint the locus of transfer-related brain activity, we used fMRI to determine the phase within dMTS wherein activation would distinguish the experimental and control groups. Specifically, if as hypothesized training on the n-back task were to confer an advantage to dMTS performance by virtue of improving the maintenance function within WM, then one should observe a neural difference between the two groups in the maintenance phase. Furthermore, the differences between the two groups during the maintenance phase should be apparent in regions known to underlie delay-period maintenance in

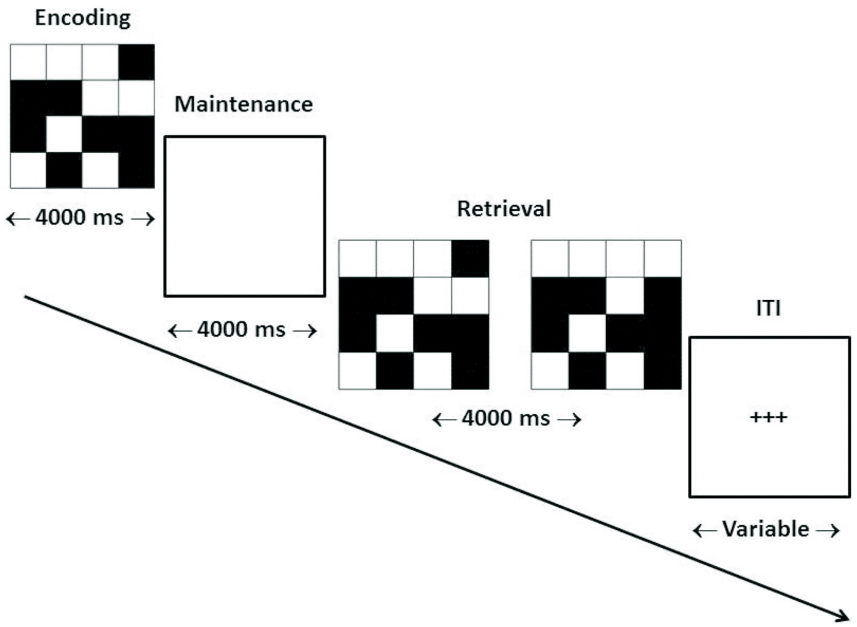

FIGURE 1 | Trial structure of dMTS. dMTS, delayed Matching-to-Sample Task. Participants completed 40 trials of identical structure. During *encoding* participants memorized the stimulus. During *maintenance* participants maintained the stimulus in working memory. During *retrieval* participants pressed the button corresponding to the stimulus (left, right) that matched the stimulus presented during *encoding*. The location (left, right) of the matching stimulus was counterbalanced across trials. The ITI varied randomly between 4,000 and 6,000 ms. ITI, inter-trial interval. Arrow indicates direction of trial.

visual WM, including primarily the dorsolateral and ventrolateral prefrontal cortex (PFC; BAs 9, 44, 45, and 47), the inferior parietal lobule (BA 40) and adjacent parietal regions (see de Zubicaray et al., 2001; Ranganath et al., 2004). Consistent with the idea that training on the n-back could also be related to variation in brain function in the encoding and retrieval phases of dMTS, we also compared the effect of training (i.e., n-back vs. 4-choice RT) within those two phases.

Materials and Methods

Participants

Our protocol was approved by Defence Research and Development Canada's Human Research Ethics Committee. The 43 participants (35 males, eight females) were neurologically healthy right-handed (Oldfield, 1971) volunteers ($M = 30.76$ years, SD = 9.71) with normal or corrected-to-normal vision. They were assigned randomly to the experimental ($N = 22$) or active control group ($N = 21$). To ensure similar expectations and motivations, participants were not informed about the existence of the two training conditions, or our hypotheses about the differential effects of training on outcome measures of interest (see Boot et al., 2011). There was no significant difference between the two groups in sex [$\chi^2(1) = 0.01$, $p = 0.94$], age [$t(36) = 0.33$, $p = 0.74$], or fluid intelligence [$t(41) = 0.16$, $p = 0.87$]—assessed by administering the 18 even or odd items of *Raven's Advanced Progressive Matrices* (Raven et al., 1998) within a time limit of 10 min (see Jaeggi et al., 2008).

Materials and Procedure

Cognitive Training

All participants completed three 20-min training sessions on separate days, administered using the *Cognitive Test Software* (Grushcow, 2008). Average lag time between successive sessions was 1.21 days (SD = 0.55). Durations and frequencies in WM training studies have varied greatly, ranging from a single 20-min session to 20 h spread over 10 weeks (see Buschkuehl et al., 2012, Table 1; Klingberg, 2010, Table 2). We focused on a short and concentrated training regimen specifically because we were interested in assessing its feasibility as an intervention strategy in applied professional and educational settings.

n-back

Participants in the experimental group completed the n-back task. Each session consisted of four blocks—two blocks of 2-back and two blocks of 3-back—administered in alternating order and always starting with 2-back. The stimuli in our variant of the n-back were letters. No vowels were used in the task, and we only used a subset of consonants (X, G, H, K, P, Q, S, and W). We did not control for interference lures. Each block contained 150 trials. On 50 trials within each block the presented letter matched the target letter presented two or three positions earlier in the sequence (depending on the block), whereas on the remaining 100 trials it did not. Each letter was presented for 500 ms. Inter-stimulus interval (ISI) was a blank screen presented for 2500 ms. Participants pressed the spacebar when they detected a match.

4-choice RT

Participants in the active control group completed the 4-choice RT task (Dollins et al., 1993). On each trial of this task, one of four adjacent locations on the computer screen was highlighted randomly. Participants pressed one of four keys corresponding to the highlighted location. We selected this task to control for task engagement not involving a WM task. Participants completed 420 trials per session. Based on normative data collected in our lab from the same population using the same task (Nakashima et al., 2011), we expected accuracy to be at ceiling across the three sessions.

dMTS

Participants completed the dMTS in the fMRI scanner 3.29 days (SD = 1.11) after the last training session (see **Figure 1**).

fMRI Acquisition

A 3-Tesla MR scanner with an 8-channel head coil (Discovery MR750, 22.0 software, GE Healthcare, Waukesha, WI, USA) was used to acquire T1 anatomical volume images (0.86 mm × 0.86 mm × 1.0 mm voxels). For functional imaging, T2*-weighted gradient echo spiral-in/out acquisitions were used to produce 26 contiguous 5 mm thick axial slices [repetition time (TR) = 2000 ms; echo time (TE) = 30 ms; flip angle (FA) = 70°; field of view (FOV) = 200 mm; 64 × 64 matrix; voxel dimensions = 3.1 mm × 3.1 mm × 5.0 mm], positioned to cover the whole brain. The first five volumes were discarded to allow for T1 equilibration effects. The number of volumes acquired was 354.

fMRI Analysis

Data were analyzed using Statistical Parametric Mapping (SPM8). Head movement was less than 2 mm. All functional volumes were spatially realigned to the first volume. A mean image created from realigned volumes was spatially normalized to the MNI EPI brain template using non-linear basis functions. The derived spatial transformation was applied to the realigned T2* volumes, and spatially smoothed with an 8 mm full-width at half-maximum isotropic Gaussian kernel. Time series across each voxel were high-pass filtered with a cut-off of 128 s, using cosine functions to remove section-specific low frequency drifts in the BOLD signal. Condition effects at each voxel were estimated according to the GLM and regionally specific effects compared using linear contrasts. The BOLD signal was modeled as a box-car, convolved with a canonical hemodynamic response function.

We applied a combination of voxel-height and cluster extent correction for multiple comparisons using AlphaSim (http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf) incorporated in REST (Song et al., 2011). Whereas originally AlphaSim was developed for use within the Analysis of Functional Neuroimages (AFNI) software (Cox, 1996), REST enables one to conduct the same analysis on a Windows platform using SPM masks. AlphaSim takes into account the size of the search space and the estimated smoothness, and using Monte Carlo simulations generates probability estimates of a random field of noise, producing a cluster of voxels of a given size for a set of voxels passing a given voxel-wise p-value threshold. Using a random-effects analysis, we report activations that survived $p < 0.05$—corrected for multiple comparisons (FWE) within the avg152T2.nii whole-brain mask from the SPM toolbox. The real smoothness in the three directions was estimated from the residuals (FWHMx = 11.699 mm, FWHMy = 11.869 mm, FWHMz = 10.992 mm). Within our mask, the Monte Carlo simulations determined that a FWE-corrected false-positive probability of $p < 0.05$ was achieved using a voxel-wise threshold of $p < 0.005$ combined with a spatial extent threshold of 249 voxels.

Results

Cognitive Training

For the experimental group we conducted a repeated-measures ANOVA with session (1, 2, and 3) and level (2-back, 3-back) as within-subjects variables. The key dependent variable was d' (sensitivity; Stanislaw and Todorov, 1999; see Kane et al., 2007). When d' is positive (and high), participants are considered to display good sensitivity, whereas when d' is negative participants are incorrectly judging matches as mismatches and vice versa. In addition, we also investigated the effects of the two independent variables on the criterion—defined as the value of the decision variable deemed sufficiently high to determine that there is a match. A liberal value for the criterion biases the participant toward responding that there is a match, whereas a conservative value biases the participant toward responding that there is no match.

For the experimental group, there was a main effect for session, demonstrating that d' improved across sessions, $F(2,42) = 10.50$, $p < 0.001$, $\eta_p^2 = 0.33$. Paired comparisons demonstrated that compared to session 1, d' was higher at sessions 2 and 3. There was no difference between sessions 2 and 3 ($p = 0.10$). There was also a main effect for level, demonstrating that d' was greater on 2-back than 3-back, $F(1,21) = 25.05$, $p < 0.001$, $\eta_p^2 = 0.54$. In addition, there was a session × level interaction such that across three sessions d' improved more for 2-back than 3-back, $F(2,42) = 5.90$, $p < 0.01$, $\eta_p^2 = 0.22$ (**Figure 2**). In contrast, when we focused on the criterion as the dependent variable, the effects of session, level and the session × level interaction were not significant (all $ps \geq 0.99$).

For the active control group we conducted an ANOVA with session (1, 2, and 3) as the within-subjects variable, and accuracy as the dependent variable. As predicted (see Nakashima et al., 2011), performance was at ceiling across sessions 1 ($M = 96.57\%$, SD = 3.10), 2 ($M = 95.93\%$, SD = 4.14), and 3 ($M = 96.86\%$, SD = 2.24), $F(2,38) = 1.48$, $p = 0.25$, $\eta_p^2 = 0.07$. We conducted an additional ANOVA with session (1, 2, and 3) as the within-subjects variable, and RT as the dependent variable. There was a main effect such that RT decreased across the first ($M = 0.45$ s, SD = 0.06), second ($M = 0.42$ s, SD = 0.05) and third sessions ($M = 0.41$ s, SD = 0.05), $F(2,38) = 15.13$, $p = < 0.001$, $\eta_p^2 = 0.44$.

dMTS

The experimental group ($M = 96.70\%$, SD = 4.72) performed marginally better than the active control group ($M = 93.81\%$,

SD = 5.22) on dMTS, $t(41) = 1.91$, $p = 0.06$, Cohen's $d = 0.58$. To directly test whether performance on dMTS would be a function of improvement in training on the n-back, for the experimental group we computed a new variable that was the difference in d' between session 1 and session 3 ($d'_{\text{difference}} = d'_{\text{session 3}} - d'_{\text{session 1}}$)—separately for 2-back and 3-back. Next, we regressed accuracy (%) in dMTS performance onto $d'_{\text{difference}}$. The results demonstrated that degree of improvement in 2-back was unrelated to dMTS performance, $\beta = 0.31$, $p = 0.16$. In contrast, degree of improvement in 3-back predicted variation in dMTS performance, $\beta = 0.46$, $p = < 0.05$. This result demonstrates that the degree of improvement in 3-back is a significant factor in dMTS performance. In fact, improvement in 3-back performance during training accounted for 21% of the variance in dMTS performance (**Figure 3A**).

To determine whether the degree of improvement in 4-choice RT was predictive of dMTS performance amongst participants in the control group, we computed a new variable that was the difference in RT between session 1 and session 3 ($RT_{\text{difference}} = RT_{\text{session 3}} - RT_{\text{session 1}}$). Next, we regressed accuracy (%) in dMTS performance onto $RT_{\text{difference}}$. Importantly, only 19 data points (rather than 21) were included in this analysis because one participant failed to complete the third session of training, and another data point was excluded because it was an outlier—determined by its deviation from the means of both distributions by approximately 3 SDs (see Wainer, 1976). Degree of improvement in RT was unrelated to dMTS performance, $\beta = -0.30$, $p = 0.21$ (**Figure 3B**).

fMRI

Using an event-related design, we specified six regressors corresponding to (1) encoding, (2) maintenance, (3) retrieval, (4) ISI, (5) ITI, and (6) motor response. ISI and motor response were modeled out of the analyses by assigning weights of 0 to their corresponding regressors in all analyses. **Table 1** lists the regions activated in the encoding (−ITI), maintenance (−ITI), and retrieval (−ITI) phases of dMTS *across all participants*. An independent-samples *t*-test demonstrated greater activation in the n-back than active control group in the maintenance phase in the left inferior frontal gyrus (IFG; $T = 3.97$, $k_E = 389$, $x = -44$, $y = 22$, $z = 16$), the right posterior parietal cortex (PPC; $T = 3.30$, $k_E = 299$, $x = 32$, $y = -74$, $z = 36$), and the cerebellum ($T = 3.56$, $k_E = 277$, $x = -10$, $y = -68$, $z = -42$; **Figure 4**). Neither the reverse contrast nor the contrasts in either direction involving the encoding or retrieval phase revealed any significant difference between the two groups. In other words, the difference in brain activation between the n-back and active control groups was limited exclusively to the maintenance phase of dMTS.

TABLE 1 | Coordinates for the observed activations in the encoding, maintenance, and retrieval phases of delayed matching-to-sample task (dMTS; vs. rest) across all participants.

Contrast	Structure	*x*	*y*	*z*	*T-score*
Encoding−ITI	Precuneus	−16	−68	50	13.52
	Precuneus	24	−68	46	11.89
	Precentral gyrus	−46	−6	46	12.27
	Parahippocampus	38	−14	−30	4.90
Maintenance−ITI	Precuneus	−24	−64	50	13.25
	Inferior parietal lobe	−36	−44	40	12.81
	Superior parietal lobe	12	−64	62	11.70
Retrieval−ITI	Anterior insula	32	26	−2	11.52
	Anterior insula	−28	24	0	9.68
	Cerebellum	0	−50	−36	5.87

The coordinates are reported in MNI space. All reported activation survived whole-brain family-wise error (FWE) correction ($p < 0.05$) as implemented by AlphaSim in REST (Song et al., 2011).

The analysis of our behavioral data had demonstrated that improvement in 3-back performance during training accounted for 21% of the variance in dMTS performance (**Figure 3A**). To explore this effect at the neural level, we conducted three separate regression analyses to see whether difference in d' for 3-back ($d'_{\text{difference}} = d'_{\text{session 3}} - d'_{\text{session 1}}$) would covary with brain activation during (1) encoding, (2) maintenance, and (3) retrieval. The results demonstrated that brain activation did not covary in relation to $d'_{\text{difference}}$ during encoding or retrieval. In contrast, during the maintenance phase brain activation in right lateral PFC ($T = 3.68$, $k_E = 260$, $x = 56$, $y = 16$, $z = 10$) and motor cortex ($T = 3.78$, $k_E = 421$, $x = 46$, $y = -22$, $z = 44$) covaried with $d'_{\text{difference}}$ (**Figure 5**).

Although our behavioral data had demonstrated that for the experimental group improvement in 2-back performance was unrelated to dMTS performance, we nevertheless explored this effect at the neural level. As with 3-back, we conducted three separate regression analyses to see whether difference in d' for 2-back ($d'_{\text{difference}} = d'_{\text{session 3}} - d'_{\text{session 1}}$) would covary with brain activation during (1) encoding, (2) maintenance, and (3) retrieval. Demonstrating a pattern similar to 3-back, brain activation did not covary in relation to $d'_{\text{difference}}$ during encoding or retrieval. However, during the maintenance phase $d'_{\text{difference}}$ covaried with activation in a distributed network in the brain, including three locations in right ($T = 6.18$, $k_E = 2164$, $x = 44$, $y = 4$, $z = 28$), left ($T = 4.66$, $k_E = 2161$, $x = -42$, $y = 2$, $z = 16$), and medial ($T = 5.51$, $k_E = 982$, $x = -8$, $y = 14$, $z = 52$)

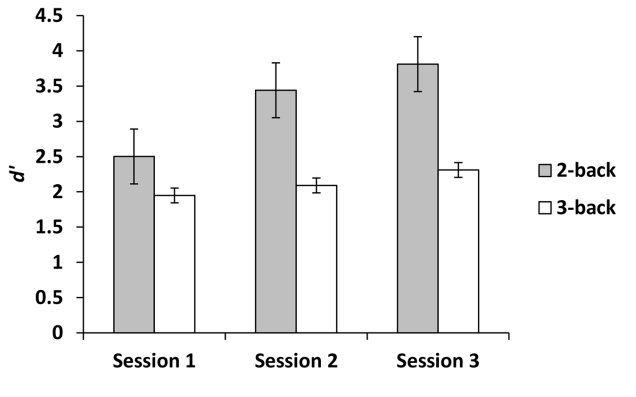

FIGURE 2 | The effects of session and level on n-back performance during training.

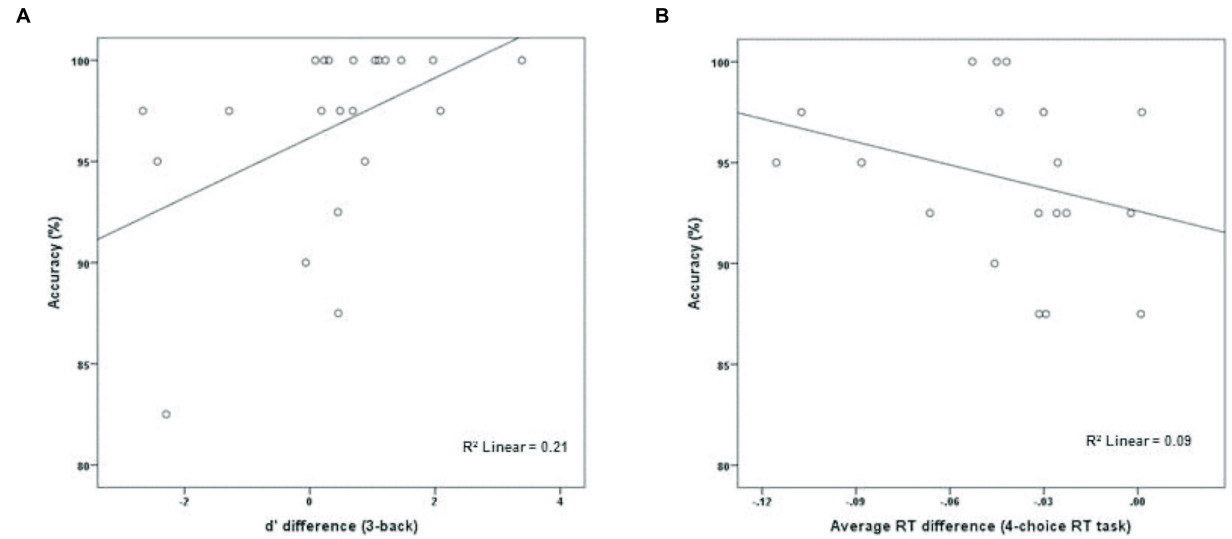

FIGURE 3 | The relationship between degree of training-related improvement in (A) n-back and (B) 4-choice RT and dMTS performance. Whereas the degree of training-related improvement in n-back predicted dMTS performance, degree of improvement in 4-choice RT did not (see text).

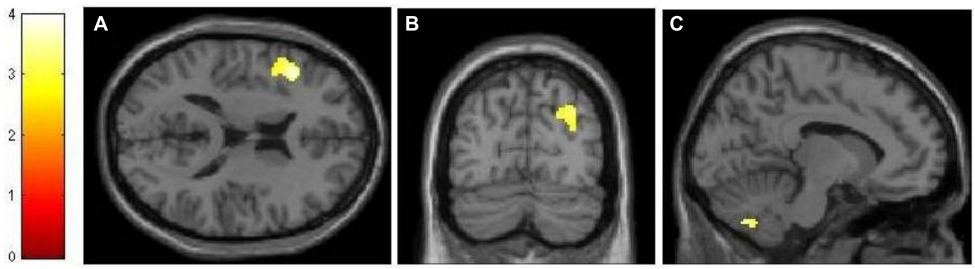

FIGURE 4 | Neural differences between experimental and control conditions during the maintenance phase of dMTS. There was greater activation in left IFG **(A)**, right PPC **(B)**, and cerebellum **(C)** in the experimental than control group during maintenance. SPMs rendered into standard stereotactic space and superimposed on to transverse **(A)**, coronal **(B)**, and saggital **(C)** MRI in standard space. Bar represents the corresponding *T*-score. dMTS, delayed Matching-to-Sample Task, IFG, inferior frontal gyrus, PPC, posterior parietal cortex.

middle frontal gyrus (BA 6), left lateral PFC ($T = 4.62$, $k_E = 940$, $x = -42$, $y = 24$, $z = 6$), right superior parietal lobule ($T = 5.06$, $k_E = 765$, $x = 36$, $y = -56$, $z = 54$), right cingulate ($T = 4.57$, $k_E = 358$, $x = 12$, $y = -10$, $z = 36$), right extrastriate cortex ($T = 4.68$, $k_E = 669$, $x = 36$, $y = -76$, $z = 20$), and three locations in left ($T = 5.52$, $k_E = 2059$, $x = -18$, $y = -74$, $z = -18$; $T = 4.60$, $k_E = 1263$, $x = -10$, $y = -50$, $z = -48$) and right ($T = 3.80$, $k_E = 504$, $x = 28$, $y = -54$, $z = -32$) cerebellum (**Figure 6**).

Finally, although our behavioral data had demonstrated that for the control group improvement in the 4-choice RT task was unrelated to dMTS performance, we nevertheless explored this effect at the neural level as well. Specifically, we conducted three separate regression analyses to see whether difference in RT in the 4-choice RT task ($RT_{difference} = RT_{session\ 3} - RT_{session\ 1}$) would covary with brain activation during (1) encoding, (2) maintenance, and (3) retrieval. Our results demonstrated that there was no relationship between brain activation and $RT_{difference}$ during encoding, maintenance or the retrieval phase.

Discussion

The n-back group performed marginally better than the active control group on dMTS, registering a medium effect size (Cohen, 1988). Importantly, although participants in the experimental condition were more likely to exhibit improvement across the three training sessions in the 2-back level than the 3-back level (**Figure 2**), it was their degree of improvement in the 3-back level that predicted variation in dMTS performance, accounting for 21% of the observed variance in dMTS performance (**Figure 3A**). Critically, degree of improvement in the 4-choice RT task in the control condition was unrelated to dMTS performance or its neural correlates, despite the fact that both are visuospatial tasks. These results demonstrate a dissociation between how training-related improvement in a WM task vs. a non-WM task is related to a target WM task. More specifically, they suggest that although performance on relatively easier levels of n-back is more likely to improve within three brief practice sessions, it is improvement in the more difficult levels that is more likely to be positively

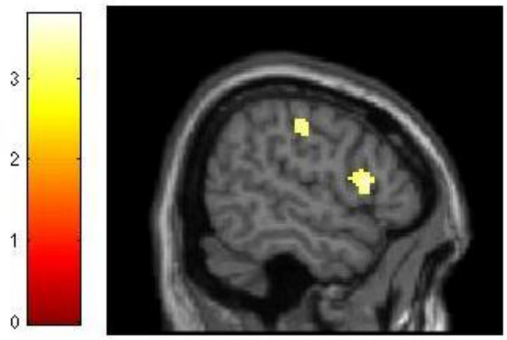

FIGURE 5 | Relationship between training-related improvement in 3-back and brain activation in the maintenance phase of dMTS. Activation in right lateral PFC and motor cortex covaried with degree of training-related improvement in 3-back. SPM rendered into standard stereotactic space and superimposed on saggital MRI in standard space. Bar represents the corresponding *T*-score. dMTS, delayed Matching-to-Sample Task.

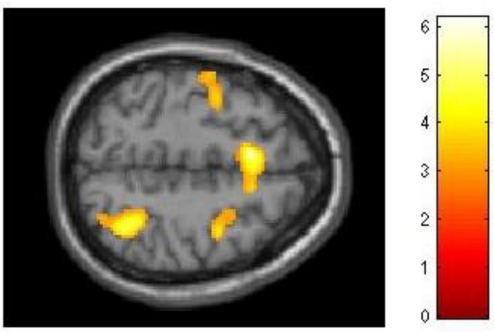

FIGURE 6 | Relationship between training-related improvement in 2-back and brain activation in the maintenance phase of dMTS. Activation in a distributed network including the superior parietal lobe and middle frontal gyrus (left, right, medial) covaried with degree of training-related improvement in 2-back. SPM rendered into standard stereotactic space and superimposed on transverse MRI in standard space. Bar represents the corresponding *T*-score. dMTS, delayed Matching-to-Sample Task.

related to performance on target tasks drawing on the same capacity.

In addition, the neural difference between the two groups was only apparent during the maintenance phase of dMTS, and localized to the left IFG, right PPC and the cerebellum. Sustained activation in the PFC has been related to maintenance in memory (Fuster, 1991). Indeed, IFG activation has been shown to be involved in the maintenance phase of the delayed non-matching-to-sample task (de Zubicaray et al., 2001). This is consistent with the involvement of the ventrolateral regions of the left PFC in delay-period maintenance in visual WM tasks (Ranganath et al., 2004). In addition, posterior parietal regions have been shown to contribute to various aspects of visual short-term mnemonic function including maintenance (Munk et al., 2002) and active maintenance of information in WM (Cohen et al., 1997). In fact, PPC activity has been shown to predict individual differences in visual short-term memory capacity (Todd and Marois, 2005; see also Todd and Marois, 2004). Our neural results suggest that the effects of n-back training on transfer-related brain function in dMTS are likely to be observed in regions that underlie capacities enhanced during training, and subsequently recruited by the untrained task. Our behavioral task analysis had led us to believe that n-back training would likely benefit dMTS performance because both tasks tax the maintenance function in WM, among others. Our neural results are generally consistent with this idea, although further experimentation is needed to determine that the regions distinguishing the two groups during maintenance indeed underlie transfer from n-back to dMTS.

Because training-related improvement in 3-back predicted dMTS performance (**Figure 3A**), we explored this effect at the neural level. Our results revealed that during the maintenance phase of dMTS, brain activation in right lateral PFC and motor cortex covaried with training-related improvement in 3-back (**Figure 5**). This region of the lateral PFC corresponds to Brodmann Area 44, and has been shown to be involved in both the storage and manipulation aspects of WM (see Wager and Smith, 2003). Our results suggest that this region is sensitive to training-related changes in relation to 3-back, and could be a region shared by both the n-back and dMTS for maintenance in WM.

In addition, although behaviourally training-related improvement in 2-back was unrelated to dMTS performance, our analyses of fMRI data demonstrated that during the maintenance phase of dMTS brain activation in a distributed network including the middle frontal gyrus, lateral PFC, superior parietal lobule, cingulate, extrastriate cortex, and the cerebellum covaried with training-related improvement in 2-back (**Figure 6**). Within this network, the frontal and parietal regions represent well-established nodes in the fronto-parietal WM network (Petrides, 2005; D'Esposito, 2008). Although these results demonstrate that brain activation during the maintenance phase of dMTS was modulated by the degree of training-related improvement in 2-back, care must be exercised in interpreting this finding given the absence of a corresponding behavioral effect (**Figure 3B**).

Limitations

Our results must be considered preliminary because our study had a number of limitations. First, our design involved randomly assigning participants to two treatment conditions, and subsequently measuring differences between the two groups on an outcome measure (i.e., dMTS) following training. As such, our results are correlational, and we cannot draw causal inferences. In addition, although the degree of training-related improvement in 3-back predicted and accounted for 21% of the variance in dMTS performance (**Figure 3A**), gain data alone cannot be used as evidence for inferring transfer effects (Tidwell et al., 2014). Rather, there is reason to further explore the possibility of a causal link between

n-back training and performance on other WM tasks, including dMTS.

Second, our active control condition was meant to control for task engagement only—defined by identical frequency and duration of training. Although in WM training studies active control conditions are preferable to passive control conditions (Shipstead et al., 2010), it would be better still to include both types of control conditions in a given design. Particularly desirable would be to use control conditions that enable one to isolate specific components of training that are believed to be related to transfer to dMTS performance (e.g., updating, maintenance, etc.). The present results lay the groundwork for implementing such a design feature in future studies, perhaps comparing different types of WM training that tax different aspects of WM function.

Third, although the durations and frequencies of training sessions in WM training studies have ranged greatly in the past, ranging from one 20- or 30-min session to 20 h spread over 10 weeks (see Buschkuehl et al., 2012, Table 1; Klingberg, 2010, Table 2), our WM training intervention was relatively short and involved a non-adaptive WM task. Future studies would benefit from implementing an adaptive WM task, possibly administered in the context of more frequent training sessions.

Fourth, it is likely that there was a ceiling effect associated with our outcome measure (dMTS). In turn, this might have made it more difficult to observe differences between the two training conditions on this task, given that there was less room for improvement. There are at least two ways to increase the difficulty level on dMTS. First, on each trial we used a stimulus consisting of a 4 × 4 matrix (**Figure 1**). Doubling the matrix dimensions (i.e., 8 × 8) reduces average accuracy rates to around 70% (Nakashima et al., 2011). Second, whereas we used a fixed delay period, dMTS paradigms can incorporate variable delay periods. This will enable one to analyze differences in performance as a function of varying delay periods. These modifications can be incorporated in future studies.

Conclusion

Our results demonstrated that a group training on the n-back task performed marginally better than an active control group on dMTS. Although the n-back group improved more on 2-back than 3-back across three training sessions, it was improvement in 3-back that predicted and accounted for 21% of the variance in dMTS performance. There was no relationship between training-related gains and dMTS performance in the control group. At the neural level, the n-back group exhibited greater activation in the left IFG, right PPC and the cerebellum during the maintenance phase within dMTS. In addition, degree of improvement in 3-back covaried with brain activation in the right lateral prefrontal and motor cortices during the maintenance phase of dMTS, as did the degree of improvement in 2-back and activation in a distributed network including fronto-parietal WM nodes. In contrast, in the control group no relationship was observed between degree of improvement on the 4-choice RT task and dMTS performance. Combined, our results suggest that n-back training is more closely associated with dMTS performance than training on a task that does not tax WM.

Acknowledgments

We thank Ruby Endre, Garry Detzler and Fred Tam for assistance with data collection, and Chao-Gan Yan for help with running AlphaSim in REST. This work was supported by a Department of National Defence *Applied Research Project* under the direction of the last author. Portions of this work were presented at the annual meeting of the Association for Psychological Science, held in San Francisco, CA, USA, May 2014.

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnsys.2015.00086/abstract

References

Boot, W. R., Blakely, D. P., and Simons, D. J. (2011). Do action video games improve perception and cognition? *Front. Psychol.* 2:226. doi: 10.3389/fpsyg.2011.00226

Buschkuehl, M., Jaeggi, S. M., and Jonides, J. (2012). Neuronal effects following working memory training. *Dev. Cogn. Neurosci.* 2(Suppl. 1), S167–S179. doi: 10.1016/j.dcn.2011.10.001

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., et al. (1997). Temporal dynamics of brain activation during a working memory task. *Nature* 386, 604–608. doi: 10.1038/386604a0

Conway, A. R., Kane, M. J., Bunting, M. F., Hambrick, D. Z., Wilhelm, O., and Engle, R. W. (2005). Working memory span tasks: a methodological review and user's guide. *Psychon. Bull. Rev.* 12, 769–786. doi: 10.3758/BF03196772

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res.* 29, 162–173. doi: 10.1006/cbmr.1996.0014

Dahlin, E., Neely, A. S., Larsson, A., Backman, L., and Nyberg, L. (2008). Transfer of learning after updating training mediated by the striatum. *Science* 320, 1510–1512. doi: 10.1126/science.1155466

D'Esposito, M. (2008). "Working memory," in *Handbook of Clinical Neurology: Neuropsychology and Behavioral Neurology*, eds G. Goldenberg and B. Miller (Amsterdam: Elsevier), 237–247.

de Zubicaray, G. I., Mcmahon, K., Wilson, S. J., and Muthiah, S. (2001). Brain activity during the encoding, retention, and retrieval of stimulus representations. *Learn. Mem.* 8, 243–251. doi: 10.1101/lm.40301

Dollins, A. B., Lynch, H. J., Wurtman, R. J., Deng, M. H., Kischka, K. U., Gleason, R. E., et al. (1993). Effect of pharmacological daytime doses of melatonin on human mood and performance. *Psychopharmacology (Berl.)* 112, 490–496. doi: 10.1007/BF02244899

Fuster, J. M. (1991). The prefrontal cortex and its relation to behavior. *Prog. Brain Res.* 87, 201–211. doi: 10.1016/j.pscychresns.2009.03.012

Grushcow, M. (2008). *Cognitive Test Software* [*Computer Software*]. North York, ON: NTT Systems Inc.

Harrison, T. L., Shipstead, Z., Hicks, K. L., Hambrick, D. Z., Redick, T. S., and Engle, R. W. (2013). Working memory training may increase working

memory capacity but not fluid intelligence. *Psychol. Sci.* 24, 2409–2419. doi: 10.1177/0956797613492984

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Kane, M. J., Conway, A. R., Miura, T. K., and Colflesh, G. J. (2007). Working memory, attention control, and the N-back task: a question of construct validity. *J. Exp. Psychol. Learn. Mem. Cogn.* 33, 615–622. doi: 10.1037/0278-7393.33.3.615

Kane, M. J., and Engle, R. W. (2002). The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. *Psychon. Bull. Rev.* 9, 637–671. doi: 10.3758/BF03196323

Karbach, J., and Kray, J. (2009). How useful is executive control training? Age differences in near and far transfer of task-switching training. *Dev. Sci.* 12, 978–990. doi: 10.1111/j.1467-7687.2009.00846.x

Klingberg, T. (2010). Training and plasticity of working memory. *Trends Cogn. Sci.* 14, 317–324. doi: 10.1016/j.tics.2010.05.002

Melby-Lervåg, M., and Hulme, C. (2013). Is working memory training effective? A meta-analytic review. *Dev. Psychol.* 49, 270–291. doi: 10.1037/a0028228

Miller, E. K., Erickson, C. A., and Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *J. Neurosci.* 16, 5154–5167.

Morrison, A. B., and Chein, J. M. (2011). Does working memory training work? The promise and challenges of enhancing cognition by training working memory. *Psychon. Bull. Rev.* 18, 46–60. doi: 10.3758/s13423-010-0034–30

Munk, M. H., Linden, D. E., Muckli, L., Lanfermann, H., Zanella, F. E., Singer, W., et al. (2002). Distributed cortical systems in visual short-term memory revealed by event-related functional magnetic resonance imaging. *Cereb. Cortex* 12, 866–876. doi: 10.1093/cercor/12.8.866

Nakashima, A., Vartanian, O., Bouak, F., Hofer, K., and Cheung, B. (2011). "A test battery for the assessment of psychological and physiological performance following primary blast wave exposure," in *Proceedings of the Military and Veterans Health Research Forum*, eds A. B. Aiken and S. A. H. Bélanger (Kingston, ON: Canadian Defence Academy Press), 134–153.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Persson, J., Welsh, K. M., Jonides, J., and Reuter-Lorenz, P. A. (2007). Cognitive fatigue of executive processes: interaction between interference resolution tasks. *Neuropsychologia* 45, 1571–1579. doi: 10.1016/j.neuropsychologia.2006.12.007

Petrides, M. (2005). Lateral prefrontal cortex: architectonic and functional organization. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 781–795. doi: 10.1098/rstb.2005.1631

Ranganath, C., Cohen, M. X., Dam, C., and D'esposito, M. (2004). Inferior temporal, prefrontal, and hippocampal contributions to visual working memory maintenance and associative memory retrieval. *J. Neurosci.* 24, 3917–3925. doi: 10.1523/JNEUROSCI.5053-03.2004

Raven, J., Raven, J. C., and Court, J. H. (1998). *Raven Manual: Section 4. Advanced Progressive Matrices*. Oxford: Oxford Psychologists Press.

Rottschy, C., Langner, R., Dogan, I., Reetz, K., Laird, A. R., Schulz, J. B., et al. (2012). Modelling neural correlates of working memory: a coordinate-based meta-analysis. *Neuroimage* 60, 830–846. doi: 10.1016/j.neuroimage.2011.11.050

Salminen, T., Frensch, P., Strobach, T., and Schubert, T. (2015). Age-specific differences of dual n-back training. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 1–22. doi: 10.1080/13825585.2015.1031723 [Epub ahead of print].

Salminen, T., Strobach, T., and Schubert, T. (2012). On the impacts of working memory training on executive functioning. *Front. Hum. Neurosci.* 6:166. doi: 10.3389/fnhum.2012.00166

Shipstead, Z., Redick, T. S., and Engle, R. W. (2010). Does working memory training generalize? *Psychol. Belg.* 50, 245–276. doi: 10.5334/pb-50-3-4-245

Song, X. W., Dong, Z. Y., Long, X. Y., Li, S. F., Zuo, X. N., Zhu, C. Z., et al. (2011). REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE* 6:e25031. doi: 10.1371/journal.pone.0025031

Sprenger, A. M., Atkins, S. M., Bolger, D. J., Harbison, J. I., Novick, J. M., Chrabaszcz, J. S., et al. (2013). Training working memory: limits of transfer. *Intelligence* 41, 638–663. doi: 10.1016/j.intell.2013.07.013

Stanislaw, H., and Todorov, N. (1999). Calculation of signal detection theory measures. *Behav. Res. Methods Instrum. Comput.* 31, 137–149. doi: 10.3758/bf03207704

Tidwell, J. W., Dougherty, M. R., Chrabaszcz, J. R., Thomas, R. P., and Mendoza, J. L. (2014). What counts as evidence for working memory training? Problems with correlated gains and dichotomization. *Psychon. Bull. Rev.* 21, 620–628. doi: 10.3758/s13423-013-0560–567

Todd, J. J., and Marois, R. (2004). Capacity limit of visual short-term memory in human posterior parietal cortex. *Nature* 428, 751–754. doi: 10.1038/nature02466

Todd, J. J., and Marois, R. (2005). Posterior parietal cortex activity predicts individual differences in visual short-term memory capacity. *Cogn. Affect. Behav. Neurosci.* 5, 144–155. doi: 10.3758/cabn.5.2.144

Wager, T. D., and Smith, E. E. (2003). Neuroimaging studies of working memory: a meta-analysis. *Cogn. Affect. Behav. Neurosci.* 3, 255–274. doi: 10.3758/CABN.3.4.255

Wainer, H. (1976). Robust statistics: a survey and some prescriptions. *J. Educ. Stat.* 1, 285–312. doi: 10.2307/1164985

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
SYSTEMS NEUROSCIENCE

HYPOTHESIS AND THEORY ARTICLE
published: 14 November 2014
doi: 10.3389/fnsys.2014.00220

Robust sequential working memory recall in heterogeneous cognitive networks

Mikhail I. Rabinovich[1], Yury Sokolov[2]* and Robert Kozma[2]

[1] *BioCircuits Institute, University of California San Diego, La Jolla, CA, USA*
[2] *Department of Mathematical Sciences, University of Memphis, Memphis, TN, USA*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Tara Thiagarajan, National Center for Biological Sciences, India
KongFatt Wong-Lin, University of Ulster, UK
Zachary P. Kilpatrick, University of Houston, USA

****Correspondence:***
Yury Sokolov, Department of Mathematical Sciences, University of Memphis, 373 Dunn Hall, Memphis, TN 38152, USA
e-mail: ysokolov@memphis.edu

Psychiatric disorders are often caused by partial heterogeneous disinhibition in cognitive networks, controlling sequential and spatial working memory (SWM). Such dynamic connectivity changes suggest that the normal relationship between the neuronal components within the network deteriorates. As a result, competitive network dynamics is qualitatively altered. This dynamics defines the robust recall of the sequential information from memory and, thus, the SWM capacity. To understand pathological and non-pathological bifurcations of the sequential memory dynamics, here we investigate the model of recurrent inhibitory-excitatory networks with heterogeneous inhibition. We consider the ensemble of units with all-to-all inhibitory connections, in which the connection strengths are monotonically distributed at some interval. Based on computer experiments and studying the Lyapunov exponents, we observed and analyzed the new phenomenon—clustered sequential dynamics. The results are interpreted in the context of the winnerless competition principle. Accordingly, clustered sequential dynamics is represented in the phase space of the model by two weakly interacting quasi-attractors. One of them is similar to the sequential heteroclinic chain—the regular image of SWM, while the other is a quasi-chaotic attractor. Coexistence of these quasi-attractors means that the recall of the normal information sequence is intermittently interrupted by episodes with chaotic dynamics. We indicate potential dynamic ways for augmenting damaged working memory and other cognitive functions.

Keywords: cognitive dynamics, memory disorders, inhibition, sequential intermittency, complex networks, heteroclinic chimeras

1. INTRODUCTION

The human brain is a complex net of functionally interconnected regions. Deeper understanding the dynamics of this network is very useful for describing how brain activities transform to task-dependent cognitive processes. This dynamical approach is providing new insights into abnormal brain organization in various psychiatric and neurological disorders. Advances in this area stimulate new discoveries on dynamical disorders related to network connectivity, such as obsessive-compulsive disorder, schizophrenia, dementia, and drug dependence (Chambers et al., 2009; Bystritsky et al., 2012; Hughes et al., 2013). Non-linear dynamical models studying psychopathology must focus on understanding how disturbances in the networks' architecture contribute to cognitive and affective dysfunctions. In particular, it is extremely important to separate emergent dynamics into pathological and non-pathological regimes concerning a specific cognitive task.

As well known, cognitive human resources are finite (Franconeri et al., 2013). When a person becomes sick or its cognitive problem worsens, the performance degrades. There are several possible mechanisms of cognitive resource limitations discuss in the literature; for a review, see Emrich et al. (2013). In this paper, we focus on sequential working memory (SWM) capacity and discuss the instability mechanism related to the length of the information items (chunks) sequence. SWM is a dynamical cognitive network that enables and sustains a set of sequentially ordered mental representations for further recall and processing. The capacity of SWM is quite limited, i.e., it is 5 ± 2 chunks of information at any time instant (Miller, 1956; Bick and Rabinovich, 2009; Rabinovich et al., 2014). The contents of SWM are generally thought to be conscious. The SWM cognitive network, as imaging experiments indicate, consists of several brain modules distributed in different areas of the frontal cortex, sensory cortical regions, hippocampus and some others. These modules interact through the attentional process (Postle, 2006), forming stimulus-dependent spatiotemporal informational modes.

In excitatory-inhibitory cognitive networks that perform SWM, these modes sequentially turn off/ turn on each other according to winnerless competition (WLC) principle. As a result, a stable time-ordered sequence of chunks is formed (Rabinovich et al., 2014). In experiments, the sequence of such switching looks like a chain of metastable states—each state corresponds to the specific mode lasting for a finite time (Stopfer et al., 2003; Jones et al., 2007; Bouchard et al., 2013). The mathematical image of the sequence of metastable states is a stable heteroclinic channel (SHC) in the phase space of the corresponding dynamical model (Afraimovich et al., 2004a; Rabinovich et al., 2012b).

If excitation is constant, cognitive inhibition plays a key role in SWM dynamics and it is the origin of WLC. Cognitive inhibition refers to the mind's ability to tune out stimuli that are irrelevant to the task/process at hand or to the mind's current state (Harnishfeger, 1995; MacLeod, 2007). Cognitive inhibition is caused by several different interacting biological factors. The first is the existence of inhibitory neurotransmitters, or chemicals emitted by brain cells to both communicate and inhibit communication between each other. GABA is an inhibitory transmitter that has been implicated in certain simple behavioral measures of inhibition and the control of behavior in normal and pathological cases; it has been identified in the cerebral cortex (Dempster and Corkill, 1999). Given the cerebral cortex's importance in many brain functions such as memory and thought, the presence of the inhibitory substance GABA supports the cognitive inhibition processes that go on in this area of the brain. Cognitive inhibition is playing a key role in schizophrenia (Westerhausen et al., 2011). The corresponding degradation of the sequence of information items stability is analyzed in this paper. In particular, we are interested in the dynamics of sequential switching in the case of heterogeneity of the SWM network as a result of decreasing cognitive inhibition.

For the description of the modes interaction in excitatory-inhibitory cognitive networks, we use here the traditional model of population dynamics and game theory—generalized Lotka-Volterra equation for N interacting agents (Hofbauer and Sigmund, 1998). In canonic form (see below) this model has N metastable states that are represented in the phase space by saddle fixed points on the axes corresponding to different agent-variables. We consider the case of all-to-all inhibitory connections between participants. Depending on the strengths of the anatomical connections of the subnetworks - motifs or clusters that are embedded in an original larger network can form. These are anatomical motifs. Here we show that in heterogeneous networks one can observe the emergence of dynamical clusters, or dynamical motifs in the phase space, which can be interpreted as temporal unification of different groups of agents.

Cognitive functions, including working memory and attention, involve interconnected networks of brain regions. Recent investigations indicated abnormalities in structural and functional networks in the case of schizophrenia and other disorders, such as depression, obsessive-compulsive disorder, and substance abuse. These conditions are associated with deficits of GABA-mediated synaptic transmission in the brain, when inhibitory connections become weaker in frontal-subcortical neuronal networks (Tekin and Cummings, 2002; Lewis et al., 2005; Murray et al., 2014a).

In contrast with GABA synaptic inhibition in neurophysiological networks, the inhibition in cognition is a concept that is based on behavioral and imaging experiments. In fact, it is a process that has been postulated and modeled by kinetic equations for the competitive cognitive modes (Rabinovich et al., 2012b). In the framework of such models, it is possible to explain changes and deteriorations in the cognitive performance in many domains of psychological and psychiatric research. There are many areas of psychology and cognitive science where the concept of inhibition in global brain networks has been used successfully (Aron, 1982; Constantinidis and Wang, 2004; Gorfein et al., 2007; Joorman et al., 2007; Engelhart et al., 2008; Baumeister et al., 2014; Deco et al., 2014).

Following previous studies, our results indicate potential dynamical ways for augmenting damaged working memory and other cognitive functions. Specifically, we hypothesize that psychiatric and cognitive conditions will express substantial changes in temporal dynamics during key cognitive functions. Furthermore, if these models are successful it would be of great interest to determine if manipulating the organization of the feedback between fMRI time series of working memory (WM) activity through repetitive transcranial stimulation targeting prefrontal cortex can modulate inhibitory WM network and thus provide some control of the chaotic dynamics.

This paper focuses on the analysis of two related cognitive processes, i.e., sequential working memory and attention sharing. The analysis of the dynamics of corresponding functional global networks with inhibitory heterogeneous connections between cognitive modes (information items) revealed a new type of network behavior, which is coined *clustering dynamics*. Clustering dynamics is a sequential activity that includes ordered switching between a few information items, interrupted by intervals with chaotic switching between some other ones. The mathematical image of such intermittent dynamics we named *heteroclinic chimera* as an analog to chimeras observed in networks of phase oscillators (see Omelchenko et al., 2013; Panaggio and Abram, 2014), inspired by neuroscience (Kozma, 1998; Henderson and Robinson, 2011). The observed phenomenon leads to decreasing capacity of the sequential working memory. Moreover, it causes serious impediments in the process of attention sharing among several objects.

2. RELATION TO OTHER MODELING STUDIES

In Loh et al. (2007), based on a statistical dynamical model of integrate-and-fire **neuronal** network, the stability of attractor states in prefrontal cortical networks has been analyzed. The authors showed that for the stability of network dynamics is important to have a balance between excitation (NMDA conductance) and inhibition (GABA conductance). In particular, decreasing inhibition reduced the basins of cognitive attractors and destabilized the cognitive task performance that models the schizophrenia symptoms. The concept of excitation-inhibition dynamical balance is supported also by other modeling (Murray et al., 2014b), and experimental studies. In their nice work Murray and coauthors have showed that with constant excitation disinhibition increases random drift and decreases memory precision.

It is important to note that dynamical modeling of sequential neural activities has a long history; for a review, see Rabinovich et al. (2006). In particular, about 30 years ago, two seminal papers have been published (Kleinfeld, 1986; Sompolinsky and Kanter, 1986), in which authors used the same idea, i.e., that sequential patterns were generated by neural networks with time-delayed connections. Recent work about robust sequential memory in networks with controllable steady-state period is related to this idea (Xia et al., 2009). Camperi and Wang (1998) analyzed visual working memory models using networks with cellular bistability. Szatmary and Izhikevich (2010) built a spike-timing network

model of working memory using associative short-term synaptic plasticity (STDP). Buonomano (2000) has trained a biophysical network model of decoding temporal information using similar principles. In Seliger et al. (2003), the problem of recalling temporal sequence was solved in the framework of WLC networks. Concluding this obviously incomplete list, we notice that memory storage on short timescales can be maintained by neural activity that passed sequentially through a chain of network states (Goldman, 2009). This mechanism reminds the information propagation along heteroclinic channel in the phase space of WLC network (Rabinovich et al., 2012a).

3. MATERIALS AND METHODS

3.1. DYNAMICAL PRINCIPLES OF COGNITION: THE SIMPLEST CANONICAL MODEL

In order to build a non-linear dynamical model of sequential working memory, attention, and other cognitive functions in normal and pathological conditions, we use the following ideas based on brain imaging and behavioral experiments (see Rabinovich et al., 2012b): (i) the equations of the model have to be written for variables that represent the evolution of the temporal coherency of the brain components, and must have solution which correspond to metastable patterns (knowledge) in the brain; (ii) the model must be based on winnerless competitive (WLC) dynamics, a non-linear process of interaction of many informational items or spatiotemporal modes, which guarantees sequential switching between metastable states and potential robustness of transient creativity dynamics; (iii) the model is an open dissipative system where inhibition is balanced by excitation; and (iv) the dynamics of the model has to be sensitive to the changes in memory and environment information.

In our study, we consider a kinetic equation, which can be written as $\dot{x}_i = x_i F(x)$, where $F(x)$ is a vector function and $x = (x_1, \ldots, x_n)$. The Generalized Lotka Volterra (GLV) equation is a specific example of the kinetic equation. Thus, GLV is a non-linear population model with a simple quadratic nonlinearity. Moreover, it is known that a system of non-linear equations can be rewritten as system of GLV equations after some suitable transformations (Hernandez-Bermejo et al., 1998). Therefore, the "simple" Lotka Volterra equations can provide a powerful tool for the description of the dynamics of complex networks. Using this approach, one can write the model in the simplest canonical form of Generalized Lotka-Volterra equations (Rabinovich et al., 2006):

$$\tau_\ell \frac{dR_\ell}{dt} = R_\ell \left(\gamma_\ell - \sum_{k=1}^{N} a_{\ell,k} R_k \right), \qquad (1)$$

where R_ℓ is the level of activity of ℓ-th mode , $\ell = 1, \ldots, N$. Information mode variable R_ℓ must be positive or equal to zero for all ℓ. N is the total number of modes describing the components that interact to perform a specific cognitive task. Time constants τ_ℓ are fixed for a given system ℓ, while parameters $a_{\ell,k}$ describe the inhibitory connections between mode ℓ and k, while $a_{\ell,\ell} = 1$ for any ℓ, and γ_ℓ is the strength of the stimulation of mode ℓ. It is important that, in general, the elements of this matrix are controlled by cognitive tasks.

The dynamics of the cognitive network is extremely sensitive to the diversity of inhibitory connections. In this work, we model cognitive diseases through heterogeneous decrease of inhibitory activity in the cortex.

It is very important to emphasize that the complexity of the corresponding model is determined by the numbers of variables (agents) and the task dependent functional hierarchical architecture of the cognitive networks (Rabinovich et al., 2014). The individual dynamics of the agents is of secondary importance and it can be selected based on a suitably simple model. While numerous models exist that explain various aspects of cortical behavior, even simple, parsimonious GLV models (Bick and Rabinovich, 2009; Rabinovich et al., 2010) can produce intermittent chaotic behavior by a change in inhibitory weights, which provide insights into dynamical behaviors that may mirror pathological conditions.

3.2. LOTKA-VOLTERRA NETWORKS. GALLERY OF THE PHENOMENA

GLV model is very important and popular model for the analysis of multi agent non-equilibrium dynamics in ecology, biochemistry, and neuroscience. There is a huge amount of publications about GLV dynamics. Here we recall the main phenomena that are described by this model, which have been observed over a wide range of the control parameters.

If the connection matrix is symmetric the GLV model is a gradient system and demonstrates monotonic dissipative dynamics (Hirsch and Smith, 2005). In particular, one can observe multistability like in associative memory neural networks (Hopfeld, 1982; Cohen and Grossberg, 1983; Yi et al., 2003). In the case of moderate inhibition, the typical regime is winner-share-all (Fukai and Tanaka, 1997). Phase portrait of such stable regime of the symmetric high-dimensional model Equation (1) is shown in **Figure 1A**.

If the connection matrix is non-symmetric, the GLV model dynamics is extremely rich. For $N > 3$ the dynamics of GLV system can be chaotic (Arneodo et al., 1982; Takeuchi, 1996; Varona et al., 2002; Rabinovich et al., 2012b). An example of chaotic behavior in the GLV model with 6-modes is shown in **Figure 1B**. Another example is the transient dynamics representing cognitive information processing such as attention switching or sequential working memory stability (Bick and Rabinovich, 2009; Rabinovich et al., 2014); stable heteroclinic transients are illustrated in **Figure 1C**.

The key issue for the present study is understanding the origin of the SHC instability in the framework of model Equation (1) with heterogeneous connections. The results are important for the description of sequential information processing; for a review, see Rabinovich et al. (2012a).

3.3. STABILITY OF THE INFORMATION SEQUENCE. SADDLE VALUES

Robust transient dynamics is organized in the phase space typically as a chain of sequentially switching of metastable states—saddle points. The mathematical image of such dynamics is a stable heteroclinic channel—the vicinity of the chain of saddles coupled sequentially by their unstable separatrices. The chain can be finite, i.e., ending at the simple attractor (stable fixed point), or it can be periodic, asymptotically reaching a heteroclinic cycle.

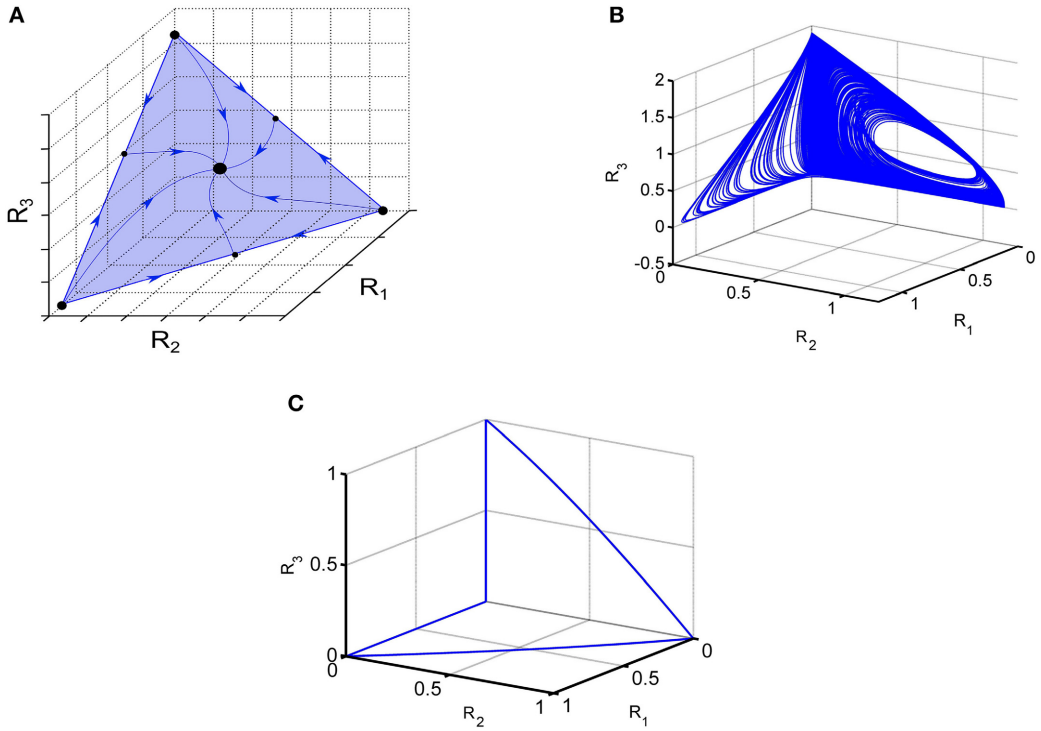

FIGURE 1 | Illustration of various dynamical regimes of the Lotka-Volterra system. (A) Example of a system when all trajectories converge to one stable fixed point, illustrating the coexistence of 3 modes. **(B)** Portrait of irregular switching in a 6-dimensional inhibitory LV network. 3D projection of the phase portrait of the observed strange attractor Equation (1). **(C)** Winnerless competitive dynamics of a network with random non-symmetric connections; 3D projection is shown; see Huerta and Rabinovich (2004).

Let $A_i = (0, \ldots, 0, \gamma_i, 0, \ldots, 0)$ be an equilibrium point of the system Equation (1), $i = 1, \ldots, N$. If $\lambda_1^{(i)}, \ldots, \lambda_N^{(i)}$ are eigenvalues of the matrix of the system linearized at A_i, that are ordered as follows $\lambda_1^{(i)} > \cdots \geq \mathrm{Re}\lambda_{k_i}^{(i)} > 0 > \mathrm{Re}\lambda_{k_i+1}^{(i)} \geq \cdots \geq \mathrm{Re}\lambda_N^{(i)}$ then A_i is a saddle with k_i-dimensional unstable manifold.

When the unstable manifolds of the saddles are one-dimensional, i.e., $k_i = 1$ for all i, the stability of a SHC depends on the ratios of the compression of the phase volume to the stretching of it in the vicinity of the channel. These ratios are called saddle values and they can be defined as $\nu_i = -\mathrm{Re}\lambda_2^{(i)}/\lambda_1^{(i)}$. Thus, if $\nu_i > 1$, the saddle is called dissipative and the trajectories get closer to the unstable manifold of the saddle after passing through its neighborhood. The mechanism of the SHC emergence in dissipative systems is the Winnerless Competition that can guarantee the sequential switching of agents activity in networks with non-symmetric inhibitory coupling (Rabinovich et al., 2006, 2012b; Bick and Rabinovich, 2010).

The conditions of the existence and stability of the heteroclinic contour with constant uniform stimulation strength $\gamma_i = 1$ for any i are given in Afraimovich et al. (2004a). The conditions of existence and stability of the heteroclinic sequence with different values of γ_i were obtained in Afraimovich et al. (2004b). To support the proposed interpretation of cognitive dynamics using heteroclinic chimera, we provide detailed mathematical analysis in the Appendix.

4. RESULTS

4.1. DEPENDENCE OF THE NETWORK DYNAMICS ON THE DISTRIBUTION OF INHIBITORY CONNECTIONS STRENGTHS

In this work, a system of 6 LV Equations (1) is studied. **Figure 2** illustrates the general structure of such system with all-to-all connections among 6 modes shown in 6 different colors; external inputs are marked by arrows pointing to each mode. We are able to select the parameters of the model to guarantee the regime of regular sequential working memory. In order to produce a system having robust heteroclinic contour, the inhibitory connections are chosen in two groups, i.e., with weights greater than one and smaller than one, respectively, while the self-inhibition weights are equal to one. Moreover, the weights in each of these subsets have a limited spread, i.e., they are concentrated around a particular value. Also, the strength of the inhibition is growing when the number of interacting modes increases; for details, see Bick and Rabinovich (2010). In the pathological case of weakened inhibition, on the other hand, we may expect that the strength of some inhibitory connections to approach zero.

4.2. DYNAMICAL CLUSTERING IN HETEROGENEOUS NETWORKS. INTERMITTENT SEQUENCES AND HETEROCLINIC CHIMERA

To analyze in detail the case of reduced/intermediate strength of coupling when quasi-periodic heteroclinic dynamics and chaos co-exist in a mutually coupled system, we performed extensive

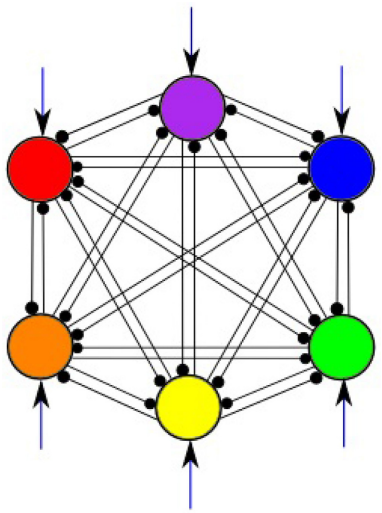

FIGURE 2 | All-to-all inhibitory interactions between 6 modes (information items) of the cognitive network; excitatory self connections are not shown. Black arrows represent incoming signals.

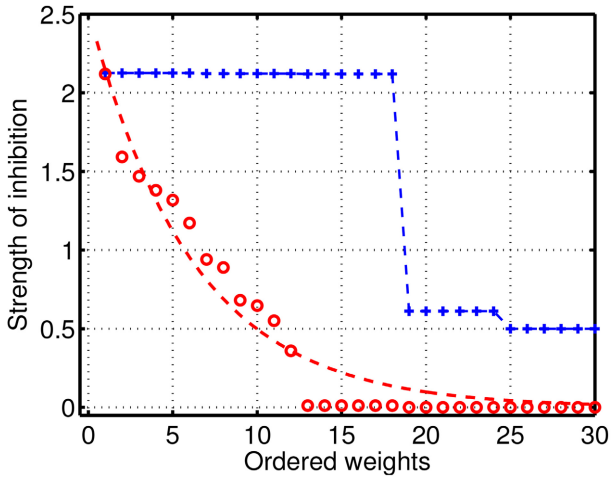

FIGURE 3 | Distribution of the strengths of the inhibitory connections in the GLV network with 6 participants. Blue: connectivity in a network producing normal SHC behavior; the large weights represent two triangle motifs with strong constant connectivity values, while there is a group of small weights describing weak connectivity between the motifs. Red: illustrates the pathological case with significantly reduced inhibitory connections with respect to normal SHC case, which are distributed over the parameter range; the dotted red line is a simple exponential fit shown for better visibility.

simulations with various sets of parameters. Examples of the distribution of the inhibitory weights in the GLV system with 6 modes are given in **Figure 3**. Blue color illustrates connectivity in a network producing normal SHC behavior, when the strong weights correspond to two triangle motifs, while there is a group of small weights describing weak connectivity between the motifs. The degraded (pathological) case is shown in red and it has significantly reduced inhibitory connection values with respect to the normal SHC case. The magnitudes of the weights are distributed over a range of parameters; the dotted red line illustrates a simple exponential fit for better visibility.

Takens' theorem (Takens, 1981) can be used to reconstruct high-dimensional attractors from the time series of a variable using time-delayed coordinate embedding. Note that time delay τ can be selected according to the given problem to produce a suitable display of the phase portrait. For example, $R_1(t)$ and its time-lagged copies $R_1(t-\tau)$ and $R_1(t-2\tau)$ are used in **Figure 4** to show the 3-dimensional phase portrait with time-lagged reconstruction. The case of $\tau = 150$ is used in this display; the direction of the trajectory is illustrated by arrows. **Figure 4A** shows the case of SHC corresponding to normal parameters. On the other hand, the attractor produced by reduced strength of coupling parameters given in **Figure 4** reflecting pathological conditions is shown in **Figure 4B**. Here a highly complex dynamics emerges resembling the Roessler strange attractor with two wings (Kennel et al., 1992). Specifically, the attractor in **Figure 4B** has a main wing and a secondary wing. The main wing is shown by overlapping red, orange, yellow, and green patches. There is a second wing, which is less pronounced and approaches but does not quite reach the blue and magenta dots. The secondary wing starts when the trajectories exit the main wing shown as *Exit* in **Figure 4B**, and later return to the main wing through the region shown as *Enter*. **Figure 4C** depicts a more detailed view of the two-wing attractor using more detailed computer simulations with about 10^5 time steps.

Quantitative evaluation of the Lyapunov exponents confirms the coexistence of heteroclinic cycles and chaos. Namely, we have two positive Lyapunov exponents, one small negative value close to zero, one small negative exponent, and two large negative exponents. The exact Lyapunov exponent values corresponding to parameters shown in **Figure 3** are as follows: $\lambda_1 = 0.0061 \pm 0.0005$, $\lambda_2 = 0.0008 \pm 0.0001$, $\lambda_3 = -0.0019 \pm 0.0015$, $\lambda_4 = -0.0127 \pm 0.0019$, $\lambda_5 = -0.6654 \pm 0.0004$, $\lambda_6 = -1.4409 \pm 0.0002$. We explored a variety of systems close and further away from the heteroclinic cycles. The above conclusions have been confirmed, i.e., we have two positive Lyapunov exponents, one close to zero, and the rest are negative. Our results show that two different dynamic regimes coexist in a single system of coupled agents with non-oscillatory intrinsic dynamics, similarly to the chimera states described recently in the literature (Abrams and Strogatz, 2004; Hagerstrom et al., 2012; Omelchenko et al., 2012). Earlier manifestations of chimera states have been in ensembles of phase oscillators. In our case of WLC, however, we observe amplitude clusterization.

Dynamical clustering means the separation of the phase flow into several qualitatively different components. In our example we have two components, one includes quasi-heteroclinic regular trajectories, while the other is a transient chaotic set. We named such complex image as **heteroclinic chimera**. We observe that the network's cooperative dynamics is dominated by the weak interaction between just a few (in our case two) dynamical sub-networks, although the monotonic heterogeneity of the connection strengths between agents would allow more distributed interaction dynamics.

We interpret this behavior as emergent granulation of the distributed dynamics into the interaction of just a few sub-networks.

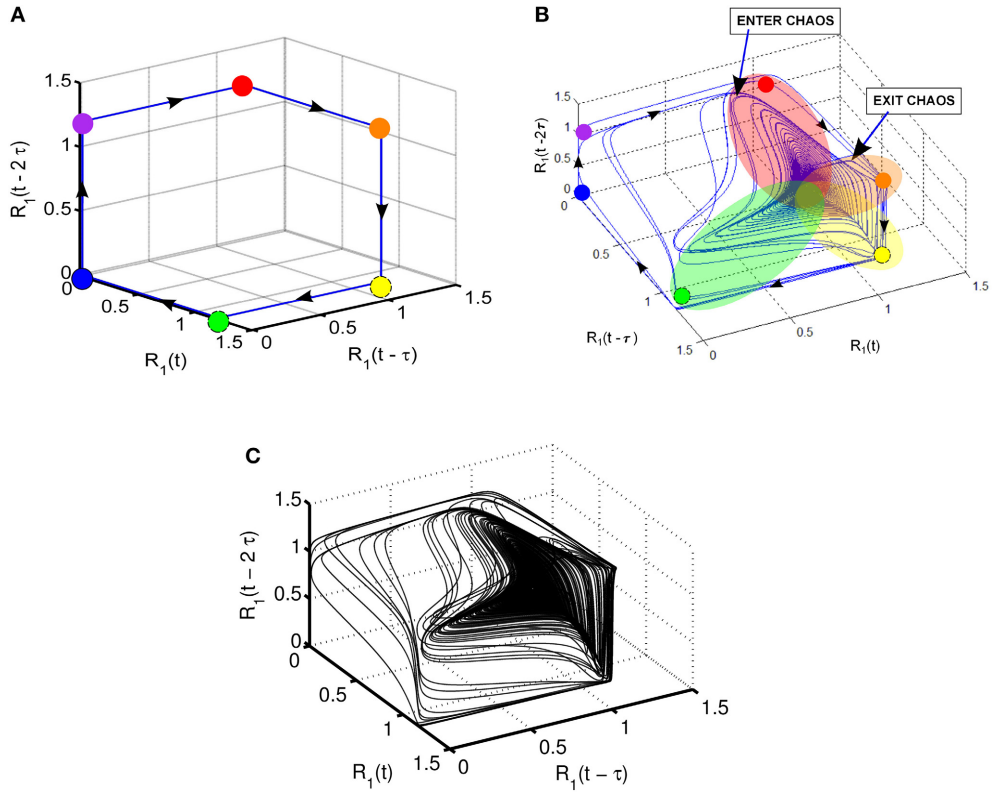

FIGURE 4 | Illustration of various dynamic regimes using 3-dimensional phase portrait with time-lagged reconstruction of R_1; (A) normal regime with stable heteroclinic channel (SHC); (B) abnormal regime: coexistence of SHC and quasi-chaotic attractor—heteroclinic chimera; (C) abnormal regime: coexisting SHC and quasi-chaotic attractor for longer time series with up to 10^5 points.

These sub-networks could be formed through the collaboration between agents $R_i, i = 1, \ldots, 6$ with the strongest mutual interaction between each others. We have tested this interpretation in the case of weak interaction between two strongly interconnected sub-networks, each with 3 modes (triangles). Our results indicate that in the case of coupled triangles we have topologically the same phase portrait of heteroclinic chimera as we observed in the original system with distributed parameters. We pointed out earlier, following Equation 1, that information mode variable R_i is either positive or zero, which determines the differences between the previously known phase chimera characterizing the dynamics of the network of phase oscillators, and the new heteroclinic chimera observed here in the case of the WLC processes. These results point to the importance of conducting a rigorous analysis in the case of weak inhibitory connections; details are given in the Appendix.

Figure 5 is the schematic image of a SHC modeling normal cognitive functions with sequential switching between various memory items. The consecutive patterns are symbolized by circles of different colors for simplicity. The appearance of the transient chaotic dynamics is illustrated in **Figure 6**, when the sequence of the patterns enters a *valley*, in which various colors are mixed in a chaotic fashion. This is the model of abnormal cognitive dynamics when the regular sequential memory dynamics breaks down. After some period of time, however, the trajectory leaves the chaotic *valley*, e.g., through the orange unit, and it resumes the regular sequential switching pattern.

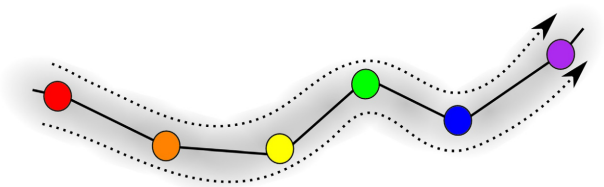

FIGURE 5 | Illustration of the trajectory in an SHC progressing through the sequence of saddle points; different memory patterns are shown in different colors, i.e., red, orange, yellow, green, blue, and violet.

Figure 7 illustrates heteroclinic chimera in a GLV network with degraded inhibitor weights. This GLV network has 6 units, from which two units are shown here, R_1 and R_2, respectively. **Figure 7A** depicts variable $R_1(t)$, which exhibits SHC regime with stable values over extended time periods. **Figure 7B** shows the temporal evolution of variable $R_2(t)$, which has frequent irregular oscillations. The determined positive Lyapunov exponent indicates the existence of chaos. The dynamics of all 6 variables $R_i, i = 1, \ldots, 6$ of the abnormal system is summarized in **Figure 8** using a raster plot. A given variable is shown in the plot

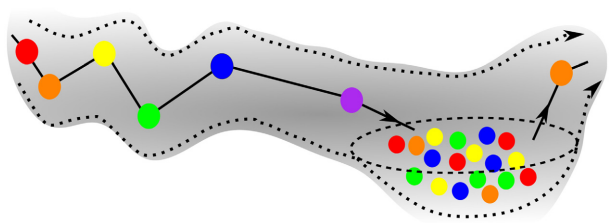

FIGURE 6 | Schematics of the trajectory of a pathological case of GLV equations, when the initial sequence enters a chaotic valley after the violet unit. The system stays in the valley for some time, but ultimately escapes it and resumes the regular SHC switching, see exit at the orange unit.

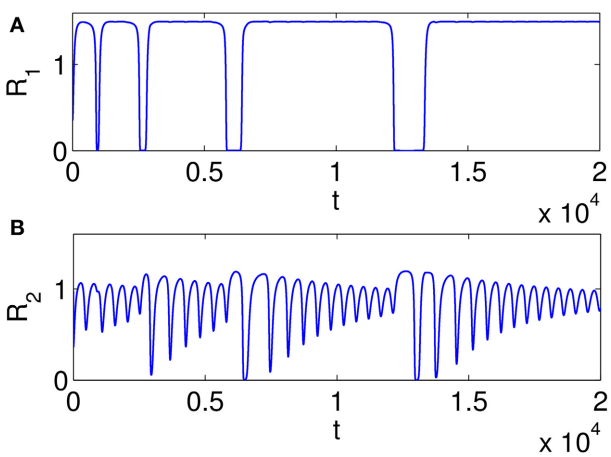

FIGURE 7 | Examples of time series illustrating heteroclinic chimera in a GLV network with degraded inhibitor weights; the GLV network has 6 units, and two units are shown here. (A) Variable $R_1(t)$ exhibits SHC regime with stable values over extended time periods; **(B)** Variable $R_2(t)$ has frequent irregular oscillations; the determined positive Lyapunov exponent indicates the existence of chaos.

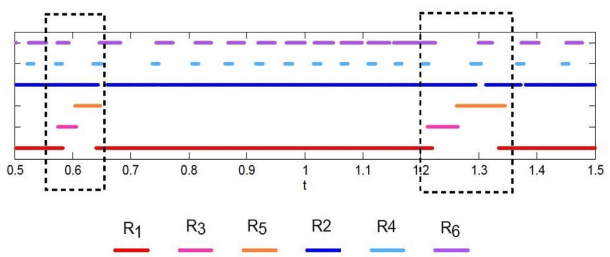

FIGURE 8 | Raster plot of intermittent sequences of the variables of the GLV system in the case of heteroclinic chimera dynamics; the 6 variables are shown as R_1, R_2, R_3, R_4, R_5, and R_6, respectively. A given variable is shown in the plot if its amplitude exceeds a threshold value; the threshold used here is 0.1. Note the preservation of sequential switching in the boxes marked by dashed lines.

if its amplitude exceeds a threshold value (of 0.1). Note the preservation of sequential switching in the boxes marked by dashed lines. While some of the variables apparently maintain stable heteroclinic trajectories, others exhibit intermittent oscillations and chaos, as a manifestation of heteroclinic chimera dynamics.

5. DISCUSSION

This paper analyzes the role of the heterogeneity of inhibitory connections in a cognitive network that models cognitive sequential information processing. The introduced model has been applied for the description and prediction of many cognitive processes like working memory, attention, and decision making. Here we suggested a plausible dynamical mechanism to study the deterioration of the working memory. Sequential order has been destroyed as the result of pathological heterogeneous decrease of some of the inhibitory connections. This mechanism is related to a new dynamical phenomenon: dynamical clustering of information items (cognitive modes) in networks with heterogeneous inhibitory connections.

The corresponding phenomenon is coined here "heteroclinic chimera." In the case of heteroclinic chimera, we observe the coexistence of chaotic and heteroclinic cycle behavior, thus the chimera property is expressed through sequential amplitude coordination. An important distinction between the previously described phase chimera and heteroclinic chimera is that phase chimera represents phase dynamics and it does not relate to the temporal sequence of items. The phenomenon of dynamical clustering described in this work in the case of heterogeneous inhibitory connections in model Equation 1 is robust. In Sokolov et al. (2014) the dynamics of the model is studied in the presence of multiplicative noise. It is shown that the noise does not change the qualitative picture of the dynamics.

Studies focusing on the analysis of sequential non-linear brain dynamics in the case of psychiatric disorders attract the interest of medical doctors. Results exist related to anxiety and depression (Bystritsky et al., 2014), and to obsessive-compulsive disorder (Bystritsky et al., 2012; Schiepek et al., 2013). Some psychiatrists anticipate that analysis of non-linear sequential dynamics will lead to changes in cognitive behavioral therapies (Kronemyer and Bystritsky, 2014).

There are several fMRI experiments that focused on the spatiotemporal analysis of the representation of informational items in short-time memory (Attout et al., 2014; D'Argembeau et al., 2014, and references therein). In addition, some experiments show the disruption of functional cortical networks in the case of psychiatric disorders including schizophrenia (Baker et al., 2014; Bittner et al., 2014). However, no comprehensive analysis have been completed yet concerning the spatiotemporal dynamics of functional cortical networks, in the case of patients with schizophrenia who do not recall ordered information from SWM. Together with our colleague Alan Simmons (UCSD Dept. of Psychiatry), we plan to conduct such analyses and compare the results with our predictions. In particular, we intend to measure the voxels in functional time series in pathological conditions, i.e., signal intensity vs. time activity in parietal and prefrontal cortexes, to characterize the performance of SWM recall. The modeling results are represented by the $R_i(t)$ time series. The mathematical image of the damaged SWM with heteroclinic chimera is in fact an intermittent chaotic dynamical regime. There

are several successful methods for controlling chaotic dynamics; see, for example Sieber et al. (2014), which is a successful approach to modulate the irregular activities by feedback.

Observations in neuroimaging studies were used to describe the neural correlates of cognitive deficits in attention, working memory and executive functions in patients with Huntington's disease (Montoya et al., 2006). The chaotic behavior of clustered sequential dynamics can serve as a model of Huntington's Chorea.

A related important cognitive problem involves abnormalities in attention switching and focusing, which can be described by the proposed dynamical model; see also Rabinovich et al. (2013). Of the many clinical features of schizophrenia, disturbances in certain cognitive processes, such as impairments in attention, memory and executive functions (that is, the ability to plan, initiate, and regulate goal directed behavior), might represent the core features of the illness (Elvevag and Goldberg, 2000). There is increasing evidence indicating that such disorders are related to decreasing level of inhibition in cortical inhibitory circuits (Lewis et al., 2005). In recent studies, schizophrenia patients has been tested to answer the question: are they impaired relative to controls in sustaining attention, switching attention, or both (Smid et al., 2013). The results supported the hypothesis that schizophrenia is associated with attention switching, while the mechanisms of sustained attention remains largely intact. Our results give a dynamical interpretation to these observations.

If the GLV model suggested is successful in characterizing the differences in temporal dynamics in normal and pathological samples then it would be of great interest in determining if this could guide a potential intervention in these dynamics to either mimic or ameliorate the core symptomologies of these pathologies. We expect that it would be very promising to provide feedback based on functional sequential cortical activity during memory recall, i.e., the time series representing sequential switching between metastable states (Polyn et al., 2005; Norman et al., 2006), by repetitive transcranial brain stimulation. This feedback may involve either repetitive magnetic stimulation (Barr et al., 2013) or ultrasound stimulation (Hameroff et al., 2013; Mueller et al., 2014). Such feedback has to support the correct sequential switching between corresponding recalled information items from SWM.

SHC is the mathematical image of regular sequential switching of attention as it is postulated in Rabinovich et al. (2013). Regular sequential switching between attention modalities is maintained in our model at normal conditions. In the case of the pathological inhibitory strength distribution (selective decrease of inhibitory weights), the regular sequential switching of attention focus is impaired. As a result, the sequential switching is intermittently interrupted by periods of irregular/chaotic dynamics, and the attention switching process becomes uncontrolled. Our modeling results are also in agreement with recent works (Colzato et al., 2007; Tomasi et al., 2010) showing that chronic cocaine use is associated with disrupted inhibitory connections in the brain. In particular, findings in Tomasi et al. (2010) suggest that decreased functional dopaminergic inhibitory connectivity of the midbrain interferes with the activation and deactivation signals associated with sustained attention in cocaine addicts.

AUTHOR CONTRIBUTIONS

All co-authors have equal contribution to all steps of preparation of this article and they approved the version to be published.

ACKNOWLEDGMENTS

Thanks to Pablo Varona, Valentin Afraimovich and Karl Friston for useful discussions. Mikhail I. Rabinovich acknowledges support from ONR grant N00014310205. This material is based upon work supported by NSF to Robert Kozma in the CRCNS Program, grant number DMS-13-11165.

REFERENCES

Abrams, D., and Strogatz, S. (2004). Chimera states for coupled oscillators. *Phys. Rev. Lett.* 93, 174102. doi: 10.1103/PhysRevLett.93.174102

Afraimovich, V., Rabinovich, M., and Varona, P. (2004a). Heteroclinic contours in neural ensembles and the winnerless competition principle. *Int. J. Bifurcat. Chaos* 14, 1195. doi: 10.1142/S0218127404009806

Afraimovich, V. S., Zhigulin, V., and Rabinovich, M. I. (2004b). On the origin of reproducible sequential activity in neural circuts. *Chaos* 14, 1123–1129. doi: 10.1063/1.1819625

Arneodo, A., Coullet, P., Peyraud, J., and Tresser, C. (1982). Strange attractors in volterra equations for species in competition. *J. Math. Biol.* 14, 153–157. doi: 10.1007/BF01832841

Aron, A. (1982). The neural basis of inhibition in cognitive control. *Neuroscientist* 13, 153–157.

Attout, L., Fias, W., Salmon, E., and Majerus, S. (2014). Common neural substrates for ordinal representation in short-term memory, numerical and alphabetical cognition. *PLoS ONE* 9:e9204. doi: 10.1371/journal.pone.009204

Baker, J., Holmes, A., Maste, G., Yeo, B., Krienen, F., Buckner, R., et al. (2014). Disruption of cortical association networks in schizophrenia and psychotic bipolar disorder. *AMA Psychiatry* 71, 109–118. doi: 10.1001/jamapsychiatry.2013.3469

Barr, M., Farzan, F., Rajji, T., Voineskos, A., Blumberger, D., Arenovich, T., et al. (2013). Can repetitive magnetic stimulation improve cognition in schizophrenia? pilot data from a randomized controlled trial. *Biol. Psychiatry* 73, 510–517. doi: 10.1016/j.biopsych.2012.08.020

Baumeister, S., Hohmann, S., Wolf, I., Plichta, M., Rechtsteiner, S., Zangl, M., et al. (2014). Sequential inhibitory control processes assessed through simultaneous eeg-fmri. *Neuroimage* 94, 349–359. doi: 10.1016/j.neuroimage.2014.01.023

Bick, C., and Rabinovich, M. I. (2009). Dynamical origin of the effective storage capacity in the brain's working memory. *Phys. Rev. Lett.* 103:218101. doi: 10.1103/PhysRevLett.103.218101

Bick, C., and Rabinovich, M. I. (2010). On the occurrence o stable heteroclinic channels in lotka-volterra models. *Dyn. Syst.* 25, 1–14. doi: 10.1080/14689360903322227

Bittner, R., Linden, D., Roebroeck, A., Hartling, F., Rotarska-Jagiela, A., Maurer, K., et al. (2014). The when and where of working memory dysfunction in early-onset schizophrenia—a functional magnetic resonance imaging study. *Cereb. Cortex*. doi: 10.1093/cercor/bhu050. [Epub ahead of print].

Bouchard, K. E., Mesgarani, N., Johnson, K., and Chang, E. F. (2013). Functional organization of human sensorimotor cortex for speech articulation. *Nature* 495, 327–332. doi: 10.1038/nature11911

Buonomano, D. V. (2000). Decoding temporal information: a model based on short-term synaptic plasticity. *J. Neurosci.* 20, 1129–1141.

Bystritsky, A., Danial, J., and Kronemeyer, D. (2014). Interactions between diabetes and anxiety and depression: implications for treatment. *Endocrinol. Metab. Clin. N Am.* 43, 269–283. doi: 10.1016/j.ecl.2013.10.001

Bystritsky, A., Nierenberg, A. A., Feusner, J. D., and Rabinovich, M. (2012). Computational non-linear dynamical psychiatry: a new methodological paradigm for diagnosis and course of illness. *J. Psychiatry Res.* 46, 428–435. doi: 10.1016/j.jpsychires.2011.10.013

Camperi, M., and Wang, X. (1998). A model of visuospatial working memory in prefrontal cortex: recurrent network and cellular bistability. *J. Comput. Neurosci.* 5, 383–405. doi: 10.1023/A:1008837311948

Chambers, C., Garavan, H., and Bellgrove, M. (2009). Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. *Neurosci. Biobehav. Rev.* 33, 631–646. doi: 10.1016/j.neubiorev.2008.08.016

Cohen, M., and Grossberg, S. (1983). Absolute stability of global pattern formation and parallel memory storage by competitive neural networks. *IEEE Trans. Syst. Man Cybernet.* 13, 288–307.

Colzato, L., van den Wildenberg, W., and Hommel, B. (2007). Impaired inhibitory control in recreational cocaine users. *PLoS ONE* 2:e1143. doi: 10.1371/journal.pone.0001143

Constantinidis, C., and Wang, X. (2004). A neural circuit basis for spatial working memory. *Neuroscientist* 10, 553–565. doi: 10.1177/1073858404268742

D'Argembeau, A., Jeunehomme, O., Majerus, S., Bastin, C., and Salmon, E. (2014). The neural basis of temporal order processing in past and future thought. *J. Cogn. Neurosci*. doi: 10.1162/jocn_a_00680. [Epub ahead of print].

Deco, G., Ponce-Alvarez, A., Hagmann, P., Romani, G., Mantini, D., and Corbetta, M. (2014). How local excitation-inhibition ratio impacts the whole brain. *Dynam. J. Neurosci.* 34, 7886–7898. doi: 10.1523/JNEUROSCI.5068-13.2014

Dempster, F., and Corkill, A. (1999). "The development of intelligence. Studies in developmental psychology," in *Chapter Neo-Interference Research and The Development of Intelligence*, ed M. Anderson (East Sussex: Psychology Press Limited), 215–243.

Elvevag, B., and Goldberg, T. E. (2000). Cognitive impairment in schizophrenia is the core of the disorder. *Crit. Rev. Neurobiol.* 14, 1–21. doi: 10.1615/CritRevNeurobiol.v14.i1.10

Emrich, S., Riggall, A., LaRocque, J., and Postle, B. (2013). Distributed patterns of activity in sensory cortex reflect the precision of multiple items maintained in visual short-term memory. *J. Neurosci.* 33, 6516–6523. doi: 10.1523/JNEUROSCI.5732-12.2013

Engelhart, P., Nigg, J., Carr, L., and Ferreia, F. (2008). Cognitive inhibition and working memory in attention-deficient/hyperactivity disorder. *J. Abnorm. Psychol.* 117, 591–605. doi: 10.1037/a0012593

Franconeri, S., Alvarez, G., and Cavanagh, P. (2013). Flexible cognitive resources: competitive content maps for attention and memory. *Trends Cogn. Sci.* 17, 134–141. doi: 10.1016/j.tics.2013.01.010

Fukai, T., and Tanaka, S. (1997). A simple neural network exhibiting selective activation of neuronal ensemblies: from winner-take-all to winners-share all. *Neural Comput.* 9, 77–97. doi: 10.1162/neco.1997.9.1.77

Goldman, M. (2009). Memory without feedback in a neural network. *Neuron* 61, 621–634. doi: 10.1016/j.neuron.2008.12.012

Gorfein, D. S., MacLeod, C. M., and Colin, M. (2007). *Inhibition in Cognition*. Washington, DC: American Psychological Association. doi: 10.1037/11587-000

Hagerstrom, A. M., Murphy, T. E., Roy, R., Hövel, P., Omelchenko, I., and Schöll, E. (2012). Experimental observation of chimeras in coupled map lattices. *Nat. Phys.* 8, 658. doi: 10.1038/nphys2372

Hameroff, S., Trakas, M., Duffield, C., Annabi, E., Gerace, M. B., Boyle, P., et al. (2013). Transcranial ultrasound (tus) effects on mental states: a pilot study. *Brain Stimul.* 6, 409–415. doi: 10.1016/j.brs.2012.05.002

Harnishfeger, K. (1995). *The Development of Cognitive Inhibition*. San Diego, CA: Academic Press, Inc.

Henderson, J. A., and Robinson, P. A. (2011). Geometric effects on complex network structure in the cortex. *Phys. Rev. Lett.* 107, 018102. doi: 10.1103/PhysRevLett.107.018102

Hernandez-Bermejo, B., Fairen, V., and Leon, B. (1998). Algebraic recasting of nonlinear systems of odes into universal formats. *J. Phys. A Math. Gen.* 31, 2415. doi: 10.1088/0305-4470/31/10/016

Hirsch, M. W., and Smith, H. (2005). *Handbook of Differential Equations: Ordinary Differential Equations*. Amsterdam: Elsevier.

Hofbauer, J., and Sigmund, K. (1998). *Evolutionary Games and Population Dynamics*. Cambridge, UK: Cambridge University Press. doi: 10.1017/CBO9781139173179

Hopfeld, J. (1982). Neural networks and physical systems with emergent collective computational abilities. *Proc. Natl. Acad. Sci. U.S.A.* 79, 2554–2558. doi: 10.1073/pnas.79.8.2554

Huerta, R., and Rabinovich, M. (2004). Reproducible sequence generation in random neural ensembles. *Phys. Rev. Lett.* 93:238104. doi: 10.1103/PhysRevLett.93.238104

Hughes, L., Ghosh, B., and Rowe, J. (2013). Reorganization of brain networks in frontotemporal dementia and progressive supranuclear palsy. *Neuroimage Clin.* 2, 459–468. doi: 10.1016/j.nicl.2013.03.009

Jones, L. M., Fontanini, A., Sadacca, B. F., Miller, P., and Katz, D. B. (2007). Natural stimuli evoke dynamic sequences of states in sensory cortical ensembles. *Proc. Natl. Acad. Sci. U.S.A.* 104, 18772–18777. doi: 10.1073/pnas.0705546104

Joorman, J., Yoon, K., and Zetsche, U. (2007). Cognitive inhibition in depression. *Appl. Prevent. Psychol.* 12, 128–139. doi: 10.1016/j.appsy.2007.09.002

Kennel, M. B., Brown, R., and Abarbanel, H. D. (1992). Determining embedding dimension for phase-space reconstruction using a geometrical construction. *Phys. Rev. A* 45, 3403. doi: 10.1103/PhysRevA.45.3403

Kleinfeld, D. (1986). Sequential state generation by model neural networks. *Proc. Nati. Acad. Sci. USA* 83, 9469–9473. doi: 10.1073/pnas.83.24.9469

Kozma, R. (1998). Intermediate-range coupling generates low-dimensional attractors deeply in the chaotic region of one-dimensional lattices. *Phys. Lett. A* 244, 85–91. doi: 10.1016/S0375-9601(98)00275-8

Kronemyer, D., and Bystritsky, A. (2014). A non-linear dynamical approach to belief revision in cognitive behavioral therapy. *Front. Comput. Neurosci.* 8:55. doi: 10.3389/fncom.2014.00055

Lewis, D., Hashimoto, T., and Volk, D. (2005). Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci*. 6, 312–324. doi: 10.1038/nrn1648

Loh, M., Rolls, E., and Deco, G. (2007). A dynamical systems hypothesis of schizophrenia. *PLoS Comput. Biol.* 3:e228. doi: 10.1371/journal.pcbi.0030228

MacLeod, C. (2007). "Inhibition in cognition," in *Chapter The Concept of Inhibition in Cognition*, eds D. S. Gorfein and C. M. MacLeod (Washington, DC: American Psychological Association), 3–23.

Miller, G. A. (1956). The magical number seven, plus or minus two: some limits on our capacity for processing information. *Psychol. Rev.* 63, 81–97. doi: 10.1037/h0043158

Montoya, A., Price, B., Menear, M., and Lepage, M. (2006). Brain imaging and cognitive dysfunctions in huntington's disease. *J. Psychiatry Neurosci.* 31, 21–29.

Mueller, J., Legon, W., Opitz, A., Sato, T., and Tyler, W. J. (2014). Transcranial focused ultrasound modulates intrinsic and evoked eeg dynamics. *Brain Stimul*. doi: 10.1016/j.brs.2014.08.008. [Epub ahead of print].

Murray, J., Anticevic, A., Gancsos, M., and Ichinose, M. (2014a). Linking microcircuit dysfunction to cognitive impairment: effects of disinhibition associated with schizophrenia in a cortical working memory model. *Cereb. Cortex* 24, 859–872. doi: 10.1093/cercor/bhs370

Murray, J., Anticevic, A., Gancsos, M., Ichinose, M., Corlett, P., Krystal, J., et al. (2014b). Linking microcircuit dysfunction to cognitive impairment: effects of disinhibition associated with schizophrenia in a cortical working memory model. *Cereb. Cortex* 24, 859–872. doi: 10.1093/cercor/bhs370

Norman, K. A., Polyn, S. M., Detre, G. J., and Haxby, J. V. (2006). Beyond mind-reading: multi-voxel pattern analysis of fmri data. *Trends Cogn. Sci.* 10, 424–430. doi: 10.1016/j.tics.2006.07.005

Omelchenko, I., Omelchenko, O., Hövel, P., and Schöll, E. (2013). When nonlocal coupling between oscillators becomes stronger: patched synchrony or multichimera states. *Phys. Rev. Lett.* 110:224101. doi: 10.1103/PhysRevLett.110.224101

Omelchenko, I., Riemenschneider, B., Hövel, P., Maistrenko, Y., and Schöll, E. (2012). Transition from spatial coherence to incoherence in coupled chaotic systems. *Phys. Rev. E* 85, 026212. doi: 10.1103/PhysRevE.85.026212

Panaggio, M., and Abram, D. (2014). Chimera states: coexistence of coherence and incoherence in networks of coupled oscillators. arXiv:1403.6204v2

Polyn, S. M., Natu, V. S., Cohen, J. D., and Norman, K. A. (2005). Category-specic cortical activity precedes retrieval during memory search. *Science* 310, 1963–1966. doi: 10.1126/science.1117645

Postle, B. (2006). Working memory as an emergent property of the mind and brain. *Neuroscience* 139, 23–38. doi: 10.1016/j.neuroscience.2005.06.005

Rabinovich, M. I., Afraimovich, V. S., Bick, C., and Varona, P. (2012a). Information flow dynamics in the brain. *Phys. Life Rev.* 9, 51–73. doi: 10.1016/j.plrev.2011.11.002

Rabinovich, M. I., Friston, K., and Varona, P. (2012b). *Principles of Brain Dynamics: Global State Interactions*. Cambridge, MA: MIT Press.

Rabinovich, M. I., Muezzinoglu, M. K., Strigo, I., and Bystritsky, A. (2010). Dynamical principles of emotion-cognition interaction: mathematical images of mental disorders. *PLoS ONE* 5:e12547. doi: 10.1371/journal.pone.0012547

Rabinovich, M. I., Tristan, I., and Varona, P. (2013). Neural dynamics of attentional cross-modality control. *PLoS ONE* 8:e64406. doi: 10.1371/journal.pone.0064406

Rabinovich, M. I., Varona, P., Tristan, I., and Afraimovich, V. S. (2014). Chunking dynamics: heteroclinics in mind. *Front. Comput. Neurosci.* 8:22. doi: 10.3389/fncom.2014.00022

Rabinovich, M. I., Varona, P., Selverston, A., and Abarbanel, H. (2006). Dynamical principles in neuroscience. *Rev. Mod. Phys.* 78, 1213–1265. doi: 10.1103/RevModPhys.78.1213

Schiepek, G., Tominschek, I., Heinzel, S., Aigner, M., and Dold, M., (2013). Discontinuous patterns of brain activation in the psychotherapy process of obsessive-compulsive disorder: converging results from repeated fmri and daily self-reports. *PLoS ONE* 8:e71863. doi: 10.1371/journal.pone.0071863

Seliger, P., Tsimring, L., and Rabinovich, M. (2003). Dynamics-based sequential memory: winnerless competition of patterns. *Phys. Rev. E* 67, 1–4. doi: 10.1103/PhysRevE.67.011905

Sieber, J., Omel'chenko, O. E., and Wolfrum, M. (2014). Controlling unstable chaos: stabilizing chimera states by feedback. *Phys. Rev. Lett.* 112:054102. doi: 10.1103/PhysRevLett.112.054102

Smid, H., Martens, S., de Witte, M., and Bruggeman, R. (2013). Inflexible minds: impaired attention switching in recent-onset schizophrenia. *PLoS ONE* 8:e78062. doi: 10.1371/journal.pone.0078062

Sokolov, Y., Kozma, R., and Rabinovich, M. (2014). "Noise effects in a dynamical model of attentional switching," in *Biomedical Science and Engineering Center Conference (BSEC), 2014 Annual Oak Ridge National Laboratory* (Oak Ridge, TN).

Sompolinsky, H., and Kanter, I. I. (1986). Temporal association in asymmetric neural networks. *Phys. Rev. Lett.* 57, 2861–2864. doi: 10.1103/PhysRevLett.57.2861

Stopfer, M., Jayaraman, V., and Laurent, G. (2003). Intensity versus identity coding in an olfactory system. *Neuron* 39, 991–1004. doi: 10.1016/j.neuron.2003.08.011

Szatmary, B., and Izhikevich, E. (2010). Spike-timing theory of working memory. *PLoS Comput. Biol.* 6:e1000879. doi: 10.1371/journal.pcbi.1000879

Takens, F. (1981). *Dynamical Systems and Turbulence*. New York, NY: Springer-Verlag.

Takeuchi, Y. (1996). *Global Dynamical Properties of Lotka-Volterra Systems*. Singapore: World Scientific.

Tekin, S., and Cummings, J. (2002). Frontal-subcortical neuronal circuits and clinical neuropsychiatry: an update. *J. Psychosom. Res.* 53, 647–654. doi: 10.1016/S0022-3999(02)00428-2

Tomasi, D., Volkow, N., Wang, R., Carrillo, J., Maloney, T., Alia-Klein, N., et al. (2010). Disrupted functional connectivity with dopaminergic midbrain in cocaine abusers. *PLoS ONE* 5:e10815. doi: 10.1371/journal.pone.0010815

Varona, P., Rabinovich, M., Selverston, A., and Arshavsky, Y. (2002). Winnerless competition between sensory neurons generates chaos: a possible mechanism for molluscan hunting behavior. *Chaos* 12, 672–677. doi: 10.1063/1.1498155

Westerhausen, R., Kompus, K., and Hugdahl, K. (2011).) impaired cognitive inhibition in schizophrenia: a meta-analysis of the stroop interference effect. *Schizophr. Res.* 133, 172–181. doi: 10.1016/j.schres.2011.08.025

Xia, M., Fang, J., Pan, F., and Bai, E. (2009). Robust sequence memory in sparsely-connected networks with controllable steady-state period. *Neurocomputing* 72, 1213–1265. doi: 10.1016/j.neucom.2009.03.004

Yi, Z., Tan, K., and Lee, T. (2003). Multistability analysis for recurrent neural networks with unsaturating piecewise linear transfer functions. *Neural Comput.* 15, 639–662. doi: 10.1162/089976603321192112

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 July 2014; accepted: 21 October 2014; published online: 14 November 2014.

Citation: Rabinovich MI, Sokolov Y and Kozma R (2014) Robust sequential working memory recall in heterogeneous cognitive networks. Front. Syst. Neurosci. ***8****:220. doi: 10.3389/fnsys.2014.00220*

This article was submitted to the journal Frontiers in Systems Neuroscience.

APPENDIX

Here the case of GLV with weak inhibitory connections are studied. For convenience, let us introduce the notation for foregoing sub-networks S_1 and S_2, respectively. Both subsystems have 3 modes, S_1 contains modes R_1, R_3, and R_5, while S_2 includes R_2, R_4, and R_6. We consider the case of weak, uniform connections between the two subsystems. Accordingly, κ_1 and κ_2 denote the connection strength from S_1 to S_2 and from S_2 to S_1, respectively. For the sake of simplicity, here we assume $\kappa_2 = 0$ and we study bifurcation only with respect to one parameter $\kappa = \kappa_1$. Note that the results of this section are readily extendable to the case $\kappa_2 \neq 0$.

Based on the definition of subsystems S_1 and S_2, clearly two independent stable heteroclinic cycles exist for $\kappa_1 = 0$. Further, it is expected that the two heteroclinic cycles are maintained for very weak coupling $0 < \kappa << 1$. Increasing κ in the small to intermediate coupling levels, complex dynamics may emerge, such as simultaneous presence of chaos and heteroclinic cycles. The upper bound of this region is marked as κ^*. Finally, the cycles must collapse if coupling exceeds a high threshold value ($\kappa >> 1$).

We start with the definition of several quantities, which allow us to separate the whole domain of coupling parameter κ into regions with different types of behavior. The threshold values are expressed as follows: $\kappa^* = max_i\{\gamma_{i+1}/\gamma_i\}$, $i = 1, \ldots, 6$, where γ_i is the strength of stimulation of mode i (see Equation 1). Further, let us characterize each equilibria. In the following considerations, all indices are written with respect to (mod 6) and we use of the following convention $\{i \in \{0, \ldots, n\} | i = n \ (\mod n)\} = n$. Let us define for each $i = 1, \ldots, 6$ the corresponding set of two numbers $\bar{i} = \{(i \pm 2) \ mod \ 6\}$.

Further notations are: $k^*_{odd} = \max\{k_1, k_3, k_5\}$ and $k^*_{even} = \max\{k_2, k_4, k_6\}$, and indexes defined as $i^*_{odd} = \arg\max\{k^*_{odd}\}$ and $i^*_{even} = \arg\max\{k^*_{even}\}$, where k_i is defined by

$$k_i = \frac{\gamma_{(i+1)}(-\sum_{k\in\bar{i}} \gamma_k a_{ki} + \gamma_i \prod_{k\in\bar{i}} a_{ki})}{\prod_{k\in\bar{i}} (\gamma_k - \gamma_i a_{ki})} \quad (2)$$

In the following considerations, we use quantities $k' = \max\{k_{i^*_{odd}-2}, k_{i^*_{odd}+2}\}$ and $k" = \max\{k_{i^*_{even}-2}, k_{i^*_{even}+2}\}$. It is easy to see that k' and $k"$ are larger than the other thresholds. In the following description, we assume that $k' < k"$ unless it is specified otherwise.

There exists a value of coupling parameter $0 < k^0 << 1$ such that for $\kappa \in (0, k^0)$ the coupled system exhibits two heteroclinic cycles. For $\kappa \in (k^o, \kappa^*)$ various complex behaviors emerge, with the possibility of co-existing chaotic attractor and heteroclinic cycle. In the case of $\kappa \in (\kappa^*, k')$ the system converges to a fixed point. For $\kappa \in (k', k")$, a heteroclinic cycle in one system coexists with zero fixed points in the other system. Finally, for $\kappa > k"$ the coupled system collapses. Thus, we have the following regions

$$0 < k^o < \kappa^* < k' \leq k" < \infty. \quad (3)$$

If $k' = k"$ then the conclusion still holds with the difference that the behavior corresponding to values between k' and $k"$ does not occur. The present results were derived based on the dissipative property of the saddle point. In other words, the equilibrium point attracts trajectories in its neighborhood if it is dissipative point. However, if the dissipative property of the saddle point changes, i.e., the saddle value is no more greater than one due to the increase of κ, we may observe that the orbits move in directions away from equilibria. For this reason, when the coupling parameter is large ($\kappa > k'$), the origin will attract the trajectories of one of the subsystems. Under this scenario, we have one subsystem (i.e., $S1$ or $S2$) embedded in 6 dimension, and this subsystem behaves as in the case $\kappa = 0$. In other words, considering the phase space $\mathbb{R}^6 = \mathbb{R}^3 \oplus \mathbb{R}^3$, if one subsystem vanishes we deal with the subspace where all coordinates of this subsystem are zero, so the other subsystem behaves like in the case $\kappa = 0$.

In the case of $\kappa \in (\kappa^*, k')$, the central eigenspace of each equilibria stays the same. However, the number of stable non-leading eigenvalues is increased to the maximum possible value, thus fixed points appear. In the most challenging case when $\kappa \in (k^0, \kappa^*)$, the equilibria which form the heteroclinic cycles have different number of stable and unstable non-leading eigenvalues. This case is possible due to the non-symmetry of the equations, which describe the coupled system with mutual inhibition between its subsystems. To put it simply, there is no symmetry group between its subsystems coupling.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 13 May 2014
doi: 10.3389/fnsys.2014.00038

Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain

Kimberly R. Urban[1] and Wen-Jun Gao[2]*

[1] *Department of Psychology, University of Delaware, Newark, DE, USA*
[2] *Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Kimberly Simpson, University of Mississippi Medical Center, USA
Christopher R. Madan, University of Alberta, Canada

Correspondence:
Wen-Jun Gao, Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, USA
e-mail: wgao@drexelmed.edu

Cognitive enhancement is perhaps one of the most intriguing and controversial topics in neuroscience today. Currently, the main classes of drugs used as potential cognitive enhancers include psychostimulants (methylphenidate (MPH), amphetamine), but wakefulness-promoting agents (modafinil) and glutamate activators (ampakine) are also frequently used. Pharmacologically, substances that enhance the components of the memory/learning circuits—dopamine, glutamate (neuronal excitation), and/or norepinephrine—stand to improve brain function in healthy individuals beyond their baseline functioning. In particular, non-medical use of prescription stimulants such as MPH and illicit use of psychostimulants for cognitive enhancement have seen a recent rise among teens and young adults in schools and college campuses. However, this enhancement likely comes with a neuronal, as well as ethical, cost. Altering glutamate function via the use of psychostimulants may impair behavioral flexibility, leading to the development and/or potentiation of addictive behaviors. Furthermore, dopamine and norepinephrine do not display linear effects; instead, their modulation of cognitive and neuronal function maps on an inverted-U curve. Healthy individuals run the risk of pushing themselves beyond optimal levels into hyperdopaminergic and hypernoradrenergic states, thus vitiating the very behaviors they are striving to improve. Finally, recent studies have begun to highlight potential damaging effects of stimulant exposure in healthy juveniles. This review explains how the main classes of cognitive enhancing drugs affect the learning and memory circuits, and highlights the potential risks and concerns in healthy individuals, particularly juveniles and adolescents. We emphasize the performance enhancement at the potential cost of brain plasticity that is associated with the neural ramifications of nootropic drugs in the healthy developing brain.

Keywords: methylphenidate, modafinil, ampakine, cognitive enhancement, synaptic plasticity, brain development

INTRODUCTION

Cognitive enhancement, and the ethical considerations that go along with it, is one of the hottest current topics in the neuroscience community. Humans have sought substances to improve our cognitive function for centuries, from ancient civilizations using hallucinogens in an attempt to raise their consciousness to commune with their gods, to the rise of coffee, to the more recent development of drugs such as stimulants and glutamate activators. Some might argue, therefore, that seeking to improve ourselves is a human trait, and therefore cognitive enhancement is nothing more than our application of new scientific approaches to meet our age-old desire for self-improvement and development. However, others argue that artificially enhancing one's cognitive abilities is unfair and gives an unbeatable advantage to the richer populations who will have more ready access to the drugs (Butcher, 2003; Cakic, 2009). The issue of cognitive enhancement has even been likened to the steroid debate in sports (Cakic, 2009). There are many comprehensive reviews and articles published on the ethical concerns of cognitive enhancement; however, literature on the safety of consuming these drugs in youth is starkly lacking despite the significant increase in teen misuse and abuse of stimulants reported in a recent national study (Goldberg, 2013). Therefore, for the purpose of this review, we will concentrate on examining potential neurobiological ramifications of the popular cognitive enhancers, and highlight recent data on these drugs' actions in developing brains. It is likely that a large proportion of the population is exposed to cognitive enhancing drugs and pressure to take them may be especially high among college and high school students; these individuals are facing more stringent college and graduate school acceptance criteria, limited job pools and an ever-increasing pressure to perform better and better if they hope to succeed (Goodman, 2010; Franke et al., 2011; Lynch et al., 2011). However, individuals in this population may be the ones most likely at risk for potential neurological consequences,

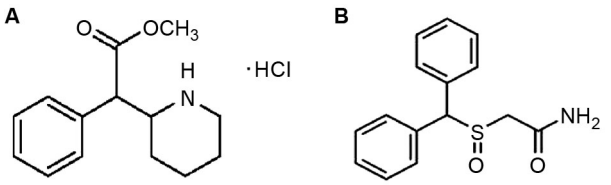

FIGURE 1 | (A) Chemical structure of methylphenidate (Ritalin©). **(B)** Chemical structure of modafinil (Provigil©). The drug bears a striking resemblance to methylphenidate and other stimulants.

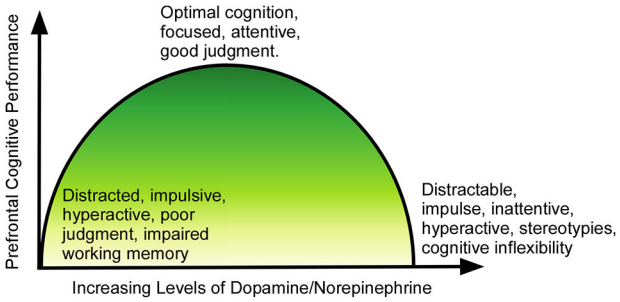

FIGURE 2 | Relationship of dopamine/norepinephrine to prefrontal function. At lower than optimal levels, the PFC is underactive, and the individual suffers from symptoms of ADHD (impulsivity, poor judgment, inattentiveness, motor hyperactivity). As levels rise, the function improves, until cognition and executive function reaches peak performance at optimal levels of dopamine/norepinephrine. As levels of the neurotransmitters continue to rise past the optimal point, cognition again becomes impaired, with the individual showing distractability, impulsivity, stereotypical behaviors and cognitive inflexibility.

due to their still-developing brains. We express regret that we are not able to cite many other good articles due to the topic specificity and sparsity of existing research; however, interesting information on cognitive enhancers that was outside the scope of this review can be found in these additional references (Dresler et al., 2013; Pang and Hannan, 2013; Ragan et al., 2013; Madan, 2014).

METHYLPHENIDATE AND THE DEVELOPING BRAIN

One of the most popular drugs under consideration for cognitive enhancement was originally developed to treat attention deficit-hyperactivity disorder (ADHD). Methylphenidate (Ritalin©; MPH; **Figure 1A**) is currently the most commonly prescribed medications for the treatment of ADHD (Challman and Lipsky, 2000; Spiller et al., 2013). MPH is a psychostimulant, related to amphetamine and cocaine and exerts its effects by blocking the transporters that reuptake dopamine and norepinephrine into the presynaptic neuron following their release; thus, it increases the levels or prolongs the availability of these neurotransmitters in the synapses to exert effects on postsynaptic neurons (Kuczenski and Segal, 2005).

However, a large proportion of literature on the safety and efficacy of MPH comes from studies performed on normal, healthy adult animals, as there is currently no sufficiently reliable animal model for ADHD. Several decades ago, studies began emerging that suggested that reduced hyperactivity and impulsivity in stimulant-treated ADHD patients were not "paradoxical" effects, but in fact also occurred in healthy individuals given the same doses (Rapoport et al., 1978, 1980). More recent MPH studies in both humans and rats have found that low doses of MPH that correspond to those given to ADHD patients in the clinic appear to enhance prefrontal-dependent functions and cognition in much the same way in healthy humans and rats as they do in ADHD patients and disease model rat strains (Mehta et al., 2001; Askenasy et al., 2007; Dow-Edwards et al., 2008; Agay et al., 2010; Linssen et al., 2012). These facts led to not only the acceptance of MPH study in normal subjects, but also the consideration of the medication as a cognitive enhancer.

The vast majority of studies on the cognitive enhancing effects of MPH and its effects on the normal brain have been performed in adult animals or humans. Higher doses (doses greater than those given to treat ADHD; 5–10 mg/kg intraperitoneal in rats) increase locomotor activity and impair attention and performance on prefrontal cortex-dependent cognitive tasks; however, lower doses (doses equivalent to the range given to ADHD patients; 0.5–2 mg/kg intraperitoneal in rats) improve cognitive performance and reduce locomotor activity in healthy individuals (Mehta et al., 2001). Likewise, lower doses of MPH (0.25–1 mg/kg, intraperitoneal, i.p.) in normal adult rats resulted in increased performance on attention tasks along with no effect on locomotor activity, while higher doses impaired performance and caused hyperactivity; doses beyond 10 mg/kg resulted in "stereotypes" (repetitive, fine motor movements similar to the tics seen in disorders like Tourette's syndrome) (Mehta et al., 2001). The low doses of MPH result in slight increases in dopamine and norepinephrine selectively in the prefrontal cortex, while not affecting other brain regions (Berridge et al., 2006). This allows for improvements in executive control and working memory (WM) without inducing locomotor activity or stereotypes.

However, the dangers of cognitive enhancement with stimulants like MPH lie in their potential effects on the regulation of dopamine and norepinephrine (**Figure 2**). At optimal doses, dopamine binds to higher-affinity D1-like receptors, and norepinephrine binds to α2 receptors, leading to an increase in prefrontal cortical signal-to-noise ratio and enhancing the flow of information and strengthening neuronal communication (Arnsten and Li, 2005). When the levels of dopamine and norepinephrine rise beyond the optimal levels, they begin to activate dopamine D2-class receptors and noradrenergic α1 and β receptors, which leads to weakening of the signal-to-noise ratio via activation of neurons that may not be involved in the current task (Arnsten and Li, 2005; Arnsten, 2009b). This nonspecific activation impairs attentional selectivity and results in a manifestation of locomotor hyperactivity, distractability and poor impulse control.

Levels of dopamine and norepinephrine in a normal, healthy brain are not universal and they may vary slightly over time within the same individual based on season, time of day, or activity (Otter and Nurmand, 1980; Petrović et al., 1980). Currently, there is no reliable method for determining optimal levels of these neurotransmitters in living human brains; thus, predicting how a certain dose of MPH will affect a particular person is largely an

educated approximation. It is also possible that, although many studies found no overt cognitive differences between the effects of low-dose MPH on normal individuals and ADHD patients, molecular or cellular differences may exist that await detection by the development of more advanced technology. Thus, one must examine the research on MPH as a cognitive enhancer and studies using normal individuals with caution.

MPH is currently most often abused and sold on the black market among adolescents, particularly in high schools and on college campuses (Goodman, 2010; Franke et al., 2011). Students look for the medication when they have exams, or need to stay awake for long hours, in order to boost their energy and memory. This rather commonplace usage among adolescents is particularly frightening in light of the developmental timeline of the prefrontal cortex. This brain region, the center of control of judgment, behavioral inhibition and emotion, WM, logical thinking and decision making, does not finish developing until young adulthood; in humans this falls around the end of the second decade or the beginning of the third decade of life (Casey et al., 2008). During adolescent development, the levels of norepinephrine and dopamine surge and wane to allow for maturation of the executive control and reward pathways (Kanitz et al., 2011). Introducing a substance that alters dopamine and norepinephrine levels, such as MPH, might disrupt the maturation of the prefrontal cortex and have lasting behavioral consequences.

Indeed, research has recently begun to shift toward understanding MPH's actions in a juvenile brain. These pioneering studies have yielded striking results, indicating that early life treatment with MPH may alter circadian rhythms, induce anxiety that persists into adulthood, and even impair object-recognition memory (Lee et al., 2009; Algahim et al., 2010). However, many of the studies have not been particularly stringent in their dosing regimens, and the reader must examine the amount of drug used in each study very carefully. In adult rats, a therapeutic, clinically-relevant dose of MPH is one that produces blood plasma levels of 8–40 ng/dL; this appears to be in the range of 0.25–1 mg/kg given in an intraperitoneal injection (i.p.) (Berridge et al., 2006). We have recently completed several studies examining the effects of a low therapeutic dose (1 mg/kg, i.p.) on juvenile rats. We reported that a single dose of MPH resulted in significant depression of neuronal excitability and synaptic transmission in the prefrontal cortex; treatment with a chronic regimen of 3 weeks resulted in even further depression (Urban et al., 2012). In adult rats, however, the same low dosage increased neuronal activity (Urban et al., 2012). These results suggest that there is an age-dependent difference in MPH's actions, and that in healthy juveniles and adolescents, the doses previously thought to be therapeutic and cognitively enhancing may in fact be inducing excessive levels of dopamine and norepinephrine and in fact impairing certain aspects of cognition. Further supporting this theory, we discovered that the depression of neuronal activity was due, at least in part, to activation of a channel known as the hyperpolarization-activated non-specific cation channel (HCN; Urban et al., 2012). The HCN channel allows for flow of positively-charged ions, particularly potassium, out of the neuron, lowering its voltage potential and making it harder for the neuron to fire action potentials. The HCN channel is also known to be activated by a hyperdopaminergic state; thus, its role in juvenile treatment with MPH suggests that the dosage is inducing excessive dopamine, and possibly norepinephrine as well (Arnsten, 2009a).

One important unique property of the prefrontal cortex is its high level of plasticity, allowing for executive functions like WM and active decision-making; this plasticity may be a product of the slow maturation of this region (Jernigan et al., 1991; Kuboshima-Amemori and Sawaguchi, 2007; Spencer-Smith and Anderson, 2009; Newman and McGaughy, 2011; Teffer and Semendeferi, 2012; Selemon, 2013). Plasticity is controlled by levels of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and n-methyl-d-aspartate (NMDA) receptors. NMDA receptors contain two NR1 subunits with a combination of either NR2A or NR2B; NR2B conveys slower kinetics to the channel, allowing it to remain partially open during multiple stimulations (Cull-Candy et al., 2001). This property results in summation of responses and the continuation of the neural activity briefly after input has stopped, which is thought to be the neuronal correlate of WM (Wang et al., 2008, 2013). In most cortical brain regions, the ratio of NR2B/NR2A is high at birth, and declines over development; however, in prefrontal cortex it remains high (Wang et al., 2008). This allows for retention of plasticity throughout life, allowing the prefrontal cortex to continually adapt to incoming information and adjust behavioral output. We recently discovered that low dose (1 mg/kg, i.p.) treatment of juvenile rats with MPH induced a selective decrease in the levels of NR2B without affecting NR2A subunits (Urban et al., 2013). This finding supports our theory that the juvenile brain may be hypersensitive to dopamine levels; excessive levels of dopamine induce internalization of NR2B receptors via activation of glycogen synthase kinase (GSK)-3β, which causes phosphorylation of β-catenin, disrupting the β-catenin-NR2B interaction that stabilizes the NR2B subunit (Li et al., 2009). With β-catenin unbound, the NR2B subunits become targeted for internalization.

What do our findings mean for the healthy adolescent taking MPH? The prefrontal cortex's uniquely high levels of NR2B subunits throughout life impart the ability of the neurons to summate responses to incoming stimuli, resulting in the short-term potentiation of neural activity necessary for WM; thus, decreasing the levels of NR2B in prefrontal cortex leads to a reduction in the summation, which should impair WM (Wang et al., 2008, 2013; Urban et al., 2013). However, long-term potentiation (LTP) was found to be enhanced following juvenile treatment with MPH (Urban et al., 2013). The exact roles of NR2A versus NR2B receptor subunits in LTP regulation in the prefrontal cortex are not well understood, but it is currently believed that the direction of plasticity in prefrontal cortex (potentiation or depression) is dependent on the ratio of NR2A/NR2B, rather than exact levels of each subunit (Massey et al., 2004; Xu et al., 2009; Foster et al., 2010). Thus, reducing NR2B levels without altering NR2A levels, as was seen following juvenile MPH treatment, was enough to alter the direction of PFC long-term plasticity (Urban et al., 2013). The behavioral ramifications of altering LTP and long-term depression (LTD) in the prefrontal cortex are unclear, as it is not known exactly what LTP is representing in this region. However, it has been hypothesized that, if short-term potentiation is a cellular constituent of WM, then LTP might be a marker of

sustained attention and long-term memory consolidation. Thus, perhaps treatment of the healthy juvenile brain with these low doses of MPH results in impaired WM and behavioral flexibility, but enhanced sustained attention and long-term memory? If this is the case, it could indicate that MPH-treated children who do not in fact have ADHD would appear successfully treated in a classroom setting—these children would be paying attention to the teacher, less hyperactive and learning might improve. However, stringent testing of their behavioral flexibility and WM might reveal subtle deficits that may affect their lives. For example, behavioral flexibility is needed for driving an automobile—the driver must be able to quickly shift attention from the road, to road signs, other approaching vehicles, back to the road and so on. Rigid attention and lack of flexibility could potentially result in inattentive or distracted driving. Behavioral flexibility is also a critical component of interpersonal skills; one must be able to adapt to different individuals and, in a work setting, shift plans and roles within the group in order to achieve the goal. Again, behavioral and cognitive inflexibility could impair the individual's function at their job and lead to reduced pay, unemployment or disciplinary action. Finally, behavioral flexibility is a critical component of resisting and recovering from drug abuse. Kalivas and Volkow identified alterations in glutamatergic signaling that result in an inability to alter one's behavior in cocaine addicts (Kalivas and Volkow, 2005, 2011; Kalivas et al., 2005). MPH has been shown to reduce the likelihood of later drug abuse in individuals diagnosed with ADHD, but, as the drug appears to affect glutamatergic signaling, it could potentially result in similar behavioral rigidity and lead to an increased likelihood for obsessive-compulsive or addictive behaviors (Newman and McGaughy, 2011).

MODAFINIL—POTENTIAL FOR STIMULANT-LIKE EFFECTS?

MPH's effects on brain development are surely interesting and potentially frightening; however, it is not the only cognitive enhancing medication that alters dopamine and glutamate transmission. Another cognitive enhancer that has begun to receive attention in the scientific community is modafinil (Provigil©), which bears a striking structural resemblance to methylphenidate (MPH) and other stimulants (**Figure 1B**). Originally developed in France in the 1970s, modafinil elevates hypothalamic histamine levels, but also appears to have a striking affinity for cell surface dopamine transporters (Engber et al., 1998; Ishizuka et al., 2008; Zolkowska et al., 2009). Its exact mechanism of action remains under debate, although arguments have been made both for its performing more as a wakefulness-promoting reagent via the hypocretin/orexin system of the hypothalamus, and as a classical psychostimulant via its blockade of the dopamine reuptake inhibitor (Ishizuka et al., 2003; Zolkowska et al., 2009). However, modafinil still promotes wakefulness in orexin knockout mice, so it appears that the orexin system is not required for therapeutic benefits (Willie et al., 2005). Whatever the mechanism, or mechanisms, of action may turn out to be, modafinil is currently a heavily studied drug with multiple uses. It is currently approved by the US Food and Drug Administration (FDA) for the treatment of narcolepsy, shift-work disorder and obstructive sleep apnea (Erman and Rosenberg, 2007; Cephalon, 2013). It has been shown to reduce jet lag and improve mood among shift workers, who often struggle with depression and chronic fatigue, forgetfulness and general cognitive impairments brought on by their work hours not allowing for a steady sleep-wake cycle (O'Connor, 2004; Hart et al., 2006). Modafinil has also been studied as an alternative to amphetamines for military usage—the military provides stimulants to soldiers in sleep-deprivation or high stress situations that require extreme alertness for long stretches of time. It is currently approved for Air Force missions in the US, and is also used in the UK and India (Taylor and Keys, 2003; Wheeler, 2006; Sharma, 2011).

Although modafinil is considered a first-line therapy for excessive daytime sleepiness (EDS) associated with narcolepsy in adults; it is also widely used in the treatment of EDS in children (Ivanenko et al., 2003; Sullivan, 2012). Caution is again the rule, especially at younger ages, due to reports of serious adverse events (such as tachycardia, insomnia, agitation, dizziness and anxiety) in elevated modafinil doses (Spiller et al., 2009), and in fact, the manufacturer recommends against use of modafinil in younger children. Despite these reports, modafinil is FDA-approved for use in children over age 16 years (Sullivan, 2012).

The usefulness of modafinil in improving alertness and wakefulness in non-sleep-deprived, healthy individuals, and its military involvement, has led to the consideration of the drug as a cognitive enhancer (Turner et al., 2003; Baranski et al., 2004; Randall et al., 2005b). Most studies agree that modafinil induces improvements in pattern recognition memory, digit span recall and mental digit manipulation (performing addition/subtraction/multiplication in one's mind), but the effects on spatial memory, attention and other aspects of executive function are more ambiguous, and appear to depend on the baseline performance of the individual in question (Turner et al., 2003; Baranski et al., 2004; Müller et al., 2004; Randall et al., 2005b). In a study of healthy student volunteers, modafinil improved target sensitivity in a rapid visual information processing (RVIP) task, and speed of color naming and drawing, but only in individuals with a "low" (mean 106 + 6) IQ; it had no significant effect on individuals with "higher" (mean 115 + 5) IQs (Randall et al., 2005a). In rats, these results are replicated, with low responding rats showing improvement on stop-signal reaction time tests after modafinil; higher performing rats showed no improvement (Eagle et al., 2007). Interestingly, MPH also shows sensitivity to baseline performance; many studies have indicated that MPH induces greater improvement in low-performing individuals than in higher performing individuals, and in some cases may actually cause deficits in higher performers (Eagle et al., 2007; Finke et al., 2010).

A recent study conducted in healthy human subjects reported that modafinil differs from other arousal-enhancing agents in chemical structure, neurochemical profile, and behavioral effects (Rasetti et al., 2010). Unlike most functional neuroimaging studies that focused on the effect of modafinil only on information processing underlying executive cognition, this study examined the effect of modafinil on neural circuits underlying affective processing and cognitive functions. They underwent

blood-oxygen-level dependent (BOLD) functional magnetic resonance imaging (MRI, or functional MRI, fMRI) while performing an emotion information-processing task that activates the amygdala and two prefrontally dependent cognitive tasks—a WM task and a variable attentional control (VAC) task. BOLD fMRI revealed significantly decreased amygdala reactivity to fearful stimuli on modafinil compared with the placebo condition. During executive cognition tasks, a WM task and a VAC task, modafinil reduced BOLD signal in the prefrontal cortex and anterior cingulate. This study suggested that modafinil in low doses has a unique physiological profile compared with stimulant drugs: it enhances the efficiency of prefrontal cortical cognitive information processing, while dampening reactivity to threatening stimuli in the amygdala, a brain region implicated in anxiety (Rasetti et al., 2010).

The baseline performance sensitivity, and dopamine reuptake transporter affinity, indicates that modafinil could induce similar effects on the brain as psychostimulants like MPH. If this is the case, cause for concern arises when modafinil is considered as a cognitive enhancer in adolescents and young adults. To enlist in the Air Force, where modafinil is currently in use for pilots, one must be between 17–27 years of age (U. S. Air Force, 2013). The prefrontal cortex, under tight regulation by levels of dopamine and norepinephrine, and the brain's main center of attention and executive processing, does not finish development until the late 20's to early 30's for humans; thus, young pilots may be at risk for modafinil inducing excessive levels of dopamine in this brain region (Casey et al., 2008). One can expect that the potential ramifications of modafinil use in healthy young adults and teenagers would be similar to those seen in juvenile/adolescent use of MPH (Urban et al., 2012, 2013). Thus, modafinil could induce changes in plasticity or behavioral rigidity, and potentially damage WM, logical thinking and decision making. It has been reported that prolonged wakefulness induces experience-dependent synaptic plasticity in mouse hypocretin/orexin neurons (Rao et al., 2007). Specifically, acute and chronic prolonged wakefulness in mice induced by modafinil treatment produced LTP of glutamatergic synapses on hypocretin/orexin neurons in the lateral hypothalamus, a well-established arousal/wake-promoting center. A similar potentiation of synaptic strength at glutamatergic synapses on hypocretin/orexin neurons was also seen when mice were sleep deprived for 4 h. These results indicate that synaptic plasticity due to prolonged wakefulness occurs in circuits responsible for arousal and may contribute to changes in the brain of animals experiencing sleep loss. It is therefore likely that misuse and abuse of modafinil in the teens will eventually result in brain plasticity, especially brain regions related to sleep and motivation such as hypothalamus and dopamine-rich prefrontal cortex, hippocampus and nucleus accumbens. Future studies will need to address these shortcomings in order to determine the safety and efficacy of modafinil as a true cognitive enhancer. Recent reviews proposed some interesting mechanisms that may explain the likelihood of cognitive enhancement (Lynch et al., 2011; Roesler and Schröder, 2011; Lynch and Gall, 2013) but experiments are warranted for further exploration. The current research is contradictory in that some studies have noted clear improvements in sustained attention in humans, while others have failed to find any effect of the drug (Turner et al., 2003; Randall et al., 2005b). Similar discrepancies can be found in rodent studies; however, more recent studies are pointing to the possibility that modafinil selectively enhances WM without affecting consolidation of memories into long-term storage (Béracochéa et al., 2002; Turner et al., 2003; Müller et al., 2004; Randall et al., 2005b; Minzenberg and Carter, 2008). These studies are interesting, and suggest striking utility of modafinil as a cognitive enhancer; however, they have been performed on adult humans and rodents. MPH has also been shown in studies of healthy adults and children with ADHD to apparently enhance WM (Mehta et al., 2004; Pietrzak et al., 2006; Kobel et al., 2009; Marquand et al., 2011), yet recent juvenile rat studies suggest that in a healthy, developing brain, the drug might actually impair WM at low doses thought to be clinically relevant, i.e., doses that produce blood plasma levels of 8–40 ng/dL (Urban et al., 2013). Modafinil's profile by showing improvements in WM in healthy adults and sleep-deprived individuals (the population the drug was originally developed for) is analogous to MPH promoting improvements for healthy adults and children with ADHD; since both drugs appear to affect dopamine levels through blockade of the reuptake transporters, and alter glutamate signaling, it stands to reason that they could result in similar effects on WM in healthy, juvenile brains. Thus, modafinil at certain doses might cause a reduction in NMDA receptor levels, impairments in short-term plasticity and alterations in long-term plasticity much as MPH does (Urban et al., 2013). Future studies of modafinil as a cognitive enhancer should examine this possibility, and establish whether the drug shows an age- and dose-dependent profile of effects like the classic psychostimulants.

AMPAKINES—DRUGS FOR TREATMENT OF ALZHEIMER'S DISEASE—TURNED COGNITIVE ENHANCERS

The final classes of medications we will discuss in this review are the ampakines, which also have potential for significant effects on the developing glutamatergic system. Ampakines are a class of drugs that bind to the glutamatergic AMPA receptor, enhancing its activity by slowing deactivation and attenuating desensitization of AMPA receptor currents, increasing synaptic responses and enhancing LTP (Arai and Kessler, 2007). AMPA receptors are critically involved in regulating cortical plasticity; trafficking of AMPA receptors to the synapse is crucial for maintenance of excitability that leads to LTP (Malinow and Malenka, 2002; Huganir and Nicoll, 2013). However, there is more to the story of how AMPA regulates excitability; it does not function alone in the process. A second class of ionotropic glutamate receptors, NMDA receptors, actually trigger the induction of LTP; however, these receptors are normally blocked by magnesium at resting membrane potentials (Dingledine et al., 1999; Cull-Candy et al., 2001; Paoletti et al., 2013). Activation of AMPA receptors induces EPSCs, which depolarize the neuron and remove the magnesium block of NMDA, allowing for the induction of LTP. Then, NMDA receptors increase trafficking of more AMPA receptors to the synapse, maintaining the LTP (Lu et al., 2001; Paoletti et al., 2013). No ampakines are currently FDA approved, but they are being investigated as treatments for Alzheimer's senility, Parkinson's disease, ADHD, Rhett syndrome,

schizophrenia, depression, autism, and Angelman syndrome (AS; Goff et al., 2001; Arai and Kessler, 2007; Ogier et al., 2007; Wezenberg et al., 2007; Simmons et al., 2009; Baudry et al., 2012; Silverman et al., 2013). However, they've also shown effectiveness at improving memory and cognition in healthy adult volunteers and rats (Ingvar et al., 1997; Hampson et al., 1998; Lynch and Gall, 2006; Wezenberg et al., 2007). Ampakines are also being studied by the US military for use as cognitive enhancers and alertness promoters for soldiers in high-stress extended combat situations; the lack of central nervous stimulation (such as would occur with modafinil, amphetamines or MPH make the ampakines very attractive (Saletan, 2008). Although ampakines have few adverse effects at therapeutically relevant concentrations and protect neurons against neurotoxic insults in adults (Arai and Kessler, 2007), the ampakine faramptor can cause headache, somnolence and nausea (Wezenberg et al., 2007).

While the ampakines represent perhaps the most promising group of pharmaceuticals for low-risk cognitive enhancement, as well as a potential relief for sufferers of psychiatric illnesses, they are likely not without danger to teens, adolescents, and young adults. First, very little is known about these drugs; the only example to reach human clinical trials is Cortex Pharmaceuticals' CX-717, which was evaluated in Phase I for the treatment of Alzheimer's disease; histological damage was seen in animal studies but Cortex claimed this was an artifact of tissue fixation (Stoll and Griesel, 2007). The FDA denied the application, and CX-717 approval halted. None of the other ampakines is known to currently be in human trials, so little can be proven about their efficacy or safety in healthy individuals. However, we can speculate based on knowledge of plasticity and the glutamate system.

The first concern when stimulating glutamate transmission in the brain is the potential for excitotoxicity. Glutamate toxicity generally occurs when excess glutamate storms the AMPA and NMDA receptors, causing a mass influx of calcium. This excess calcium in the cells activates a number of enzymes like proteases and phospholipases, which induce damage to organelles, the cell membrane, and DNA (Manev et al., 1989; Ankarcrona et al., 1995). However, activating AMPA receptors directly would cause a similar mass influx of cations and could also induce excitotoxicity. A recent study reported that ampakines promote spine actin polymerization, LTP, and learning in a mouse model of AS (Baudry et al., 2012). AS is a neurodevelopmental disorder largely due to abnormal maternal expression of the UBE3A gene leading to the deletion of E6-associated protein. AS subjects have severe cognitive impairments for which there are no therapeutic interventions. Mouse models (knockouts of the maternal UBE3A gene: "AS mice") of the disorder have substantial deficits in LTP and learning. Baudry et al reported that ampakine CX929 significantly enhanced LTP and notably, reduced dendritic spine abnormality and learning impairments (Baudry et al., 2012). This minimally invasive drug treatment is certainly promising for AS, and probably other neurodevelopmental disorders such as fragile X syndrome and autism (Rueda et al., 2009; Silverman et al., 2013) as well. However, such a magnitude of effects on synaptic plasticity and dendritic spine integrity also raises serious concern for immature brains of young children using ampakines as cognitive enhancers. It is not difficult to imagine that ampakines would have similar effects on the synaptic transmission and neuronal communication in the normal brain, eventually eliciting brain plasticity in the regions that are associated with emotional and affective functions. This could potentially lead to poor emotional regulation and impaired behavioral inhibition if plasticity is excessive and unregulated. Indeed, one of the important mechanisms by which the brain connections are maintained and tuned is through synaptic pruning, whereby highly active synapses are strengthened and less active synapses are removed through axon retraction (Luo and O'Leary, 2005; Gazzaniga and Mangum, 2009; Kolb et al., 2012). At first thought, heightened plasticity might seem to be a benefit—translating to faster learning and improved cognitive function; however, the excessive plasticity could also lead to high activity in all synapses and therefore reduce synaptic pruning. Impairments in synaptic pruning have in fact been associated with autistic spectrum disorders (Belmonte et al., 2004). The excessive connectivity leads to a heightened overall brain activation but the reduction in selectivity of activation is such that the signal-to-noise ratio is greatly lowered (Belmonte, 2000; Belmonte and Yurgelun-Todd, 2003). Thus, one can clearly see the potential dangers associated with unregulated plasticity, and how ampakines (which strengthen synapses and heighten plasticity by promoting dendritic spine growth) might lead to autism-like syndromes.

Although no studies have yet noted this in humans, doses of ampakines given to humans thus far have been tightly controlled. If the drug became available as a cognitive enhancer, or reached the black market, individuals could easily exceed safe doses and suffer neuronal damage from glutamate toxicity. Furthermore, the main purported therapeutic action of the ampakines is an alteration of plasticity; they are known to lower the threshold for induction of LTP and also increase the magnitude of LTP achieved (Lynch and Gall, 2006). While this alteration of plasticity may improve many aspects of learning and cognition, such as alertness, enhancement of LTP will likely come with a concomitant decrease in the opposite direction of plasticity, i.e., LTD. LTD is crucial for formation of spatial maps, and might play a role in cerebellar motor learning as well (although studies of motor performance after LTD impairment have been somewhat contradictory) (Aiba et al., 1994; Manahan-Vaughan, 2005; Kemp and Manahan-Vaughan, 2007). Thus, shifting plasticity in favor of LTP could lead to impairments in spatial memory and perhaps motor function. Careful determination of a dose-response curve, excitotoxic effects and species differences in metabolism/reaction to ampakines will need to be completed in the future in order to determine their true utility as cognitive enhancers.

CONCLUSION AND FUTURE PERSPECTIVE

In this review, we have examined three major pharmaceuticals under consideration as cognitive enhancers—MPH, modafinil and the ampakines. We have reported striking and deeply concerning effects of clinically relevant doses of MPH on the juvenile prefrontal cortex function and plasticity, compared them to the potential ramifications of modafinil treatment, and suggested several potential risks of ampakine exposure in healthy individuals. It is clear from the current lack of research in the field that

much work needs to be done in order to determine the safety of cognitive enhancers, particularly among adolescents, the population most likely to take advantage of these drugs should they become available. There is already a high demand on college campuses and in high schools for MPH; thus, many healthy adolescents and young adults are already being exposed to unregulated doses of this substance. Understanding the behavioral and functional ramifications in cellular and molecular changes in the yet immature brains is paramount to mitigating risks for potential brain plasticity and consequent emotional and behavioral changes (Urban and Gao, 2012, 2013).

It is currently unclear if the dose range of stimulants that translates to effective ADHD symptom alleviation and cognitive enhancement in the healthy adult will translate to the same behavioral effects in juveniles; however, our recent studies suggest that the juvenile brain is hypersensitive to the effects of MPH (Urban et al., 2012). Thus, even a low, purportedly clinically relevant dose is likely to cause excessive levels of dopamine and norepinephrine, and impair executive functions and WM. This excessive dopamine/norepinephrine is likely also a potential risk of juvenile treatment with modafinil. It is far less clear how the ampakines might affect juvenile brain function, but their effects on plasticity through the glutamatergic system warrants further exploration. The desire for development of cognitive enhancing substances is unlikely to diminish with time; it may represent the next stage in evolution—man's desire for self-improvement driving artificial enhancement of innate abilities. It is therefore the responsibility of scientists and the medical community to stringently evaluate and research each new candidate substance, furthering our understanding of the brain in the process. Perhaps most importantly, the role of age and developmental stage in individual responses to cognitive enhancing substances needs to be thoroughly examined. Juvenile metabolic rates compared to adult are not clear in humans or rodent models; the dose-response curve for juveniles compared to adults for MPH, modafinil and the ampakines, as well as many other psychoactive medications, has not been examined. Finally, a potential long-term ramification of early life exposure of the healthy juvenile brain to these substances is only a very recent emerging topic of research, and much care needs to be taken to answer the questions expediently. Cognitive enhancement is no longer a scientific fiction; we must consider the unique dynamics of the developing brain and proceed cautiously until thorough safety and efficacy parameters have been established.

ACKNOWLEDGMENTS

This study was supported by grant R01MH085666 to Wen-Jun Gao from the National Institutes of Health, USA.

REFERENCES

Agay, N., Yechiam, E., Carmel, Z., and Levkovitz, Y. (2010). Non-specific effects of methylphenidate (Ritalin) on cognitive ability and decision-making of ADHD and healthy adults. *Psychopharmacology (Berl)* 210, 511–519. doi: 10.1007/s00213-010-1853-4

Aiba, A., Kano, M., Chen, C., Stanton, M. E., Fox, G. D., Herrup, K., et al. (1994). Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 79, 377–388. doi: 10.1016/0092-8674(94)90205-4

Algahim, M. F., Yang, P. B., Burau, K. D., Swann, A. C., and Dafny, N. (2010). Repetitive ritalin treatment modulates the diurnal activity pattern of young SD male rats. *Cent. Nerv. Syst. Agents Med. Chem.* 10, 247–257. doi: 10.2174/1871524911006030247

Ankarcrona, M., Dypbukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A., et al. (1995). Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* 15, 961–973. doi: 10.1016/0896-6273(95)90186-8

Arai, A. C., and Kessler, M. (2007). Pharmacology of ampakine modulators: from AMPA receptors to synapses and behavior. *Curr. Drug Targets* 8, 583–602. doi: 10.2174/138945007780618490

Arnsten, A. F. (2009a). Stress signalling pathways that impair prefrontal cortex structure and function. *Nat. Rev. Neurosci.* 10, 410–422. doi: 10.1038/nrn2648

Arnsten, A. F. (2009b). Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs* 1(Suppl. 1), 33–41. doi: 10.2165/00023210-200923000-00005

Arnsten, A. F., and Li, B. M. (2005). Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions. *Biol. Psychiatry* 57, 1377–1384. doi: 10.1016/j.biopsych.2004.08.019

Askenasy, E. P., Taber, K. H., Yang, P. B., and Dafny, N. (2007). Methylphenidate (Ritalin): behavioral studies in the rat. *Int. J. Neurosci.* 117, 757–794. doi: 10.1080/00207450600910176

Baranski, J. V., Pigeau, R., Dinich, P., and Jacobs, I. (2004). Effects of modafinil on cognitive and meta-cognitive performance. *Hum. Psychopharmacol.* 19, 323–332. doi: 10.1002/hup.596

Baudry, M., Kramar, E., Xu, X., Zadran, H., Moreno, S., Lynch, G., et al. (2012). Ampakines promote spine actin polymerization, long-term potentiation and learning in a mouse model of Angelman syndrome. *Neurobiol. Dis.* 47, 210–215. doi: 10.1016/j.nbd.2012.04.002

Belmonte, M. (2000). Abnormal attention in autism shown by steady-state visual evoked potentials. *Autism* 4, 269–285. doi: 10.1177/1362361300004003004

Belmonte, M. K., Allen, G., Bekel-Mitchener, A., Boulanger, L. M., Carper, R. A., and Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *J. Neurosci.* 24, 9228–9231. doi: 10.1523/jneurosci.3340-04.2004

Belmonte, M. K., and Yurgelun-Todd, D. A. (2003). Functional anatomy of impaired selective attention and compensatory processing in autism. *Brain Res. Cogn. Brain Res.* 17, 651–664. doi: 10.1016/s0926-6410(03)00189-7

Béracochéa, D., Celerier, A., Borde, N., Valleau, M., Peres, M., and Pierard, C. (2002). Improvement of learning processes following chronic systemic administration of modafinil in mice. *Pharmacol. Biochem. Behav.* 73, 723–728. doi: 10.1016/s0091-3057(02)00877-8

Berridge, C. W. D., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol. Psychiatry* 60, 1111–1120. doi: 10.1016/j.biopsych.2006.04.022

Butcher, J. (2003). Cognitive enhancement raises ethical concerns. Academics urge pre-emptive debate on neurotechnologies. *Lancet* 362, 132–133. doi: 10.1016/s0140-6736(03)13897-4

Cakic, V. (2009). Smart drugs for cognitive enhancement: ethical and pragmatic considerations in the era of cosmetic neurology. *J. Med. Ethics* 35, 611–615. doi: 10.1136/jme.2009.030882

Casey, B. J., Jones, R. M., and Hare, T. A. (2008). The adolescent brain. *Ann. N Y Acad. Sci.* 1124, 111–126. doi: 10.1196/annals.1440.010

Cephalon, I. (2013). Provigil(R)-modafinil. As accessed 10-22-2013. www.provigil.com

Challman, T. D., and Lipsky, J. J. (2000). Methylphenidate: its pharmacology and uses. *Mayo Clin. Proc.* 75, 711–721. doi: 10.4065/75.7.711

Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335. doi: 10.1016/s0959-4388(00)00215-4

Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61.

Dow-Edwards, D. L., Weedon, J. C., and Hellmann, E. (2008). Methylphenidate improves performance on the radial arm maze in periadolescent rats. *Neurotoxicol. Teratol.* 30, 419–427. doi: 10.1016/j.ntt.2008.04.001

Dresler, M., Sandberg, A., Ohla, K., Bublitz, C., Trenado, C., Mroczko-Wasowicz, A., et al. (2013). Non-pharmacological cognitive enhancement. *Neuropharmacology* 64, 529–543. doi: 10.1016/j.neuropharm.2012.07.002

Eagle, D. M., Tufft, M. R., Goodchild, H. L., and Robbins, T. W. (2007). Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology (Berl)* 192, 193–206. doi: 10.1007/s00213-007-0701-7

Engber, T. M., Koury, E. J., Dennis, S. A., Miller, M. S., Contreras, P. C., and Bhat, R. V. (1998). Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. *Neurosci. Lett.* 241, 95–98. doi: 10.1016/s0304-3940(97)00962-2

Erman, M. K., and Rosenberg, R., Modafinil Shift Work Sleep Disorder Study Group. (2007). Modafinil for excessive sleepiness associated with chronic shift work sleep disorder: effects on patient functioning and health-related quality of life. *Prim. Care Companion J. Clin. Psychiatry* 9, 188–194. doi: 10.4088/pcc.v09n0304

Finke, K., Dodds, C. M., Bublak, P., Regenthal, R., Baumann, F., Manly, T., et al. (2010). Effects of modafinil and methylphenidate on visual attention capacity: a TVA-based study. *Psychopharmacology (Berl)* 210, 317–329. doi: 10.1007/s00213-010-1823-x

Foster, K. A., McLaughlin, N., Edbauer, D., Phillips, M., Bolton, A., Constantine-Paton, M., et al. (2010). Distinct roles of NR2A and NR2B cytoplasmic tails in long-term potentiation. *J. Neurosci.* 30, 2676–2685. doi: 10.1523/jneurosci.4022-09.2010

Franke, A. G., Bonertz, C., Christmann, M., Huss, M., Fellgiebel, A., Hildt, E., et al. (2011). Non-medical use of prescription stimulants and illicit use of stimulants for cognitive enhancement in pupils and students in Germany. *Pharmacopsychiatry* 44, 60–66. doi: 10.1055/s-0030-1268417

Gazzaniga, M. S., and Mangum, G. R. (2009). *Cognitive Neuroscience: The Biology of the Mind*. New York, NY: W. W. Norton and Company.

Goff, D. C., Leahy, L., Berman, I., Posever, T., Herz, L., Leon, A. C., et al. (2001). A placebo-controlled pilot study of the ampakine CX516 added to clozapine in schizophrenia. *J. Clin. Psychopharmacol.* 21, 484–487. doi: 10.1097/00004714-200110000-00005

Goldberg, C. (2013). National study: teen misuse and abuse of prescription drugs up 33 percent since 2008 stimulants contributing to sustained Rx epidemic. Newsroom, The Partnership at DrugFree.org. Accessed 11-1-2013. http://www.drugfree.org/newsroom

Goodman, R. (2010). Cognitive enhancement, cheating, and accomplishment. *Kennedy Inst. Ethics J.* 20, 145–160. doi: 10.1353/ken.0.0309

Hampson, R. E., Rogers, G., Lynch, G., and Deadwyler, S. A. (1998). Facilitative effects of the ampakine CX516 on short-term memory in rats: enhancement of delayed-nonmatch-to-sample performance. *J. Neurosci.* 18, 2740–2747.

Hart, C. L., Haney, M., Vosburg, S. K., Comer, S. D., Gunderson, E., and Foltin, R. W. (2006). Modafinil attenuates disruptions in cognitive performance during simulated night-shift work. *Neuropsychopharmacology* 31, 1526–1536. doi: 10.1038/sj.npp.1300991

Huganir, R. L., and Nicoll, R. A. (2013). AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80, 704–717. doi: 10.1016/j.neuron.2013.10.025

Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G. A., et al. (1997). Enhancement by an ampakine of memory encoding in humans. *Exp. Neurol.* 146, 553–559. doi: 10.1006/exnr.1997.6581

Ishizuka, T., Murakami, M., and Yamatodani, A. (2008). Involvement of central histaminergic systems in modafinil-induced but not methylphenidate-induced increases in locomotor activity in rats. *Eur. J. Pharmacol.* 578, 209–215. doi: 10.1016/j.ejphar.2007.09.009

Ishizuka, T., Sakamoto, Y., Sakurai, T., and Yamatodani, A. (2003). Modafinil increases histamine release in the anterior hypothalamus of rats. *Neurosci. Lett.* 339, 143–146. doi: 10.1016/s0304-3940(03)00006-5

Ivanenko, A., Tauman, R., and Gozal, D. (2003). Modafinil in the treatment of excessive daytime sleepiness in children. *Sleep Med.* 4, 579–582. doi: 10.1016/s1389-9457(03)00162-x

Jernigan, T. L., Trauner, D. A., Hesselink, J. R., and Tallal, P. A. (1991). Maturation of human cerebrum observed in vivo during adolescence. *Brain* 114(Pt. 5), 2037–2049. doi: 10.1093/brain/114.5.2037

Kalivas, P. W., and Volkow, N. D. (2005). The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* 162, 1403–1413. doi: 10.1176/appi.ajp.162.8.1403

Kalivas, P. W., and Volkow, N. D. (2011). New medications for drug addiction hiding in glutamatergic neuroplasticity. *Mol. Psychiatry* 16, 974–986. doi: 10.1038/mp.2011.46

Kanitz, E., Otten, W., Hameister, T., Tuchscherer, M., Puppe, B., and Tuchscherer, A. (2011). Age-related changes in corticosteroid receptor expression and monoamine neurotransmitter concentrations in various brain regions of postnatal pigs. *J. Neurosci. Res.* 89, 1134–1141. doi: 10.1002/jnr.22621

Kalivas, P. W., Volkow, N., and Seamans, J. (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron* 45, 647–650. doi: 10.1016/j.neuron.2005.02.005

Kemp, A., and Manahan-Vaughan, D. (2007). Hippocampal long-term depression: master or minion in declarative memory processes? *Trends Neurosci.* 30, 111–118. doi: 10.1016/j.tins.2007.01.002

Kobel, M., Bechtel, N., Weber, P., Specht, K., Klarhofer, M., Scheffler, K., et al. (2009). Effects of methylphenidate on working memory functioning in children with attention deficit/hyperactivity disorder. *Eur. J. Paediatr. Neurol.* 13, 516–523. doi: 10.1016/j.ejpn.2008.10.008

Kolb, B., Mychasiuk, R., Muhammad, A., Li, Y., Frost, D. O., and Gibb, R. (2012). Experience and the developing prefrontal cortex. *Proc. Natl. Acad. Sci. U S A* 109, 17186–17193. doi: 10.1073/pnas.1121251109

Kuboshima-Amemori, S., and Sawaguchi, T. (2007). Plasticity of the primate prefrontal cortex. *Neuroscientist* 13, 229–240. doi: 10.1177/1073858406298554

Kuczenski, R., and Segal, D. S. (2005). Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse. *Biol. Psychiatry* 57, 1391–1396. doi: 10.1016/j.biopsych.2004.12.036

Lee, M. J., Yang, P. B., Wilcox, V. T., Burau, K. D., Swann, A. C., and Dafny, N. (2009). Does repetitive Ritalin injection produce long-term effects on SD female adolescent rats? *Neuropharmacology* 57, 201–207. doi: 10.1016/j.neuropharm.2009.06.008

Li, Y. C., Xi, D., Roman, J., Huang, Y. Q., and Gao, W. J. (2009). Activation of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibition of synaptic NMDA receptor function in the rat prefrontal cortex. *J. Neurosci.* 29, 15551–15563. doi: 10.1523/JNEUROSCI.3336-09.2009

Linssen, A. M., Vuurman, E. F., Sambeth, A., and Riedel, W. J. (2012). Methylphenidate produces selective enhancement of declarative memory consolidation in healthy volunteers. *Psychopharmacology (Berl)* 221, 611–619. doi: 10.1007/s00213-011-2605-9

Lu, W., Man, H., Ju, W., Trimble, W. S., MacDonald, J. F., and Wang, Y. T. (2001). Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* 29, 243–254. doi: 10.1016/s0896-6273(01)00194-5

Luo, L., and O'Leary, D. D. (2005). Axon retraction and degeneration in development and disease. *Annu. Rev. Neurosci.* 28, 127–156. doi: 10.1146/annurev.neuro.28.061604.135632

Lynch, G., and Gall, C. M. (2006). Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci.* 29, 554–562. doi: 10.1016/j.tins.2006.07.007

Lynch, G., and Gall, C. M. (2013). Mechanism based approaches for rescuing and enhancing cognition. *Front. Neurosci.* 7:143. doi: 10.3389/fnins.2013.00143

Lynch, G., Palmer, L. C., and Gall, C. M. (2011). The likelihood of cognitive enhancement. *Pharmacol. Biochem. Behav.* 99, 116–129. doi: 10.1016/j.pbb.2010.12.024

Madan, C. R. (2014). Augmented memory: a survey of the approaches to remembering more. *Front. Syst. Neurosci.* 8:30. doi: 10.3389/fnsys.2014.00030

Malinow, R., and Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* 25, 103–126. doi: 10.1146/annurev.neuro.25.112701.142758

Manahan-Vaughan, D. (2005). "Hippocampal long-term depression as a declarative memory mechanism. in: H.E.S.," in *Synaptic Plasticity and Transsynaptic Signaling Scharfman*, eds P. K. Scharfman and C. Bramham (Springer: Berlin), 305–319.

Manev, H., Favaron, M., Guidotti, A., and Costa, E. (1989). Delayed increase of Ca2+ influx elicited by glutamate: role in neuronal death. *Mol. Pharmacol.* 36, 106–112.

Marquand, A. F., De Simoni, S., O'Daly, O. G., Williams, S. C., Mourao-Miranda, J., and Mehta, M. A. (2011). Pattern classification of working memory networks reveals differential effects of methylphenidate, atomoxetine and placebo in healthy volunteers. *Neuropsychopharmacology* 36, 1237–1247. doi: 10.1038/npp.2011.9

Massey, P. V. J., Johnson, B. E., Moult, P. R., Auberson, Y. P., Brown, M. W., Molnar, E., et al. (2004). Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J. Neurosci.* 24, 7821–7828. doi: 10.1523/jneurosci.1697-04.2004

Mehta, M. A., Goodyer, I. M., and Sahakian, B. J. (2004). Methylphenidate improves working memory and set-shifting in AD/HD: relationships to baseline memory capacity. *J. Child Psychol. Psychiatry* 45, 293–305. doi: 10.1111/j.1469-7610.2004.00221.x

Mehta, M. A., Sahakian, B. J., Mavaddat, N., Pickard, J. D., and Robbins, T. W. (2001). "Comparative psychopharmacology of methylpyhenidate and related drugs in human volunteers, patients with ADHD and experimental animals," in *Stimulant Drugs and ADHD: Basic and Clinical Neuroscience.*, eds M. V. A. Solanto, A. F. T. Arnsten and F. X. Castellanos (New York: Oxford University Press), 303–331.

Minzenberg, M. J., and Carter, C. S. (2008). Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology* 33, 1477–1502. doi: 10.1038/sj.npp.1301534

Müller, U., Steffenhagen, N., Regenthal, R., and Bublak, P. (2004). Effects of modafinil on working memory processes in humans. *Psychopharmacology (Berl)* 177, 161–169. doi: 10.1007/s00213-004-1926-3

Newman, L. A., and McGaughy, J. (2011). Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Dev. Psychobiol.* 53, 391–401. doi: 10.1002/dev.20537

O'Connor, A. (2004). *Wakefulness Finds a Powerful Ally.* New York: The New York Times.

Ogier, M., Wang, H., Hong, E., Wang, Q., Greenberg, M. E., and Katz, D. M. (2007). Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. *J. Neurosci.* 27, 10912–10917. doi: 10.1523/jneurosci.1869-07.2007

Otter, M., and Nurmand, L. B. (1980). Seasonal fluctuations in the catecholamine content in the brain of white rats. *Biull. Eksp. Biol. Med.* 89, 215–217.

Pang, T. Y. C., and Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology* 64, 515–528. doi: 10.1016/j.neuropharm.2012.06.029

Paoletti, P., Bellone, C., and Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat. Rev. Neurosci.* 14, 383–400. doi: 10.1038/nrn3504

Petrović, V. M., Maksimović, K., and Davidović, V. (1980). Chemical sympathectomy and diurnal fluctuations of noradrenaline calorigenic action in the rat. *Arch. Int. Physiol. Biochim.* 88, 273–276. doi: 10.3109/13813458009104866

Pietrzak, R. H., Mollica, C. M., Maruff, P., and Snyder, P. J. (2006). Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. *Neurosci. Biobehav. Rev.* 30, 1225–1245. doi: 10.1016/j.neubiorev.2006.10.002

Ragan, C. I., Bard, I., and Singh, I. (2013). What should we do about student use of cognitive enhancers? An analysis of current evidence. *Neuropharmacology* 64, 588–595. doi: 10.1016/j.neuropharm.2012.06.016

Randall, D. C., Shneerson, J. M., and File, S. E. (2005a). Cognitive effects of modafinil in student volunteers may depend on IQ. *Pharmacol. Biochem. Behav.* 82, 133–139. doi: 10.1016/j.pbb.2005.07.019

Randall, D. C., Viswanath, A., Bharania, P., Elsabagh, S. M., Hartley, D. E., Shneerson, J. M., et al. (2005b). Does modafinil enhance cognitive performance in young volunteers who are not sleep-deprived? *J. Clin. Psychopharmacol.* 25, 175–179. doi: 10.1097/01.jcp.0000155816.21467.25

Rao, Y., Liu, Z. W., Borok, E., Rabenstein, R. L., Shanabrough, M., Lu, M., et al. (2007). Prolonged wakefulness induces experience-dependent synaptic plasticity in mouse hypocretin/orexin neurons. *J. Clin. Invest.* 117, 4022–4033. doi: 10.1172/jci32829

Rapoport, J. L., Buchsbaum, M. S., Zahn, T. P., Weingartner, H., Ludlow, C., and Mikkelsen, E. J. (1978). Dextroamphetamine: cognitive and behavioral effects in normal prepubertal boys. *Science* 199, 560–563. doi: 10.1126/science.341313

Rapoport, J. L. B., Buchsbaum, M. S., Weingartner, H., Zahn, T. P., Ludlow, C., and Mikkelsen, E. J. (1980). Dextroamphetamine- its cognitive and behavioral effects in normal and hyperactive boys and normal men. *Arch. Gen. Psychiatry* 37, 933–943. doi: 10.1001/archpsyc.1980.01780210091010

Rasetti, R., Mattay, V. S., Stankevich, B., Skjei, K., Blasi, G., Sambataro, F., et al. (2010). Modulatory effects of modafinil on neural circuits regulating emotion and cognition. *Neuropsychopharmacology* 35, 2101–2109. doi: 10.1038/npp.2010.83

Roesler, R., and Schröder, N. (2011). Cognitive enhancers: focus on modulatory signaling influencing memory consolidation. *Pharmacol. Biochem. Behav.* 99, 155–163. doi: 10.1016/j.pbb.2010.12.028

Rueda, J. R., Ballesteros, J., and Tejada, M. I. (2009). Systematic review of pharmacological treatments in fragile X syndrome. *BMC Neurol.* 9:53. doi: 10.1186/1471-2377-9-53

Saletan, W. (2008). Night of the living meds: the US military's sleep-reduction program, slate magazine. *The Slate Group*. Accessed 11-1-2013. http://www.slate.com/articles/health_and_science/human_nature/2008/07/night_of_the_living_meds.html

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Transl. Psychiatry* 3:e238. doi: 10.1038/tp.2013.7

Sharma, V. (2011). Pilot pill project, pruneMirror.in. Accessed 10-22-2013. http://www.punemirror.in/article/2/2011021620110216063316445877l4a65/Pilot-pill-project.html

Silverman, J. L., Oliver, C. F., Karras, M. N., Gastrell, P. T., and Crawley, J. N. (2013). AMPAKINE enhancement of social interaction in the BTBR mouse model of autism. *Neuropharmacology* 64, 268–282. doi: 10.1016/j.neuropharm.2012.07.013

Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M., et al. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proc. Natl. Acad. Sci. U S A* 106, 4906–4911. doi: 10.1073/pnas.0811228106

Spencer-Smith, M., and Anderson, V. (2009). Healthy and abnormal development of the prefrontal cortex. *Dev. Neurorehabil.* 12, 279–297. doi: 10.3109/17518420903090701

Spiller, H. A., Borys, D., Griffith, J. R., Klein-Schwartz, W., Aleguas, A., Sollee, D., et al. (2009). Toxicity from modafinil ingestion. *Clin. Toxicol. (Phila)* 47, 153–156. doi: 10.1080/15563650802175595

Spiller, H. A., Hays, H. L., and Aleguas, A. Jr. (2013). Overdose of drugs for attention-deficit hyperactivity disorder: clinical presentation, mechanisms of toxicity and management. *CNS Drugs* 27, 531–543. doi: 10.1007/s40263-013-0084-8

Stoll, R. G. M. E., and Griesel, D. (2007). Cortex's AMPAKINE CX717 toxicoloy data package submitted to the FDA, cortex pharmaceuticals.

Sullivan, S. S. (2012). Current treatment of selected pediatric sleep disorders. *Neurotherapeutics* 9, 791–800. doi: 10.1007/s13311-012-0149-2

Taylor, G. P. Jr., and Keys, R. E. (2003). *Modafinil and Management of Aircrew Fatigue*. Washington, DC: United States Department of the Air Force.

Teffer, K., and Semendeferi, K. (2012). Human prefrontal cortex: evolution, development, and pathology. *Prog. Brain Res.* 195, 191–218. doi: 10.1016/B978-0-444-53860-4.00009-X

Turner, D. C., Robbins, T. W., Clark, L., Aron, A. R., Dowson, J., and Sahakian, B. J. (2003). Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacology (Berl)* 165, 260–269. doi: 10.1007/s00213-002-1250-8

Urban, K. R., and Gao, W. J. (2012). Evolution of the study of methylphenidate and its actions on the adult versus juvenile brain. *J. Atten. Disord.* 24, 65–74. doi: 10.1177/1087054712455504

Urban, K. R., and Gao, W.-J. (2013). Methylphenidate and the juvenile brain: enhancement of attention at the expense of cortical plasticity? *Med. Hypotheses* 81, 988–994. doi: 10.1016/j.mehy.2013.09.009

Urban, K. R., Li, Y. C., and Gao, W. J. (2013). Treatment with a clinically-relevant dose of methylphenidate alters NMDA receptor composition and synaptic plasticity in the juvenile rat prefrontal cortex. *Neurobiol. Learn. Mem.* 101, 65–74. doi: 10.1016/j.nlm.2013.01.004

Urban, K. R., Waterhouse, B. D., and Gao, W. J. (2012). Distinct age-dependent effects of methylphenidate on developing and adult prefrontal neurons. *Biol. Psychiatry* 72, 880–888. doi: 10.1016/j.biopsych.2012.04.018

U. S. Air Force. (2013). Joining the air force-enlisted overview. Accessed 10-22-2013. http://www.airforce.com/joining-the-air-force/enlisted-overview/

Wang, H., Stradtman, G. G. 3rd., Wang, X. J., and Gao, W. J. (2008). A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proc. Natl. Acad. Sci. U S A* 105, 16791–16796. doi: 10.1073/pnas.0804318105

Wang, M., Yang, Y., Wang, C.-J., Gamo, N. J., Jin, L. E., Mazer, J. A., et al. (2013). NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron* 77, 736–749. doi: 10.1016/j.neuron.2012.12.032

Wezenberg, E., Verkes, R. J., Ruigt, G. S., Hulstijn, W., and Sabbe, B. G. (2007). Acute effects of the ampakine farampator on memory and information processing in healthy elderly volunteers. *Neuropsychopharmacology* 32, 1272–1283. doi: 10.1038/sj.npp.1301257

Wheeler, B. (2006). *UK Army Tested "Stay Awake" Pills*. London: BBC News.

Willie, J. T., Renthal, W., Chemelli, R. M., Miller, M. S., Scammell, T. E., Yanagisawa, M., et al. (2005). Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. *Neuroscience* 130, 983–995. doi: 10.1016/j.neuroscience.2004.10.005

Xu, Z., Chen, R. Q., Gu, Q. H., Yan, J. Z., Wang, S. H., Liu, S. Y., et al. (2009). Metaplastic regulation of long-term potentiation/long-term depression threshold by activity-dependent changes of NR2A/NR2B ratio. *J. Neurosci.* 29, 8764–8773. doi: 10.1523/jneurosci.1014-09.2009

Zolkowska, D., Jain, R., Rothman, R. B., Partilla, J. S., Roth, B. L., Setola, V., et al. (2009). Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J. Pharmacol. Exp. Ther.* 329, 738–746. doi: 10.1124/jpet.108.146142

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 24 November 2013; accepted: 03 March 2014; published online: 13 May 2014.

Citation: Urban KR and Gao W-J (2014) Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain. Front. Syst. Neurosci. ***8****:38. doi: 10.3389/fnsys.2014.00038*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 17 April 2014
doi: 10.3389/fnsys.2014.00053

Attitudes toward pharmacological cognitive enhancement—a review

Kimberly J. Schelle[1], Nadira Faulmüller[2,3]*, Lucius Caviola[2] and Miles Hewstone[2]

[1] Department of Industrial Design, Eindhoven University of Technology, Eindhoven, Netherlands
[2] Department of Experimental Psychology, University of Oxford, Oxford, UK
[3] Department of Values, Technology and Innovation, Delft University of Technology, Delft, Netherlands

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Veljko Dubljevic, McGill University, Canada
Wayne Denis Hall, University of Queensland, Australia

***Correspondence:**
Nadira Faulmüller, Department of Experimental Psychology, University of Oxford, 9 South Parks Road, Oxford, OX1 3UD, UK
e-mail: nadira.faulmueller@psy.ox.ac.uk

A primary means for the augmentation of cognitive brain functions is "pharmacological cognitive enhancement" (PCE). The term usually refers to the off-label use of medical substances to improve mental performance in healthy individuals. With the final aim to advance the normative debate taking place on that topic, several empirical studies have been conducted to assess the attitudes toward PCE in the public, i.e., in groups outside of the academic debate. In this review, we provide an overview of the 40 empirical studies published so far, reporting both their methodology and results. Overall, we find that several concerns about the use of PCE are prevalent in the public. These concerns largely match those discussed in the normative academic debate. We present our findings structured around the three most common concerns: medical safety, coercion, and fairness. Fairness is divided into three subthemes: equality of opportunity, honesty, and authenticity. Attitudes regarding some concerns are coherent across studies (e.g., coercion), whereas for others we find mixed results (e.g., authenticity). Moreover, we find differences in how specific groups—such as users, nonusers, students, parents, and health care providers—perceive PCE: a coherent finding is that nonusers display more concerns regarding medical safety and fairness than users. We discuss potential psychological explanations for these differences.

Keywords: cognitive enhancement, neuroenhancement, brain function augmentation, medical safety, coercion, fairness, authenticity, smart drugs

INTRODUCTION

Brain enhancement is a topic of huge interest in the media and the academic literature. An often discussed form of brain enhancement is *pharmacological cognitive enhancement* (PCE) which usually refers to the use of certain prescription substances. Schermer et al. (2009) define these sorts of enhancements as "pharmacological interventions that are intended to improve certain mental functions and that go beyond currently accepted medical indications" (p. 77). PCE is not only a topic that is debated in academia—mainly in neuroscience, law, and ethics—but also a reality: several surveys show that students, but also other groups such as academics and surgeons, use substances to enhance their cognitive performance (e.g., Maher, 2008; Smith and Farah, 2011; Franke et al., 2013). Examples of seemingly common pharmacological enhancers are methylphenidate (e.g., Ritalin®), mixed amphetamine salts (e.g., Aderall®), and modafinil (e.g., Provigil®). Originally, the first two were developed as treatment for Attention Deficit Hyperactivity Disorder and the latter as treatment for Narcolepsy, but now they are being used to enhance performance in healthy individuals.

Although there are considerable intra- and interpersonal differences in the effects of these substances (e.g., Husain and Mehta, 2011; Van Der Schaaf et al., 2013), average effects have been reported. In their overview of findings and meta-analysis of the effects of methylphenidate and modafinil in healthy individuals, Repantis et al. (2010) assessed mood, motivation and four categories of cognitive processes: wakefulness, attention and vigilance, memory and learning, and executive functions and information processing. The available data about methylphenidate did not provide enough information to draw a firm conclusion about the effects of methylphenidate on enhancing or maintaining performance, although evidence for a positive effect on memory was found. An enhancing effect on attention was not verified, conflicting with the goal of increasing concentration and alertness that users of enhancement substances often have (Teter et al., 2005). The aggregated findings of potential enhancing effects of modafinil indicate that it improves attention for well-rested individuals. Furthermore, it helps in maintaining a higher degree of wakefulness, memory and executive functions over a period of sleep deprivation (Repantis et al., 2010). The authors suggest that these effects led to a growing popularity of modafinil, and strongly recommend a public debate on the ethics of the use of PCE which takes this into account. However, other reviews did not find any effect or even suggested that the non-cognitive effects of the substances, e.g., on confidence and motivation, might be responsible for enhanced performance (Farah et al., 2014).

In the normative debate about whether or not PCE is to be endorsed, certain concerns about its use are often raised and have been discussed by several authors (e.g., Farah et al., 2004; Bostrom and Sandberg, 2009; Schermer et al., 2009; Hyman, 2011). In this review, we focus on three concerns that are most often emphasized by the public, but are also discussed in the normative debate.

First, the *medical safety* of the aforementioned substances has been discussed, in particular in terms of short- or long-term side effects. A second topic of discussion is *coercion*, relating to concerns about the explicit and implicit pressures that can arise from the availability of PCE, forcing people to use these substances to be able to compete, for example at the workplace or in school. The third concern relates to the *fairness* of the use of PCE. This concern includes several subtopics such as a possible unequal distribution of access to such enhancers, their use in competitive environments being seen as cheating, and also to what degree performance brought about under PCE can be seen as authentic.

There are good reasons to examine the *attitudes of the public* toward PCE in addition to this normative debate. In their plea for the inclusion of public views on developments in biomedicine and technology, Schicktanz et al. (2012) underline a view already suggested a few years ago by Sarewitz (2010) in a column in *Nature.* They propose three general arguments for the inclusion of the public in ethical reasoning. First, views and attitudes of the public can point to remote or emerging moral problems. Second, empirical research can be used to examine premises about human behavior and social consequences of actions that underlie several applied ethical arguments. Third, research on public opinions can increase the context-sensitivity of ethical reasoning by pointing out consequences of concrete decisions in social policy. They argue that any bioethical discussion that avoids a confrontation with public opinions "not only runs the risk of missing important aspects, ideas, and arguments. It also arouses strong suspicion of being indeed one-sided, biased or ideological—thus illegitimate." (Schicktanz et al., 2012, p. 136). Apart from the aforementioned general arguments to include the public's attitude in certain bioethical discussions, specific arguments for the case of PCE are also made. Faulmüller et al. (2013) describe how PCE, as long as it is perceived negatively by the public, might give rise to indirect psychological costs for users: they might be treated in ways that damage their psychological well-being, for example by a misattribution of their success to the enhancer rather than themselves, or by dehumanization or ostracism. More insight into the public's view can lead to more insight into these potential indirect psychological costs. In addition, Banjo et al. (2010) argue that physicians are and will be "gatekeepers" in dispensing at least a portion of PCE substances. Although there is discussion on if and how their views might affect social policy or legal regulation on PCE (Delaney and Martin, 2011), PCE is part of their clinical reality. Therefore their views can provide specific insights that are of importance to the general debate (Ott et al., 2012).

The current article is based on an extensive literature search. We used two databases, Web of Science and Scopus, and included all articles published in English between 1990 and 2014. The search terms we used were [("cognitive enhancer" OR "cognitive enhancement" OR "pharmacological enhancement" OR "prescription drug" OR "performance enhancing drug" OR neuroenhancement OR "human enhancement") AND (view OR perspective OR opinion OR attitude OR judgment OR motive OR justification)] as part of the title, abstract or keyword of the text. This search resulted in 447 articles in Web of Science and 4162 articles in Scopus. Based on a careful check of titles and abstracts, we selected all articles that reported empirical studies on the opinions of groups outside the normative academic debate (such as students or physicians) on the use of PCE. We also cross-checked the reference lists of the studies found this way to identify additional relevant literature matching our inclusion criteria. Moreover, we checked Google Scholar and asked colleagues publishing in this area to direct us to relevant publications. Overall, this search resulted in 40 publications reporting qualitative or quantitative examinations of attitudes, opinions, and views of the general public and more often specific sub-populations toward PCE, which we included in this review. These 40 publications are marked with an asterisk in the reference list.

Our overarching finding is that in the public several concerns about the use of PCE substances are prevalent: they agree on several concerns when being asked specifically and raise concerns themselves when being asked for their opinion. These concerns widely match the normative debate. Hence, this review is structured around the common concerns reflected in the academic and public debate: (1) medical safety, (2) coercion, and (3) fairness. Each section in this review will be introduced with a short description of the academic debate on the respective concern. Then, the relevant empirical studies are reported. We also try to pinpoint some shortcomings in the current literature and suggest potential paths for future research. **Table 1** provides an overview of key methodological aspects of all studies reviewed, containing information about the concerns investigated, the research method by which the data were obtained, the country where the study took place, and the sample (occupation of participants, sample size, sampling method, and response rate).

MEDICAL SAFETY

The normative debate about the safety of PCE usually focuses on potential trade-offs between benefits and risks. As with all medical procedures, there might be side effects and yet-unknown long-term health risks with substances such as methylphenidate or modafinil (King et al., 2006). Schermer et al. (2009) point out that the harm-benefit ratio of PCE deserves special consideration, as the life-improving benefits might not outweigh the potential risks for consumers who use them for enhancement purposes, instead of the therapeutic purposes for which the substances were originally developed. However, Dubljevic (2013) emphasizes the importance of analyzing different substances in a case-by-case approach, as some entail greater health and addiction risks than others (Kociancic et al., 2004). Several studies have examined people's health concerns about such cognitive enhancers. Interestingly both users and nonusers systematically overestimate the cognitive-enhancing effects of PCE (Finger et al., 2013; Ilieva et al., 2013), but a sharp discrepancy between their risk estimations is revealed. While nonusers generally have strong concerns regarding the safety of PCE, users show less concern.

Nonusers tend to believe that such substances are addictive, might induce sleep disorders and may even lead to mental health problems, which was shown in an interview study with 19 Australian students (Partridge et al., 2013). In two surveys conducted on a UK university campus with a total of 357 students, Scheske and Schnall (2012) observed that moral reservations against PCE stem mostly from these safety concerns: the riskier the substances, the more student respondents morally object to

Table 1 | Overview of the empirical studies discussed in this review.

Authors	Country	Occupation of participants	Sampling method	Research method	Response rate	Sample size	Concerns
Aikins, 2011	USA	University students	Purposive sampling	Semi-structured interview	n/a	12	Safety, fairness
Asscher and Schermer, 2013	The Netherlands	General public	Purposive sampling	Focus groups	n/a	37	Safety
Ball and Wolbring, 2014	Canada	Parents	Purposive sampling	Semi-structured interview	n/a	12	Safety
Banjo et al., 2010	USA and Canada	Physicians	Convenience sampling	Web-based survey	n/a	212	Safety, coercion, Fairness
Bell et al., 2013	Australia	University students	Convenience sampling	Interview	n/a	19	Safety, coercion
Bergström and Lynöe, 2008	Sweden	General public	Random sampling	Paper and pencil questionnaire	52%	517	Safety
		Physicians			39%	108	
Bossaer et al., 2013	USA	University students	All students at one university invited	Web-based survey	59.9%	372	Safety, fairness
Desantis and Hane, 2010	USA	University students	Convenience sampling	Interview	n/a	175	Safety
Dodge et al., 2012	USA	University students	All students at one university invited	Web-based survey	37%	±1200	Fairness
Dubljević et al., 2013*	Germany	University students	Three stage cluster sampling (universities, disciplines, students)	Web-based survey	First wave 53.5%	5882	Fairness
					Second wave 69.1%	3486	
Eickenhorst et al., 2012	Germany	University students	Convenience sampling	Web-based survey	n/a	1218	Safety
		University graduates				106	
European Citizens Panel, 2006	Belgium, Denmark, France, Germany, Greece, Hungary, Italy, the Netherlands, UK	General public	Stratified random sampling (age, profession, gender)	Citizen's deliberation	n/a	126	Coercion
Fitz et al., 2013	USA and Canada	General public	Convenience sampling, Amazon's Mechanical Turk recruitment	Web-based survey	n/a	4011	Safety, coercion, fairness

(Continued)

Table 1 | Continued

Authors	Country	Occupation of participants	Sampling method	Research method	Response rate	Sample size	Concerns
Forlini and Racine, 2009	Canada	University students	Purposive sampling	Focus groups	n/a	29	Coercion
		Parents				21	
		Health care providers				15	
Forlini and Racine, 2012a	Canada	University students	Purposive sampling	Focus groups	n/a	29	Safety, coercion, fairness
		Parents				21	
		Health care providers				15	
Forlini and Racine, 2012b	Canada	University students	Purposive sampling	Focus groups	n/a	29	Safety, fairness
		Parents				21	
		Health care providers				15	
Franke et al., 2012a*	Germany	High school students	All students at 12 public grammar and vocational schools, and students of three departments of one university invited	Paper and pencil questionnaire	83%	1035	Safety, coercion, fairness
		University students				512	
Franke et al., 2012b	Germany	University students	Convenience sampling	Interview	n/a	22	Safety, coercion, fairness
Franke et al., 2014	Germany	Physicians	All primary care physicians in one state invited	Paper and pencil questionnaire	30.2%	832	Safety
Hotze et al., 2011	USA	Physicians	Random sampling	Paper and pencil questionnaire	46.6%	633	Fairness
Judson and Langdon, 2009	USA	University students	All students at two colleges invited	Paper and pencil questionnaire	10%	333	Safety
Kudlow et al., 2013	Canada	University students	All medical students at one medical school invited	Web-based survey	50%	326	Safety
Maier et al., 2013	Switzerland	University students	All students at three educational institutions invited	Web-based survey	22.3%	6275	Coercion
Maslen et al., in press	Germany	University students	Convenience sampling	Paper and pencil questionnaire	n/a	80	Coercion
Mazanov et al., 2013	Australian	University students	Convenience sampling	Web-based survey	n/a	1729	General, fairness
Ott and Biller-Andorno, 2013	Switzerland	University students	Convenience sampling	Web-based survey and separate paper and pencil questionnaire	n/a	1765	Safety, fairness

(Continued)

Table 1 | Continued

Authors	Country	Occupation of participants	Sampling method	Research method	Response rate	Sample size	Concerns
Ott et al., 2012	Switzerland	Physicians	Stratified random sampling (profession, gender, years of training, language)	Paper and pencil questionnaire	23.9%	379	Safety
Partridge et al., 2012	Australia	General public	Random sampling	Telephone interview	31.9%	1265	General
Partridge et al., 2013	Australia	University students	Convenience sampling	Interview	n/a	19	Safety
Riis et al., 2008	USA	University students	*No information provided*	Web-based survey	n/a	357	Fairness
Sabini and Monterosso, 2005	USA	University students	Convenience sampling	Paper and pencil questionnaire	n/a	185	Fairness
Santoni de Sio et al., in press	United Kingdom	University students	Convenience sampling	Paper and pencil questionnaire	n/a	102	Safety, fairness
Sattler et al., 2013a*	Germany	University students	Three stage cluster sampling (universities, disciplines, students)	Web-based survey	87.1%	1852	Safety, fairness
(Sattler et al., 2013b)*	Germany	University teachers	Three stage cluster sampling (universities, disciplines, students/teachers)	Web-based survey	33.5%	1402	Safety
		University students			69.1%	3486	
Sattler et al., 2014*	Germany	University students	Three stage cluster sampling (universities, disciplines, students); only second time wave	Web-based survey	69.1%	3486	Safety, coercion, fairness
Sattler and Wiegel, 2013*	Germany	University students	Three stage cluster sampling (universities, disciplines, students); only second time wave	Web-based survey	First wave 53.5%	5882	Safety
					Second wave 69.1%	3486	
Scheske and Schnall, 2012	UK	University students	Convenience sampling, two studies - two samples	Paper and pencil questionnaire	n/a	50	Safety, fairness
						306	
Schildmann et al., 2013	Germany	University students	*No information provided*	Survey	n/a	1026	Coercion, fairness

(Continued)

Table 1 | Continued

Authors	Country	Occupation of participants	Sampling method	Research method	Response rate	Sample size	Concerns
Schuijff and Brom, 2013	The Netherlands	All	Purposive sampling	Focus groups	n/a	38	Safety, coercion, fairness
Sweeney, 2010	USA	University students	Convenience sampling	Paper and pencil questionnaire	n/a	100	Safety, fairness

Convenience sampling and purposive sampling require no random selection of participants, whereas random sampling, stratified random sampling and cluster sampling do. Purposive sampling requires obtaining a sample of people who meet a predetermined criterion, whereas convenience sampling does not. For stratified random sampling, a population is divided in strata (subgroups) from which participants are randomly selected to make sure all strata are represented in the sample in proportion to their prevalence in the population. Cluster sampling requires a list of clusters, e.g., disciplines in a university, from which a few clusters are randomly chosen. Instead of randomly selecting participants from a list of potential participants, e.g., all students of the university, every member of the selected cluster is invited to participate (Cozby, 2009).

**The authors explicitly state that N is not equal for each analysis due to missing data or specific criteria employed.*

their non-medical use. Similarly, in a survey involving 102 UK Science students, Santoni de Sio et al. (in press) found that the concern against enhancement use that was raised most often by respondents related to potential unintended side-effects.

A plausible consequence is that students are less willing to engage in PCE the higher the severity and risk of the resulting health issues are perceived to be. This was shown in two German factorial design online surveys based on a large pool of vignettes with a sample of 1852 students in the first study (Sattler et al., 2013a) and 3486 students and 1402 university teachers in the second study (Sattler et al., 2013b; these are results of the second wave of a biannual project; also see Sattler and Wiegel, 2013 and Dubljević et al., 2013 for findings on the first and second wave, and (Sattler et al., 2014) for other results on the second wave). Moreover, Franke et al. (2012a) showed in an extensive paper-and-pencil questionnaire study with a sample of 1547 German students that the majority would consider taking PCE substances only if their safety could be assured.

The relation between willingness to use PCE and the perceived risk of PCE might also be an explanation for the results from a focus group study on several human enhancement technologies conducted with 38 Dutch participants divided into five groups (Schuijff and Brom, 2013). (A focus group study is a qualitative research technique in which a group of participants discuss their opinions on a given topic.) After more information on the effects and risks of using methylphenidate as an enhancer was provided, fewer participants stated they would consider using the substance for enhancement purposes than before receiving the information. Participants in this study were not familiar with the concept of human enhancement, in contrast to participants in most of the other studies, and therefore might have underestimated the risks accompanying the use of PCE. In another Dutch focus group study with 37 participants divided into five groups, several examples of the use of medical means to fulfill non-medical wishes were investigated. An example related to PCE, taking β-blockers during a driving test, raised several concerns about medical risks (Asscher and Schermer, 2013). In a semi-structured interview study with twelve Canadian parents of either cognitively disabled or non-disabled children, Ball and Wolbring (2014) observed that all parents unanimously agreed that medical safety has to be insured in order to administer PCE to their children.

Sattler and Wiegel (2013) provided the first evidence on the influence of the perceived severity of side effects and risk attitudes on actual PCE use. In a large- scale online survey with 5882, in a first wave, and 3486 respondents, in a second wave, a lower proneness to risk and an expectation of more severe drug-related side effects were associated with more PCE use at the first time point and increased use of PCE over a 6-months period. However, other results in the second wave of the same project did not show the relation between the expected severity of side effects or risk attitudes and willingness to use PCE (Sattler et al., 2014). The authors did, though, find in the second wave that a higher expected likelihood of side effects decreased the willingness to use PCE.

Other findings suggest that natural remedies are perceived as less harmful than PCE substances, as shown in a questionnaire study involving the Swedish general public and physicians with a sample size of 625 in total (Bergström and Lynöe, 2008). Furthermore, people morally object more to the application of PCE substances if they are artificial rather than natural, and if they are taken in the form of injections rather than pills (Scheske and Schnall, 2012). Focus group participants in the Netherlands referred to natural remedies, placebo, and psychological treatment as alternatives to a specific example of PCE (taking β-blockers during a driving test; Asscher and Schermer, 2013). However, these results may depend on the familiarity of the sample with medical substances. An online survey among 326 Canadian medical students showed that there were no significant differences between attitudes toward pharmacological or natural supplements for cognitive enhancement (Kudlow et al., 2013).

Furthermore, 212 US American and Canadian physicians, reported in an online survey, being less comfortable prescribing PCE substances for non-medical use to young adults, compared to older patients (Banjo et al., 2010). Franke et al. (2014) confirmed these findings in a paper and pencil questionnaire study among 832 German physicians. In both studies, physicians worried about misuse and deemed PCE for young people to be unnecessary. Yet, this applied less to the treatment (i.e., medical

use) of older patients. Consistent with these findings, an experimental vignette study conducted online with 4011 respondents found that the US American and Canadian public is more tolerant of side effects when they can be seen as the result of necessary therapy instead of enhancement (Fitz et al., 2013). This touches upon a big debate in medical ethics about the distinction between treatment and enhancement, since this is not a distinction based on biological facts, but, rather, reflecting subjective valuation (Parens, 1998; Daniels, 2000; Hyman, 2011). This is also reflected in a survey by Ott et al. (2012), who concluded that subjective suffering is taken into account as a criterion for disease. From the respondents, 379 Swiss general practitioners and psychiatrists, 88% reported being influenced by the degree of subjective suffering in prescribing an enhancer in four different scenarios. When asked if they would prescribe a PCE substance if a student requested a prescription to stay awake to study more, only 15% confirmed without any doubt, although 54% would prescribe if there were no therapeutic alternative. Around half of the respondents reported being confronted in their practice with such requests for prescriptions.

In contrast to these consistent findings on nonusers, *users* of PCE differ in their estimation of the safety of PCE substances. In an online survey with 1324 German students, users tended to rate the health consequences of PCE as less dangerous than nonusers did (Eickenhorst et al., 2012). This could be explained by users' higher ratings of willingness to take risks, found in a large-scale online survey involving 1765 Swiss students (Ott and Biller-Andorno, 2013). Moreover, fewer individuals (63.9%) from this latter survey's "user group" of 108 students reported worrying about side effects than individuals from the "nonuser group" of 1689 students (81.9%). Similarly, in a semi-structured interview study with 12 US American students who were illicitly or licitly using PCE, most participants believed that the benefits of PCE outweighed the potential negative side effects (Aikins, 2011). Another study with 333 US American students revealed that students who were illicitly using PCE had even more positive attitudes to the use of PCE with regard to medical safety issues compared to licit users (Judson and Langdon, 2009). In another interview study with 22 German students who used both caffeine and PCE, participants saw differences between the two forms of enhancement: in particular they estimated both the desired effects and the negative side effects as more pronounced in the case of PCE (Franke et al., 2012b). Also, Sweeney (2010) presents in her thesis results of a campus survey with a sample of 100 US American students, which demonstrate that students who are illicitly using cognitive enhancers seem to be more likely than nonusers to believe that the substances are harmless.

The aforementioned findings concerning differences between users and nonusers in their estimation of risks and harmfulness might also explain the large difference between the attitudes of users and nonusers found in other studies. A study on attitudes toward the acceptableness of PCE amongst 1265 members of the general public in Australia found that respondents who were familiar with PCE—either by using it themselves or by knowing somebody who used PCE—were twice as likely to find PCE acceptable than respondents who were not familiar with it (Partridge et al., 2012). Also users of PCE among a group of Canadian medical students tended to have more favorable attitudes toward PCE than nonusers (Kudlow et al., 2013). Lastly, a survey of 1729 students from Australian universities revealed differences in attitudes between users and nonusers of PCE, with users more often finding the use of study drugs "moral" than nonusers (Mazanov et al., 2013). The latter three studies did not specifically present the reason why respondents had a certain attitude toward PCE, and thus their opinions might not be related to medical safety. Still, these results are in line with a common finding in drug epidemiology, revealing that positive attitudes toward a drug, in particular low perceived risk, correlate with its use (e.g., Bachman et al., 1998; Bavarian et al., 2013; Cabriales et al., 2013).

In addition, users justify their practice and underestimate potential health risks for themselves and for others as shown in an interview-based survey with 175 US American students (Desantis and Hane, 2010). Conversely, Franke et al. (2012a) found that users and nonusers estimate addictive risks similarly, perhaps because there is a greater likelihood of substance dependency for users (Kroutil et al., 2006). However, Franke et al. (2012a) did not specifically test for PCE dependency in their study. In the abovementioned study by Desantis and Hane (2010), it was revealed that users argue for the substances' safety and downplay potential health risks by contrasting PCE to street-drugs (e.g., cocaine, heroin, etc.) and by pointing toward their acceptance within the medical establishment. This relates to the observation that students who believe that they know enough in order to safely use PCE are more likely to state that PCE is harmless (Sweeney, 2010). The fact that users tend to estimate PCE usage as harmless might explain why they find its application to be morally and socially acceptable (Desantis and Hane, 2010).

Hence, there is a general discrepancy between the views of users and nonusers with regard to the associated health risks of PCE and its moral acceptance, with users being less concerned than nonusers.

COERCION

The question of coercion relates to autonomy, i.e. the freedom to decide about one's personal life, and is a central issue in the normative debate on PCE. A main concern is that people are being pressured or even coerced into enhancing themselves. This might happen either indirectly in the form of peer pressure (Warren et al., 2009), or potentially directly in certain workplaces with long working hours and high demands on cognitive functioning, such as in the military or in surgery (Schoomaker, 2007; Maslen et al., in press). While opponents of enhancement consider it a "threat to the responsibility one bears for one's own life" (Habermas, 2003, p. 61), proponents instead focus on its advantages. They point out that PCE in particular entails the possibility of enhancing autonomy itself by increasing the reasoning abilities required to engage in such autonomous decisions (Schaefer et al., 2013).

The participants in Schuijff and Brom's (2013) focus group study indicated implicit peer pressure and explicit demand by employers to use human enhancement technologies to be a major concern for them. Maslen et al. (in press) found in a survey of 80 UK students clear and strong objection against the idea that people in professions with high responsibility, such as pilots and

physicians, might even have a moral obligation to enhance their performance, with only one respondent agreeing to such an obligation and 44% completely disagreeing. Two other surveys among German students found that the majority of students did not approve of PCE in jobs with high responsibilities: in one survey only 26% of the 1026 respondents approved of the use of PCE in highly responsible jobs (Schildmann et al., 2013), the second found that approximate 20% of their 1547 respondents approved the use of PCE for individuals with high responsibility (Franke et al., 2012a).

Forlini and Racine (2009) conducted one of the (very few) studies investigating people's attitudes on autonomy and coercion specifically with regards to PCE, again with the use of focus groups. Their participants, 65 Canadians assigned to one of nine groups consisting of either students, parents, or healthcare providers, agreed that PCE should be a matter of personal choice. This seems in line with one of the recommendations of a "European citizens' panel" held in 2005 and 2006, a citizens' deliberation on brain science involving 126 individuals from nine European countries. (A citizen's deliberation is a form of public participation in consultation about science, but can be less structured than a focus group study because groups can change during the deliberation.) One of the topics touched upon briefly was human enhancement. The participants' highest ranked recommendation was that people should be given the right to take "whatever drug they want," but enough information about the effects and dangers should be available; however, they did not support use of PCE in situations in which people have to pass exams (European Citizens Panel, 2006).

Participants in Forlini and Racine's (2009) study held the descriptive view that users are generally deciding to take such substances as a result of a voluntary decision. At the same time, however, they believed that this decision can be influenced by perceived social pressure or by competitive environments, such as academia or the job market, where people are striving for success and feel they have to perform better than average. Health care providers amongst the participants admitted that students who don't take enhancers may be disadvantaged because demands are getting higher and PCE is becoming more prevalent. They regarded peer pressure as an important contributing factor to the perceived need to take cognitive enhancers. Parents, in contrast, were aware of the pressure being put on students and consequently felt worry and sadness. They feared that the use of PCE may become a new standard.

However, peer pressure seems to be a more complex phenomenon than one might assume. Sattler et al. (2014) show that willingness to take PCE drugs does not increase when others encourage it, but it decreases when disapproval of the use of PCE by others rises. Furthermore, on the one hand, Forlini and Racine (2009) observe the desire of students not to be at a disadvantage, while on the other hand, less than 10% of 1547 German students stated in a survey by Franke et al. (2012a) that they would use PCE if others did so. In an online study amongst 6275 Swiss students less than 3% agreed that other people's use of substances would justify the use of PCE, compared to over 66% who agreed that increased learning would justify the use (Maier et al., 2013). One methodological reason for these diverging findings might be that even though a qualitative approach as used by Forlini and Racine (2009) can reveal aspects that might stay undiscovered in quantitative approaches like surveys, the small sample size might limit the generalizability of the findings. Going beyond methodology, we might speculate that it is not other people using PCE *per se*, but other people performing better, that puts pressure on students and leads them to consider taking such substances. In general, student participants in Forlini and Racine's (2009) focus groups viewed PCE as a personal lifestyle choice and emphasized the importance of personal integrity, i.e., they accepted the use of PCE conditional on the fact that one remains faithful to one's personal values. At the same time, they recognized the difficulty of that endeavor when social pressure is high and when abstinence could lead to personal disadvantage. Parents of university students, on the other hand, maintained a paternalistic view: students should be informed about cognitive enhancement, and, as a consequence, they should be held responsible and accountable for the decision to engage in PCE.

Thus, people consider the role of peer pressure as problematic and agree on the importance of deciding autonomously whether to engage in PCE. However, since the few studies reported here reveal mixed results, more research is needed to investigate the topic in greater depth.

FAIRNESS

The normative debate around the fairness of PCE is perhaps the least clearly defined. The term "fairness" seems to raise different distinct concepts in the lay mind, and thus creates a difficulty in comparing different studies that ask for opinions on the fairness of PCE use without defining what is meant by "fair." Overall, fairness related concerns seem to play an important role in the public, since they have been the second most common argument, after unintended side effects, against the use of enhancement raised by participants in Santoni de Sio et al.'s (in press) survey. Forlini and Racine's (2012a also c.f. Forlini and Racine, 2009) focus-group study explored lay statements about fairness of PCE in greater detail. They developed a model to describe three different subthemes: they suggest that judgments of fairness can (apart from external factors like legislation) be defined by a relationship between *equality of opportunity*, *honesty*, and *authenticity*. Participants who valued the equality of opportunity described the importance of an equal distribution of opportunities to obtain PCE substances and opportunities deriving from their use. Honesty and authenticity are both related to effort that has to be invested to achieve a certain task. The underlying assumption is that when high performance is achieved with less effort—as might be the case when PCE substances are taken—this might be less fair compared to performance that is achieved with substantial effort. Honesty relates to the social aspect of this assumption and reflects the effect of PCE use on other individuals, for example in a competitive environment where PCE use might be seen as cheating. Authenticity relates to the individual PCE user and questions whether his/her performance under PCE, often seen as a situation in which effort is discounted, is an authentic performance. It is based on the underlying belief that putting in effort shapes the experience of an individual and thus affects a "future" individual that does not gain the same

experience while using PCE. Although the above separation of concepts is too coarse to fully reflect the depth of the academic normative debate, the concerns of the public can be grouped around these subthemes of fairness.

EQUALITY OF OPPORTUNITY

First, we discuss equality of opportunity, the fear that inequality in access to enhancement substances might increase inequalities in society. Farah et al. (2004) describe how certain groups might experience cost barriers and social barriers to access PCE. (In this section, we do not consider concerns about restrictions of freedom to follow personal preferences, as these are discussed in the previous section about coercion). Equality of opportunity is also referred to as *distributive fairness* (e.g., Scheske and Schnall, 2012), *distributive justice* (Farah et al., 2004), or the *concern of inequality* (e.g., Bostrom and Sandberg, 2009). Note that the notion of equality of opportunity relates to a certain underlying theory of justice (cf. Rawls, 1971), which can be contrasted, for example, to the notion of equality of outcome. However, these underlying theories are not distinguished yet in empirical research on the public's opinions about PCE.

Although healthcare providers, students and parents who participated in the focus group study by Forlini and Racine (2012a) believed that, currently, everybody who wanted could find PCE substances one way or another, they did emphasize the importance of the value of equality of opportunity as part of their judgment on the fairness of the use of PCE. Correspondingly, in survey research by Sattler et al. (2013a) amongst 1852 respondents, a lower score on both willingness-to-use a PCE and moral acceptability of PCE substances was reported when judging an imaginary situation where no other students take this PCE substance, compared to situations in which half of the other students or all fellow students were taking the PCE substance. Possibly, they read the "no other student" situation as one where there is inequality in opportunity with them having, and other students not having, access and found this to be morally unacceptable. However, Sattler et al.'s (2013a) finding could also point to the experience of a "social norm," following from the prevalence of use that influences the judgment of moral acceptability.

In a survey, Hotze et al. (2011) presented 633 US American general practitioners with two statements related to this topic: a slight majority agreed that society should prevent economic advantages turning into biological advantages (57%), and that everyone should have equal access to medical enhancers (55%). Scheske and Schnall (2012) showed that the use of PCE substances is perceived as more wrong if not everybody can afford them, compared to situations in which everybody can. Fitz et al. (2013) investigated fairness by using the contrastive vignette technique online. Their 4011 respondents, recruited via Amazon's Mechanical Turk, were randomly assigned to one of 22 different vignettes that described the use of PCE diverging in terms of alleged safety, societal and peer pressure, fairness and authenticity. Respondents saw it as less fair if a student obtained an enhancer with the help of money given by his parents rather than with money earned by own work. The 185 US American respondents in Sabini and Monterosso's (2005) study endorsed the so-called "interaction view" (p. 91): their judgments of fairness depended on the group that was affected by the drug. Although their fairness ratings of PCE use were always close to or lower than the midpoint of the scale—thus generally regarding it as rather unfair—respondents believed the use by the worst performing 10% of students to be fairer than the use by everybody or the top 10%. The "interaction view" corresponds to what John Rawls (1971, 1985) calls the "difference principle": inequality is acceptable only if the current situation for those least advantaged is improved.

A survey amongst 1026 German students demonstrated that 27% of the respondents approved the use of PCE by worse performing classmates, while 57% approved the use for elderly with declining cognitive performance (Schildmann et al., 2013). Correspondingly, over a quarter of the respondents of another study among German students reported that classmates with low academic performance should be allowed to use PCE, while 50% indicated that the use of PCE by cognitively impaired elderly should be allowed (Franke et al., 2012a). The percentage of respondents who endorsed the use of PCE by classmates with low academic performance was higher among PCE users than nonusers. It is clear that although the interaction view is endorsed, previous performance is of less influence on judgments of fairness of the use of PCE than age. However, this is perhaps because the samples are both young students who are in competition with other young students, so that age and performance variables could be said to be confounded in the sample. Future studies need to investigate the attitudes of young students in competition with elderly students, and compare elderly students with elderly non-students, so that attitudes toward these factors could be separated. Sabini and Monterosso (2005) explain the interaction view by arguing that a substance that would affect the worst performing 10% only can better be seen as a normalizer instead of an enhancer, suggesting that in this case it might be closer to a treatment than to an enhancement. If this explanation is correct, it would seem that both the acceptance of side effects as well as the acceptance of a certain unequal distribution is greater in the case of a treatment compared to enhancement.

In general, healthcare providers, students, and parents seem to agree that an unequal distribution of PCE is unfair, if the unequal distribution is related to factors that are changeable, such as wealth. If the unequal distribution exists due to biological dispositions, such as having a low attention span, an unequal distribution is seen as less relevant to moral judgments. This is related to the distinction between treatment and enhancement, in which the former is generally believed to be seen as more acceptable (Parens, 1998; Daniels, 2000; Hyman, 2011). Users find it more acceptable than nonusers that fellow students with low academic performance use PCE, but research investigating the reasons for these diverging views of users and nonusers is still lacking.

HONESTY

Honesty relates to the question of whether the use of PCE might give a user an unfair advantage over people who do not use PCE, and thus might need to exert more effort to achieve the same result. Scheske and Schnall (2012) refer to honesty as *competitive*

fairness and Bostrom and Sandberg (2009) discuss it in relation to *cheating*. Using an example and taking a normative stance, Bostrom and Sandberg (2009) describe how goals and rules define whether an act qualifies as cheating: if the primary goal of schooling is acquiring knowledge, PCE might be legitimate. In the case of a competition for grades or admission, however, PCE could be seen as cheating if it were against the rules or if access were unequally distributed.

Quantitative accounts of the public's opinion on honesty related to PCE use can be found in several studies. Students of a highly competitive UK university deemed the competitive advantage PCE can give as one of the most important concerns regarding its use: an advantage due to PCE use was found most morally wrong when no other competitors were taking the substance, relating competitive to distributive fairness and peer pressure (Scheske and Schnall, 2012). Moreover, an online survey by Bossaer et al. (2013) demonstrated that 60% of the 372 student respondents agreed that PCE provides users with an unfair advantage over other students. An almost identical amount of just over half of the respondents (56%) believed that PCE use for study purposes could be seen as academic dishonesty. In a large-scale online survey, with respectively 5882 and 3486 participants in the first and second wave, (Dubljević et al., 2013) found that German students deem the use of PCE with the intention to increase study performance to be morally less acceptable than traditional forms of academic misconduct, such as cheating in exams, fabrication, or plagiarism. Schildmann et al. (2013) reported that half of the respondents of their questionnaire study thought the use of PCE by others was unfair, another quarter was unsure of their thoughts about the statement, and only a quarter "rather" or "absolutely" agreed that the use of PCE by others was fair. Over half of the respondents in Franke et al. (2012a) indicated in a questionnaire that they found PCE fair "under no circumstances" or "probably not." This percentage was higher among females and among nonusers. Although exact percentages are not provided, Sweeney (2010) also discusses a survey amongst 100 students in which nonusers felt more troubled than users by academic advantages obtained by PCE. This difference between users and nonusers was also found in the online survey by Ott and Biller-Andorno (2013). They show that a little over 40% of nonusers agree that with using PCE, one would be betraying others who do not use PCE, while less than 20% of users agree to this statement. An online study with 1200 male US American student participants revealed that the misuse of performance enhancement substances in the sporting domain received a higher rating on a scale that measured the degree of cheating than the use of PCE in academia (Dodge et al., 2012).

As described above, Fitz et al. (2013) showed that when obtaining PCE takes less effort, it is seen as more unfair. This was reflected in reduced fairness ratings in a scenario in which one individual could use PCE and another could not. The effort needed to obtain PCE was manipulated, as well as a second variable, that of the effect of the PCE as either reducing the effort needed to study or increasing the number of hours that one could study for. The combination of diminished effort in obtainment and reduced study effort produced the lowest ratings of fairness. Thus, any variation of the description that would result in a reduction of effort for the user of PCE in comparison to a nonuser resulted in respondents judging PCE as less fair.

Qualitative studies provide a more elaborate but also more ambiguous perspective on honesty in relation to PCE. In an interview study amongst 19 Australian students by Bell et al. (2013 also c.f. Partridge et al., 2013) fairness appeared at the top of the list of concerns mentioned. More than half of the participants described PCE as a form of cheating, while most of the others explicitly reported that they did not find it unfair in comparison to other available methods for performance improvement (e.g., coffee). In contrast to coffee, focus group participants saw PCE as a form of cheating similar to the use of steroids (Forlini and Racine, 2012b). Also, participants showed dissent and indecision about whether PCE use should be seen as cheating or not (Forlini and Racine, 2012a). Each subgroup of students, parents, and health care providers included some individuals who saw PCE as an unfair shortcut, putting others at a disadvantage, but also individuals who considered PCE a study tool like any other.

To summarize, a little over half of the public believes that the use of PCE provides an unfair advantage to users, a situation that is seen as cheating, especially in highly competitive environments. Nonusers provide lower ratings of honesty than users in quantitative studies. In general there seems to be dissent on whether PCE qualifies as cheating or not. Qualitative studies give more insight into the different perspectives on this question.

AUTHENTICITY

In the normative debate, critics of PCE argue that users, compared with nonusers, of enhancement substances experience the value of exerting effort on a task less. This makes activities less meaningful and facilitates fewer experiences of self-development (see Schermer, 2008, for an analysis of this argument). Part of the debate therefore relates to authentic performance, for which effort is seen as a necessary condition (e.g., President's Council on Bioethics, 2003). Goodman (2010) specifies that this is true for what he calls process goods, for which the activity itself is seen as central. For so-called outcome goods, however, for which the result of an activity counts, effort is less relevant.

We can apply the distinction between process goods and outcome goods to the list of 19 traits rated by 357 respondents in an online study by Riis et al. (2008). For each trait they had to rate their willingness to enhance it using pharmacological means, if they could. Outcome related traits, such as memory, received higher scores on willingness-to-enhance than more process related traits, such as mood and social comfort. These higher ratings for what we deem outcome related traits might be explained by respondents valuing the notion of effort. Furthermore, respondents were less willing to enhance traits that were rated as more fundamental to the self, such as kindness and empathy.

Participants in the focus group study by Forlini and Racine (2012a) explicitly related effort to judgments of the fairness of PCE. Students, parents, and healthcare providers commented on effort and authenticity. They displayed both positive (nonproblematizing) and negative (problematizing) views toward PCE in relation to effort and authenticity in equal proportions, as can be seen in the following examples. Participants of each target

group (students, parents, and healthcare providers) noted that even with the use of PCE, effort still had to be put in to complete certain tasks. However, participants also discussed how enhancement may compromise certain social and personal values that shape an individual's behavior. Also some of the student participants in the interview study by Bell et al. (2013) described PCE as a quick fix rather than a true reflection of an individual's abilities, while other participants neither reported effort nor authenticity as being corrupted by the use of PCE. Two thirds of the student respondents in the survey by Schildmann et al. (2013) agreed that performances done with PCE were "less commendable than comparable performances that are done without these substances" (p. 25). In addition, 63% of the 644 physicians who completed the survey by Hotze et al. (2011) viewed PCE as a threat to the value of human achievement. Moreover, an online survey by Banjo et al. (2010) showed that the respondents, 212 physicians, ranked "PCE undermines the values of personal effort" as the fourth most important reason (of 13 presented) to feel uncomfortable about prescribing PCE to non-elderly people. However, an online survey among 1729 Australian students showed that users of prescription amphetamines had fewer concerns regarding the integrity of authentic and moral actions than did nonusers (Mazanov et al., 2013).

Finally, in their experimental vignette study Fitz et al. (2013) found that respondents rated an individual's performance as significantly less authentic when PCE was used, compared to when it was not used. However, this opinion did not completely transfer to judgments of worthiness for a promotion when the character would be assigned a new project in his job. Respondents judged a PCE user as significantly more worthy when successful without enhancement compared to with enhancement, but they also indicated that people who succeeded with the help of PCE were more worthy of promotion than people who did not use PCE and failed.

To summarize, in general people are more likely to enhance outcome related traits than process related traits. However, in direct discussions about effort and authenticity in relation to fairness, individuals display divergent opinions on the importance of these topics. The proportion of people who believe effort is discounted and authenticity violated when using PCE is a little over half of the respondents in most studies. This implies that little less than half of the public is not that concerned about effort and authenticity in relation to PCE. One reason that is given is that effort still has to be invested to achieve certain goals, even when PCE is used.

CONCLUSION

This review provides an overview of 40 studies on the public's opinion about PCE. Our main finding is that in groups outside the normative academic debate several concerns about the use of pharmacological substances for performance enhancement are either raised or endorsed. These concerns that are discussed in the current review of research on opinions about PCE widely match the normative debate. These similarities between the public's opinion and the normative debate are also found in studies on other ethical issues, for example the sex selection of embryos (Banks et al., 2006) and life extension technologies (Partridge et al., 2009). The findings we present are divided between three concerns regarding PCE use which are common in both the ethical and lay debate: medical safety, coercion, and fairness.

Several studies have shown that *medical safety* is regarded as central by nonusers of PCE, and insecurity about it provides a reason for them to refuse the use of PCE. Related to this concern are findings that point toward a preference for natural over artificial enhancers. A similar preference can be seen for interventions that might be closer to treatment than to enhancement. Users, on the other hand, do not display these preferences and indicate conflicting results on judgments of (subjective) health risks associated with PCE. They are concerned about addiction, but do not worry about other health risks, and deem PCE more often harmless than nonusers do. A more convergent view can be found on the theme of *coercion*. Different subgroups agree that PCE should be a matter of personal choice. They believe that decisions concerning use are, in general, made voluntarily, although they can be influenced by perceived social pressure or by competitive environments. It is shown that peer pressure is a complex phenomenon, as students might not always be influenced by other people's PCE use itself, but only when these others achieve a higher performance compared to their own. However, only a few studies have investigated coercion to date and we call for future research to fill this gap. Finally, we discussed *fairness*, divided into three subthemes: equality of opportunity, honesty, and authenticity. An unequal distribution of PCE substances that might develop due to changeable factors—such as wealth—is seen as unfair, while an unequal distribution due to biological dispositions—such as a low attention span—is seen as less relevant to judgments of fairness. This might relate to a general finding that treatments are seen as more acceptable than enhancements. The public's opinion on the subthemes of honesty and authenticity shows a more complex pattern. Nonusers believe more often than users that the use of PCE provides an unfair advantage, although in general only half of the public raises concerns about this topic of honesty and cheating. Several studies show that around the theme of authenticity both problematizing and non-problematizing views on PCE arise in equal proportions. While some respondents indicate that "the work has still to be done" even when PCE is used, others believe that PCE is a quick fix and undermines an authentic performance.

One important distinction within the public can be found between users and nonusers, who tend to differ in their perspectives on the medical safety and subthemes of the fairness of PCE: while users generally deem PCE to be safe and fair, nonusers do not. These results imply that users are either more willing to engage in PCE because of their positive attitude toward it, or that they adopted their positive attitude as a result of personal usage. In either case, the differences in users' and nonusers' attitudes toward PCE might be driven by cognitive biases. It is possible, for example, that nonusers display a more negative view toward PCE because they experience the so-called status quo bias, which describes the irrational preference for an option only because it preserves the current state of affairs (Kahneman and Tversky, 2000). As currently PCE use is not seen as a common way to improve cognitive performance, this bias might result in the preference to not use PCE. This tendency, to use the

status quo as a reference point, might explain why people prefer interventions when they are seen as treatments as opposed to enhancements (Bostrom and Ord, 2006). It is also possible, however, that users adopt a more positive view toward PCE in order to reduce their cognitive dissonance, the discomfort experienced when one's actions don't reflect one's beliefs (Festinger, 1957). This would reflect a situation in which users adapt their attitudes toward their PCE use, that is, their behavior. Future research is called to examine in greater depth which biases might influence people's attitudes toward PCE and the causal direction explaining the attitudes currently prevalent. Further, future research might also reveal whether differences between users and nonusers also hold for other concerns, such as coercion and authenticity for which data on this distinction is currently lacking.

It is important to note that the current literature focuses mostly on opinions of students, with only a few exceptions provided by studies on health care providers and the general public. Furthermore, several studies were conducted with a non-random sample of participants, or obtained a low response rate in the case of random sampling. This might have biased the results. In addition, even the studies that were conducted with random sampling of students at specific universities can create a biased population overview in our review, because differences between colleges on the use of PCE are found (e.g., McCabe et al., 2005). Furthermore, future research should provide more insight into the opinions of populations other than students, such as the general population or more specifically people active in the workforce. This would add to a more accurate picture of opinions of the general population and of potential users in those areas where use is to be expected.

Moreover, future research is called for to reveal more fine-grained differences in the public opinion for certain concerns. In the current literature, different concerns can have the same name, as is seen with fairness, while different names may also be used for the same concern, as was described for equality of opportunity. This makes it harder to draw precise conclusions on the state of research on public attitudes toward PCE and to systematically compare it with arguments from the normative debate. Although more fine-grained studies are needed to reflect the depth of the normative debate, it can be said that, thus far, concerns of the public regarding the use of PCE reflect the main issues fiercely debated in academia.

REFERENCES

*Aikins, R. D. (2011). Academic performance enhancement: a qualitative study of the perceptions and habits of prescription stimulant-using college students. *J. Coll. Stud. Dev.* 52, 560–576. doi: 10.1353/csd.2011.0064

*Asscher, E. C. A., and Schermer, M. (2013). Wish-fulfilling medicine in practice: the opinions and arguments of lay people. *J. Med. Ethics.* doi: 10.1136/medethics-2013-101480. [Epub ahead of print].

Bachman, J. G., Johnson, L. D., and O'Malley, P. M. (1998). Explaining recent increases in students' marijuana use: impacts of perceived risks and disapproval, 1976 through 1996. *Am. J. Public Health* 88, 887–892. doi: 10.2105/AJPH.88.6.887

*Ball, N., and Wolbring, G. (2014). Cognitive enhancement: perceptions among parents of children with disabilities. *Neuroethics* 1–20. doi: 10.1007/s12152-014-9201-8. [Epub ahead of print].

*Banjo, O. C., Nadler, R., and Reiner, P. B. (2010). Physician attitudes towards pharmacological cognitive enhancement: safety concerns are paramount. *PLoS ONE* 5:e14322. doi: 10.1371/journal.pone.0014322

Banks, S., Scully, J. L., and Shakespeare, T. (2006). Ordinary ethics: lay people's deliberations on social sex selection. *New Genet. Soc.* 25, 289–303. doi: 10.1080/14636770601032890

Bavarian, N., Flay, B. R., Ketcham, P. L., and Smit, E. (2013). Illicit use of prescription stimulants in a college student sample: a theory-guided analysis. *Drug Alcohol Depend.* 132, 665–673. doi: 10.1016/j.drugalcdep.2013.04.024

*Bell, S., Partridge, B., Lucke, J., and Hall, W. (2013). Australian university students' attitudes towards the acceptability and regulation of pharmaceuticals to improve academic performance. *Neuroethics* 6, 197–205. doi: 10.1007/s12152-012-9153-9

*Bergström, L. S., and Lynöe, N. (2008). Enhancing concentration, mood and memory in healthy individuals: an empirical study of attitudes among general practitioners and the general population. *Scand. J. Public Health*. 36, 532–537. doi: 10.1177/1403494807087558

*Bossaer, J. B., Gray, J. A., Miller, S. E., Enck, G., Gaddipati, V. C., and Enck, R. E. (2013). The use and misuse of prescription stimulants as "cognitive enhancers" by students at one academic health sciences center. *Acad. Med.* 88, 967–971. doi: 10.1097/Acm.0b013e318294fc7b

Bostrom, N., and Ord, T. (2006). The reversal test: eliminating status quo bias in applied ethics. *Ethics* 116, 656–679. doi: 10.1086/505233

Bostrom, N., and Sandberg, A. (2009). Cognitive enhancement: methods, ethics, regulatory challenges. *Sci. Eng. Ethics* 15, 311–341. doi: 10.1007/s11948-009-9142-5

Cabriales, J. A., Cooper, T. V., and Taylor, T. (2013). Prescription drug misuse, illicit drug use, and their potential risk and protective correlates in a Hispanic college student sample. *Exp. Clin. Psychopharmacol.* 21, 235–244. doi: 10.1037/a0031973

Cozby, P. C. (2009). *Methods in Behavioral Research.* New York, NY: McGraw-Hill.

Daniels, N. (2000). Normal functioning and the treatment-enhancement distinction. *Camb. Q. Healthc. Ethics* 9, 309–322. doi: 10.1017/S0963180100903037

Delaney, J. J., and Martin, D. P. (2011). The role of physician opinion in human enhancement. *Am. J. Bioeth.* 11, 19–20. doi: 10.1080/15265161.2010.534537

*Desantis, A. D., and Hane, A. C. (2010). "Adderall is definitely not a drug": justifications for the illegal use of ADHD stimulants. *Subst. Use Misuse* 45, 31–46. doi: 10.3109/10826080902858334

*Dodge, T., Williams, K. J., Marzell, M., and Turrisi, R. (2012). Judging cheaters: is substance misuse viewed similarly in the athletic and academic domains? *Psychol. Addict. Behav.* 26, 678–682. doi: 10.1037/A0027872

Dubljevic, V. (2013). Cognitive enhancement, rational choice and justification. *Neuroethics* 6, 179–187. doi: 10.1007/s12152-012-9173-5

*Dubljević, V., Sattler, S., and Racine, E. (2013). Cognitive enhancement and academic misconduct: a study exploring their frequency and relationship. *Ethics Behav.* doi: 10.1080/10508422.2013.869747. [Epub ahead of print].

*Eickenhorst, P., Vitzthum, K., Klapp, B. F., Groneberg, D., and Mache, S. (2012). Neuroenhancement among German university students: motives, expectations, and relationship with psychoactive lifestyle drugs. *J. Psychoactive Drugs* 44, 418–427. doi: 10.1080/02791072.2012.736845

*European Citizens' Panel. (2006). *Meeting of Minds, European Citizens' Deliberation on Brain Science*. Available online at: http://www.kbs-frb.be/otheractivity.aspx?id=193934&langtype=1033 (Accessed January 15, 2014).

Farah, M. J., Illes, J., Cook-Deegan, R., Gardner, H., Kandel, E., King, P., et al. (2004). Neurocognitive enhancement: what can we do and what should we do? *Nat. Rev. Neurosci.* 5, 421–425. doi: 10.1038/nrn1390

Farah, M. J., Smith, M. E., Ilieva, I., and Hamilton, R. H. (2014). Cognitive enhancement. *WIREs Cogn Sci.* 5, 95–103. doi: 10.1002/wcs.1250

Faulmüller, N., Maslen, H., and Santoni de Sio, F. (2013). The indirect psychological costs of cognitive enhancement. *Am. J. Bioeth.*. 13, 45–47. doi: 10.1080/15265161.2013.794880

Festinger, L. (1957). *A Theory of Cognitive Dissonance*. Stanford, CA: Stanford University Press.

Finger, G., da Silva, E. R., and Falavigna, A. (2013). Use of methylphenidate among medical students: a systematic review. *Rev. Assoc. Med. Bras.* 59, 285–289. doi: 10.1016/J.Ramb.2012.10.007

*Fitz, N. S., Nadler, R., Manogaran, P., Chong, E. W. J., and Reiner, P. B. (2013). Public attitudes toward cognitive enhancement. *Neuroethics*. doi: 10.1007/s12152-013-9190-z

*Forlini, C., and Racine, E. (2009). Autonomy and coercion in academic "Cognitive Enhancement" using methylphenidate: perspectives

of key stakeholders. *Neuroethics* 2, 163–177. doi: 10.1007/s12152-009-9043-y

*Forlini, C., and Racine, E. (2012a). Added stakeholders, added value(s) to the cognitive enhancement debate: are academic discourse and professional policies sidestepping values of stakeholders? *AJOB Prim. Res.* 3, 33–47. doi: 10.1080/21507716.2011.645116

*Forlini, C., and Racine, E. (2012b). Stakeholder perspectives and reactions to "academic" cognitive enhancement: unsuspected meaning of ambivalence and analogies. *Public Underst. Sci.* 21, 606–625. doi: 10.1177/0963662510385062

Franke, A. G., Bagusat, C., Dietz, P., Hoffmann, I., Simon, P., Ulrich, R., et al. (2013). Use of illicit and prescription drugs for cognitive or mood enhancement among surgeons. *BMC Med.* 11:102. doi: 10.1186/1741-7015-11-102

*Franke, A. G., Bonertz, C., Christmann, M., Engeser, S., and Lieb, K. (2012a). Attitudes toward cognitive enhancement in users and nonusers of stimulants for cognitive enhancement: a pilot study. *AJOB Prim. Res.* 3, 48–57. doi: 10.1080/21507716.2011.608411

*Franke, A. G., Lieb, K., and Hildt, E. (2012b). What users think about the differences between caffeine and illicit/prescription stimulants for cognitive enhancement. *PLoS ONE* 7:e40047. doi: 10.1371/journal.pone.0040047

*Franke, A. G., Papenburg, C., Schotten, E., Reiner, P. B., and Lieb, K. (2014). Attitudes towards prescribing cognitive enhancers among primary care physicians in Germany. *BMC Fam. Pract.* 15:3. doi: 10.1186/1471-2296-15-3

Goodman, R. (2010). Cognitive enhancement, cheating, and accomplishment. *Kennedy Inst. Ethics J.* 20, 145–160. doi: 10.1353/ken.0.0309

Habermas, J. (2003). *The Future of Human Nature*. Cambridge: Polity Press.

*Hotze, T. D., Shah, K., Anderson, E. E., and Wynia, M. K. (2011). "Doctor, would you prescribe a pill to help me …?" a national survey of physicians on using medicine for human enhancement. *Am. J. Bioeth.* 11, 3–13. doi: 10.1080/15265161.2011.534957

Husain, M., and Mehta, M. A. (2011). Cognitive enhancement by drugs in health and disease. *Trends Cogn. Sci.* 15, 28–36. doi: 10.1016/j.tics.2010.11.002

Hyman, S. E. (2011). Cognitive enhancement: promises and perils. *Neuron* 69, 595–598. doi: 10.1016/j.neuron.2011.02.012

Ilieva, I., Boland, J., and Farah, M. J. (2013). Objective and subjective cognitive enhancing effects of mixed amphetamine salts in healthy people. *Neuropharmacology* 64, 496–505. doi: 10.1016/j.neuropharm.2012.07.021

*Judson, R., and Langdon, S. W. (2009). Illicit use of prescription stimulants among college students: prescription status, motives, theory of planned behaviour, knowledge and self-diagnostic tendencies. *Psychol. Health Med.* 14, 97–104. doi: 10.1080/13548500802126723

Kahneman, D., and Tversky, A. (2000). *Choices, Values, and Frames*. New York, NY: Cambridge University Press.

King, S., Griffin, S., Hodges, Z., Weatherly, H., Asseburg, C., Richardson, G., et al. (2006). A systematic review and economic model of the effectiveness and cost-effectiveness of methylphenidate, dexamfetamine and atomoxetine for the treatment of attention deficit hyperactivity disorder in children and adolescents. *Health Technol. Assess* 10, iii–iv, xiii–146.

Kociancic, T., Reed, M. D., and Findling, R. L. (2004). Evaluation of risks associated with short- and long-term psychostimulant therapy for treatment of ADHD in children. *Expert Opin. Drug Saf.* 3, 93–100. doi: 10.1517/eods.3.2.93.27337

Kroutil, L. A., Van Brunt, D. L., Herman-Stahl, M. A., Heller, D. C., Bray, R. M., and Penne, M. A. (2006). Nonmedical use of prescription stimulants in the United States. *Drug Alcohol Depend.* 84, 135–143. doi: 10.1016/j.drugalcdep.2005.12.011

*Kudlow, P. A., Treurnicht Naylor, K., Xie, B., and McIntyre, R. S. (2013). Cognitive enhancement in Canadian medical students. *J. Psychoactive Drugs*45, 360–365. doi: 10.1080/02791072.2013.825033

Maher, B. (2008). Poll results: look who's doping. *Nature* 452, 674–675. doi: 10.1038/452674a

*Maier, L. J., Liechti, M. E., Herzig, F., and Schaub, M. P. (2013). To dope or not to dope: neuroenhancement with prescription drugs and drugs of abuse among Swiss university students. *PLoS ONE* 8:e77967. doi:10.1371/journal.pone.0077967

*Maslen, H., Santoni de Sio, F., and Faulmüller, N. (in press). "With cognitive enhancement comes great responsibility?," in *Responsible Innovation*. Vol. 2, eds B. J. Koops et al. (Dordrecht: Springer).

*Mazanov, J., Dunn, M., Connor, J., and Fielding, M. L. (2013). Substance use to enhance academic performance among Australian university students. *Perform. Enhance. Health* 2, 110–118. doi: 10.1016/j.peh.2013.08.017

McCabe, S. E., Knight, J. R., Teter, C. J., and Wechsler, H. (2005). Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction* 99, 96–106. doi:10.1111/j.1360-0443.2004.00944.x

*Ott, R., and Biller-Andorno, N. (2013). Neuroenhancement among Swiss students - a comparison of users and non-users. *Pharmacopsychiatry* 47, 22–28. doi: 10.1055/s-0033-1358682

*Ott, R., Lenk, C., Miller, N., Neuhaus Buhler, R., and Biller-Andorno, N. (2012). Neuroenhancement - perspectives of Swiss psychiatrists and general practitioners. *Swiss Med. Wkly.* 142, w13707. doi: 10.4414/smw.2012.13707

Parens, E. (1998). *Enhancing Human Traits: Ethical and Social Implications.* Washington, DC: Georgetown University Press.

*Partridge, B., Bell, S., Lucke, J., and Hall, W. (2013). Australian university students' attitudes towards the use of prescription stimulants as cognitive enhancers: perceived patterns of use, efficacy and safety. *Drug Alcohol Rev.* 32, 295–302. doi: 10.1111/dar.12005

*Partridge, B., Lucke, J., and Hall, W. (2012). A comparison of attitudes toward cognitive enhancement and legalized doping in sport in a community sample of Australian adults. *AJOB Prim. Res.* 3, 81–86. doi: 10.1080/21507716.2012.720639

Partridge, B., Underwood, M., Lucke, J., Bartlett, H., and Hall, W. (2009). Ethical concerns in the community about technologies to extend human life span. *Am. J. Bioeth.* 9, 68–76. doi: 10.1080/15265160903318368

President's Council on Bioethics. (2003). *Beyond Therapy. Biotechnology and the Pursuit of Happiness*. Available online at: https://bioethicsarchive.georgetown.edu/pcbe/reports/beyondtherapy/index.html (Accessed March 31, 2014).

Rawls, J. A. (1971). *A Theory of Justice*. Cambridge, MA: Harvard University Press.

Rawls, J. A. (1985). Justice as fairness: political not metaphysical. *Philos. Public Aff.* 14, 223–251.

Repantis, D., Schlattmann, P., Laisney, O., and Heuser, I. (2010). Modafinil and methylphenidate for neuroenhancement in healthy individuals: a systematic review. *Pharmacol. Res.* 62, 187–206. doi: 10.1016/j.phrs.2010.04.002

*Riis, J., Simmons, J. P., and Goodwin, G. P. (2008). Preferences for enhancement pharmaceuticals: the reluctance to enhance fundamental traits. *J. Cons. Res.* 35. doi: 10.1086/588746

*Sabini, J., and Monterosso, J. (2005). Judgments of the fairness of using performance enhancing drugs. *Ethics Behav.* 15, 81–94. doi: 10.1207/s15327019eb1501_6

*Santoni de Sio, F., Faulmüller, N., Savulescu, J., and Vincent, N. A. (in press). "Moving beyond responsibility-shifting, authenticity, and cheating, towards a nature-of-activities approach," in *Cognitive Enhancement: Ethical and Policy Implications in International Perspectives*, eds F. Jotterand, and V. Dubljevic (Oxford: Oxford University Press).

Sarewitz, D. (2010). World view: not by experts alone. *Nature* 466, 688. doi: 10.1038/466688a

*Sattler, S., Forlini, C., Racine, E., and Sauer, C. (2013a). Impact of contextual factors and substance characteristics on perspectives toward cognitive enhancement. *PLoS ONE* 8:e71452. doi: 10.1371/journal.pone.0071452

*Sattler, S., Mehlkop, G., Graeff, P., and Sauer, C. (2014). Evaluating the drivers of and obstacles to the willingness to use cognitive enhancement drugs: the influence of drug characteristics, social environment, and personal characteristics. *Subst. Abuse Treat. Prev. Policy* 9, 1–14. doi: 10.1186/1747-597X-9-8

*Sattler, S., Sauer, C., Mehlkop, G., and Graeff, P. (2013b). The rationale for consuming cognitive enhancement drugs in university students and teachers. *PLoS ONE* 8:e68821. doi: 10.1371/journal.pone.0068821

Sattler, W., and Wiegel, C. (2013). Cognitive test anxiety and cognitive enhancement: the influence of students' worries on their use of performance-enhancing drugs. *Subst. Use Misuse* 48, 220–232. doi: 10.3109/10826084.2012.751426

*Schaefer, G. O., Kahane, G., and Savulescu, J. (2013). Autonomy and enhancement. *Neuroethics.* doi: 10.1007/s12152-013-9189-5. [Epub ahead of print].

Schermer, M. (2008). Enhancements, easy shortcuts, and the richness of human activities. *Bioethics* 22, 355–363. doi: 10.1111/j.1467-8519.2008.00657.x

Schermer, M., Bolt, I., De Jongh, R., and Olivier, B. (2009). The future of psychopharmacological enhancements: expectations and policies. *Neuroethics* 2, 75–87. doi: 10.1007/s12152-009-9032-1

*Scheske, C., and Schnall, S. (2012). The ethics of 'smart drugs': moral judgments about healthy people's use of cognitive-enhancing drugs. *Basic Appl. Soc. Psych.* 34, 508–515. doi: 10.1080/01973533.2012.711692

Schicktanz, S., Schweda, M., and Wynne, B. (2012). The ethics of 'public understanding of ethics'–why and how bioethics expertise should include public and patients' voices. *Med. Health Care Philos.* 15, 129–139. doi: 10.1007/s11019-011-9321-4

*Schildmann, J., Beranek, R., and Roser, P. (2013). "Prevalence and attitudes to neurenhancement among students., results of a survey at the UAMR Universities" in *Conference Presentation* (Bochum: Ruhr University Bochum, Bochum).

Schoomaker, E. B. (2007). Military medical research on cognitive performance: the warfighter's competitive edge. *Aviat. Space Environ. Med.* 78, B4–B6.

*Schuijff, M., and Brom, F. W. A. (2013). "The dynamics of citizen deliberation regarding human enhancement in the Netherlands," in *Beyond Therapy v. Enhancement? Multidisciplinary Analysis of a Heated Debate,* ed F. Lucivero and A. Vedder (Pisa: Pisa University Press), 143–161.

Smith, M. E., and Farah, M. J. (2011). Are prescription stimulants "smart pills"? The epidemiology and cognitive neuroscience of prescription stimulant use by normal healthy individuals. *Psychol. Bull.* 137, 717–741. doi: 10.1037/a0023825

*Sweeney, S. (2010). The use of prescription drugs for academic performance enhancement in college aged students. *Social Work Theses* Paper 48.

Teter, C. J., McCabe, S. E., Cranford, J. A., Boyd, C. J., and Guthrie, S. K. (2005). Prevalence and motives for illicit use of prescription stimulants in an undergraduate student sample. *J Am. Coll. Health.* 53, 253–262. doi: 10.3200/Jach.53.6.253-262

Van Der Schaaf, M. E., Fallon, S. J., Ter Huurne, N., Buitelaar, J., and Cools, R. (2013). Working memory capacity predicts effects of methylphenidate on reversal learning. *Neuropsychopharmacology* 38, 2011–2018. doi: 10.1038/npp.2013.100

Warren, O. J., Leff, D. R., Athanasiou, T., Kennard, C., and Darzi, A. (2009). The neurocognitive enhancement of surgeons: an ethical perspective. *J. Surg. Res.* 152, 167–172. doi: 10.1016/j.jss.2007.12.761

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 January 2014; accepted: 21 March 2014; published online: 17 April 2014.
*Citation: Schelle KJ, Faulmüller N, Caviola L and Hewstone M (2014) Attitudes toward pharmacological cognitive enhancement—a review. Front. Syst. Neurosci. **8**:53. doi: 10.3389/fnsys.2014.00053*
This article was submitted to the journal Frontiers in Systems Neuroscience.

*References marked with an asterisk indicate studies of the public's opinion on PCE; their results are discussed in this review.

frontiers in
SYSTEMS NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 17 February 2015
doi: 10.3389/fnsys.2015.00010

A survey of substance use for cognitive enhancement by university students in the Netherlands

Kimberly J. Schelle[1,2]*, Bas M. J. Olthof[1,3], Wesley Reintjes[1,4], Carsten Bundt[1,5], Joyce Gusman-Vermeer[1] and Anke C. C. M. van Mil[1,6]

[1] *Radboud Honours Academy, Radboud University, Nijmegen, Netherlands*
[2] *Department of Industrial Design, Eindhoven University of Technology, Eindhoven, Netherlands*
[3] *Faculty of Medical Sciences, Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK*
[4] *Department of Neurology, Isala Clinics, Zwolle, Netherlands*
[5] *Department of Experimental Psychology, Ghent University, Ghent, Belgium*
[6] *Department of Physiology, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Wanja Wolff, University of Potsdam, Germany
Eshetu Haileselassie Engeda, University of Gondar, Ethiopia

****Correspondence:***
Kimberly J. Schelle, Radboud Honours Academy, Radboud University, Comeniuslaan 4, 6525 HP Nijmegen, Netherlands
e-mail: kimberlyschelle@gmail.com

Background: Pharmacological cognitive enhancement, using chemicals to change cellular processes in the brain in order to enhance one's cognitive capacities, is an often discussed phenomenon. The prevalence among Dutch university students is unknown.

Methods: The study set out to achieve the following goals: (1) give an overview of different methods in order to assess the prevalence of use of prescription, illicit and lifestyle drugs for cognitive enhancement (2) investigate whether polydrug use and stress have a relationship with cognitive enhancement substance use (3) assessing opinions about cognitive enhancement prescription drug use. A nationwide survey was conducted among 1572 student respondents of all government supported Dutch universities.

Results: The most detailed level of analysis—use of specific substances without a prescription and with the intention of cognitive enhancement—shows that prescription drugs, illicit drugs and lifestyle drugs are respectively used by 1.7, 1.3, and 45.6% of the sample. The use of prescription drugs and illicit drugs is low compared to other countries. We have found evidence of polydrug use in relation to cognitive enhancement. A relation between stress and the use of lifestyle drugs for cognitive enhancement was observed. We report the findings of several operationalizations of cognitive enhancement drug use to enable comparison with a wider variety of previous and upcoming research.

Conclusions: Results of this first study among university students in the Netherlands revealed a low prevalence of cognitive enhancement drug use compared to other countries. Multiple explanations, such as a difference in awareness of pharmacological cognitive enhancement among students, accessibility of drugs in the student population and inclusion criteria of enhancement substances are discussed. We urge enhancement researchers to take the different operationalizations and their effects on the prevalence numbers into account.

Keywords: cognitive enhancement, neuroenhancement, smart drugs, prescription stimulants, non-medical use, illicit drugs, lifestyle drugs, self-report

INTRODUCTION

Recently the topic of cognitive enhancement has received much attention, both in popular media (e.g., Schwarz, 2012; Sharrett, 2012; Shiner, 2013; Monks, 2014) and academic literature (e.g., a special issue on cognitive enhancers by Neuropharmacology Lynch et al., 2013). Cognitive enhancement can be accomplished in a variety of ways. Some methods use advanced technology to modulate brain activity, e.g., deep brain stimulation, whilst other methods are based on chemicals that are aimed at changing the cellular processes in the brain, also named pharmacological cognitive enhancement. Pharmacological cognitive enhancement refers to boosting cognitive capacities by using substances, with the aim to enhance one's performance above baseline levels (e.g., Bostrom and Sandberg, 2009). Most substances regularly referred to in the discussion about pharmacological cognitive enhancement were originally developed to treat neuropsychiatric disorders that are often accompanied by cognitive deficits. A drug widely reported to be used with the purpose of pharmacological cognitive enhancement is methylphenidate (e.g., Ritalin, Concerta), which was developed as a treatment for attention deficit hyperactivity disorder (ADHD). Other drugs often used to boost cognitive capacities include modafinil and amphetamines (Ragan et al., 2013). However, recently also other substances, such as caffeine and nicotine as well as several illicit drugs, are included in the discussion of the cognitive enhancement literature (e.g., Franke et al., 2011b, 2012; Wolff et al., 2014).

The expectations of the effect prescription drugs for cognitive enhancement exert in healthy individuals often exceed their real effect (Repantis et al., 2010). Whether effective or not, the prevalence studies have shown that some people do experiment with drugs to boost their (cognitive) performance (Smith and Farah, 2011).

Herman-Stahl et al. (2007) suggested that the use of prescription drugs to improve one's cognitive performance is especially common among individuals in cognitively demanding environments, such as schools and universities. The authors showed that college students are more likely to use prescription stimulants for nonmedical purposes than young adults who are not enrolled in college, although no intentions of use (e.g., recreational, or to enhance performance) were examined in this study. In addition, most of the prevalence studies conducted so far have focused on the university student population. Smith and Farah (2011) give an overview of 28 epidemiological studies on the prevalence of nonmedical prescription drug use that were conducted among secondary and post-secondary students in the US and Canada before July 2010. The prevalence numbers of lifetime nonmedical stimulant use among university students ranged from 5.3% in an online study reaching over 2000 respondents (DuPont et al., 2008) to 55% in a study among 307 fraternity members (DeSantis et al., 2009). Smith and Farah (2011) conclude that it is hard to draw quantitative conclusions from the list of studies, as they differ in many ways. For example, there are marked differences in the way the prevalence numbers are calculated, where some studies include all nonmedical use, others only include nonmedical use with the intention to enhance ones cognitive performance. This means that in the latter condition users with solely recreational or experimental intentions are excluded from the prevalence numbers that are presented. Moreover, some studies focus solely on the intention, meaning that also individuals with a prescription are counted as a cognitive enhancement drug user when they report to use these drugs for nonmedical reasons, while others exclude all users with a prescription regardless of their intention of use. Smith and Farah (2011) also describe a large variation between studies in sampling method and demographic characteristics, ranging from studies examining a single department at a single institution to nationwide epidemiological surveys on nonmedical drug use. These differences have a major influence on the outcome of the studies because of several factors that can vary between institutions or regions. In a national cross-section study of over 10,000 students enrolled at 4-year colleges McCabe et al. (2005) demonstrated, that the nonmedical use of prescription stimulants was higher in the North-Eastern region of the US, compared to other regions, and higher for institutions with more competitive admission criteria compared to less competitive admission criteria. Finally, a difference can be found in the way that cognitive enhancement drugs are addressed in the questionnaire, for example by either questioning general drug use for the purpose of enhancement or questioning the use of specific types of stimulants (e.g., Ritalin) and/or active ingredient (e.g., methylphenidate) for this purpose. The latter limits the number of stimulants and/or active ingredients that are taken into account in the survey, but leads to more specific questions for the respondent.

Recently more and more studies have been conducted on the university student population in (mostly Western) Europe. The aforementioned variety in definitions and demographic characteristics cause difficulty in comparing the prevalence numbers of these studies as well. For example, Holloway and Bennett (2012) are sometimes mentioned in the discussion on cognitive enhancement drugs, while they focus on general nonmedical use and only briefly mention the intention of cognitive enhancement. From the sample of 1614 students 33% reported to use prescription drugs nonmedical, but only three students reported the reason "to study." Castaldi et al. (2012) on the other hand, focus solely on the intention, and thus include two students who have a prescription in their prevalence number of 16% of students who use cognitive enhancement drugs in a sample of 77 Italian medical students. Also Pustovrh and Mali (2014) did a (pilot) study in only a single institution, in Slovenia, resulting in a prevalence of 11 out of 211 students (5.21%) who had ever used prescription drugs for cognitive enhancement.

In contrast to the aforementioned studies, the focus of studies seems to shift from prevalence of substance use for cognitive enhancement toward broader topics, such as the motives behind the use, differences between users and non-users, or theoretically grounded accounts of why certain students choose to use drugs for cognitive enhancement while others do not (Eickenhorst et al., 2012; Sattler and Wiegel, 2013; Wolff and Brand, 2013; Wolff et al., 2013, 2014; Ott and Biller-Andorno, 2014). However, prevalence numbers are usually presented as well and will be reviewed here even though they are not always the primary aim of study. Larger studies in Germany and Switzerland demonstrate differences in targeting specific substances or a more general group of pharmacological cognitive enhancers. Sattler and Wiegel (2013), for example, examined prior use by asking whether respondents had ever used a prescription medicine without medical necessity to enhance cognitive efficiency, resulting in a lifetime prevalence of 4.56%. Ott and Biller-Andorno (2014) examined the particular use of Ritalin, Adderall or Modasomil among 1765 students at one Swiss university, and found a prevalence of 6.2% of 1765 students who use prescription drugs without a prescription to increase concentration or alertness, of which 4.7% more specifically reported study purposes. However, they claim the questionnaire is not designed to be representative for their population. Maier et al. (2013) examined the use of 17 specific substances with the intention to enhance cognitive performance, categorized as prescription drugs (e.g., methylphenidate), drugs of abuse (e.g., alcohol) and other substances (e.g., caffeine). They found a prevalence of 7.6% for nonmedical prescription drug use for cognitive enhancement and 7.8% for drugs of abuse for cognitive enhancement among their sample of 6275 students from three Swiss universities. This points to a more widespread phenomenon among several surveys in Germany, to not only include prescription drugs as potential enhancers, but also illicit drugs and/or more commonly available substances (also: lifestyle drugs) such as coffee and energy drinks. Most studies clearly separate them, such as Wolff et al. (2014), who demonstrated the use of lifestyle, prescription and illicit drugs for cognitive enhancement among approximately 1000 students all over Germany, and found a lifetime prevalence of respectively, 83.2, 5.8, and 3.5%.

Franke et al. (2011a,b) surveyed the use of coffee, caffeinated drinks, caffeine tablets, prescription drugs, and illicit drugs for cognitive enhancement among high school and undergraduate students and found a prevalence of respectively, 53.2, 39, 10.5, 1.3, and 2.6%. However, other researchers in Germany diverge from this distinction between lifestyle, prescription and illicit drugs, and group them in one general class of cognitive enhancers. Dietz et al. (2013), for instance, report a 12-month prevalence of use of cognitive-enhancing drugs of 20%. In this number the authors include the use of prescription drugs, illicit drugs and caffeine tablets. Eickenhorst et al. (2012) grouped prescription drug use and illicit drug use to improve cognitive performance or mood, and found a prevalence of 7% among 1324 students and graduates.

The current study does not aim to solve the problems that arise due to different definitions, sampling methods or questions, nor do we aim to provide a theoretical framework for pharmacological cognitive enhancement drug use. We aim to contribute to the discussion by examining different study methods in the same sample. This creates the possibility to directly compare the influence of certain questions and inclusion criteria on the resulting prevalence number. Furthermore, the present study sets out to assess the prevalence of pharmacological cognitive enhancement by means of a web-based survey administered to university students in the Netherlands, a sample that to our knowledge has not been examined before. There are only a few studies investigating the use of cognitive enhancers in the Dutch population. Ganpat et al. (2009) surveyed the illicit use of prescription drugs with the intention to improve performance in sport and study among adolescents between 14 and 17 years old and found an overall prevalence rate of 1.7%. A second study assessed the quantity of psychopharmacological enhancement among Dutch psychiatrists and other physicians working in psychiatry (Timmer and Glas, 2012). The results demonstrated a lifetime prevalence of 11%. This latter number is comparable to recent results of a survey among German surgeons (Franke et al., 2013).

In an attempt to reach a representative sample our survey has been advertised online and offline among students of all 14 government supported universities in the Netherlands. The survey examines the prevalence of general nonmedical use of prescription drugs, and the use of particular prescription drugs (methylphenidate, modafinil, beta blockers, and rivastigmine) with the specific purpose of cognitive enhancement with and without a prescription. Furthermore, we will assess the use of prescription drugs for cognitive enhancement with a question not targeted at particular drugs, but at the general group of prescription drugs. Furthermore, we will describe the use of lifestyle and illicit drugs with the purpose of cognitive enhancement. It is impossible to make a comparison of all definitions and study methods in previous studies, but in this way many differences of prior studies are addressed, which makes it possible to directly compare prevalence numbers by different methods within this study and to studies with similar methods abroad.

In addition of giving an overview of different prevalence numbers of the use of substances for cognitive enhancement in the student population in the Netherlands, we also examine two topics that have been found to relate to pharmacological cognitive enhancement, being polydrug use and the relationship between using cognitive enhancement substances and stress. Polydrug use considers the finding that there is a positive relationship between the use of prescription drugs for cognitive enhancement and the use of other substances in general (Barret et al., 2005; McCabe et al., 2005; Eickenhorst et al., 2012; Mazanov et al., 2013). Furthermore, it regards a positive relationship between the use of prescription drugs for cognitive enhancement, the use of illicit drugs for cognitive enhancement and the use of lifestyle drugs for cognitive enhancement, meaning that if somebody uses one of these for cognitive enhancement, there is a higher chance of using substances from the other groups with the purpose of cognitive enhancement as well (Maier et al., 2013; Wolff and Brand, 2013; Ott and Biller-Andorno, 2014). We hypothesize that university students in the Netherlands will also display polydrug use, meaning that (1) users of prescription drugs for the purpose of cognitive enhancement are more likely to use other substances than non-users and (2) there is a correlation between the use of prescription, illicit and lifestyle drugs for cognitive enhancement.

Second, Maier et al. (2013) demonstrated that prescription and illicit drug use for cognitive enhancement is related to perceived pressure to perform. Wolff et al. (2014) found a similar relation for the use of prescription and lifestyle drug use for cognitive enhancement and self-reported strain. Wolff and Brand (2013) demonstrated that overwhelming demands in school could predict the use of prescription drugs and lifestyle drugs for cognitive enhancement. With a longitudinal design Sattler and Wiegel (2013) demonstrated that increased cognitive test anxiety increased the prevalence of nonmedical prescription drug use for cognitive enhancement over time. We hypothesize that a similar relation between the use of cognitive enhancement substances and stress will be prevalent among students in the Netherlands; and thus that students who use substances for the purpose of cognitive enhancement will report more stress than students who do not use substances for the purpose of cognitive enhancement.

Finally, several surveys have examined attitudes toward the use of prescription drugs for cognitive enhancement. In general, the public displays similar concerns as described in the academic literature on the topic, concerning topics such as the safety of using prescription drugs by healthy people, the possibility of being coerced in using a drug for cognitive enhancement and the fairness of using drugs to enhance ones cognitive performance (Schelle et al., 2014). To add to this discussion we assessed opinions in the current sample toward several statements regarding the safety and policy surrounding the use of prescription drugs for cognitive enhancement.

To sum up, the survey aimed at (1) giving an overview of different methods to assess the prevalence of use of substances for cognitive enhancement and apply these methods to a new sample of university students in the Netherlands (2) investigating whether findings of polydrug use and the relationship between cognitive enhancement substance use and stress can also be applied to this population and (3) assessing opinions about the use of prescription drugs for cognitive enhancement.

METHODS

RESPONDENTS

In the current study, 1572 respondents of a total population of approximately 245,000 students registered at Dutch universities, replied to a nationwide poster spread, social media advertisements and a letter to student organizations. Prior to analysis 69 respondents were excluded [exclusion criteria were not being a student at a Dutch university, technical difficulties with the online questionnaire and an affirmative answer on a control question (see below)], resulting in a final sample of 1503 respondents with a mean age of 21.8 years (± sd 2.8 years; 70% women). The sample included students of all 14 government supported Dutch universities, although the relative distribution of respondents was not equal for different universities. Respondents were stimulated to complete the questionnaire by raffling one tablet PC, 30 shopping vouchers (€15,-) and 20 cinema vouchers (€7, 50).

PROCEDURE

The questionnaire was an anonymous online survey in Dutch, which could only be accessed after signing a digital informed consent form. This form was followed by the questionnaire as described in a next section. After submission of the questionnaire a new non-related website opened where the respondent was offered the opportunity to enter in the lottery for the tablet PC and vouchers. Data were stored in an offline database for later analysis. Care was taken not to store IP addresses from the respondents in the dataset. Contact information needed for distribution of the prizes was stored in a separate data file. The procedure and questionnaire are approved by the Ethics Committee Faculty of Social Sciences (ECSS) of the Radboud University, Nijmegen, the Netherlands.

QUESTIONNAIRE

The first section of our questionnaire assessed demographics and background characteristics. Furthermore, we assessed study behavior (e.g., time spent studying) and study outcome to be able to further characterize the cognitive enhancement substance user. After that, we translated and adapted the "Perceived Stress Scale" (Cohen et al., 1983) to a version assessing "Perceived Study Stress" by inserting the word "study" in front of the word "stress" each time this was mentioned in the original scale. The remainder of the survey focused on the use of and the opinion about several substances, the main outcome measures of this study.

The length of the survey depended on the amount of substances used, because respondents were routed toward more specific questions about substances. All questions had a forced response format, meaning that respondents could not skip the question. For most respondents the survey was about 80 questions long. On average, respondents took approximately 18 min to complete the survey.

MAIN OUTCOME MEASURES

Type of substances

The main outcome measures concern the use of prescription drugs, illicit drugs (stimulants, soft drugs and hard drugs) and lifestyle drugs (alcohol, nicotine, and caffeine). We asked respondents to indicate their use of prescription drugs in two different ways, namely with questions about four prescription drugs in particular (methylphenidate, modafinil, beta blockers, and rivastigmine) and questions about the use of prescription drugs in general.

The use of specific prescription drugs was part of a single question in which we asked respondents to indicate whether they had used one or more of the following substances since the start of their university studies, followed by examples as shown in the following overview: alcohol (e.g., in beer, wine, liquor), nicotine (e.g., in cigarettes, roll-ups), caffeine (e.g., in coffee, energy drinks), pharmacy products (such as painkillers, nutritional supplements), soft drugs (such as marihuana), smart shop products (such as memory boosters, herb pills), stimulants (such as amphetamine, cocaine), hard drugs (such as lysergic acid diethylamide (LSD), heroine), methylphenidate (e.g., in Ritalin), modafinil (e.g., in Provigil), beta blockers (e.g., in Propanolol) and rivastigmine (e.g., in Exelon). We added a non-existent drug to the list of possible substances respondents could indicate to have used. This drug was named "Hoxazine (e.g., in Hypersotaline)" and was placed there to detect untrustworthy results. Respondents who admitted to have used this non-existent drug were excluded from further analysis.

Because we only included four often discussed prescription drugs that could potentially be used for cognitive enhancement we also asked a question regarding general prescription drug use. Respondents were asked to indicate whether they had ever used a prescription drug without having a prescription themselves. If they confirmed this question they were routed toward a question about their reasons for using prescription drugs.

Use of substances

If respondents indicated to have been in contact with a substance they were routed toward questions about the specific substance(s) to indicate amount, times and reasons of usage. In the case of prescription drugs they were also asked to indicate whether they had a prescription or not. The answer options for amount, times and reasons of usage were based on questions that have previously been developed for the questionnaire "Family and Health 2003/2004" of the project "Family and Health" of the Radboud University Nijmegen (Heatherton et al., 1991; Engels et al., 1999; Engels and Knibbe, 2000; Harakeh et al., 2005). Specific categories and answer options for amount and times of usage were different for certain substance categories. Therefore, they will be discussed in the following sections regarding the specific substances. Answer options for amounts and times of usage of substances which were not present in the "Family and Health 2003/2004" were created based on questions asked on a similar substance in the questionnaire.

The "Family and Health 2003/2004" questionnaire discusses four general reasons for substance usage: coping with stress, conformity to peers, enhancement (feeling good) and social substance use, related to the four-factor model of alcohol use (Cooper, 1994). We added four answer options in the question about reasons for usage for each substance: (1) "to enhance study performance" as this was our main target of interest, (2) "medical reasons" and (3) "to lose weight" as it applied to some of the

Box 1 | Description of the term "smart pills" (translated from Dutch).

Some students use drugs which are only available with a prescription, without having a prescription themselves. Some other students do have a prescription, but they use a higher dose than prescribed. When they use those drugs to improve their study results, these drugs are called smart pills. The statements below are all about smart pills. Please fill in to what extent you agree with these statements.

Table 1 | The number of respondents using specific routes of obtainment for prescription drugs with the purpose of cognitive enhancement from the group of respondents who indicated to "less than often" have a prescription for the drug themselves.

Ways of obtainment	Friends, free	Friends, paid	Online	Stolen	Work/School	Other
Methylphenidate ($N = 21$)	17	8	5	2	0	0
Beta blocker ($N = 7$)	2	2	2	0	0	2

substances different than alcohol on which the four-factor model was based, and (4) "other" to provide respondents with an answer option in case their answer was not in the list.

From these questions specific measures per substance (category) were derived. The amount of nonmedical users was calculated by including all users of the substance (category) who reported to at least sometimes (on a four-point scale of seldom; sometimes; regularly; often) use the substance for any other than "medical reasons" (exception: general prescription drug use, for which this measure could only be calculated for users who do not have a prescription themselves). The amount of users using a substance (category) without a prescription was calculated by including all users of the substance (category) minus the users who reported to use the substance without "regularly" or "often" (similar four-point scale of seldom; sometimes; regularly; often) to have a prescription. The amount of users using the substance (category) with the purpose of cognitive enhancement was calculated by including all users of the substance who reported to use the substance at least sometimes "to enhance study performance" (similar four-point scale).

Opinions: prescription drug use for cognitive enhancement

Further, we were interested in the attitude of our respondents toward the use of prescription drugs for the purpose of cognitive enhancement. Respondents were presented with 17 statements and consequently asked to provide a response on a five-point Likert scale, indicating their (dis) agreement with the statement at hand (see **Table 2** for the list of statements). Before reading these statements we presented a short description of the term "smart pills" to the respondents (see **Box 1** for a translated version of the Dutch text).

DATA ANALYSIS

The first hypothesis, regarding polydrug use, has two components. Chi-square analyses were conducted to examine whether users of prescription drugs are more or less likely to use other substances than non-users. Secondly, Chi-square analyses were conducted to examine the association between the use of prescription drugs, illicit drugs, and lifestyle drugs for the purpose of cognitive enhancement. The second hypothesis, regarding the relationship between substance use for cognitive enhancement and stress, was examined with One-Way ANOVA's for the differences in perceived study stress scores between users and non-users according to our different operationalizations. P-values <0.05 (two-tailed) were considered statistically significant. The analyses were conducted with IBM SPSS Statistics version 22.0.

RESULTS

PRESCRIPTION DRUG USE

Use of specific prescription drugs

None of the respondents reported to use rivastigmine or modafinil. In our sample, 52 students indicated to have used methylphenidate, while 36 students indicated to have used beta blockers. Nonmedical use (at least "sometimes" use for any other reason than medical) of methylphenidate was self-reported by 80.8% of methylphenidate users (2.8% of the total sample of respondents) and 61.1% of beta blockers users (1.5% of the total sample of respondents). Total nonmedical use of specific prescription drugs in the sample is 4.0% (due to four users using both beta blockers and methylphenidate at least sometimes for nonmedical purposes). Excluding the respondents who indicated to "regularly" or "often have a prescription" results in 2.4% of respondents reporting to use prescription drugs at least sometimes for a nonmedical purpose without having prescription.

From the methylphenidate users 73.1% (2.5% of the total sample of respondents) reported to use methylphenidate at least sometimes for the specific purposes of improving ones study results (cognitive enhancement). From the beta blocker users 38.9% (0.9% of the total sample of respondents) reported to use beta blockers at least sometimes for the specific purpose of improving ones study results. Total specific prescription drug use with the intention of cognitive enhancement in the sample is 3.2%.

Furthermore, to examine the prevalence of the use of prescription drugs for the purpose of cognitive enhancement without having a prescription, we excluded respondents who indicated to use prescription drugs regularly or often with a prescription. A group of 40.4% of the methylphenidate users reported to use methylphenidate less than often with a prescription, and at least sometimes for the purpose of cognitive enhancement (1.4% of the total sample of respondents). For beta blockers this prevalence was 19.4% (0.5% of the total sample of respondents). Total use of specific prescription drugs with (at least sometimes) the intention of cognitive enhancement without (regularly or often) having a prescription is 1.7%. **Table 1** shows via which other methods these respondents obtained their prescription drug. Most users without a prescription acquire the drugs via people they know.

Use of a general group of prescription drugs

Sixty respondents (4.0% of the total sample of respondents) admitted to use a prescription drug without having a prescription for the drug. Forty-six respondents (3.1% of the total sample of respondents) reported nonmedical use without a prescription (at least "sometimes" use for any other reason than medical). From the 60 respondents, 40% used these prescription drugs at least sometimes to improve their study results (2.7% of the total sample of respondents).

Opinions about prescriptions drug use for cognitive enhancement

The percentage of respondents that (dis)agreed with 17 statements about the use of "smart pills" are presented in **Table 2**. Most respondents disagree with statements regarding the respondent being aware of the use of smart pills. There is less agreement about statements regarding the risks related to the use of smart pills, the fairness of the use of smart pills, and the regulation of the use of smart pills.

ILLICIT DRUG USE

Soft drugs, hard drugs and stimulants are considered illicit drugs. **Table 3** displays the prevalence of the use of the three categories of illicit drugs, the use of illicit drugs for nonmedical reasons (at least "sometimes" use for any other reason than medical) and the use of illicit drugs with the specific purpose of cognitive enhancement. Total use of illicit drugs is 20.5%. The use of illicit drugs for nonmedical reasons is 20.4% and with the specific purpose of cognitive enhancement (CE) 1.3%.

LIFESTYLE DRUG USE

Alcohol, nicotine, caffeine, over the counter pharmacy products, and (legal) smart shop products are considered lifestyle drugs. **Table 4** displays the prevalence of the use of the categories of lifestyle drugs, the use of lifestyle drugs for nonmedical reasons (at least "sometimes" use for any other reason than medical) and the use of lifestyle drugs with the specific purpose of cognitive enhancement. Total use of lifestyle drugs is 92.8%. The use of lifestyle drugs for nonmedical reasons is 90.5% and with the specific purpose of cognitive enhancement 45.6%.

Table 5 provides an overview of the prevalence of the use of certain drugs according to the above described definitions.

POLYDRUG USE

Users of specific prescription drugs with/without a prescription reported more often the use of soft drugs, stimulants, nicotine and over the counter pharmacy products. Users of prescription drugs without a prescription were more likely than non-users to report use of soft drugs, nicotine, caffeine and over the counter pharmacy drugs. **Table 6** provides the test statistics for these tests. For most substances the data confirm the hypothesis that users of prescription drugs for the purpose of cognitive enhancement are more likely to use other substances than non-users of prescription drugs for the purpose of cognitive enhancement.

Furthermore, Chi square tests of the association between prescription drugs, illicit drugs, and lifestyle drugs for the

Table 3 | Prevalence of the use of soft drugs, hard drugs and stimulants, calculated from the total sample.

	Soft drugs (%)	Hard drugs (%)	Stimulants (%)
Use	19.1	1.9	4.9
Nonmedical use	19.0	1.9	4.8
Use for CE	0.7	0.1	0.6

Table 2 | Percentages of agreement toward statements about smart pills ($N = 1503$).

Options	Strongly disagree	Disagree	Neutral	Agree	Strongly Agree
1. I am aware of students using "smart pills" regularly.	85.5	4.6	2.9	5.1	1.9
2. I am aware of students using "smart pills" during finals week.	83.3	4.8	2.5	6.9	2.5
3. I have spoken about "smart pills" with other students.	78.2	5.1	3.0	9.0	4.7
4. I have been offered stimulant drugs by another student.	92.6	1.7	1.3	2.8	1.6
5. I know students that I can get "smart pills" from.	84.1	3.2	2.8	5.9	4.1
6. "Smart pills" are easily accessible on this campus.	70.1	4.9	22.2	1.8	1.0
7. "Smart pills" should be freely accessible.	73.6	11.0	11.9	2.6	0.9
8. I think that it is harmless to use "smart pills."	57.6	16.8	19.9	4.0	1.7
9. I think students consider the risks of using "smart pills" before taking them.	36.5	25.1	33.9	3.9	0.5
10. I know enough about "smart pills" to safely use them.	67.7	13.0	11.4	4.7	3.3
11. Students should be informed about the risks and possibilities of "smart pills."	12.3	8.0	20.4	35.0	24.3
12. I think that "smart pills" provide an unfair advantage for students compared to those that don't take the drugs.	32.5	19.4	27.1	15.0	5.9
13. I think that the university board on the campus are aware of the use of "smart pills,"	16.2	14.0	64.7	3.8	1.3
14. I am aware of a policy on campus that bans the use of "smart pills."	67.4	15.9	13.2	2.1	1.4
15. The use of "smart pills" should be prohibited on this campus.	10.6	11.2	38.9	21.0	18.3
16. A policy surrounding "smart pills" would allow a fair academic standard for students.	23.8	15.4	36.3	14.9	9.6
17. I think that it is illegal to take "smart pills."	11.2	10.6	51.8	15.1	11.2

Table 4 | Prevalence of the use of alcohol, nicotine, caffeine, over the counter pharmacy products and (legal) smart shop products, calculated from the total sample.

	Alcohol (%)	Nicotine (%)	Caffeine (%)	Pharmacy (%)	Smart shop (%)
Use	84.3	20.3	69.1	59.6	3.7
Nonmedical use	83.2	19.8	66.2	36.8	3.2
Use for CE	1.8	3.0	41.7	9.0	0.5

Table 5 | An overview of the prevalence of the use of drugs for CE.

Nonmedical use of specific prescription drugs with/without prescription	4.0%
Nonmedical use of specific prescription drugs without a prescription	2.4%
Use of specific prescription drugs with/without prescription with the intention of CE	3.2%
Use of specific prescription drugs without a prescription with the intention of CE	1.7%
Nonmedical use of general group of prescription drugs without a prescription	3.1%
Use of general group of prescription drugs without a prescription with the intention of CE	1.6%
Nonmedical use of illicit drug	20.4%
Use of illicit drugs with the intention of CE	1.3%
Nonmedical use of lifestyle drugs	90.5%
Use of lifestyle drugs with the intention of CE	45.6%

Table 6 | Results of the Chi square comparisons of substance use between users and nonusers of prescription drugs, with/without and only without a prescription.

	With and without a prescription ($N = 48$)		Without a prescription ($N = 25$)	
	χ^2	Odds ratio	χ^2	Odds ratio
Alcohol	2.03		2.36	
Nicotine	30.98**	4.56	35.77**	8.78
Caffeine	3.41		4.24*	3.32
Pharmacy	7.87**	2.64	6.28*	3.62
Soft drugs	13.47**	2.90	22.41**	5.62
Smart shop products	X		X	
Stimulant drugs	20.71**	5.02	X	
Hard drugs	X		X	

*Significance levels are indicated with *P < 0.05 and **P < 0.01. X is used to indicate that a statistical test was not performed as small cell sizes precluded significance testing.*

purpose of cognitive enhancement demonstrate that users of lifestyle drugs for the purpose of cognitive enhancement are more likely to use prescription drugs and illicit drugs for the purpose of cognitive enhancement. This partly confirms the hypothesis that there is an association between the use of prescription, illicit, and lifestyle drugs for cognitive enhancement. **Table 7** provides the test statistics for these tests. Similar tests for the relation between the use of illicit drugs for the purpose of cognitive enhancement and prescription drugs for the purpose of cognitive enhancement could not be performed because small cell sizes precluded significance testing.

COGNITIVE ENHANCEMENT AND STRESS

Users of specific prescription drugs with/without a prescription for the purpose of cognitive enhancement and users of lifestyle drugs for the purpose of cognitive enhancement report more study related stress than their respective non-user groups. This means that our hypothesis that students who use substances for the purpose of cognitive enhancement will report more stress than students who do not use substances for the purpose of cognitive enhancement is only confirmed for certain definitions of cognitive enhancement drug use. **Table 8** provides the according test statistics.

DISCUSSION

The present study demonstrates the prevalence of the nonmedical use and the use with the specific purpose of cognitive enhancement of prescription drugs, illicit drugs, and lifestyle drugs among university students in the Netherlands. General use and nonmedical use is larger for lifestyle drugs than illicit drugs, and larger for illicit drugs than prescription drugs. Even though prevalence numbers differ for general and nonmedical use, the use of prescription drugs and illicit drugs with the purpose of cognitive enhancement is rather similar and low in occurrence in the current sample. However, almost half of the respondents use lifestyle drugs with the intention to cognitively enhance themselves. Users of prescription drugs for the purpose of cognitive enhancement are more likely to use other substances than non-users. There is a relation between the use of prescription drugs and the use of illicit drugs, but not with the use of lifestyle drugs when it comes to using these substances to cognitively enhance oneself. The hypothesis, that respondents who use substances for cognitive enhancement experience more stress than non-users, is confirmed for the group of users of specific prescription drugs that include both users with and without a prescription and for users of lifestyle drugs.

The use of substances for cognitive enhancement has not previously been examined in the university student population in the Netherlands. However, the prevalence of the use of prescription drugs without a prescription for the purpose of cognitive enhancement, is in line with a study within a younger Dutch population (14–17 year) in which 1.7% reported to use prescription drugs—which were not prescribed to themselves or in a different way than prescribed to them—with the intention the

Table 7 | Results of the Chi square association between the use of prescription drugs (specific prescription drugs with/without a prescription; specific prescription drugs without a prescription; general prescription drugs without a prescription), illicit and lifestyle drugs for cognitive enhancement.

	Specific with/without		Specific without		General without		Illicit	
	χ^2	OR	χ^2	OR	χ^2	OR	χ^2	OR
Lifestyle	22.56**	4.75	12.15**	4.89	8.51**	3.65	9.68**	4.87

Significance levels are indicated with $^*P < 0.05$ and $^{**}P < 0.01$. The odds ratio (OR) is calculated as a measure of the effect size.

Table 8 | Results of One-Way ANOVAs for the relation between the use of prescription drugs (specific prescription drugs with/without a prescription; specific prescription drugs without a prescription; general prescription drugs without a prescription), illicit and lifestyle drugs for cognitive enhancement and study related stress.

	Users M (sd)	Nonusers M (sd)	F (df 1,1501)	*r*
Specific w/wo	39.29 (7.74)	36.67 (6.81)	35.75*	0.067
Specific wo	38.68 (8.61)	36.72 (6.82)	2.02	
General wo	38.25 (8.56)	36.73 (6.82)	1.17	
Illicit	38.00 (6.61)	36.73 (6.86)	0.67	
Lifestyle	37.89 (6.99)	35.79 (6.59)	35.75**	0.153

Significance levels are indicated with $^*P < 0.05$ and $^{**}P < 0.01$.

enhance performance (Ganpat et al., 2009). Compared to the nonmedical use of prescription drugs in Wales, our result of nonmedical use is far lower (Holloway and Bennett, 2012). The same trend is observed for the use of substances for the specific purpose of cognitive enhancement. The use of specific prescription drugs and general prescription drugs with this intention (1.7 and 1.6%) is lower than most lifetime prevalence numbers in Europe (ranging from 4.6 to 16%) (Castaldi et al., 2012; Maier et al., 2013; Sattler and Wiegel, 2013; Ott and Biller-Andorno, 2014; Pustovrh and Mali, 2014; Wolff et al., 2014). It might be suggested that our numbers are lower due to their timeframe being based on "during respondent's university studies" instead of lifetime prevalence. However, Eickenhorst et al. (2012) also examined use "during studies" and found a prevalence of 7%, similar to other lifetime reports. Furthermore, the only two studies with a low prevalence more similar to our prevalence are conducted in Germany by Franke et al. (2011a) with a lifetime prevalence of 0.8% and by Mache et al. (2012) with a lifetime prevalence up to 2%.

The use of illicit drugs to enhance cognitive performance in our sample (1.3%) is a little lower than previous prevalence numbers of 2.9% (Franke et al., 2011a) and 3.5% in Germany (Wolff et al., 2014; however, a smaller difference is found when comparing to their finding of the point prevalence of 1.7%). When we combine our prevalence of the use of illicit drugs and alcohol for cognitive enhancement and compare it with the category of drugs of abuse (7.8%) by Maier et al. (2013), the resulting prevalence of our study is clearly lower. The use of lifestyle drugs to enhance cognitive performance (45.6%) is lower than found by Wolff et al. (2014; 83.2%), but more in line with the prevalence of the use of coffee for this purpose by 53% respondents of Franke et al. (2011a) and what the authors report as soft enhancers (e.g., coffee) by about half of the respondents of Maier et al. (2013). Again, when looking at the point prevalence instead of lifetime prevalence of the use of lifestyle drugs for cognitive enhancement (52.3%) by Wolff et al. (2014), the difference is smaller. In general our prevalence numbers of prescription drugs and illicit drugs appear to be low compared to research in other European countries, whilst our results for lifestyle drugs are more in line.

One potential reason that explains the low prevalence of the use of substances for cognitive enhancement among university students in the Netherlands can be found by taking a closer look at responses to statements regarding the awareness about the use of prescription drugs for cognitive enhancement purposes. Whereas in a Swiss sample 93.7% of the respondents and in a German sample almost 60% knew that substances could and are being used for the purpose of cognitive enhancement, only 13.7% of our current sample has ever spoken about the use of prescription drugs for cognitive enhancement (Franke et al., 2011a; Maier et al., 2013). Lesser awareness of the possibility to use substances for the purpose of cognitive enhancement among peers can have an impact on the prevalence, in particular for prescription drugs which, when not obtained by a prescription, are often obtained via peers. This is in line with previous research (McCabe and Boyd, 2005; Maier et al., 2013; Ott and Biller-Andorno, 2014).

Although awareness of the use of cognitive enhancement drugs by others is rather low (10%), it is still considerably higher than the actual prevalence of the use of prescription drugs for cognitive enhancement (1.6–3.2%). It remains unclear whether this difference is based on the fact that respondents know the same users, whether students overestimate the use of prescription drugs for cognitive enhancement by other students, whether there is a bias in sampling toward more non-users, or whether they did not report their own use truthfully. Recent research suggests that prevalence rates vary depending on the type of questions. Previous research by Franke et al. (2011a) showed a prevalence in Germany of below 1% while a more recent study, using an altered version of the Randomized Response Technique estimated a prevalence of 20% prescription drugs use for cognitive enhancement in the German student population (Dietz et al., 2013). Dietz et al. (2013) relates this difference to the stigmatized nature of the topic of enhancement drug use, which would indicate that our results demonstrate an underestimated prevalence rate due to non-truthful answers. However, other researchers emphasize the overestimation of the use of cognitive enhancement drugs by peers, which would suggest that the number of 9%, of students who know other students to be users, is unreliable, while our actual prevalence rate is more reliable (Lucke et al., 2011).

In spite of the low prevalence numbers, the majority of the students believe they ought to be receiving adequate information about the risks and opportunities of using prescription drugs for cognitive enhancement. This is in line with findings from previous studies on the opinions about pharmacological cognitive enhancement in which people argued that it was important to be able to make autonomous decisions about the use of such substances (Schelle et al., 2014). However, deciding whether or not to give students or the general population more information about the use of substances for cognitive enhancement is a difficult task. Sandberg (2013) argues that on the one hand the lack of information can create irrational demand and employer coercion when hype will dominate, but at the same time there is not enough information yet—especially on the long term effects—to create a proper risk/benefit analysis.

Modafinil and rivastigmine were not reported as being used by the students in our sample, while being proposed as two of four specific prescription drugs that could be used for cognitive enhancement. One explanation for this discrepancy could be that these two drugs are not as often prescribed in the Netherlands to students compared to methylphenidate and beta blockers. For example, while modafinil is only 10,500 times provided by the public pharmacies in the Netherlands in 2009, the amount of times methylphenidate was provided was already rising to a million prescriptions per year, which it passed in 2011 (Stichting Farmaceutische Kengetallen, 2010, 2012). In contrast, rivastigmine is often prescribed to older people, which may lead to its accessibility being scarce in the student population, especially when compared to the amount of methylphenidate prescriptions in the population. This explanation based on the amount of prescriptions in the corresponding age population is supported by the fact that most prescription drugs which are not obtained by a prescription are obtained via peers, either freely or paid. This corroborates previous studies in other countries (McCabe and Boyd, 2005). It would be interesting to further examine whether different populations in the Netherlands do use prescription drugs like rivastigmine and modafinil for the purpose of cognitive enhancement, especially focusing on those populations for whom the specific effect of the substance is more desirable, or the substance is more accessible. Examples of specific target populations are academics, pilots and surgeons (see e.g., Franke et al., 2013).

As hypothesized, users of prescription drugs for cognitive enhancement reported more often the use of other substances such as soft drugs, nicotine and stimulants than non-users. This polydrug use is consistent with previous studies (Barret et al., 2005; McCabe et al., 2005; Teter et al., 2006; Eickenhorst et al., 2012; Mazanov et al., 2013). Polydrug use is also found in other domains, such as doping in sport (Dunn et al., 2009; Backhouse et al., 2013). Based on their co-occurrence Backhouse et al. (2013) suggest that athletes who engage in legal performance enhancement are an "at-risk" group for transition toward doping, the gateway hypothesis. Our findings display that users of lifestyle drugs for the purpose of cognitive enhancement are more likely to use illicit drugs and prescription drugs for the purpose of cognitive enhancement and thus support previous findings in suggesting that this hypothesis not only plays a role in physical performance enhancement but also for cognitive performance enhancement.

Furthermore, in accordance with previous studies there is an association between the use of prescription drugs and illicit drugs for cognitive enhancement purposes. In contrast to previous findings, no such relation was found between the use of prescription drugs and lifestyle drugs for cognitive enhancement purposes (Maier et al., 2013; Wolff and Brand, 2013; Ott and Biller-Andorno, 2014). An explanation of this discrepancy can be found in the way that the category of lifestyle drugs is approached and measured. Ott and Biller-Andorno (2014) found that use of cigarettes could explain variance in their logistic regression model about cognitive enhancement drug use, while coffee did not. Another explanation might be found in the low awareness about the use of prescription drugs for cognitive enhancement in the current sample. There might be a higher discrepancy between the awareness of the use of prescription drugs for cognitive enhancement and the use of lifestyle drugs for cognitive enhancement than in other countries, resulting in less association between the two types of cognitive enhancement drug use.

Finally, it was proposed that students who use substances for the purpose of cognitive enhancement report more stress than non-users. This hypothesis is confirmed for the group of users of specific prescription drugs that include users with and without a prescription, and for the group of users of lifestyle drugs for cognitive enhancement. Lifestyle drugs are most commonly used for cognitive enhancement in our sample, by almost half of all respondents. Our findings suggest that stress might be a predicting factor for using lifestyle drugs for cognitive enhancement. A similar longitudinal study as Sattler and Wiegel (2013) conducted on the relation between cognitive test anxiety and the use of prescription drugs for cognitive enhancement would inform whether the use of lifestyle drug use is a coping mechanism for (study related) stress or a potential cause of stress. The finding that there is no relation between the use of illicit drugs for cognitive enhancement and stress is supported by previous findings of Wolff et al. (2014) and Wolff and Brand (2013) but not Maier et al. (2013). More research is needed to explore these contrasting findings. Furthermore, future research can also give more insight in why students in the Netherlands who indicate to use a general group of prescription drugs for cognitive enhancement, or specific prescription drugs without having a prescription, do not confirm the previous associations between prescription drugs use for cognitive enhancement and stress (Maier et al., 2013; Sattler and Wiegel, 2013; Wolff et al., 2014).

In this study, a differentiation between different operationalizations of cognitive enhancement substance use is presented. By displaying results based on different operationalizations we aim to create the possibility to compare our results with as many studies as possible and inform the debate about differences in the result due to different research methods. As several of the prevalence numbers of the use of substances for cognitive enhancement in our sample are rather low, large differences between the operationalizations have not arisen. However, our findings suggest that there is less influence of asking about specific prescription drugs vs. a general kind of prescription drugs than there is influence of including or not including users with a prescription for a

prescription drug. Furthermore, our findings demonstrate a large difference between asking nonmedical use of any substance or asking about use for the specific purpose of cognitive enhancement. This supports the importance of a means-to-end relation as proposed by Wolff and Brand (2013) and Wolff et al. (2014).

In addition to the different operationalizations that were proposed in an attempt to tackle most differences between studies on the prevalence of the use of substances for cognitive enhancement, a further topic of interest is found. Recently, more and more researchers start including not only the use of prescription drugs, but also the use of illicit and lifestyle drugs in the debate about cognitive enhancement. It was hard to define which substances belong to which overarching categories, as several frameworks can be chosen to underlie these categories (e.g., legal; normative acceptance; accessibility). Therefore, different categories are used, such as lifestyle drugs vs. soft enhancers (Maier et al., 2013; Wolff et al., 2014) and illicit drugs vs. drugs of abuse (Franke et al., 2011a; Maier et al., 2013; Wolff et al., 2014). When one wants to relate the use of a certain category of substances to for example stress, it is clear that these relations depend on the type of substances included in a certain category. We aimed to create transparency about this topic by providing the prevalence number for each specific substance within each category separately.

LIMITATIONS

A limitation to this study is the sample which, due to convenience sampling, constituted only an approximate representation of the student population in the Netherlands. Women, for example, were oversampled. In addition, the sample was not equally distributed for different universities, as well as not distributed in line with the absolute difference in amount of students of the 14 Dutch government supported universities. However, the percentages of recreational substance use obtained in our sample are lower, but within normal limits, than numbers specified in the National Drug Monitor in the Netherlands (Van Laar et al., 2012). This indicates that, regarding our main topic of drug use, our sample is representative for the population. Therefore, no weighting strategies are applied.

Furthermore, it is clear that although the use of substances for cognitive enhancement is mostly examined in student populations, it is probably not possible to generalize this to other populations. This is supported by the prevalence rate of prescription drug use for cognitive enhancement of 11% among Dutch psychiatrists, which is rather high compared to the prevalence in the current sample (Timmer and Glas, 2012). Even similar aged non-student populations may demonstrate a different pattern of use (Herman-Stahl et al., 2007). Future studies will need to give more insight in the use of substances for cognitive enhancement in other target groups such as in specific occupations, or specific age groups, both within the Netherlands as abroad.

A final limitation regards the study methods. The research is conducted by an online large-scale self-report questionnaire with many questions which might have given rise to a decrease of the feeling of anonymity, a burden in the time that was needed to complete the questionnaire, or for example a lack of memory about certain situations and feelings during the use of certain substances. It is possible that the use of certain substances is stigmatized (Dietz et al., 2013), which may lead to a different reported prevalence rate compared to the actual prevalence rates.

CONCLUSION

To sum up, the present study indicates that the use of prescription drugs and illicit drugs to increase cognitive performance among students in Dutch universities is rather low compared to other European countries, while the use of lifestyle drugs for cognitive enhancement fits the European context better. We have found further evidence of polydrug use in relation to cognitive enhancement, while previous findings of the relation between cognitive enhancement drug use and stress have not been confirmed consistently. We have decided to report the findings of several operationalizations of cognitive enhancement drug use to enable comparison with a wider variety of previous and upcoming research. We urge future researchers to take the discussion about these different operationalizations and the effects that they have on the prevalence numbers into account in designing and reporting future experiments on the use of substances for cognitive enhancement.

ACKNOWLEDGMENTS

We thank prof. dr. H. Zwart and prof. dr. R. Cools for their feedback on previous versions of this article.

REFERENCES

Backhouse, S. H., Whitaker, L., and Petróczi, A. (2013). Gateway to doping? Supplement use in the context of preferred competitive situations, doping attitude, beliefs, and norms. *Scand. J. Med. Sci. Sports* 23, 244–252. doi: 10.1111/j.1600-0838.2011.01374.x

Barret, S. P., Darredeau, C., and Bordy, L. E. (2005). Characteristics of methylphenidate misuse in a university sample. *Can. J. Psychiatry* 50, 457–461.

Bostrom, N., and Sandberg, A. (2009). Cognitive enhancement: methods, ethics, regulatory challenges. *Sci. Eng. Ethics* 15, 311–341. doi: 10.1007/s11948-009-9142-5

Castaldi, S., Gelatti, U., Orizio, G., Hartung, U., Moreno-Londono, A. M., Schulz M., et al. (2012). Use of cognitive enhancement medication among Northern Italian university students. *J. Addict. Med.* 6, 112–117. doi: 10.1097/ADM.0b013e3182479584

Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *J. Health Soc. Behav*. 24, 4, 385–396. doi: 10.2307/2136404

Cooper, M. L. (1994). Motivations for alcohol use among adolescents: development and validation of a four-factor model. *Pscyhol. Assess*. 6, 117–128.

DeSantis, A., Noar S. M., and, Webb, E. M. (2009). Nonmedical ADHD stimulant use in fraternities. *J. Stud. Alcohol Drugs* 70, 952–954. doi: 10.3200/JACH.57.3.315-324

Dietz, P., Striegel, H., Franke, A. G., Lieb, K., Simon, P., and Ulrich, R. (2013). Randomized response estimates for the 12-month prevalence of cognitive-enhancing drug use in university students. *Pharmacotherapy* 33, 44–50. doi: 10.1002/phar.1166

Dunn, M., Mazanov, J., and Sitharthan, G. (2009). Predicting future anabolic-androgenic steroid use intentions with current substance use: findings from an internet-based suvery. *Clin. J. Sport Med.* 19, 222–227. doi: 10.1097/JSM.0b013e31819d65ad.

DuPont, R. L., Coleman, J. J., Bucher, R. H., and Wilford, B. B. (2008). Characteristics and motives of college students who engage in nonmedical use of methylphenidate. *Am. J. Addict.* 17, 167–171. doi: 10.1080/10550490802019642

Eickenhorst, P., Vitzthum, K., Klapp, B. F., Groneberg, D., and Mache, S. (2012). Neuroenhancement among German University students: motives, expectations, and relationship with psychoactive lifestyle drugs. *J. Psychoactive Drugs* 44, 418–427. doi: 10.1080/02791072.2012.736845

Engels, R. C. M. E., and Knibbe, R. A. (2000). Alcohol use and intimate relationships in adolescence: when love comes to town. *Addict. Behav.* 25, 435–439. doi: 10.1016/S0306-4603(98)00123-3

Engels, R. C. M. E., Knibbe, R. A., and Drop, M. J. (1999). Why do late adolescents drink at home? A study on psychological well-being, social integration and drinking context. *Addict Res.* 7, 31–46. doi: 10.3109/16066359909004373

Franke, A. G., Bagusat, C., Dietz, P., Hoffmann, I., Simon, P., Ulrich, R., et al. (2013). Use of illicit and prescription drugs for cognitive or mood enhancement among surgeons. *BMC Med.* 11:102. doi: 10.1186/1741-7015-11-102

Franke, A. G., Bonertz, C., Christmann, M., Huss, M., Fellgiebel, A., Hildt, E., et al. (2011a). Non-medical use of prescription stimulants and illicit use of stimulants for cognitive enhancement in pupils and students in Germany. *Pharmacopsychiatry* 44, 60–66. doi: 10.1055/s-0030-1268417

Franke, A. G., Christmann, M., Bonertz, C., Fellgiebel, A., Huss, M., and Lieb, K. (2011b). Use of coffee, caffeinated drinks and caffeine tablets for cognitive enhancement in pupils and students in Germany. *Pharmacopsychiatry* 44, 331–338. doi: 10.1055/s-0031-1286347

Franke, A. G., Lieb, K., and Hildt, E. (2012). What users think about the differences between caffeine and illicit/prescription stimulants for cognitive enhancement. *PLoS ONE* 7:e40047. doi: 10.1371/journal.pone.0040047

Ganpat, S., Kleinjan, M., and Van de Mheen, D. (2009). *Oneigenlijk Medicijngebruik Onder Nederlandse Jongeren: Aard en Omvang*. Rotterdam: Instituut voor Verslavingsonderzoek (IVO).

Harakeh, Z., Scholte, R. H., De Vries, H., and Engels, R. C. (2005). Parental rules and communication: their association with adolescent smoking. *Addiction* 100, 862–870. doi: 10.1111/j.1360-0443.2005.01067.x

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., and Fagerström, K. O. (1991). The Fagerström test for nicotine dependence: a revision of the Fagerström Tolerance Questionnaire. *Br. J. Addict.* 86, 1119–1127. doi: 10.1111/j.1360-0443.1991.tb01879.x

Herman-Stahl, M. A., Krebs, C. P., Kroutil, L. A., and Heller, D. C. (2007). Risk and protective factors for methamphetamine use and nonmedical use of prescription stimulants among young adults aged 18 to 25. *Addict. Behav.* 32, 1003–1015. doi: 10.1016/j.addbeh.2006.07.010

Holloway, K., and Bennett, T. (2012). Prescription drug misuse among university staff and students: a survey of motives, nature and extent. *Drug Educ. Prev. Policy* 19, 137–144. doi: 10.3109/09687637.2011.594114

Lucke, J. C., Bell, S., Partridge, B., and Hall, W. D. (2011). Deflating the neuroenhancement bubble. *AJOB Neurosci.* 2, 38–43. doi: 10.1080/21507740.2011.611122

Lynch, G., Sahakian, B., Collingridge, G. L., Crawley, J. N., Nye, J. S., and Frenguelli, B. G. (2013). Cognitive enhancers: molecules, mechanisms and minds, 22nd neuropharmacology conference: cognitive enhancers [special issue]. *Neuropharmacology* 64, 1–596. doi: 10.1016/j.neuropharm.2012.08.005

Mache, S., Eickenhorst, P., Vitzthum, K., Klapp, B. F., and Groneberg, D. A. (2012). Cognitive-enhancing substance use at German universities: frequency, reasons and gender differences. *Wien. Med. Wochenschr.* 162, 262–271. doi: 10.1007/s10354-012-0115-y

Maier, L. J., Liechti, M. E., Herzig, F., and Schaub, M. P. (2013). To dope or not to dope: neuroenhancement with prescription drugs and drugs of abuse among Swiss university students. *PLoS ONE* 8:e77967. doi: 10.1371/journal.pone.0077967

Mazanov, J., Dunn, M., Connor, J., and Fielding, M. L. (2013). Substance use to enhance academic performance among Australian university students. *Perform. Enhanc. Health* 2, 110–118. doi: 10.1016/j.peh.2013.08.017

McCabe, S. E., and Boyd, C. J. (2005). Sources of prescription drugs for illicit use. *Addict. Behav.* 30, 1342–1350. doi: 10.1016/j.addbeh.2005.01.012

McCabe, S. E., Knight, J. R., Teter, C. J., and Wechsler, H. (2005). Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction* 99, 96–106. doi: 10.1111/j.1360-0443.2005.00944.x

Monks, K. (2014). *Pimp My Brain: A Week on Smart Drugs. Metro World News.* Available online at: http://www.metro.us/newyork/news/national/2014/02/12/pimp-my-brain-a-week-on-smart-drugs/ (June 1, 2014).

Ott, R., and Biller-Andorno, N. (2014). Neuroenhancement among swiss students - A comparison of users and non-users. *Pharmacopsychiatry* 47, 22–28. doi: 10.1055/s-0033-1358682

Pustovrh, T., and Mali, F. (2014). Exploring some challenges of the pharmaceutical cognitive enhancement discourse: users and policy recommendations. *Neuroethics* 7, 137–158. doi: 10.1007/s12152-013-9192-x

Ragan, C. I., Bard, I., and Singh, I. (2013). What should we do about student use of cognitive enhancers? An analysis of current evidence. *Neuropharmacology* 64, 588–595. doi: 10.1016/j.neuropharm.2012.06.016

Repantis, D., Schlattmann, P., Laisney, O., and Heuser, I. (2010). Modafinil and methylphenidate for neuroenhancement in healthy individuals: a systematic review. *Pharmacol. Res.* 62, 187–206. doi: 10.1016/j.phrs.2010.04.002

Sandberg, A. (2013). Enhancement policy and the value of information. *Am. J. Bioeth.* 13, 34–55. doi: 10.1080/15265161.2013.795826

Sattler, S., and Wiegel, C. (2013). Cognitive test anxiety and cognitive enhancement: the influence of students' worries on their use of performance-enhancing drugs. *Subst. Use Misuse* 48, 220–232. doi: 10.3109/10826084.2012.751426

Schelle, K. J., Faulmüller, N., Caviola, L., and Hewstone, M. (2014). Attitudes toward pharmacological cognitive enhancement - a review. *Front. Syst. Neurosci.* 8:53. doi: 10.3389/fnsys.2014.00053

Schwarz, A. (2012). *Risky Rise of the Good-Grade Pill*. The New York Times. Available online at: http://www.nytimes.com/ (Accessed July 23, 2013).

Sharrett, L. (2012). *In Their Own Words: Study Drugs*. The New York Times. Available online at: http://www.nytimes.com/ (Accessed July 23, 2013).

Shiner, F. (2013). *Drug test Students, Says Scientist Barbara Sahakian, But is it Right? The Huffington Post UK.* Available online at: http://www.huffingtonpost.co.uk/2013/07/02/drug-testing-students-barbara-sahakian n3533065.html (Accessed June 1, 2014).

Smith, M. E., and Farah, M. J. (2011). Are prescription stimulants smart pills? The epidemiology and cognitive neuroscience of prescription stimulant use by normal healthy individuals. *Psychol. Bull.* 137, 717–741. doi: 10.1037/a0023825

Stichting Farmaceutische Kengetallen. (2010). Nog geen rem op ADHD. *Pharmaceutisch Weekblad.* 145, 16–17.

Stichting Farmaceutische Kengetallen. (2012). Gebruik ADHD-middelen niet in te tomen. *Pharmaceutisch Weekblad.* 147, 30–31.

Teter, C. J., McCabe, S. E., LaGrange, M. S. W. K., Cranford, J. A., and Boyd, C. J. (2006). Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. *Pharmacotherapy* 26, 1501–1510. doi: 10.1592/phco.26.10.1501

Timmer, S. J., and Glas, G. (2012). Pillen voor de psych(e); een exploratief onderzoek naar neuro-enhancement onder Nederlandse psychiaters en artsen. *Tijdschr Psychiatry* 54, 371–376.

Van Laar, M. W., Cruts, A. A. N., van Ooyen-Houben, M. M. J., Meijer, R. F., Croes, E. A., Ketelaars, A. P. M., et al. (eds) (2012). *Nationale Drug Monitor*, *Jaarbericht*. Den Haag: Trimbos Instituut, 2013.

Wolff, W., Baumgarten, F., and Brand, R. (2013). Reduced self-control leads to disregard of an unfamiliar behavioral option: an experimental approach to the study of neuroenhancement. *Subst. Abuse Treat. Prev. Policy* 8:41. doi: 10.1186/1747-597X-8-41

Wolff, W., and Brand, R. (2013). Subjective stressors in school and their relation to neuroenhancement: a behavioral perspective on students' everyday life "doping." *Subst. Abuse Treat. Prev. Policy* 8:23. doi: 10.1186/1747-597X-8-23

Wolff, W., Brand, R., Baumgarten, F., Lösel, J., and Ziegler, M. (2014). Modeling students instrumental (mis-) use of substances to enhance cognitive performance: neuroenhancement in the light of job demands-resources theory. *Biopsychosoc. Med.* 8:12. doi: 10.1186/1751-0759-8-12

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 June 2014; accepted: 20 January 2015; published online: 17 February 2015.

Citation: Schelle KJ, Olthof BMJ, Reintjes W, Bundt C, Gusman-Vermeer J and van Mil ACCM (2015) A survey of substance use for cognitive enhancement by university students in the Netherlands. Front. Syst. Neurosci. ***9****:10. doi: 10.3389/fnsys.2015.00010 This article was submitted to the journal Frontiers in Systems Neuroscience.*

frontiers in
SYSTEMS NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 25 February 2015
doi: 10.3389/fnsys.2015.00013

Performance enhancement in the workplace: why and when healthy individuals should disclose their reliance on pharmaceutical cognitive enhancers

Mirko D. Garasic[1]* and Andrea Lavazza[2]

[1] *The Federmann School of Public Policy and Government Campus, The Hebrew University of Jerusalem, Jerusalem, Israel*
[2] *Centro Universitario Internazionale, Arezzo, Italy*

Edited by:
Manuel Fernando Casanova, University of Louisville, USA

Reviewed by:
Vittorio Alessandro Sironi, Research Center on History of Biomedical Thought, Italy
Markus Christen, University of Zurich, Switzerland

****Correspondence:***
Mirko D. Garasic, The Federmann School of Public Policy and Government Campus, The Hebrew University of Jerusalem, Mount Scopus Jerusalem, 9190501 Jerusalem, Israel
e-mail: mdgarasic@fulbrightmail.org

The use of pharmaceuticals cognitive enhancers (PCE) has been stirring growing interest, not only in the scientific domain but also in the popular media, and has probably had some increase recently in academic, professional and military quarters. So this phenomenon is deemed as a normal procedure aimed at improving the performance of an individual as well as the overall standards of an organization. Although the vast majority of countries have some kind of restrictions to reduce the wide non-medical usage of PCE, these can be overcome quite easily. In arguing for our explicit claim that, in many contexts, the use of cognitive enhancers should be disclosed—as a moral and socially relevant duty—we maintain that PCE present typical, or at least not rare, properties. The features are the following: (a) the enhancer has acute and/or chronic effects. In the first case, shortly after taking the drug the performance is significantly better than average; in the second case, there is a growing or lasting effect, which, however, is poised to diminish when one stops taking the drug; (b) those effects are significant (there is a difference in the outcome considered between taking and not taking the drug) and sometimes dramatic; and (c) a third feature, not directly related to enhancers as such, is their varying safety, availability, and legal permissibility, which might either induce people to take them or refrain them from doing so. We will consider the issue of fairness due to "unenhanced" people as well as the potentially dysfunctional social consequences of an undisclosed PCE use.

Keywords: pharmaceutical cognitive enhancers, privacy, fairness, autonomy, performance-enhancing substances, moral duty, social duty

INTRODUCTION

In recent years, the use of pharmaceuticals cognitive enhancers (PCE) has been stirring growing interest, not only in the scientific domain but also in the popular media, and has probably had some increase in academic, professional and military quarters, although such increase is very difficult to assess (McCabe et al., 2005, 2007; Teter et al., 2005; Sahakian and Morein-Zamir, 2007; Russo et al., 2008; Franke et al., 2014). PCE can be tentatively defined as drugs which have been shown to improve to some degree some features of human cognition, namely attention, executive functioning (planning, inhibition, and problem-solving), memory and learning, via altering specific neurotransmitters. The most investigated and probably used PEC are off-label drugs primarily aimed at treating neurodegenerative diseases, ADHD, or narcolepsy, which are taken by the healthy to enhance their performance (Greely et al., 2008; Lanni et al., 2008; Marchant et al., 2009; Advokat and Scheithauer, 2013; Mereu et al., 2013; Sattler et al., 2013; Wood et al., 2013; Urban and Gao, 2014) across university campuses (Babcock and Byrne, 2000; Shillington et al., 2006; DeSantis et al., 2008; here we talk about "academic doping", see Cakic, 2009) as well as other competitive contexts (Sahakian and Morein-Zamir, 2007; Chandler, 2012). For reason of space, arguments related to the current restrictions in place (Smith and Farah, 2011) concerning the purchase of these "smart drugs" (Mehlman, 2004)[1]—and their very limited impact—(Farah et al., 2004; Herman-Stahl et al., 2007) will not be discussed here. Instead, this paper aims at analyzing a specific problem related to PCE use. In particular, it is our goal to show the tension between the *local effect* (the enhancement of the individual performance) and the *global effects* (the unwanted social results deriving from the spreading of the use of PCE). Those effects are to be related to two distinct concepts, which— in that case—are at odds: (1) autonomy, and consequently privacy, due to people in their choice of enhancing themselves; and (2) fairness, which is a socially appreciated value. In the following sections we shall considered them separately, starting with the former and later considering its implications on the latter.

LOCAL EFFECT AS PRIVACY

In arguing for our explicit claim that the use of cognitive enhancers should be disclosed, we maintain that such enhancers

[1] Maxwell Mehlman provides a useful account of the use, effects and contexts of these drugs (Mehlman, 2004).

present typical, or at least not rare, properties. The features are the following: (a) the enhancer has acute and/or chronic effects. In the first case, shortly after taking the drug the performance is significantly better than average; in the second case, there is a growing or lasting effect, which, however, is poised to diminish when one stops taking the drug; and (b) those effects are significant (the outcome is different depending on whether the drug was taken or not) and sometimes dramatic; (c) a third feature, not directly related to enhancers as such, is their varying safety, availability, and legal permissibility, which might either induce people to take them or refrain them from doing so. Some recent review studies (Lucke et al., 2011; Smith and Farah, 2011) show that a number of scholars are inclined to say that today's enhancers present those features in a small percentage. Yet, recent developments in the public acknowledgement of PCE use in workplaces put doubts over the accuracy of these statements.[2]

Since some scholars argue that current enhancers have little effect on cognition, it is helpful to highlight their overall effects. A recent qualitative study conducted by Scott Vrecko showed that Ritalin and Adderall do affect intellectual capacities (such as executive functions, working memory and information process), but they *also* heavily affect the user's emotional states. "Such alterations appear to be an important dimension of the drug effects that users perceive to enable improved academic performance" (Vrecko, 2013). Participants said that the perception of better emotional or affective states was the most important feature of the enhancers, leading to improvements in the sense of having augmented skills in doing academic work. Emotional dynamics are a salient dimension of the use of stimulant-based medications. Altered emotional states caused by cognitive enhancers are "part of what makes stimulant drugs useful in relation to academic work". This explains why the specifically cognitive effect appears limited. Instead, Ritalin and Adderall have an important action on the dopaminergic system: they affect the attention, the system of pleasure and that of emotions, including a euphoric effect (Racine and Forlini, 2010; Volkow et al., 2012). Therefore, if, as it seems, enhancers are increasingly spreading among intellectual professionals, it means that the users expect a positive and significant effect, at least at the level of subjective perception. Yet, even though research in the field is at the outset, psychostimulants seem to have very complex, dose and context-dependent effects (Konrad et al., 2004; Wood et al., 2013). The role of age, gender, and ethnic groups in drug efficacy is not clear as well, and such factors play a role in creating differences in neurotransmitter systems of individuals. Thus, the effectivity of PCE is deemed to be unreliable. People relying on PCE for their ordinary or extraordinary performances cannot adequately assess the effects of taking different doses of PCE in different contexts and they would often think they are better than they actually are. And that turns in further reason for disclosing the use of PCE beyond those we expose below.

There are no doubts however, that one of the issues that appear more problematic about the idea of disclosing one's use of PCE is that of entering the private sphere of one's (moral) conduct. As Warren and Brandeis famously put it (Warren and Brandeis, 1890), privacy is part of a more general right to immunity of the person and—through the principle of "inviolate personality"—it preserves a space of non-interference by others. The right to privacy seems to suggest that creating publicly embarrassing or accusatory situations against the individual should not be tolerated (Prosser, 1960). The enhancement of individual performances through legal drugs seems to pertain to such a scenario. If we are to accept a "public intrusion" in such a choice of lifestyle, it is thus necessary to provide a counterbalancing moral reason that could legitimize the request for a disclosure of such information. When is it possible to identify, then, a moral duty or a social interest that goes against individual privacy in the case of PCE? We shall look into this question in the next section.

GLOBAL EFFECTS AS UNFAIRNESS

Interestingly, indeed, it has been recently suggested that PCE might represent a shortcut to the redistribution of (cognitive) wealth (Kohn, 2014). Starting from another study conducted by Mullainathan and Shafir (2013) (in which they underlined that external inputs related to the participants' income substantially affect their cognitive response in the experiment), Marek Kohn argues that—while not being an ideal solution—taking a pill to reduce the impact of the external variables related to the contingent economical disparities between individuals might be the way to go. We will focus on fairness in competitive contexts. As we shall see, in general those situations are such that some researchers, workers or students who use PCE can be considered more skilled and better at performing their task than they really are. But this is not the main problem *per se*. The potentially dysfunctional social consequences are due to the undisclosedness of the PCE use. If one does not have her drug dose available, she will not be capable of the same performance as usual and, in some professions, might even put her colleagues at risk. If standards are set based on PCE-using workers, this may lead to an organizational dysfunction. Some brief examples can present realistic scenarios in which the principle of the duty of disclosure seems perfectly suitable and reasonable. These examples will highlight the argument for the moral and socially relevant duty to disclose the use of cognitive enhancers, insofar as it is sufficiently harmful or unfair to third parties, though not sufficiently harmful or unfair to justify a legal or organizational ban.

John and Susan are researchers in the same university department. They are good friends but are now competing to get a new position opening up in the department. The university committee has decided to put them to a test to prepare for which they are given 2 weeks off work. What they do not know is that the committee will be evaluating both the *content* of their answers, and the time they will take to complete the test. John chooses to take a 15-mg Adderall tablet, so as to increase his capacity to concentrate and maximize the efforts of his studies. To be fair to Susan, he offers her the same tablet, but she refuses, claiming that it would be unfair to alter her performance for the sole purpose of passing the test. In the end, John gets the

[2]In a recent interview for example, an Australian Public Servant admitted that in May 2014 there was an extensive (and widespread) use of modafinil in order to complete the Federal Budget on time (Farr, 2014).

job. Although he gave one correct answer less than Susan, he did so in a much shorter time, and so the committee opted for him: they want knowledge, but *also* efficiency. In discovering this, Susan regards the results as clearly unfair: had she taken Adderall as well—she believes—her reaction time in answering the questions would have been comparable to John's. In this way, she would have won for the higher number of correct answers. She thus approaches John urging him to publicly acknowledge that he took Adderall before the test. He refuses to do so for two reasons: first, he *did* give her the chance to have "completely equal" starting conditions, it was only *her choice* not to take the tablet. Second, there is no official requirement to acknowledge the use of any legal substance: why did she not state that she had three coffees that morning?[3] Susan objects that, first, the starting conditions would have been unfair in any case because—unless they decided to take Adderall for the rest of their lives—their performance would still not correspond to their real, normal capacities. Second, coffee is well known by the committee, and they probably assume that people under examination would take some. On the contrary, Adderall's effects are little known, and they vary for each person.

Now consider Robert. He wishes to help his community and has decided to become a professional nurse in the emergency unit of the local hospital. As he approaches the day of the entrance test, he becomes more and more nervous: he knows that if he could only concentrate fully during the written test, he would have a very good chance to pass. He is told by a friend that Ritalin, a legal drug, would increase his alertness, so he decides to give it a try. On the day of the test, he takes his "smart pill" and enters the room of the written exam. After having performed in a way that he deems very close to perfect, he discovers that the practical test is going to follow in 1 h. Given that he still has a few pills left, he takes another one. Even in this context, he can tell that his performance has improved. In line with what he expected, Robert is accepted as a nurse in the emergency unit. Wanting to "help more", however, he decides that he will take Ritalin regularly when in service. He does so for 6 months, until he runs out of tablets. This happens just on the evening when the local stadium in town crumples down. At both the psychological and the psychical level, Robert is shaken by this "deprivation" and his colleagues have to help him stay focused a few times during the night: this has never happened before. Luckily the wounded are taken care of properly and in a relatively short time, and Robert's "under-performance" does not cause any particular damage. However, his boss notices the change and asks him what happened. Having a clean conscience and thinking he had done nothing wrong, Robert tells the truth. His boss is extremely angry at his words: notwithstanding the moral questionability of taking PCE for the tests, the main issue is that he needs to know who can perform what, and under what circumstances. They are dealing with life and death: anything preventing him from having the actual picture of the people working under him potentially jeopardizes the success of their medical assistance. For this reason, Robert's boss asks him to resign. Robert is shocked and objects that he only thought he was going to be more useful, and certainly he did not perceive this as a "secret". It simply never came out in a conversation.[4]

With short notice, the Ministry of Education occasionally sample tests high school students. The goal is to assess the quality of education, in order to have quantitative data to make comparisons between different towns and regions, identifying effective methods to improve the performance of both teachers and students where needed. Let us assume that standardized student assessment tests are also submitted to those who are old enough to legally take PCE. Even if the tests are anonymous, situations could arise in which students take PCE to improve their results, perhaps encouraged by the teachers themselves, who are likely to aspire to high rankings for their classes. In this way, certain classes or schools in certain towns and regions may end up having very high scores, which, however, would have nothing to do with the teaching methods used or with the students' abilities. This would cause evident biases in the interpretation of the results and lead to inefficient decisions (let us not forget that we are dealing with sample tests, and that a handful of classes, picked using statistically valid methods, can be used as indicators for vast geographic areas). On the basis of the analysis of the data collected, school authorities may well think that, in those classes in which students took PCE, the quality of the education was higher due to the teaching methods used, and decide to experiment with these methods elsewhere as well. This would result in a waste of public resources and hinder research on truly better teaching methods. Additionally, it could hide actual differences between geographic areas, resulting in a lack of intervention where school performance is actually poor.

In all the cases considered, there would appear to be both a moral duty and a social interest in reporting the use of PCE. Indeed, the use of PCE could be deemed fully legitimate and in fact, if openly reported, it could become an additional element in the assessment of ways to improve one's performance. To focus on this last case, by knowing what classes, in what geographic areas, took cognitive enhancers, it will be possible to assess their effectiveness without introducing biases in the overall results. This is certainly of social interest as it helps with the efficient allocation of resources. Yet, there also seems to be a moral duty, since teachers who encouraged their students to take PCE without reporting it might be trying to hide their shortcomings as educators. In other contexts, instead, taking PCE unbeknownst to evaluators may result in the failure to identify situations of economic or cultural distress, because school performance has improved only thanks to the use of cognitive enhancers.

LOCAL VS. GLOBAL EFFECTS

The duty to disclose the use of cognitive enhancers (and maybe also of mood affecting drugs) is not just intended to prevent *local effects* from prevailing on *global effects*—with undesirable social

[3]It should be noted that this argument is often used by bio-liberals: given that caffeine is also a cognitive enhancing substance, why should we limit the use of one enhancer over another? For a more precise analysis see Julien (2001), especially pages 145–164.

[4]A similar motivation could be applied to the renowned cases of cocaine use among surgeons around the world. However, our intention is to focus on PCE precisely because—unlike cocaine—they are *not* illegal drugs.

outcomes deriving from unpredictable composition effects. This is certainly the most important consequentialist reasoning that can justify the duty of disclosure. What is at stake, however, is also an ethical principle of fairness that seems to prevail over the right to privacy as we described it earlier. In fact, those who make use of cognitive enhancers in single occasions or non-repeatable competitive situations seem to be required to report the sudden difference that has arisen in their abilities without this enhancement depending on something they can be credited for. Suppose a candidate for a test, on her way to the university, finds the answer sheet that the examiner has lost from her pocket; or that the computer on which a test is run is defective and shows the correct answers together with the questions; or that, even a single time, one gets help from a renowned scientist in conducting a difficult experiment (but claiming full credit for it); or that a junior broker can rely on the advice of a knowledgeable friend who is well introduced in the sphere of finance.

These are situations in which fairness in the competition is lost without violating any rules or prohibitions, but in which the performance has little to do with the actual skills that a candidate may manifest later and with what he has done to improve them. Therefore, fairness in respecting the equality of the starting conditions as a principle of respect for the others leads to reveal aspects related to chance or to the sporadic and extrinsic improvement of one's skills. It is a much needed principle, weaker than the prohibition to resort to means extraneous to the competition or the duty to waive potential favorable conditions (the latter principle would severely limit individual autonomy). As our first example shows, if all relevant information is made available, the competition is fair and everyone can choose how to act within the framework of the existing legislation.

The duty to disclose the use of PCE has a final consequence that one should consider. We have talked mainly about a moral duty to disclose, but such a duty could also carry a legal obligation and a number of consequences. For example, would that disclosure justify that a person is not hired for a position? We think that a simple answer is not available. In some cases, the hirer could judge that the person is perfectly suitable for the position and the variability of her performance based also on PCE is always satisfactory. In other cases, if the person's performance with PCE is near the lower threshold of the minimum performance, considering the dose and context-dependent effects, it could be a risk to rely on her, so the hirer would have good reasons not to hire her. In light of these considerations, there might be a question about the effective incentives (perhaps stressing the moral praiseworthiness of disclosing) to declare the use of PCE. Such incentives deserve attention, but they cannot be analyzed here.

CONCLUSION

The aim of this paper—including the use of realistic examples—has been to shed light over the existing (but overlooked) tension between the *local* and *global effects* of the use of PCE. Even if it is perhaps problematic to accept in a liberal society at first, a partial reduction of our right to privacy might be necessary in order to preserve the equally important principle of fairness (as well as social safety and efficiency) that an unregulated use of PCE could threaten. The legitimacy of this step derives from other values deeply entrenched in liberal societies, such as the restriction of individual autonomy if this puts in jeopardy that of others. Reasons related to equity and social interests, therefore, can suggest that there is a moral duty to publicly acknowledge the use of PCE in order to limit the potential damages. There have already been a number of scientific and moral assessments of PCE in the literature, and we do not expect to have provided an exhaustive account on how to legislate further on PCE with this work, but we do hope to have offered some innovative ways of conceptualizing the debate evolving around the increasing use of PCE.

REFERENCES

Advokat, C., and Scheithauer, M. (2013). Attention-deficit hyperactivity disorder (ADHD) stimulant medications as cognitive enhancers. *Front. Neurosci.* 7:82. doi: 10.3389/fnins.2013.00082

Babcock, Q., and Byrne, T. (2000). Student perceptions of methylphenidate abuse at a public liberal arts college. *J. Am. Coll. Health* 49, 143–145. doi: 10.1080/07448480009596296

Cakic, V. (2009). Smart drugs for cognitive enhancement: ethical and pragmatic considerations in the era of cosmetic neurology. *J. Med. Ethics* 35, 611–615. doi: 10.1136/jme.2009.030882

Chandler, J. A. (2012). "Obligatory Technologies": explaining why people feel compelled to use certain technologies. *Bull. Sci. Technol. Soc.* 32, 255–264. doi: 10.1177/0270467612459924

DeSantis, D. A., Webb, E. M., and Noar, S. M. (2008). Illicit use of prescription ADHD medications on a college campus: a multimethodological approach. *J. Am. Coll. Health* 57, 315–323. doi: 10.3200/JACH.57.3.315-324

Farah, M. J., Illes, J., Cook-Deegan, R., Gardner, H., Kandel, E., King, P., et al. (2004). Neurocognitive enhancement: what can we do and what should we do?. *Nat. Rev. Neurosci.* 5, 421–425. doi: 10.1038/nrn1390

Farr, M. (2014). Public servants used drug, modafinil, to stay awake to complete the federal budget on time. *The Courier Mail*. Available online at: http://www.couriermail.com.au/news/national/public-servants-used-drug-modafinil-to-stay-awake-to-complete-the-federal-budget-on-time/story-fnihslxi-1226932640759. Accessed on 27 May 2014.

Franke, A. G., Bagusat, C., Rust, S., Engel, A., and Lieb, K. (2014). Substances used and prevalence rates of pharmacological cognitive enhancement among healthy subjects. *Eur. Arch. Psychiatry Clin. Neurosci.* 264, S83–S90. doi: 10.1007/s00406-014-0537-1

Greely, H., Sahakian, B., Harris, J., Kessler, R. C., Gazzaniga, M., Campbell, P., et al. (2008). Towards responsible use of cognitive enhancing drugs by the healthy. *Nature* 456, 702–705. doi: 10.1038/456702a

Herman-Stahl, M. A., Krebs, C. P., Kroutil, L. A., and Heller, D. C. (2007). Risk and protective factors for methamphetamine use and nonmedical use of prescription stimulants among young adults aged 18 to 25. *Addict. Behav.* 32, 1003–1015. doi: 10.1016/j.addbeh.2006.07.010

Julien, R. M. (2001). *A Primer of Drug Action.* New York, NY: Holt.

Kohn, M. (2014). Smart and smarter drugs. *Mosaic: The Science of Life*. Available online at: http://mosaicscience.com/story/smart-and-smarter-drugs

Konrad, K., Gunther, T., Hanisch, C., and Herpertz-Dahlmann, B. (2004). Differential effects of methylphenidate on attentional functions in children with attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 43, 191–198. doi: 10.1097/00004583-200402000-00015

Lanni, C., Lenzken, S. C., Pascale, A., Del Vecchio, I., Racchi, M., Pistoia, F., et al. (2008). Cognition enhancers between treating and doping the mind. *Pharmacol. Res.* 57, 196–213. doi: 10.1016/j.phrs.2008.02.004

Lucke, J. C., Bell, S., Partridge, B., and Hall, W. D. (2011). Deflating the neuroenhancement bubble. *Am. J. Bioeth. Neurosci.* 2, 38–43. doi: 10.1080/21507740.2011.611122

Marchant, N. L., Kamel, F., Echlin, K., Grice, J., Lewis, M., and Rusted, J. M. (2009). Modafinil improves rapid shifts of attention. *Psychopharmacology (Berl)* 202, 487–495. doi: 10.1007/s00213-008-1395-1

McCabe, S. E., Knight, J. R., Teter, C. J., and Wechsler, H. (2005). Non-medical use of prescription stimulants among US college students: prevalence and correlates

from a national survey. *Addiction* 100, 96–106. doi: 10.1111/j.1360-0443.2005.00944.x

McCabe, S. E., West, B. T., and Wechsler, H. (2007). Trends and college-level characteristics associated with the non-medical use of prescription drugs among US college students from 1993 to 2001. *Addiction* 102, 455–465. doi: 10.1111/j.1360-0443.2006.01733.x

Mehlman, M. J. (2004). Cognition-enhancing drugs. *Milbank Q.* 82, 483–506. doi: 10.1111/j.0887-378X.2004.00319.x

Mereu, M., Bonci, A., Newman, A. H., and Tanda, G. (2013). The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. *Psychopharmacology (Berl)* 229, 415–434. doi: 10.1007/s00213-013-3232-4

Mullainathan, S., and Shafir, E. (2013). *Scarcity: Why Having Too Little Means So Much.* New York: Times Book.

Prosser, W. (1960). Privacy. *Calif. Law Rev.* 48, 383–423. doi: 10.2307/3478805

Racine, E., and Forlini, C. (2010). Cognitive enhancement, lifestyle choice or misuse of prescription drugs? *Neuroethics* 3, 1–4. doi: 10.1007/s12152-008-9023-7

Russo, M. B., Arnett, M. V., Thomas, M. L., and Caldwell, J. A. (2008). Ethical use of cogniceuticals in the militaries of democratic nations. *Am. J. Bioeth.* 8, 39–41. doi: 10.1080/15265160802015016

Sahakian, B. J., and Morein-Zamir, S. (2007). Professor's little helper. *Nature* 450, 1157–1159. doi: 10.1038/4501157a

Sattler, S., Sauer, C., Mehlkop, G., and Graeff, P. (2013). The rationale for consuming cognitive enhancement drugs in university students and teachers. *PLoS One* 8:e68821. doi: 10.1371/journal.pone.0068821

Shillington, A. M., Reed, M. B., Lange, J. E., Clapp, J. D., and Henry, S. (2006). College undergraduate ritalin abusers in southwestern california: protective and risk factors. *J. Drug Issues* 36, 999–1014. doi: 10.1177/002204260603600411

Smith, M. E., and Farah, M. J. (2011). Are prescription stimulants "smart pills"? The epidemiology and cognitive neuroscience of prescription stimulant use by normal healthy individuals. *Psychol. Bull.* 137, 717–741. doi: 10.1037/a0023825

Teter, C. J., McCabe, S. E., Cranford, J. A., Boyd, C. J., and Guthrie, S. K. (2005). Prevalence and motives for illicit use of prescription stimulants in an undergraduate student sample. *J. Am. Coll. Health* 53, 253–262. doi: 10.3200/jach.53.6.253-262

Urban, K. R., and Gao, W. J. (2014). Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain. *Front. Syst. Neurosci.* 8:38. doi: 10.3389/fnsys.2014.00038

Volkow, N. D., Wang, G. J., Tomasi, D., Kollins, S. H., Wigal, T. L., Newcorn, J. H., et al. (2012). Metylphenidate-elicited dopamine increases in ventral striatum are associated with long-term symptom improvements in adults with attention deficit hyperactivity disorder. *J. Neurosci.* 32, 841–849. doi: 10.1523/JNEUROSCI.4461-11.2012

Vrecko, S. (2013). Just how cognitive is "cognitive enhancement"? On the significance of emotions in university students' experiences with study drugs. *AJOB Neurosci.* 4, 4–12. doi: 10.1080/21507740.2012.740141

Warren, S. D., and Brandeis, L. D. (1890). The right to privacy. *Harv. Law Rev.* 4, 193–220. doi: 10.2307/1321160

Wood, S., Sage, J. R., Shuman, T., and Anagnostaras, S. G. (2013). Psychostimulants and cognition: a continuum of behavioral and cognitive activation. *Pharmacol. Rev.* 66, 193–221. doi: 10.1124/pr.112.007054

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 August 2014; accepted: 27 January 2015; published online: 25 February 2015.

Citation: Garasic MD and Lavazza A (2015) Performance enhancement in the workplace: why and when healthy individuals should disclose their reliance on pharmaceutical cognitive enhancers. Front. Syst. Neurosci. ***9****:13. doi: 10.3389/fnsys.2015.00013*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 06 February 2015
doi: 10.3389/fnsys.2015.00004

Screening and personalizing nootropic drugs and cognitive modulator regimens *in silico*

Leslie C. Jellen[1], Alexander Aliper[2], Anton Buzdin[3]* and Alex Zhavoronkov[2,4]*

[1] *Department of Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, Memphis, TN, USA*
[2] *Aging Research, Insilico Medicine, Emerging Technology Center, Johns Hopkins University Eastern, Baltimore, MD, USA*
[3] *Personalized Medicine, Pathway Pharmaceuticals Ltd, Wan Chai, Hong Kong*
[4] *Research, Biogerontology Research Foundation, Truro, UK*

Edited by:
Ioan Opris, Wake Forest University, USA

Reviewed by:
Mikhail Lebedev, Duke University, USA
Christopher R. Madan, Boston College, USA
Stuart Richard Gilbert Calimport, Aston University, UK

****Correspondence:***
Anton Buzdin, Personalized Medicine, Pathway Pharmaceuticals Ltd, 2702-3, 56 Gloucester Road, Wan Chai, Hong Kong
e-mail: buzdin@pathway pharmaceuticals.com;
Alex Zhavoronkov, Aging Research, Insilico Medicine, Emerging Technology Center, Johns Hopkins University Eastern, B301, 1101 East 33rd Street, Baltimore, MD, USA
e-mail: alex@biogerontology.org

The go-to cognitive enhancers of today are those that are widely available rather than optimal for the user, including drugs typically prescribed for treatment of ADHD (e.g., methylphenidate) and sleep disturbances such as narcolepsy (modafinil). While highly effective in their intended therapeutic role, performance gains in healthy populations are modest at best and profoundly inconsistent across subgroups and individuals. We propose a method for *in silico* screening of possible novel cognitive enhancers followed by high-throughput *in vivo* and *in vitro* validation. The proposed method uses gene expression data to evaluate the the collection of activated or suppressed signaling pathways in tissues or neurons of the cognitively enhanced brain. An algorithm maps expression data onto signaling pathways and quantifies their individual activation strength. The collective pathways and their activation form what we term the signaling pathway cloud, a biological fingerprint of cognitive enhancement (or any other condition of interest). Drugs can then be screened and ranked based on their ability to minimize, mimic, or exaggerate pathway activation or suppression within that cloud. Using this approach, one may predict the efficacy of many drugs that may enhance various aspects of cognition before costly preclinical studies and clinical trials are undertaken.

Keywords: nootropic, cognitive enhancement, personalized medicine, drug repositioning, oncofinder, *in silico* medicine, signalome, signalome profiling

INTRODUCTION

The concept of cognitive enhancement is age-old, but despite modern advances in our understanding of the cellular and molecular bases of cognition, much remains unknown. Development of new cognitive enhancers with increased specificity, safety, and effectiveness must outpace increasing demand, particularly as off-label and illicit use of current drugs has become common in certain population subgroups (Maher, 2008) and as the aging segment of the population expands with accompanying increases in rates of cognitive decline and neurodegenerative disease (Wallace et al., 2011).

Cognition is complex and involves multiple domains, from learning and memory to attention, and cognitive enhancers can target one or more. At present, two of the most widely cited cognitive enhancers are the dopaminergic stimulants methylphenidate, prescribed for treatment of ADHD, and modafinil, prescribed for treatment of sleep disorders, such as narcolepsy. While both of these drugs can enhance memory and attention in healthy individuals (Elliott et al., 1997), their application as nootropics is secondary to their original, therapeutic purpose, and effects are disagreed upon and modest at best (Repantis et al., 2010).

A number of other potential nootropics have been developed for the treatment of cognitive deficits in aging and neurodegenerative or neuropsychiatric disease, from the FDA approved acetylcholinesterase inhibitors (e.g., donepezil) to those still under investigation: ampakines, nicotinic receptor agonists, glutamate receptor agonists, glycine inhibitors, and PDE inhibitors (for review, Wallace et al., 2011; Pieramico et al., 2014). Drugs that may play a more modulatory role target histamine, serotonin, glucocorticoid, and neuropeptide receptors and epigenetic mechanisms (Roesler and Schröder, 2011; Wallace et al., 2011). Many other drugs positioned as nootropic agents like piracetam and piracetam-like compounds are well-tolerated, but their effects and mechanisms of action are poorly understood and widely debated (Gouliaev and Senning, 1994; Gualtieri et al., 2002). While some nootropics have made it to clinical trial, others still await or have been withdrawn, and each has various drawbacks or only modest effects that are disease specific.

Development of novel nootropics is hampered by research, validation and regulatory challenges. The very definition of cognitive enhancement is difficult to pin down (Lynch et al., 2014). The road from lab to FDA approval is difficult, long, and costly. Pharmacological enhancement of healthy populations is fraught with ethical and philosophical pushback (Maslen et al., 2014). Enhancement in aging and neurodegenerative disease is less controversial, but perhaps more complex

(Pieramico et al., 2014). Moreover, therapeutic effects often contradict those in healthy populations (Kimberg et al., 1997; Belmonte and Yurgelun-Todd, 2003; Beglinger et al., 2005; Gibbs and D'Esposito, 2005, 2006; Randall et al., 2005; Frank and O'Reilly, 2006; Finke et al., 2010; Esposito et al., 2013). The U-shaped curve effect, wherein treatment effects benefit low-baseline performers but impair high-performers, is a problem with at least the dopaminergic drugs (Gibbs and D'Esposito, 2006; Finke et al., 2010; Esposito et al., 2013). Even drugs FDA-approved for therapeutic use have issues with side effects, trade-offs (one process is enhanced while another is impaired), loss of authenticity (one's true "self"), and large individual differences (Maslen et al., 2014). Long term effects are typically unknown. Finally, and most importantly, there is still much to be learned about the cellular and molecular basis for the various aspects of cognition.

For the field of neuroenhancement to advance, the benefit-to-risk ratio of pharmacological treatment must improve. This will require (a) a better understanding of the neurobiological basis of cognition; and (b) drugs with higher specificity, greater effectiveness, and fewer side effects. To this end we propose an expedited path to understanding the molecular basis of cognition and to subsequent drug discovery via a combination of advanced gene expression analysis, signaling pathway analysis, and *in silico* screening of potential nootropics. In our approach, current drugs will simply be the launching pad on the quest to find new, better performing nootropics.

SIGNALING PATHWAY ACTIVATION PROFILES AS DRUG TARGETS FOR PREDICTING NOOTROPIC EFFECTS *IN SILICO*

Enhancing cognition is a challenge as it consists of various processes each influenced by many factors and each with a distinct neurobiological framework. While single-gene studies have been an effective first step in identifying individual elements necessary for learning and memory to occur (Lee and Silva, 2009), in moving forward, integration of many other factors and influences, including the relatively few signaling pathways involved, may be more beneficial than investigating each of many potential individual network elements.

Intracellular signaling pathways show promise for complex trait analysis. At the cellular level, any two physiological states can be distinguished by changes in a set of signaling pathways, each individually activated or repressed to some extent. This collection of disturbed pathways, termed the signaling pathway cloud, is a powerful and unique biological fingerprint (Zhavoronkov and Cantor, 2011; Makarev et al., 2014; Aliper et al., 2015).

Until recently, signaling pathway analysis was impeded by the inability to quantify individual signaling pathway activation strength (PAS), owing to the complexity of protein interactions within signaling pathways and lack of experimental data to determine the importance factor for each member of a given pathway. This problem was addressed, however, with the development of Oncofinder[1], a biomathematical method that simplifies calculation of PAS and signaling pathway cloud disturbance (SPCD), as detailed below (Buzdin et al., 2014b). With the ability to calculate PAS, one can quantitatively characterize a biological condition by its associated signaling activation profile.

PAS analysis involves (1) mapping relevant pathways from the gene expression profiles for a given condition; (2) calculating individual PAS values; (3) constructing the signaling pathway cloud (net activated and repressed pathways); (4) high throughput *in silico* screening to predict and rate drugs that target these pathways, depending on the application; and (5) *in vitro* and *in vivo* validation.

The pair of conditions to be compared in signaling pathway cloud analysis is flexible. In the past, we have compared tumor biopsies to healthy tissue, revealing signaling pathway biomarkers that outperform those of single genes (Kuzmin et al., 2010; Mityaev et al., 2010; Zabolotneva et al., 2012a,b), and tissue from old vs. young patients, illustrating how this method may be useful for the screening of potential geroprotectors (Zhavoronkov and Cantor, 2011). Other applications include drug discovery and drug repurposing, as well as applications in manipulating cell differentiation.

Here, we suggest that these methods could also be applied to cognitive enhancement, first by defining what aspect of cognition is to be targeted, then by mapping signaling pathways altered in transcription profiles of the "enhanced brain" vs. control (e.g., in mouse models showing performance gains in the target cognitive domain, whether via genetic manipulation, selective breeding or inbreeding, or treatment with current nootropics) and finally by calculating disturbance of the associated signaling pathway cloud (**Figure 1**).

High throughput screening for drugs that activate or repress key pathways could then uncover drugs that replicate cloud disturbance in "enhanced" models and thus mimic or exaggerate the effects of the current drugs or genetic manipulations. These drugs could then be ranked and prioritized for validation of cognitive effects *in vivo* or *in vitro*.

These methods can be used for general screening of cognitive enhancers at present but in the future may also be used to develop personalized cognitive enhancement plans for individual patients.

The idea of using differential gene expression patterns for drug discovery is not new. The broad variability of as well as the error rates introduced by the microarray and next generation sequencing (NGS) equipment led to many early failures, impeded progress and diminished the potential of this approach. However, recent algorithmic solutions reducing complexity and smoothing variability of the gene expression mapped onto signaling pathways allow us to minimize errors and perform cross-platform analysis (Buzdin et al., 2014b). These solutions allow us to tap into legacy databases and compare hundreds of thousands of data sets in microarray and NGS repositories as well as utilize the drug effects on gene expression published in publically available databases.

STEP 1: IDENTIFYING THE PAIR OF CONDITIONS

Enhanced cognition can refer to augmented function across one or several domains and can be demonstrated in a number of highly specific paradigms or tests, thus one must first

[1]www.oncofinder.com

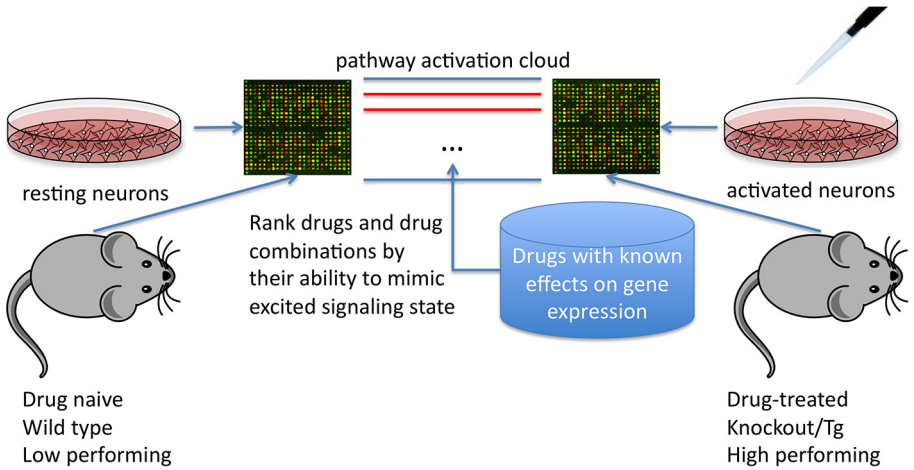

FIGURE 1 | Using differential signaling pathway activation profiles between cell/tissue culture or mice treated and untreated with known cognitive modulators or genetic models of cognitive enhancement to screen for potential nootropic compounds.

specify the goals of enhancement and choose models/tissues that demonstrate enhancement in that respect.

Ethical considerations preclude the use of human studies for this application, thus animal models would be required. Fortunately, a number of animal models are highly useful in investigating mechanisms of cognition (Roesler and Schröder, 2011). Specific aspects of attention, learning, and memory can be reliably measured in a wide variety of behavioral paradigms. Many of these have been developed for use in mice, but rats, monkeys, zebrafish, and drosophila are also potential models. For the current approach, mouse models are promising, and there are several different options within this species for comparison of the "enhanced" vs. "non-enhanced" brain.

MUTANT vs. WILD TYPE

Mice are a classic model for learning and memory and a number of models involve mutations that induce cognitive enhancement, including knockout and transgenic mutations (Lee and Silva, 2009). These model systems have revealed many genes involved in learning and memory. Hypothetically, the human orthologs of these genes would also be involved in cognition. Mutations that enhance cognition are varied in mechanism, and include enhancing excitation, dampening inhibition, regulating gene expression, regulating translation, epigenetics, microRNA biogenesis, and extracellular molecules (Lee and Silva, 2009). Generally speaking, mutations that increase the activity of cAMP response element-binding protein (CREB), a basic leucine zipper transcription factor, enhance long-term potentiation (LTP) and both long and short term memory, while those that downregulate CREB activity impair LTP and memory (Lee and Silva, 2009; Suzuki et al., 2011; Kida and Serita, 2014). In their 2009 review, Lee and Silva reported that 26 of the 29 mouse models reviewed showed enhanced LTP. In several cases, LTP was enhanced, but cognition was either not improved or was actually impaired; thus, other signaling pathways and cellular processes are involved (Lee and Silva, 2009), underscoring the potential role for signaling pathway analysis in elucidating global effects on these mice and thereby enabling pharmacological replication.

STRAIN vs. STRAIN

An alternative to single gene mutation models is selective breeding or inbreeding to exploit "natural" genetic variation among mouse strains in cognitive performance. Strains or groups of strains that perform high on paradigms of learning and memory can be compared to other low-performing strains within a genetic reference panel. The BXD strains are a good example (Williams et al., 2003). This panel of recombinant inbred strains has been extensively phenotyped for behaviors related to learning and memory and drug treatment effects as well as profiled for basal gene expression, and the data are publicly available for rapid statistical analysis on genenetwork.org (Wang et al., 2003). In minutes, known phenotypic differences can be correlated to known gene expression levels (and thus signaling pathway activation), without the unwanted or exaggerated effects of knockout or transgenic manipulation.

DRUG-TREATED vs. DRUG-NAïVE

A third alternative for comparisons in the analysis would be *in vivo* or *in vitro* expression in organisms, tissues, or cells that have been pharmacologically treated with current nootropics vs. control. The effect of nootropics on gene expression in the brain can be large; in the case of methylphenidate treatment, over 2000 genes have been shown to be differentially expressed in the caudate putamen of mice (Adriani et al., 2006a,b). This comparison would enable screening of new nootropics that mimic current nootropics, but that are more effective, more or less specific in targeted cognitive domains, and/or have fewer side effects.

STEP 2: IDENTIFYING TARGET SIGNALING PATHWAYS

Once a specific comparison is decided upon, a predicted set of relevant pathways can be assembled from the literature

or mapped from gene expression data. Here, we reviewed the literature for mouse models of cognitive enhancement, in which overexpression, knockout, loss-of-function mutation, deletion, or RNA interference of a particular gene led to significant gains in cognitive performance as measured in at least one behavioral paradigm for measuring learning and/or memory. In 2009, Lee and Silva reviewed these models and compiled list of target genes and effects of mutation. We reduced this list to only 40 genes, for which human orthologs exist. Gene set enrichment analyses of these listed entries against Insilico Cloud Intelligence signaling pathway database (Buzdin et al., 2014a) were performed using Fisher's exact test and the most enriched pathways are shown in **Table 1**. Results revealed several overrepresented signaling pathways, including IP3, IGF1R, and cAMP molecular signalization, potentially important for learning and memory in humans. Key activators/repressors of these pathways can therefore be used in further experimental assays together with the mathematical apparatus we provide in the following section.

Importantly, many of the genes and pathways identified during *in silico* screening of nootropics are also implicated in aging and longevity (Zhavoronkov and Cantor, 2011; Moskalev et al., 2014) thus suggesting that geroprotector drugs may act as nootropic agents and vice versa.

STEP 3: CALCULATING SIGNALING PATHWAY CLOUD DISTURBANCE

The work we propose involves calculating signaling activation and methods are based on our prior work with cell signaling pathways (Kiyatkin et al., 2006; Kuzmina and Borisov, 2011; Borisov et al., 2014). In the past, calculation of signaling pathway activation has been avoided because of the lack of experimental data measuring the correlation between expression and activation at the protein level. In our observation, most signal transduction proteins are far from saturation even at the peak concentrations of the activated form, in comparison with total protein abundance. From this, we suggest all activator/repressor gene products/proteins have equal importance for pathway activation/downregulation. We then arrive at the following assessment: function for overall signal pathway cloud disturbance outcome (SPCD) is proportional to the following estimator function:

$$SPCD = \frac{\prod_{i=1}^{N} [AGEL]_i}{\prod_{j=1}^{M} [RGEL]_j}$$

Here, the multiplication is performed over all possible activator and repressor proteins in the pathway, and $[AGEL]_i$ and $[RGEL]_j$ are gene expression levels of an activator i and repressor j, respectively. To obtain an additive (not multiplicative) value, one can simply switch from using absolute values of expression levels to their logarithms, arriving at the *PAS* value for each pathway

$$PAS_p = \sum_n ARR_{np} \cdot BTIF_n \cdot \lg(ECR_n)$$

In the case of cognitive enhancement, to obtain the values of *enhanced-to-control ratio (ECR)*, one divides the expression levels for a gene n in the sample taken for the enhanced group by the same average value for the normalized control group. The discrete value of activator/repressor role (ARR) is the relative strength of the target activation or repression.

The information about the activator/repressor role of a particular gene product/protein may be obtained from the analysis of open-access or customized pathway databases and from the literature. The Boolean flag of *BTIF* (beyond tolerance interval flag) equals to zero when the ECR value lies within the tolerance limit, and to one when otherwise. We determined that the ECR lies beyond the tolerance limit if it satisfies simultaneously the two criteria. First, it should be either higher than 3/2 or lower than 2/3 of the corresponding gene expression level in normal group of samples, and, second, the expression level of a gene should differ by more than two standard deviations from the average expression level for the same gene in a control group of samples.

LIMITATIONS OF DIFFERENTIAL SIGNALING PATHWAY ANALYSIS-BASED DRUG SCREENING METHODS

Acquiring gene expression data from the various regions of human brain during excitation is difficult and human data

Table 1 | Signaling pathways associated with cognitive enhancement in animal models.

Pathway name	Overlap with gene list (%)	Odds ratio	*p*-value
IP3 pathway (gene expression)	25.9	0.346	$1.53E-08$
IGF1R pathway (cell survival)	17.9	0.215	$2.39E-07$
cAMP pathway (axonal growth)	20.8	0.245	$6.88E-06$
IGF1R main pathway	6.6	0.073	$2.09E-05$
IP3 main pathway	5.2	0.058	0.000142525
IL-2 main pathway	4.4	0.044	0.002495586
Wnt main pathway	2.8	0.029	0.028947377
GPCR main pathway	2.5	0.025	0.033190669
cAMP main pathway	2.1	0.021	0.034326773

Gene set enrichment analyses of the genes implicated in cognitive performance against Insilico Cloud Intelligence signaling pathway database performed using Fisher's exact test and the most enriched pathways.

would have to be obtained from post mortem tissues or cultured cells or tissues. Gene expression data from mouse brain is easier to obtain, but further studies are required to evaluate the correlations between signaling pathway activation profiles in mice and humans. Tissue selection is also important, gene expression in one region or cell type may or may not be representative of other areas of the brain and may not capture all effects of a given condition. Also, gene expression alone may not provide the complete picture of the state of the tissue, and while the pathway activation analysis approach may be used to analyze differences in genomic DNA (Spirin et al., 2014) there may be epigenetic regulation of cognitive states that may require other analytical methods to be performed. Aside from tissue selection, the complexity of cognition presents other challenge. The proposed approach evaluates the various drugs and drug combinations that mimic or enhance the effects of already known nootropics or genetic manipulations; however, no current nootropic agents optimally enhance specific aspects of cognitive function without side effects, and even genetic models of cognitive enhancement can produce unintended impairments (Lee and Silva, 2009). In this paper, we have referred to the "enhanced" brain vs. control in describing the comparisons one would use to perform signaling pathway analysis. However, "enhanced" is a simplified, general term that does not specify effects on the various cognitive domains, and defining enhancement remains an important issue in developing new nootropics (Lynch et al., 2014).

CONCLUSION

Cognitive enhancement is in demand, whether in healthy populations or those with cognitive deficits, but current pharmacological enhancers offer only modest benefits. Testing of new cognitive enhancers is costly and time consuming. Even predicting the nootropic candidates out of the hundreds of thousands of drugs and drug combinations remains a major challenge. A screening process that would predict the efficacy of novel cognitive enhancers that may outperform current options would save time and money, both of which are limiting as the aging segment of the population explodes over the upcoming decades, with increasing rates of cognitive decline and neurodegenerative disease. Here we propose a method for *in silico* screening and ranking of drugs and other factors that act on signaling pathways involved in cognition. Predicting their efficacy would involve calculating their potential to maximize the difference in signaling pathway activation between cells or tissues of cognitively enhanced animal models, including mutant "smart" vs. wild type mice, high-performing vs. low-performing strains of mice, or drug-treated vs. drug naïve mice. This would be followed by *in vitro* and *in vivo* validation leading to a short list of promising components.

REFERENCES

Adriani, W., Leo, D., Greco, D., Rea, M., di Porzio, U., Laviola, G., et al. (2006a). Methylphenidate administration to adolescent rats determines plastic changes in reward-related behavior and striatal gene expression. *Neuropsychopharmacology* 31, 1946–1956. doi: 10.1038/sj.npp.1300962

Adriani, W., Leo, D., Guarino, M., Natoli, A., Di Consiglio, E., De Angelis, G., et al. (2006b). Short-term effects of adolescent methylphenidate exposure on brain striatal gene expression and sexual/endocrine parameters in male rats. *Ann. N Y Acad. Sci.* 1074, 52–57. doi: 10.1196/annals.1369.005

Aliper, A., Csoka, A. B., Buzdin, A., Jetka, T., Roumiantsev, S., Moskalev, A., et al. (2015). Signaling pathway activation drift during aging: Hutchinson-Gilford progeria syndrome fibroblasts are comparable to normal middle-age and old-age cells. *Aging (Albany NY)* [Epub ahead of print].

Beglinger, L. J., Tangphao-Daniels, O., Kareken, D. A., Zhang, L., Mohs, R., and Siemers, E. R. (2005). Neuropsychological test performance in healthy elderly volunteers before and after donepezil administration: a randomized, controlled study. *J. Clin. Psychopharmacol.* 25, 159–165. doi: 10.1097/01.jcp.0000155822.51962.b4

Belmonte, M. K., and Yurgelun-Todd, D. A. (2003). Functional anatomy of impaired selective attention and compensatory processing in autism. *Brain Res. Cogn. Brain Res.* 17, 651–664. doi: 10.1016/s0926-6410(03)00189-7

Borisov, N. M., Terekhanova, N. V., Aliper, A. M., Venkova, L. S., Smirnov, P. Y., Roumiantsev, S., et al. (2014). Signaling pathway activation profiles make better markers of cancer than expression of individual genes. *Oncotarget* 5, 10198–10205.

Buzdin, A. A., Zhavoronkov, A. A., Korzinkin, M. B., Roumiantsev, S. A., Aliper, A. M., Venkova, L. S., et al. (2014a). The OncoFinder algorithm for minimizing the errors introduced by the high-throughput methods of transcriptome analysis. *Front. Mol. Biosci.* 1:8. doi: 10.3389/fmolb.2014.00008

Buzdin, A. A., Zhavoronkov, A. A., Korzinkin, M. B., Vankova, L. S., Zenin, A. A., Smimov, P. Y., et al. (2014b). Oncofinder, a new method for the analysis of intracellular signaling pathway activation using transcriptomic data. *Front. Genet.* 5:55. doi: 10.3389/fgene.2014.00055

Elliott, R., Sahakian, B. J., Matthews, K., Bannerjea, A., Rimmer, J., and Robbins, T. W. (1997). Effects of methylphenidate on spatial working memory and planning in healthy young adults. *Psychopharmacology (Berl)* 131, 196–206. doi: 10.1007/s002130050284

Esposito, Pieramico, V., Ferretti, A., Macchia, A., Tommasi, M., Saggino, A., Ciavardelli, D., et al. (2013). Acute effects of modafinil on brain resting state networks in young healthy subjects. *PLoS One* 8:e69224. doi: 10.1371/journal.pone.0069224

Finke, K., Dodds, C. M., Bublak, P., Regenthal, R., Baumann, F., Manly, T., et al. (2010). Effects of modafinil and methylphenidate on visual attention capacity: a TVA-based study. *Psychopharmacology (Berl)* 210, 317–329. doi: 10.1007/s00213-010-1823-x

Frank, M. J., and O'Reilly, R. C. (2006). A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. *Behav. Neurosci.* 120, 497–517. doi: 10.1037/0735-7044.120.3.497

Gibbs, S. E., and D'Esposito, M. (2005). Individual capacity differences predict working memory performance and prefrontal activity following dopamine receptor stimulation. *Cogn. Affect Behav. Neurosci.* 5, 212–221. doi: 10.3758/cabn.5.2.212

Gibbs, S. E., and D'Esposito, M. (2006). A functional magnetic resonance imaging study of the effects of pergolide, a dopamine receptor agonist, on component processes of working memory. *Neuroscience* 139, 359–371. doi: 10.1016/j.neuroscience.2005.11.055

Gouliaev, A. H., and Senning, A. (1994). Piracetam and other structurally related nootropics. *Brain Res. Rev.* 19, 180–222. doi: 10.1016/0165-0173(94)90011-6

Gualtieri, F., Manetti, D., Romanelli, M., and Ghelardini, C. (2002). Design and study of piracetam-like nootropics, controversial members of the problematic class of cognition-enhancing drugs. *Curr. Pharm. Des.* 8, 125–138. doi: 10.2174/1381612023396582

Kida, S., and Serita, T. (2014). Functional roles of CREB as a positive regulator in the formation and enhancement of memory. *Brain Res. Bull.* 105, 17–24. doi: 10.1016/j.brainresbull.2014.04.011

Kimberg, D. Y., D'Esposito, M., and Farah, M. J. (1997). Effects of bromocriptine on human subjects depend on working memory capacity. *Neuroreport* 8, 3581–3585. doi: 10.1097/00001756-199711100-00032

Kiyatkin, A., Aksamitiene, E., Markevich, N. I., Borisov, N. M., Hoek, J. B., and Kholodenko, B. N. (2006). Scaffolding protein Grb2-associated binder 1 sustains epidermal growth factor-induced mitogenic and survival signaling by multiple positive feedback loops. *J. Biol. Chem.* 281, 19925–19938. doi: 10.1074/jbc.m600482200

Kuzmin, D., Gogvadze, E., Kholodenko, R., Grzela, D. P., Mityaev, M., Vinogradova, T., et al. (2010). Novel strong tissue specific promoter for gene expression in human germ cells. *BMC Biotechnol.* 10:58. doi: 10.1186/1472-6750-10-58

Kuzmina, N. B., and Borisov, N. M. (2011). Handling complex rule-based models of mitogenic cell signaling (on the example of ERK activation upon EGF Stimulation). *Int. Proc. Chem. Biol. Environ. Eng.* 5, 67–82.

Lee, Y. S., and Silva, A. J. (2009). The molecular and cellular biology of enhanced cognition. *Nat. Rev. Neurosc.* 10, 126–140. doi: 10.1038/nrn2572

Lynch, G., Cox, C. D., and Gall, C. M. (2014). Pharmacological enhancement of memory or cognition in normal subjects. *Front. Syst. Neurosci.* 8:90. doi: 10.3389/fnsys.2014.00090

Maher, B. (2008). Poll results: look who's doping. *Nature* 452, 674–675. doi: 10.1038/452674a

Makarev, E., Cantor, C., Zhavoronkov, A., Buzdin, A., Aliper, A., and Csoka, A. B. (2014). Pathway activation profiling reveals new insights into age-related macular degeneration and provides avenues for therapeutic interventions. *Aging (Albany NY)* 6, 1064–1075.

Maslen, H., Faulmuller, N., and Savulescu, J. (2014). Pharmacological cognitive enhancement—how neuroscientific research could advance ethical debate. *Front. Syst. Neurosci.* 8:107. doi: 10.3389/fnsys.2014.00107

Mityaev, M. V., Kopantzev, E. P., Buzdin, A. A., Vinogradova, T. V., and Sverdlov, E. D. (2010). Enhancer element potentially involved in human survivin gene promoter regulation in lung cancer cell lines. *Biochemistry (Mosc)* 75, 182–191. doi: 10.1134/s0006297910020082

Moskalev, A. A., Aliper, A. M., Smit-McBride, Z., Buzdin, A., and Zhavoronkov, A. (2014). Genetics and epigenetics of aging and longevity. *Cell Cycle* 13, 1063–1077. doi: 10.4161/cc.28433

Pieramico, V., Esposito, R., Cesinaro, S., Frazzini, V., and Sensi, S. (2014). Effects of non-pharmacological or pharmacological interventions on cognition and brain plasticity of aging individuals. *Front. Syst. Neurosci.* 8:153. doi: 10.3389/fnsys.2014.00153

Randall, D. C., Shneerson, J. M., and File, S. E. (2005). Cognitive effects of modafinil in student volunteers may depend on IQ. *Pharmacol. Biochem. Behav.* 82, 133–139. doi: 10.1016/j.pbb.2005.07.019

Repantis, D., Schlattmann, P., Laisney, O., and Heuser, I. (2010). Modafinil and methylphenidate for neuroenhancement in healthy individuals: a systematic review. *Pharmacol. Res.* 62, 187–206. doi: 10.1016/j.phrs.2010.04.002

Roesler, R., and Schröder, N. (2011). Cognitive enhancers: focus on modulatory signaling influencing memory consolidation. *Pharmacol. Biochem. Behav.* 99, 155–163. doi: 10.1016/j.pbb.2010.12.028

Spirin, P. V., Lebedev, T. D., Orlova, N. N., Gornostaeva, A. S., Prokofjeva, M. M., Nikitenko, N. A., et al. (2014). Silencing AML1-ETO gene expression leads to simultaneous activation of both pro-apoptotic and proliferation signaling. *Leukemia* 28, 2222–2228. doi: 10.1038/leu.2014.130

Suzuki, A., Fukushima, H., Mukawa, T., Toyoda, H., Wu, L. J., Zhao, M. G., et al. (2011). Upregulation of CREB-mediated transcription enhances both short- and long-term memory. *J. Neurosci.* 31, 8786–8802. doi: 10.1523/jneurosci.3257-10.2011

Wallace, T. L., Ballard, T. M., Pouzet, B., Riedel, W. J., and Wettstein, J. G. (2011). Drug targets for cognitive enhancement in neuropsychiatric disorders. *Pharmacol. Biochem. Behav.* 99, 130–145. doi: 10.1016/j.pbb.2011.03.022

Wang, J., Williams, R. W., and Manly, K. F. (2003). WebQTL: web-based complex trait analysis. *Neuroinformatics* 1, 299–308. doi: 10.1385/ni:1:4:299

Williams, R. W., Gu, J., Qi, S., and Lu, L. (2003). The genetic structure of recombinant inbred mice: high-resolution consensus maps for complex trait analysis. *Genome Biol.* 2:RESEARCH0046. doi: 10.1186/gb-2001-2-11-research0046

Zabolotneva, A. A., Bantysh, O., Suntsova, M. V., Efimova, N., Malakhova, G. V., Schumann, G. G., et al. (2012a). Transcriptional regulation of human-specific SVAF(1) retrotransposons by cis-regulatory MAST2 sequences. *Gene* 505, 128–136. doi: 10.1016/j.gene.2012.05.016

Zabolotneva, A. A., Zhavoronkov, A., Garazha, A. V., Roumiantsev, S. A., and Buzdin, A. A. (2012b). Characteristic patterns of microRNA expression in human bladder cancer. *Front. Genet.* 3:310. doi: 10.3389/fgene.2012.00310

Zhavoronkov, A., and Cantor, C. R. (2011). Methods for structuring scientific knowledge from many areas related to aging research. *PLoS One* 6:e22597. doi: 10.1371/journal.pone.0022597

Conflict of Interest Statement: Alex Zhavoronkov, Alex Aliper and Anton Buzdin are affiliated with the commercial companies searching for cognitive modulators and nootropics. The intent of this paper is to provide insight into the methods explored by these companies and shortlist the pathways implicated in cognitive performance.

Received: 02 December 2014; accepted: 10 January 2015; published online: 06 February 2015.

*Citation: Jellen LC, Aliper A, Buzdin A and Zhavoronkov A (2015) Screening and personalizing nootropic drugs and cognitive modulator regimens in silico. Front. Syst. Neurosci. **9**:4. doi: 10.3389/fnsys.2015.00004*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 02 September 2014
doi: 10.3389/fnsys.2014.00153

Effects of non-pharmacological or pharmacological interventions on cognition and brain plasticity of aging individuals

*Valentina Pieramico[1†], Roberto Esposito[2†], Stefano Cesinaro[1], Valerio Frazzini[1] and Stefano L. Sensi[1,2,3]**

[1] Molecular Neurology Unit, Center of Excellence on Aging, University "G. d'Annunzio", Chieti-Pescara, Chieti, Italy
[2] Department of Neuroscience, Imaging and Clinical Sciences, University "G. d'Annunzio", Chieti-Pescara, Chieti, Italy
[3] Departments of Neurology and Pharmacology, Institute for Memory Impairments and Neurological Disorders, University of California-Irvine, Irvine, CA, USA

Edited by:
Ioan Opris, Wake Forest University, USA

Reviewed by:
Preston E. Garraghty, Indiana University, USA
Mark John Ferris, Wake Forest School of Medicine, USA

***Correspondence:**
Stefano L. Sensi, Molecular Neurology Unit, Center of Excellence on Aging, University "G. d'Annunzio," Via dei Vestini, 31 Chieti-Pescara, Chieti 66100, Italy
e-mail: ssensi@uci.edu

[†] These authors have contributed equally to this work.

Brain aging and aging-related neurodegenerative disorders are major health challenges faced by modern societies. Brain aging is associated with cognitive and functional decline and represents the favourable background for the onset and development of dementia. Brain aging is associated with early and subtle anatomo-functional physiological changes that often precede the appearance of clinical signs of cognitive decline. Neuroimaging approaches unveiled the functional correlates of these alterations and helped in the identification of therapeutic targets that can be potentially useful in counteracting age-dependent cognitive decline. A growing body of evidence supports the notion that cognitive stimulation and aerobic training can preserve and enhance operational skills in elderly individuals as well as reduce the incidence of dementia. This review aims at providing an extensive and critical overview of the most recent data that support the efficacy of non-pharmacological and pharmacological interventions aimed at enhancing cognition and brain plasticity in healthy elderly individuals as well as delaying the cognitive decline associated with dementia.

Keywords: brain aging, neuronal plasticity, cognitive enhancing drugs, non-pharmacological interventions, cognitive enrichment

INTRODUCTION

Brain aging is associated with cognitive and functional decline and represents the favorable background for the onset and development of Alzheimer's disease (AD). AD is becoming a major socio-economical challenge for society (Hurd et al., 2013). Early use of imaging and biological markers can identify at-risk for AD subjects decades before the appearance of cognitive decline. It is therefore imperative to act during this extended pre-clinical phase and promote endogenous responses in brains that still have a large cognitive reserve and optimal levels of neural plasticity. Beside pharmacological intervention, a great deal of interest resides on ways that allow modulation of brain plasticity in the elderly by acting on cognitive stimulation and/or aerobic exercise. Cognitive enrichment as well as physical activity can in fact promote neural plasticity, increase neurotrofism and reduce AD incidence. This review aims at providing an extensive and critical overview of the most recent data that support the efficacy of non-pharmacological and pharmacological interventions aimed at enhancing cognition in healthy elderly individuals. This line of intervention can be also critical in delaying the cognitive decline associated with dementia or other widespread neurodegenerative conditions like Parkinson's disease (PD).

COGNITIVE AND NEURAL RESERVE

The central nervous system (CNS) is a highly dynamic structure that undergoes continuous functional remodeling. Salient experience promotes widespread changes within neuronal circuits and brain areas, a process that results in the harmonization of cognitive skills that are set to meet and cope with demands of everyday life. In this context, CNS plasticity is the ability to adopt functional or structural responses (Ganguly and Poo, 2013) aimed at promoting successful adaptive behavior (Xerri, 2008; Wittenberg, 2010). Activity-dependent neural plasticity is not only required for optimal brain development but is also occurring through adulthood and senescence (Buonomano and Merzenich, 1998; Feldman and Brecht, 2005; Feldman, 2009). Plasticity requires a complex process of molecular, structural, and functional integrations that are carried out in neurons, glia, and subcellular compartments like dendrites, spines, and synapses. These biochemical and morphological changes go along with spatiotemporal synchronization of large neuronal populations across different brain regions. While neuronal plasticity has been studied mostly in the neocortex and hippocampus, it is important to underline that the phenomenon is common to almost all the CNS structures. The last two decades have produced a dramatic increase in the availability of techniques that allow the extensive investigation of CNS from its subcellular components to neural circuits.

Advancements in fluorescent microscopy, molecular biology, and electrophysiological techniques have helped to unravel many molecular determinants of neuronal plasticity. On the other hand, the availability of neuroimaging tools such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and magnetoencephalography (MEG) have enhanced the capability to detect spatiotemporal patterns of brain activation that occur within networks, nodes, and neuronal pathways (Pascual-Leone et al., 2005; Raichle and Mintun, 2006; Grefkes and Ward, 2014). These technical advancements, along with the notion that the aging brain retains the capacity to reorganize its morphological and functional architecture, have promoted strong interest and leaps forward in the knowledge of the physiology of the aging brain and aging-related cognitive processes as well as in the exploration of strategies aimed at enhancing or maintaining cognitive skills in the elderly. This area of investigation is particularly relevant as molecular, structural and functional derangement of physiological aging represents the pathological pabulum for the onset and development of neurodegenerative processes that underlay conditions like dementia or PD.

Cognitive reserve is the concept that has been proposed to explain the functional adaptation occurring in conditions that are associated with brain damage, but where structural impairment does not translate in patent cognitive deficits. When discussing this process, a clear distinction should be made between brain reserve and cognitive reserve. Brain reserve refers to the amount of preserved CNS "hardware", namely the integrity of synapses, dendrites, neurons, glia, and pathways while cognitive reserve indicates the CNS "software", in short the brain capability to reorganize its activity by setting in motion compensatory cognitive mechanisms. Thus, the cognitive reserve concept imposes to shift the attention from brain morphological integrity to functioning (Stern, 2009).

Despite recent great progress occurring in the field, the relative contribution of cognitive and brain reserve to the maintaining of brain functioning throughout aging is not completely clear. The two processes interplay and it is well known that rehabilitative interventions can positively act on both phenomena (Voss et al., 2013).

Cognitive reserve is positively influenced by educational, environmental, nutritional as well as genetic factors (Mora, 2013). The modulation of cognitive reserve greatly influences the vulnerability to dementia and affects the clinical course of the disease. Cognitive reserve has been indicated as a resource to be has tapped on in AD patients who despite the presence of large amount of AD-related pathology show relative paucity of clinical symptoms and signs. The process is thought to require the formation of new synapses as well as the plastic remodelling and functioning of brain networks (Amieva et al., 2014).

The drawback is that these patients can go undetected for long time and then, when exhaustion of the cognitive reserve occurs, eventually show a precipitous cognitive decline (Amieva et al., 2014). This rapid progression is considered to be related to high pathological loads that were compensated by a high cognitive reserve that eventually have been subdued and collapsed (Wilson et al., 2004; Amieva et al., 2014).

Neuroimaging evidence support this idea and indicate that AD patients with greater cognitive reserve often show underlying greater AD-related pathology. In a subset of AD patients, PET measurements of resting regional cerebral blood flow (rCBF) have shown an inverse relationship between resting cerebral blood volume (rCBV) and years of education (Friedland et al., 1985; McGeer et al., 1990). These studies indicate that patients with high levels of education manifest more pronounced reductions in parieto-temporal rCBV when compared to less educated subjects presenting similar clinical features. Similar results were observed in studies evaluating protective effects of rehabilitative activities (Stern et al., 1995; Scarmeas et al., 2003). fMRI studies have revealed patterns of brain network activity that supports the concept of cognitive reserve (Stern et al., 2005). Neuroimaging studies revealed that elderly people engage additional brain regions to compensate for impaired functioning (Fjell et al., 2014). For instance, upon completion of a memory task, experimental data indicate that, compared to young people, elderly individuals show increased recruitment of the prefrontal cortex (PFC), a process that possibly compensates for the reduced activity of brain regions that support visual processing of information (Ansado et al., 2013). The phenomenon has been labelled as "age-related posterior-anterior shift", or PASA (Davis et al., 2008). PASA seems to optimize cognition by inducing the inhibition of pre-potent responses (Corbetta et al., 2008; Vincent et al., 2008; Vallesi et al., 2011). Moreover, a recent study indicates that subjects performing attention tasks show an increased activity in bilateral PFC that positively correlates with enhanced performance (Davis et al., 2012).

NON-PHARMACOLOGICAL INTERVENTIONS

Age-related modulations and variations of cognitive and neural systems are dynamic and not irreversible processes. Physical or cognitive training can promote positive and lasting cognitive effects in elderly people and behavioral gains that may translate in more efficient procedural skills that help to cope with daily life activities. Non-pharmacological intervention can be divided in two approaches: aerobic training and cognitive stimulation (Jack, 2011; Vaughan et al., 2012).

AEROBIC TRAINING

Aerobic exercise is associated with increased preservation of cognitive functions (Colcombe et al., 2004). Exercise induces a cascade of molecular and sub/cellular processes that favor angiogenesis, neurogenesis, synaptogenesis as well as enhanced production of neurotrophins specially, the increased synthesis and release of brain-derived neurotrophic factor, BDNF (Deslandes et al., 2009; Coelho et al., 2013). The role of neurotrophins is crucial as decreases in BDNF levels are associated with age-related neuronal loss and BDNF reduction is also observed in patients affected by neurodegenerative or psychiatric disorders. Aerobic exercise elevates BDNF concentrations, increases hippocampal volumes (with the control group showing a reduction of volume of ∼1.4% while the trained group showed a volume increase of ∼2% over a 1-year period), and promotes better cognitive functioning in terms of executive functions, thereby suggesting that the trophin is a critical factor in mediating the

protective and cognitive effects of this type of training (Erickson et al., 2013). Regular aerobic exercise improves executive function, attention, processing speed, memory, learning processes, and helps to preserve mental abilities throughout life span (Colcombe and Kramer, 2003; Curlik and Shors, 2013; Dresler et al., 2013). Exercise is also beneficial for subjects suffering from Mild Cognitive Impairment (MCI) or early-stage dementia (Smith et al., 2010).

Longitudinal studies suggest that engagement in exercise at young age leads to better cognitive performance upon senescence. The process seems to follow a dose- response effect as, in analogy with pharmacological intervention, more robust effects are obtained in individuals who in their youth have exercised more intensively (Middleton et al., 2010). Exercise appears to preferentially target selected brain regions and/or cognitive domains (Kramer et al., 2003; Colcombe et al., 2006; Erickson et al., 2013). Neuroimaging supports the idea of greater effects of exercise in the prefrontal and medial temporal cortices (Berchicci et al., 2013). The hippocampus appears to be particularly sensitive to exercise with trophic responses leading to increased hippocampal volume by ∼2% in subjects undergoing training (Kerr et al., 2010; Erickson et al., 2011, 2013).

Overall, exercise-driven effects on specific cognitive domains like executive functions match morpho-functional changes in related brain regions (hippocampus, PFC, and basal ganglia) and neural networks. Elderly people who regularly exercise show brain volumes increases by ∼2% in these critical areas while age-matched individuals who are less physically active undergo a 1.4% decrease in volumes (Erickson et al., 2010, 2011; Esposito et al., 2013).

COGNITIVE TRAINING

Cognitive stimulation by employing interventions that make use of ecological or virtual environments is also effective to compensate age-related cognitive decline (Curlik and Shors, 2013; Park and Bischof, 2013). To date, several types of cognitive trainings are available. Several programs are aimed at improving memory (Richmond et al., 2011; Dresler et al., 2013), learning (Bailey et al., 2010), attention (Mozolic et al., 2011), executive functions (Basak et al., 2008), fluid intelligence (Jaeggi et al., 2008), mnemonic techniques, or global cognition (Klusmann et al., 2010).

Shedding some light on the molecular targets of cognitive stimulation, preclinical models have shown that enriched environments improve cognitive performances by inducing enhanced expression of genes encoding for neurotrophins or promoting synaptogenesis, dendrite formation and arborization (van Praag et al., 2000; Fratiglioni et al., 2004). The same mechanisms also occur in brains of elderly people (Mora, 2013).

Neuroimaging data show that memory-targeted training can induce positive fMRI changes as well as modification in cortical thickness of hippocampal subfields and increases in the volumetry of left hippocampal subregions like the CA3, CA4, and dentate gyrus (Engvig et al., 2012). Memory training also increases the thickness of the right fusiform and lateral orbitofrontal cortex, a phenomenon that has been positively correlated with enhanced memory performances (Engvig et al., 2010). Unfortunately, only few fMRI studies have unveil functional connectivity effects associated with cognitive intervention (Burhan et al., 2012; Engvig et al., 2014).

PC-BASED TRAINING

Video games are not just teenager leisure material, they are gaining a place in the field of rehabilitation as tools to improve cognition in the elderly (Clark et al., 1987; Dustman et al., 1992; Goldstein et al., 1997; Basak et al., 2008). Results in this area of intervention are, however, often contradictory. Computer-based cognitive training for 6 months has been shown to stimulate multi-domain abilities like immediate memory and delayed memory or language (Miller et al., 2013). Game-based cognitive training, on the other hand, has shown no group effects on visuo-spatial navigational abilities and memory, though attention improvements have been found (Whitlock et al., 2012).

Interestingly, a recent study showed that computerized training targeted at amelioration of reaction times, inspection times, short-term memory for words, executive functions, visuo-spatial acuity, arithmetic competence, visuo-spatial memory, visual scanning, visual discrimination, and n-back working memory promoted no enhancement in these domains, but resulted in global improvement of processing speed (Simpson et al., 2012). The phenomenon is known as transfer effect. Moreover, evidence suggest that computer games designed to improve processing speed can enhance short-term memory and attention (Szelag and Skolimowska, 2012).

A very intriguing recent study has shown that cognitive capabilities can be significantly rejuvenated in the elderly (Anguera et al., 2013). Upon playing a video game targeted at increasing multitasking performance (a domain that show a physiological age-dependent decline), study subjects showed benefits not just in multitasking but also in untrained domains like sustained attention and working memory. The behavioral result was associated EEG evidence that show enhanced midline frontal theta power and frontal–posterior theta coherence in the trained group. More importantly, by simply undergoing a training period that spanned for just 12 h over a 4 week period, elderly subjects showed improved multitasking capability and matched performance levels of untrained young (20-year-old) subjects. Improved cognitive performances were maintained for 6 months after suspension of training, thereby suggesting long-lasting functional effects. Thus, the study supports the idea that age-related decline can be, not only halted, but actually reversed.

The field of PC-based cognitive training is still in its infancy, but, nevertheless, promising. Studies have started to analyze and debug areas that can potentially hamper the efficacy of this type of intervention. For instance, off the shelf, commercially available games appear to be less effective than personalized games (Peretz et al., 2011). Unfortunately, online games designed to enhance cognition in the elderly have generally failed mostly because of lack of motivation or ceiling effects in trainees who were already highly functioning and/or highly exposed to digital settings like virtual reality environments and games (Bozoki et al., 2013). Factors greatly affecting the cognitive outcome of PC-based trainings are the intensity and frequency of exposure to the training; however, a clear cut linear relationship between these factors and the cognitive output has, surprisingly, been difficult to be drawn

(Whitlock et al., 2012; Wild-Wall et al., 2012). Better study designs are therefore needed (Green and Bavelier, 2008; Nouchi et al., 2012; Whitlock et al., 2012). Finally, in a recent comparative study, a custom-made computerized intervention was found to improve overall memory and attention performances in a group of elderly subjects. The study showed that training effects were still present 3 month after training withdraw (Zelinski et al., 2011).

COMBINED TRAINING

Interest in multimodal interventions has been increased in recent years. Evidence, unfortunately still too few, suggest that strategies that combine aerobic training and cognitive stimulation have a significant synergistic value (Schneider and Yvon, 2013).

In a recent study, healthy elderly subjects were exposed to four different training conditions (Shatil, 2013). The first group was exposed to cognitive training, the second group engaged itself in moderate aerobic activity, the third group followed a combination of cognitive and aerobic training, while the control group was simply exposed to book-reading. Results of the study indicated that the third group, exposed to combined training, showed the best improvement in eye-hand coordination, working memory, long-term memory, and reaction times.

Interesting effects were also found in case of PC-based training combined with exercise. Cycling sessions performed in a virtual reality environment enhanced executive functions more effectively that regular cycling in a gym on stationary bikes (Anderson-Hanley et al., 2012). Positive effects of trained cybercyclists correlated with increase of BDNF. Important effects, in the cybercycling group, were also seen in MCI subjects. In this group, training reduced by 23% AD conversion (Anderson-Hanley et al., 2012). Another study (Maillot et al., 2012) found a significant improvement in executive control and processing speed after a 12 week aerobic training that employed the Nintendo Wii sports software console.

Other studies have supported the idea of the synergistic value of combining exercise and cognitive trainings to promote memory performance (Fabre et al., 2002; Oswald et al., 2006). Unfortunately, no data are available to verify whether these training-driven benefits translate in better performances in daily living activities. A previous study, from our group, has tried to fill the gap (Pieramico et al., 2012). We evaluated effects of 6-month combination training on cognition, functional connectivity and daily living activities. Thirty healthy elderly divided in two groups of 15 subjects were exposed to either no changes in their daily living routine (control group) or to a combination training consisting in exposure to cognitive, motor, and sensorial stimulations (trained group). Groups were evaluated, before and after the end of the training period, with neuropsychological and occupational tests, resting state fMRI and analysis of cortical thickness. Combination training improved cognitive and occupational performances, reorganized the functional connectivity of Default Mode (DMN) and Dorsal Attention (DAN) networks, and promoted lower cortical thinning in several brain regions (Pieramico et al., 2012). Interestingly, analysis of dopamine-related genes indicated that carriers of DRD3 ser9gly and catechol-O-methyltransferase (COMT) val158met polymorphisms had the greatest benefits from exposure to training, thereby suggesting a genetically-driven susceptibility to the positive effects of this type of combined intervention.

CRITICAL POINTS IN NON-PHARMACOLOGICAL INTERVENTIONS

Many unknowns are still present when dealing with training programs. For instance, the role played by intensity of intervention and knowledge on which is the most beneficial set of exercises to be employed are still largely unclear and missing. Also uncertain is what concerns the specificity and selectivity of factors that can promote significant, reproducible, and durable effects on cognition. Few studies have investigated correlations between frequency and duration of aerobic and cognitive trainings along with the overall morpho-functional changes and effects on brain functioning upon aging.

Furthermore, still not completely defined is for how long positive effects are maintained and therefore, robust well-designed longitudinal studies are needed.

A limiting factor that should be not overlooked is individual variability, an aspect that mandates more accurate customization of any given set of intervention. For instance, in analogy with the field of pharmacogenomics, a "cognitogenomic" approach, in which genetic differences are explored in relation to response to cognitive trainings, is definitely necessary (Pieramico et al., 2012).

PHARMACOLOGICAL INTERVENTIONS

Pharmacological intervention aimed at enhancing or maintaining cognitive functions has been the focus of intensive research in recent years (Lanni et al., 2008; Hussain and Mehta, 2011; Lynch et al., 2011; Lynch and Mills, 2012; Wood et al., 2013; Urban and Gao, 2014). An intuitive definition of Cognitive Enhancer Drugs (CEDs) qualifies these molecules as able to enhance cognitive skills; however, an exhaustive definition of CEDs is, perhaps, not so simple. According to Lynch et coll., there is a need for a "dimensional" system to successfully define and classify CED candidates (Lynch and Gall, 2006; Lynch et al., 2011).

The first level, or dimension, is the target of action. Does the CED candidate mainly act on "general" psychological states like arousal or attentional level or does it work by potentiating single and/or selective cognitive domains? If the action is global, CED effects should be obtained through an optimization of the processing and information flux that operate within several brain nodes and networks. In case of selective activity, the drug should instead affect only specific and distinct subsets of cognitive domains.

Another important CED level/dimension is the neurobiological one or, in other words, the molecular mechanisms of action. Which neurotransmitter systems, receptor subpopulations, neuronal circuits, and/or brain structures are modified by CED activity?

Finally, a third dimensional level is represented by the distinction between drugs that enable to perform complex tasks in a more rapid or efficient way (efficiency augmentation) vs. molecules that promote enhanced performances (cognitive capability augmentation; Lynch, 2004; Lynch et al., 2011).

In accordance with this systematization, CEDs can be classified as drugs mainly acting on general or psychological aspects of cognition, compounds aimed at potentiating more specific cognitive domains or, at least in theory, drugs that are aiming at enhancing the global cognitive capability of healthy and already well performing individuals.

Most CED candidates are evaluated in preclinical models, sets where standardized behavioral outcomes are easily assessed. Although the translation of preclinical data in valid clinical outputs poses several limitations (see Sarter, 2006), animal models remain extremely valuable to identify CED neurobiology (Roesler, 2011).

In that respect, methylphenidate and modafinil, are good examples of a category of molecules that, across the board, induces changes of psychological variables such as vigilance, attention, and memory. Drug effects on arousal have been extensively described; however, a recent study that examined human subjects required to discriminate significant stimuli from distractors raised some question about the validity of the mechanism (Agay et al., 2010). Methylphenidate increases attention or "vigilance" by stimulating the cortical dopaminergic and noradrenergic transmission systems, a mechanism of action first described in preclinical settings and then confirmed by PET in human studies. Oral methylphenidate administration (0.25 mg/Kg) has been shown to promote a 50% blockage of the dopamine transporter (Volkow et al., 1998; Hannestad et al., 2010) and significantly increase extracellular dopamine concentrations. However, oral methylphenidate (at therapeutic ranges; 0.14 mg/kg) was also shown to bind with high affinity to the norepinephrine transporter. Methylphenidate-mediated effects on attention seem quite specific for tasks that need sustained vigilance; however, the drug appears to be less efficient when subjects are dealing with situations with levels of complexity that require selective attention (Advokat, 2010; Advokat et al., 2011). The compound can also improve spatial working memory in healthy individuals in conditions in which subjects are challenged with ex novo spatial problems (Elliott et al., 1997). Interestingly, experimental data confirmed drug-dependent improvements in spatial working memory. However, the study indicated that such benefits only occurred in subjects who were poorly performing at baseline, thereby suggesting that the drug can work only when significant amounts of cognitive reserve are available to be recruited (McGeer et al., 1990).

Modafinil is another example of drugs operating on vigilance or "attentional" states. The compound was first introduced as agent that counteracts excessive sleepiness associated with narcolepsy. Modafinil binds to forebrain dopamine transporters (Volkow et al., 2009; Zolkowska et al., 2009; Andersen et al., 2010) and promotes an increase in extracellular dopamine concentrations. The molecule can also bind to norepinephrine transporters in the human thalamus (Madras et al., 2006) and modulate orexinergic neurons in the hypothalamus (Ishizuka et al., 2010). Modafinil has been shown to promote marked increases in motor activity in mice (Simon et al., 1995; Stone et al., 2002), however, the effects are modest in rats and monkeys (Edgar and Seidel, 1997; Andersen et al., 2010). Finally, some studies have indicated drug-dependent improvement in sustained attention in modafinil-treated healthy subjects (Randall et al., 2005; Esposito et al., 2013).

An important bias to be considered when evaluating CEDs is represented by the fact that most studies, in animal models (Béracochéa et al., 2002) or humans (Turner et al., 2003; Baranski et al., 2004; Muller et al., 2004; Randall et al., 2005), are focused on effects on attention and working memory while other cognitive domains (such as long-term memory) are often neglected (Minzenberg and Carter, 2008). CEDs that selectively act on specific cognitive domains (memory and attention) belong to two classes of drugs that target the fast excitatory synaptic transmission responsible for communication within cortical networks. The first class acts on nicotinic receptors (alpha7 and alpha4 agonists) and modulates glutamate release while the second one, the ampakine family, facilitates fast glutamatergic transmission by acting on α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR).

The cholinergic system is involved in several important aspects of cognitive functions including attention, learning, and memory. Classical CEDs enhance brain cholinergic tone by inhibiting acetylcolinecolinesterases (AChEIs), the enzymes responsible of acetylcholine degradation. AChEIs (rivastigmine, donepezil, galantamine) are approved for the treatment of AD patients; however, these compounds have failed in patients suffering from MCI (Raschetti et al., 2007), and very little data are available on cognitive effects on healthy adults. Rivastigmine has been shown to negatively affect episodic memory and improve motor learning and visuospatial functions in healthy elderly subjects (Wezenberg et al., 2005). Donepezil has produced mixed results, the drug improves cognitive performance in healthy young subjects (Yesavage et al., 2002) but has also been shown to cause attention and short-term memory deficits in healthy elderly subjects (Beglinger et al., 2005). Selective α4β2 nicotinic receptor agonists represent a new class of drugs acting on the cholinergic system and found to induce attentional and cognitive improvements in animal models (Howe et al., 2010). Agonists of α4β2 and α7 nicotinic receptors represent interesting examples of drugs that can potentiate the prefrontal cholinergic activity. These drugs promote an increase in fast glutamatergic transmission in the cortex, thereby enhancing attentional processes (Dunbar et al., 2007; Jiang and Role, 2008; Loughead et al., 2010). The proposed mechanism of action for α4β2 agonists is based on the hypothesis that the prefrontal cholinergic system plays a crucial role in the detection of novel and relevant stimuli, thereby increasing attentional performances (Bloem et al., 2014). The drug leads to amplitude augmentation of signal-evoked cholinergic transients when a relevant stimulus is administered to a subject.

Ampakines are positive allosteric AMPAR modulators that enhance fast excitatory transmission, promote induction and enhanced strength of long-term potentiation (LTP; Lynch, 2002). These compounds also increase the production of BDNF and related proteins (Lauterborn et al., 2000, 2003; Legutko et al., 2001; Lynch, 2002; O'Neill et al., 2004, 2005; Wezenberg et al., 2007). A recent study has described a role for the CX546 ampakine in promoting the proliferation and neuronal differentiation of stem/progenitor cells originating from the subventricular zone

(Schitine et al., 2012), thereby underlying the role of these molecules in the differentiation of newborn neurons and in the shaping of neuronal circuits. The CX929 ampakine has been reported to promote LTP and counteract learning impairments in mice models of neurodevelopmental disorders. In one study, a 5 day treatment with CX929 in a mouse model of the Angelman syndrome, was reported to enhance fast EPSCs in hippocampal slices and increase long-term memory scores by 50% (Baudry et al., 2012). Ampakine are reported to improve performance in monkeys challenged with complex cognitive tasks (Porrino et al., 2005; Hampson et al., 2009) and brain imaging has demonstrated increased activity in the frontal and temporal cortices upon treatment (Porrino et al., 2005). The study revealed drug-induced activation of the precuneus, a cortical area usually less involved in task response (Porrino et al., 2005). This observation strongly suggests that ampakine administration may promote functional recruitment of additional brain areas, thereby modifying the neural substrates of cognition. Unfortunately, no ampakines are currently approved by FDA. The only molecule that reached clinical trials is CX-717. The compound was evaluated in Phase I for AD treatment but withdraw upon additional controversial results. Potential novel candidates are nevertheless being investigated for treatment of several neurologic and psychiatric disorders such as PD, schizophrenia, autism, and Attention Deficit Hyperactivity Disorder (ADHD).

Pharmaco-genomic approaches to be implemented for CED development and administration represent a promising new avenue in the field of cognitive augmentation. Traditionally these approaches have been mainly focused on the identification of genetic polymorphisms that can predict more effective drug responses in subjects already affected by neuropsychiatric disorders. However, in the past few years, studies have started to unravel the pharmacogenomic basis of individual response to CEDs occurring in healthy subjects. Factors to be considered are polymorphisms of genes regulating drug metabolizing enzymes, transporters, and receptors. These factors may profoundly influence the dose–response relationship to drugs (Roses, 2008). Differences in COMT genotypes (Val-Val vs. Met-Met) have been associated with variability in working memory performances and cognitive responses following amphetamine administration (Bilder et al., 2004). Apoliprotein E4 (apoE4), an allele of apolipoprotein E, and a risk factor for AD, is an interesting case. Quite paradoxically, ApoE polymorphism in young healthy carriers (i.e., subjects who have therefore a higher risk of developing AD later on in life) have the tendency to perform decision-making and prospective memory tasks better than, ApoE3 carriers (who are instead protected, by virtue of this genotype, from AD; Marchant et al., 2010).

CRITICAL POINTS IN PHARMACOLOGICAL INTERVENTIONS

A critical point in the clinical output of CEDs is represented by the great variability of cognitive or behavioral responses of healthy individuals to these drugs. Several studies have begun to question whether such differences depend on genotypes and/or baseline levels of cognitive function (Kimberg et al., 1997; Mehta et al., 2000; Gibbs and D'Esposito, 2005; Frank and O'Reilly, 2006; Cools et al., 2007). For example, dopaminergic drugs have been shown to improve working memory only low performing subjects (Gibbs and D'Esposito, 2006). Methylphenidate or bromocriptine seem to improve working memory in low performers but impair performance in subjects with high baseline spans (Kimberg et al., 1997; Gibbs and D'Esposito, 2006). These contradictory results have been explained by the presence of an inverted U-shaped relationship between cognitive performance and dopamine receptor stimulation (Yerkes and Dodson, 1908). Indicating some potential neurobiological determinant of the phenomenon, dopamine synthesis has been shown to be lower in in the caudate nucleus of individuals with low working memory spans when compared to synthesis in subjects with high spans (Cools et al., 2008). Accordingly, subjects with low dopamine synthesis in the basal ganglia performed better in reversal learning tasks after bromocriptine administration when compared to treated subjects who were already showing high basal ganglia synthesis (Cools et al., 2009). Modafinil administration has also been shown to have most effective cognitive effects in low performing subjects at baseline (Randall et al., 2005; Finke et al., 2010; Esposito et al., 2013).

Baseline cognitive levels also affect the cholinergic modulation of cognition in healthy individuals. Donepezil increases cognition of healthy subjects whose performance declined after sleep deprivation but the drug induced cognitive impairments in subject without sleep loss (Chuah and Chee, 2008; Chuah et al., 2009).

Finally, it should be underlined that optimal brain functioning results from a tight control of synaptic function, circuits remodeling, and connectivity modulation. In response to extrinsic stimuli, neurons accordingly change the strength of their connections in order to avoid excessive excitation or inhibition (Pozo and Goda, 2010). Therefore, CED-driven excessive connectivity can be sometimes potentially problematic. In theory, increased excitatory synaptic transmission, along with reduced synaptic pruning, may induce unregulated synaptic plasticity and neuronal circuit overactivation, thereby promoting a low signal-to-noise ratio that actually may lead to impaired cognition (Belmonte and Yurgelun-Todd, 2003).

CONCLUSION AND FUTURE DIRECTIONS

In this review, we have summarized effects on cognition and brain plasticity of non-pharmacological and pharmacological interventions targeted on elderly individuals. The field is blooming but many issues remain unresolved. Areas that are in great need of advancement concern a better understanding of the role played by genes in the modulation of senescence and/or response to intervention. Disclosure of more detailed genetic and epigenetic roadmaps will help the design and implementation of tailored and personalized training programs. A more extensive implementation of "omics" (proteomics, lipidomics, metabolomics) approaches in combination with neuroimaging will also promote a better assessment of morpho-functional changes that are occurring in the trained brain as well as whole body.

Recent progress in digital technologies leads to foresee, in the near future, a more user-friendly implementation of trainings that can avoid personal computers and make instead use of smartphones, virtual or augmented reality portable devices. In that respect, the come of age of digitally-native generations

will undoubtedly promote an extraordinary acceleration in that direction.

All these, pharmacological and non pharmacological, interventions can be envisioned to be effective not only in maintaining optimal levels of cognitive capabilities in healthy elderly individuals, but also as preventive measures to counteract the development of AD or PD as well as therapeutic tools that can be employed in the early stage of these neurodegenerative conditions.

ACKNOWLEDGMENTS

Stefano L. Sensi is supported by funds from the Italian Department of Education (PRIN 2011) and the "Marisa Trampuz von Langendorf" Fellowship for Neuroscience.

REFERENCES

Advokat, C. (2010). What are the cognitive effects of stimulant medications? Emphasis on adults with attention-deficit/hyperactivity disorder (ADHD). *Neurosci. Biobehav. Rev.* 34, 1256–1266. doi: 10.1016/j.neubiorev.2010.03.006

Advokat, C., Lane, S. M., and Luo, C. (2011). College students with and without ADHD: comparison of self-report of medication usage, study habits and academic achievement. *J. Atten. Disord.* 15, 656–666. doi: 10.1177/1087054710371168

Agay, N., Yechiam, E., Carmel, Z., and Levkovitz, Y. (2010). Non-specific effects of methylphenidate (Ritalin) on cognitive ability and decision-making of ADHD and healthy adults. *Psychopharmacology (Berl)* 210, 511–519. doi: 10.1007/s00213-010-1853-4

Amieva, H., Mokri, H., Le Goff, M., Meillon, C., Jacqmin-Gadda, H., Foubert-Samier, A., et al. (2014). Compensatory mechanisms in higher-educated subjects with Alzheimer's disease: a study of 20 years of cognitive decline. *Brain.* 137(Pt 4), 1167–1175. doi: 10.1093/brain/awu035

Andersen, M. L., Kessler, E., Murnane, K. S., McClung, J. C., Tufik, S., and Howell, L. L. (2010). Dopamine transporter-related effects of modafinil in rhesus monkeys. *Psychopharmacology (Berl)* 210, 439–448. doi: 10.1007/s00213-010-1839-2

Anderson-Hanley, C., Arciero, P. J., Brickman, A. M., Nimon, J. P., Okuma, N., Westen, S. C., et al. (2012). Exergaming and older adult cognition: a cluster randomized clinical trial. *Am. J. Prev. Med.* 42, 109–119. doi: 10.1016/j.amepre.2011.10.016

Anguera, J. A., Boccanfuso, J., Rintoul, J. L., Al-Hashimi, O., Faraji, F., Janowich, J., et al. (2013). Video game training enhances cognitive control in older adults. *Nature* 501, 97–101. doi: 10.1038/nature12486

Ansado, J., Marsolais, Y., Methqal, I., Alary, F., and Joanette, Y. (2013). The adaptive aging brain: evidence from the preservation of communication abilities with age. *Eur. J. Neurosci.* 37, 1887–1895. doi: 10.1111/ejn.12252

Bailey, H., Dunlosky, J., and Hertzog, C. (2010). Metacognitive training at home: does it improve older adults' learning? *Gerontology* 56, 414–420. doi: 10.1159/000266030

Baranski, J. V., Pigeau, R., Dinich, P., and Jacobs, I. (2004). Effects of modafinil on cognitive and metacognitive performance. *Hum. Psychopharmacol.* 19, 323–332. doi: 10.1002/hup.596

Basak, C., Boot, W. R., Voss, M. W., and Kramer, A. F. (2008). Can training in a real-time strategy video game attenuate cognitive decline in older adults? *Psychol. Aging* 23, 765–777. doi: 10.1037/a0013494

Baudry, M., Kramar, E., Xu, X., Zadran, H., Moreno, S., Lynch, G., et al. (2012). Ampakines promote spine actin polymerization, long-term potentiation and learning in a mouse model of Angelman syndrome. *Neurobiol. Dis.* 47, 210–215. doi: 10.1016/j.nbd.2012.04.002

Beglinger, L. J., Tangphao-Daniels, O., Kareken, D. A., Zhang, L., Mohs, R., and Siemers, E. R. (2005). Neuropsychological test performance in healthy elderly volunteers before and after donepezil administration: a randomized, controlled study. *J. Clin. Psychopharmacol.* 25, 159–165. doi: 10.1097/01.jcp.0000155822.51962.b4

Belmonte, M. K., and Yurgelun-Todd, D. A. (2003). Functional anatomy of impaired selective attention and compensatory processing in autism. *Brain Res. Cogn. Brain Res.* 17, 651–664. doi: 10.1016/s0926-6410(03)00189-7

Béracochéa, D., Celerier, A., Borde, N., Valleau, M., Peres, M., and Pierard, C. (2002). Improvement of learning processes following chronic systemic administration of modafinil in mice. *Pharmacol. Biochem. Behav.* 73, 723–728. doi: 10.1016/s0091-3057(02)00877-8

Berchicci, M., Lucci, G., and Di Russo, F. (2013). Benefits of physical exercise on the aging brain: the role of the prefrontal cortex. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 1337–1341. doi: 10.1093/gerona/glt094

Bilder, R. M., Volavka, J., Lachman, H. M., and Grace, A. A. (2004). The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* 29, 1943–1961. doi: 10.1038/sj.npp.1300542

Bloem, B., Poorthuis, R. B., and Mansvelder, H. D. (2014). Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. *Front Neural Circuits* 8:17. doi: 10.3389/fncir.2014.00017

Bozoki, A., Radovanovic, M., Winn, B., Heeter, C., and Anthony, J. C. (2013). Effects of a computer-based cognitive exercise program on age-related cognitive decline. *Arch. Gerontol. Geriatr.* 57, 1–7. doi: 10.1016/j.archger.2013.02.009

Buonomano, D. V., and Merzenich, M. M. (1998). Cortical plasticity: from synapses to maps. *Annu. Rev. Neurosci.* 21, 149–186. doi: 10.1146/annurev.neuro.21.1.149

Burhan, A. M., Bartha, R., Bocti, C., Borrie, M., Laforce, R., Rosa-Neto, P., et al. (2012). Role of emerging neuroimaging modalities in patients with cognitive impairment: a review from the Canadian Consensus Conference on the Diagnosis and Treatment of Dementia. *Alzheimers Res. Ther.* 5(Suppl. 1):S4. doi: 10.1186/alzrt200

Chuah, L. Y. M., and Chee, M. W. L. (2008). Cholinergic augmentation modulates visual task performance in sleep-deprived young adults. *J. Neurosci.* 28, 11369–11377. doi: 10.1523/jneurosci.4045-08.2008

Chuah, L. Y., Chong, D. L., Chen, A. K., Rekshan, W. R., Tan, J. C., Zheng, H., et al. (2009). Donepezil improves episodic memory in young individuals vulnerable to the effects of sleep deprivation. *Sleep* 32, 999–1010.

Clark, J. E., Lanphear, A. K., and Riddick, C. C. (1987). The effects of videogame playing on the response selection processing of elderly adults. *J. Gerontol.* 42, 82–85. doi: 10.1093/geronj/42.1.82

Coelho, F. G., Gobbi, S., Andreatto, C. A., Corazza, D. I., Pedroso, R. V., and Santos-Galduróz, R. F. (2013). Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): a systematic review of experimental studies in the elderly. *Arch. Gerontol. Geriatr.* 56, 10–15. doi: 10.1016/j.archger.2012.06.003

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 1166–1170. doi: 10.1093/gerona/61.11.1166

Colcombe, S., and Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol. Sci.* 14, 125–130. doi: 10.1111/1467-9280.t01-1-01430

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., et al. (2004). Cardiovascular fitness, cortical plasticity and aging. *Proc. Natl. Acad. Sci. U S A* 101, 3316–3321. doi: 10.1073/pnas.0400266101

Cools, R., Frank, M. J., Gibbs, S. E., Miyakawa, A., Jagust, W., and D'Esposito, M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *J. Neurosci.* 29, 1538–1543. doi: 10.1523/jneurosci.4467-08.2009

Cools, R., Gibbs, S. E., Miyakawa, A., Jagust, W., and D'Esposito, M. (2008). Working memory capacity predicts dopamine synthesis capacity in the human striatum. *J. Neurosci.* 28, 1208–1212. doi: 10.1523/JNEUROSCI.4475-07.2008

Cools, R., Sheridan, M., Jacobs, E., and D'Esposito, M. (2007). Impulsive personality predicts dopamine-dependent changes in frontostriatal activity during component processes of working memory. *J. Neurosci.* 27, 5506–5514. doi: 10.1523/jneurosci.0601-07.2007

Corbetta, M., Patel, G., and Shulman, G. L. (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron* 58, 306–324. doi: 10.1016/j.neuron.2008.04.017

Curlik, D. M., and Shors, T. J. (2013). Training your brain: do mental and physical (MAP) training enhance cognition through the process of neurogenesis in the hippocampus? *Neuropharmacology* 64, 506–514. doi: 10.1016/j.neuropharm.2012.07.027

Davis, S. W., Dennis, N. A., Daselaar, S. M., Fleck, M. S., and Cabeza, R. (2008). Que PASA? The posterior-anterior shift in aging. *Cereb. Cortex* 18, 1201–1209. doi: 10.1093/cercor/bhm155

Davis, S. W., Kragel, J. E., Madden, D. J., and Cabeza, R. (2012). The architecture of cross-hemispheric communication in the aging brain: linking behavior to functional and structural connectivity. *Cereb. Cortex* 22, 232–242. doi: 10.1093/cercor/bhr123

Deslandes, A., Moraes, H., Ferreira, C., Veiga, H., Silveira, H., Mouta, R., et al. (2009). Exercise and mental health: many reasons to move. *Neuropsychobiology* 59, 191–198. doi: 10.1159/000223730

Dresler, M., Sandberg, A., Ohla, K., Bublitz, C., Trenado, C., Mroczko-Wasowicz, A., et al. (2013). Non-pharmacological cognitive enhancement. *Neuropharmacology* 64, 529–543. doi: 10.1016/j.neuropharm.2012.07.002

Dunbar, G. C., Inglis, F., Kuchibhatla, R., Sharma, T., Tomlinson, M., and Wamsley, J. (2007). Effect of ispronicline, a neuronal nicotinic acetylcholine receptor partial agonist, in subjects with age associated memory impairment (AAMI). *J. Psychopharmacol.* 21, 171–178. doi: 10.1177/0269881107066855

Dustman, R. E., Emmerson, R. Y., Steinhaus, L. A., Shearer, D. E., and Dustman, T. J. (1992). The effects of videogame playing on neuropsychological performance of elderly individuals. *J. Gerontol.* 47, 168–171. doi: 10.1093/geronj/47.3.p168

Edgar, D. M., and Seidel, W. F. (1997). Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. *J. Pharmacol. Exp. Ther.* 283, 757–769.

Elliott, R., Sahakian, B. J., Matthews, K., Bannerjea, A., Rimmer, J., and Robbins, T. W. (1997). Effects of methylphenidate on spatial working memory and planning in healthy young adults. *Psychopharmacology (Berl)* 131, 196–206. doi: 10.1007/s002130050284

Engvig, A., Fjell, A. M., Westlye, L. T., Moberget, T., Sundseth, Ø., Larsen, V. A., et al. (2010). Effects of memory training on corticalthickness in the elderly. *Neuroimage* 52, 1667–1676. doi: 10.1016/j.neuroimage.2010.05.041

Engvig, A., Fjell, A. M., Westlye, L. T., Skaane, N. V., Dale, A. M., Holland, D., et al. (2014). Effects of cognitive training on gray matter volumes in memory clinic patients with subjective memory impairment. *J. Alzheimers Dis.* 41, 779–791. doi: 10.3233/JAD-131889

Engvig, A., Fjell, A. M., Westlye, L. T., Skaane, N. V., Sundseth, Ø., and Walhovd, K. B. (2012). Hippocampal subfield volumes correlate with memory training benefit in subjective memory impairment. *Neuroimage* 61, 188–194. doi: 10.1016/j.neuroimage.2012.02.072

Erickson, K. I., Gildengers, A. G., and Butters, M. A. (2013). Physical activity and brain plasticity in late adulthood. *Dialogues Clin. Neurosci.* 15, 99–108.

Erickson, K. I., Raji, C. A., Lopez, O. L., Becker, J. T., Rosano, C., Newman, A. B., et al. (2010). Physical activity predicts grey matter volume in late adulthood: the Cardiovascular Health Study. *Neurology* 75, 1415–1422. doi: 10.1212/wnl.0b013e3181f88359

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U S A* 108, 3017–3022. doi: 10.1073/pnas.1015950108

Esposito, R., Cilli, F., Pieramico, V., Ferretti, A., Macchia, A., Tommasi, M., et al. (2013). Acute effects of modafinil on brain resting state networks in young healthy subjects. *PLoS One* 8:e69224. doi: 10.1371/journal.pone.0069224

Fabre, C., Chamari, K., and Mucci, P. (2002). Improvement of cognitive function via mental and/or individualised aerobic training in healthy elderly subject. *Int. J. Sports Med.* 23, 415–421. doi: 10.1055/s-2002-33735

Feldman, D. E. (2009). Synaptic mechanisms for plasticity in neocortex. *Annu. Rev. Neurosci.* 32, 33–55. doi: 10.1146/annurev.neuro.051508.135516

Feldman, D. E., and Brecht, M. (2005). Map plasticity in somatosensory cortex. *Science* 310, 810–815. doi: 10.1126/science.1115807

Finke, K., Dodds, C. M., Bublak, P., Regenthal, R., Baumann, F., Manly, T., et al. (2010). Effects of modafinil and methylphenidate on visual attention capacity: a TVA-based study. *Psychopharmacology (Berl)* 210, 317–329. doi: 10.1007/s00213-010-1823-x

Fjell, A. M., McEvoy, L., Holland, D., Dale, A. M., and Walhovd, K. B. (2014). Alzheimer's disease neuroimaging initiative. What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Prog. Neurobiol.* 117, 20–40. doi: 10.1016/j.pneurobio.2014.02.004

Frank, M. J., and O'Reilly, R. C. (2006). A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. *Behav. Neurosci.* 120, 497–517. doi: 10.1037/0735-7044.120.3.497

Fratiglioni, L., Paillard-Borg, S., and Winblad, B. (2004). An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol.* 3, 343–353. doi: 10.1016/s1474-4422(04)00767-7

Friedland, R. P., Brun, A., and Budinger, T. F. (1985). Pathological and positron emission tomographic correlations in Alzheimer's disease. *Lancet* 1, 228. doi: 10.1016/s0140-6736(85)92074-4

Ganguly, K., and Poo, M. M. (2013). Activity-dependent neural plasticity from bench to bedside. *Neuron* 80, 729–741. doi: 10.1016/j.neuron.2013.10.028

Gibbs, S. E. B., and D'Esposito, M. (2005). Individual capacity differences predict working memory performance and prefrontal activity following dopamine receptor stimulation. *Cogn. Affect Behav. Neurosci.* 5, 212–221. doi: 10.3758/cabn.5.2.212

Gibbs, S. E., and D'Esposito, M. (2006). A functional magnetic resonance imaging study of the effects of pergolide, a dopamine receptor agonist, on component processes of working memory. *Neuroscience* 139, 359–371. doi: 10.1016/j.neuroscience.2005.11.055

Goldstein, J., Cajko, L., Oosterbroek, M., Michielsen, M., and Van Houten, O. (1997). Video game and the elderly. *Soc. Behav. Pers. Int. J.* 25, 345–352.

Green, C. S., and Bavelier, D. (2008). Exercising your brain: a review of human brain plasticity and training-induced learning. *Psychol. Aging* 23, 692–701. doi: 10.1037/a0014345

Grefkes, C., and Ward, N. S. (2014). Cortical reorganization after stroke: how much and how functional? *Neuroscientist* 20, 56–70. doi: 10.1177/1073858413491147

Hampson, R. E., España, R. A., Rogers, G. A., Porrino, L. J., and Deadwyler, S. A. (2009). Mechanisms underlying cognitive enhancement and reversal of cognitive deficits in nonhuman primates by the ampakine CX717. *Psychopharmacology (Berl)* 202, 355–369. doi: 10.1007/s00213-008-1360-z

Hannestad, J., Gallezot, J. D., Planeta-Wilson, B., Lin, S. F., Williams, W. A., van Dyck, C. H., et al. (2010). Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. *Biol. Psychiatry* 68, 854–860. doi: 10.1016/j.biopsych.2010.06.017

Howe, W. M., Ji, J., Parikh, V., Williams, S., Mocaër, E., Trocmé-Thibierge, C., et al. (2010). Enhancement of attentional performance by selective stimulation of alpha4beta2(*) nAChRs: underlying cholinergic mechanisms. *Neuropsychopharmacology* 35, 1391–1401. doi: 10.1038/npp.2010.9

Hurd, M. D., Martorell, P., and Langa, K. M. (2013). Monetary costs of dementia in the United States. *N. Engl. J. Med.* 369, 489–490. doi: 10.1056/nejmc1305541

Hussain, M., and Mehta, M. A. (2011). Cognitive enhancement by drugs in health and disease. *Trends Cogn. Sci.* 15, 28–36. doi: 10.1016/j.tics.2010.11.002

Ishizuka, T., Murotani, T., and Yamatodani, A. (2010). Modanifil activates the histaminergic system through the orexinergic neurons. *Neurosci. Lett.* 483, 193–196. doi: 10.1016/j.neulet.2010.08.005

Jack, A. J. (2011). The impact of physical and mental activity on cognitive aging. *Curr. Top. Behav. Neurosci.* 10, 273–291. doi: 10.1007/7854_2011_141

Jaeggi, S. M., Buschkuehl, M., Jonides, J., and Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proc. Natl. Acad. Sci. U S A* 105, 6829–6833. doi: 10.1073/pnas.0801268105

Jiang, L., and Role, L. W. (2008). Facilitation of cortico-amygdala synapses by nicotine: activitydependent modulation of glutamatergic transmission. *J. Neurophysiol.* 99, 1988–1999. doi: 10.1152/jn.00933.2007

Kerr, A. L., Steuer, E. L., Pochtarev, V., and Swain, R. A. (2010). Angiogenesis but not neurogenesis is critical for normal learning and memory acquisition. *Neuroscience* 171, 214–226. doi: 10.1016/j.neuroscience.2010.08.008

Kimberg, D. Y., D'Esposito, M., and Farah, M. J. (1997). Effects of bromocriptine on human subjects depend on working memory capacity. *Neuroreport* 8, 3581–3585. doi: 10.1097/00001756-199711100-00032

Klusmann, V., Evers, A., Schwarzer, R., Schlattmann, P., Reischies, F. M., Heuser, I., et al. (2010). Complex mental and physical activity in older women and cognitive performance: a 6-month randomized controlled trial. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 680–688. doi: 10.1093/gerona/glq053

Kramer, A. F., Colcombe, S. J., McAuley, E., Eriksen, K. I., Scalf, P., Jerome, G. J., et al. (2003). Enhancing brain and cognitive function of older adults through fitness training. *J. Mol. Neurosci.* 20, 213–221. doi: 10.1385/jmn:20:3:213

Lanni, C., Racchi, M., Uberti, D., Mazzini, G., Stanga, S., Sinforiani, E., et al. (2008). Pharmacogenetics and pharmagenomics, trends in normal and pathological aging studies: focus on p53. *Curr. Pharm. Des.* 14, 2665–2671. doi: 10.2174/138161208786264133

Lauterborn, J. C., Lynch, G., Vanderklish, P., Arai, A., and Gall, C. M. (2000). Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J. Neurosci.* 20, 8–21.

Lauterborn, J. C., Truong, G. S., Baudry, M., Bi, X., Lynch, G., and Gall, C. M. (2003). Chronic elevation of brain-derived neurotrophic factor by ampakines. *J. Pharmacol. Exp. Ther.* 307, 297–305. doi: 10.1124/jpet.103.053694

Legutko, B., Li, X., and Skolnick, P. (2001). Regulation of BDNF expression in primary neuron culture by LY392098, a novel AMPA receptor potentiator. *Neuropharmacology* 40, 1019–1027. doi: 10.1016/s0028-3908(01)00006-5

Loughead, J., Ray, R., Wileyto, E. P., Ruparel, K., Sanborn, P., Siegel, S., et al. (2010). Effects of the alpha4beta2 partial agonist varenicline on brain activity and working memory in abstinent smokers. *Biol. Psychiatry* 67, 715–721. doi: 10.1016/j.biopsych.2010.01.016

Lynch, G. (2002). Memory enhancement: the search for mechanism-based drugs. *Nat. Neurosci.* 5(Suppl. 1), 1035–1038. doi: 10.1038/nn935

Lynch, G. (2004). AMPA receptor modulators as cognitive enhancers. *Curr. Opin. Pharmacol.* 4, 4–11. doi: 10.1016/j.coph.2003.09.009

Lynch, G., and Gall, C. M. (2006). Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci.* 29, 554–562. doi: 10.1016/j.tins.2006.07.007

Lynch, M. A., and Mills, K. H. (2012). Immunology meets neuroscience–opportunities for immune intervention in neurodegenerative diseases. *Brain Behav. Immun.* 26, 1–10. doi: 10.1016/j.bbi.2011.05.013

Lynch, G., Palmer, L. C., and Gall, C. M. (2011). The likelihood of cognitive enhancement. *Pharmacol. Biochem. Behav.* 99, 116–129. doi: 10.1016/j.pbb.2010.12.024

Madras, B. K., Xie, Z., Lin, Z., Jassen, A., Panas, H., Lynch, L., et al. (2006). Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J. Pharmacol. Exp. Ther.* 319, 561–569. doi: 10.1124/jpet.106.106583

Maillot, P., Perrot, A., and Hartley, A. (2012). Effects of interactive physical-activity video-game training on physical and cognitive function in older adults. *Psychol. Aging* 27, 589–600. doi: 10.1037/a0026268

Marchant, N. L., King, S. L., Tabet, N., and Rusted, J. M. (2010). Positive effects of cholinergic stimulation favor young APOE epsilon4 carriers. *Neuropsychopharmacology* 35, 1090–1096. doi: 10.1038/npp.2009.214

McGeer, E. G., Peppard, R. P., McGeer, P. L., Tuokko, H., Crockett, D., Parks, R., et al. (1990). 18Fluorodeoxyglucose positron emission tomography studies in presumed Alzheimer cases, including 13 serial scans. *Can. J. Neurol. Sci.* 17, 1–11.

Mehta, M. A., Owen, A. M., Sahakian, B. J., Mavaddat, N., Pickard, J. D., and Robbins, T. W. (2000). Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *J. Neurosci.* 20:RC65.

Middleton, L. E., Barnes, D. E., Lui, L. Y., and Yaffe, K. (2010). Physical activity over the life course and its association with cognitive performance and impairment in old age. *J. Am. Geriatr. Soc.* 58, 1322–1326. doi: 10.1111/j.1532-5415.2010.02903.x

Miller, K. J., Dye, R. V., Kim, J., Jennings, J. L., O'Toole, E., Wong, J., et al. (2013). Effect of a computerized brain exercise program on cognitive performance in older adults. *Am. J. Geriatr. Psychiatry* 21, 655–663. doi: 10.1016/j.jagp.2013.01.077

Minzenberg, M. J., and Carter, C. S. (2008). Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology* 33, 1477–1502. doi: 10.1038/sj.npp.1301534

Mora, F. (2013). Successful brain aging: plasticity, environmental enrichment and lifestyle. *Dialogues Clin. Neurosci.* 15, 45–52.

Mozolic, J. L., Long, A. B., Morgan, A. R., Rawley-Payne, M., and Laurienti, P. J. (2011). A cognitive training intervention improves modality-specific attention in a randomized controlled trial of healthy older adults. *Neurobiol. Aging* 32, 655–668. doi: 10.1016/j.neurobiolaging.2009.04.013

Muller, U., Steffenhagen, N., Regenthal, R., and Bublak, P. (2004). Effects of modafinil on working memory processes in humans. *Psychopharmacology (Berl)* 177, 161–169. doi: 10.1007/s00213-004-1926-3

Nouchi, R., Taki, Y., Takeuchi, H., Hashizume, H., Nozawa, T., Kambara, T., et al. (2012). Brain training game improves executive functions and processing speed in the elderly: a randomized controlled trial. *PLoS One* 7:e29676. doi: 10.1371/journal.pone.0029676

O'Neill, M. J., Bleakman, D., Zimmerman, D. M., and Nisenbaum, E. S. (2004). AMPA receptor potentiators for the treatment of CNS disorders. *Curr. Drug Targets CNS Neurol. Disord.* 3, 181–194. doi: 10.2174/1568007043337508

O'Neill, M. J., Murray, T. K., Clay, M. P., Lindstrom, T., Yang, C. R., and Nisenbaum, E. S. (2005). LY503430: pharmacology, pharmacokinetics and effects in rodent models of Parkinson's disease. *CNS Drug Rev.* 11, 77–96. doi: 10.1111/j.1527-3458.2005.tb00037.x

Oswald, W., Gunzelmann, T., Rupprecht, R., and Hagen, B. (2006). Differential effects of single versus combined cognitive and physical training with older adults: the SimA study in a 5-year perspective. *Eur. J. Ageing* 3, 179–192. doi: 10.1007/s10433-006-0035-z

Park, D. C., and Bischof, G. N. (2013). The aging mind: neuroplasticity in response to cognitive training. *Dialogues Clin. Neurosci.* 15, 109–119. doi: 10.1016/b978-0-12-380882-0.00007-3

Pascual-Leone, A., Amedi, A., Fregni, F., and Merabet, L. B. (2005). The plastic human brain cortex. *Annu. Rev. Neurosci.* 28, 377–401. doi: 10.1016/j.cortex.2014.06.001

Peretz, C., Korczyn, A. D., Shatil, E., Aharonson, V., Birnboim, S., and Giladi, N. (2011). Computer-based, personalized cognitive training versus classical computer games: a randomized double-blind prospective trial of cognitive stimulation. *Neuroepidemiology* 36, 91–99. doi: 10.1159/000323950

Pieramico, V., Esposito, R., Sensi, F., Cilli, F., Mantini, D., Mattei, P. A., et al. (2012). Combination training in aging individuals modifies functional connectivity and cognition and is potentially affected by dopamine-related genes. *PLoS One* 7:e43901. doi: 10.1371/journal.pone.0043901

Porrino, L. J., Daunais, J. B., Rogers, G. A., Hampson, R. E., and Deadwyler, S. A. (2005). Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. *PLoS Biol.* 3:e299. doi: 10.1371/journal.pbio.0030299

Pozo, K., and Goda, Y. (2010). Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* 66, 337–351. doi: 10.1016/j.neuron.2010.04.028

Raichle, M. E., and Mintun, M. A. (2006). Brain work and brain imaging. *Annu. Rev. Neurosci.* 29, 449–476. doi: 10.1146/annurev.neuro.29.051605.112819

Randall, D. C., Shneerson, J. M., and File, S. E. (2005). Cognitive effects of modafinil in student volunteers may depend on IQ. *Pharmacol. Biochem. Behav.* 82, 133–139. doi: 10.1016/j.pbb.2005.07.019

Raschetti, R., Albanese, E., Vanacore, N., and Maggini, M. (2007). Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials. *PLoS Med.* 4:e338. doi: 10.1371/journal.pmed.0040338

Richmond, L. L., Morrison, A. B., Chein, J. M., and Olson, I. R. (2011). Working memory training and transfer in older adults. *Psychol. Aging* 26, 813–822. doi: 10.1037/a0023631

Roesler, R. (2011). Cognitive enhancers: focus on modulatory signaling influencing memory consolidation. *Pharmacol. Biochem. Behav.* 99, 155–163. doi: 10.1016/j.pbb.2010.12.028

Roses, A. D. (2008). Pharmacogenetics in drug discovery and development: a translational perspective. *Nat. Rev. Drug Discov.* 7, 807–817. doi: 10.1038/nrd2593

Sarter, M. (2006). Preclinical research into cognition enhancers. *Trends Pharmacol. Sci.* 27, 602–608. doi: 10.1016/j.tips.2006.09.004

Scarmeas, N., Zarahn, E., Anderson, K. E., Habeck, C. G., Hilton, J., Flynn, J., et al. (2003). Association of life activities with cerebral blood flow in Alzheimer disease: implications for the cognitive reserve hypothesis. *Arch. Neurol.* 60, 359–365. doi: 10.1001/archneur.60.3.359

Schitine, C., Xapelli, S., Agasse, F., Sardà-Arroyo, L., Silva, A. P., De Melo Reis, R. A., et al. (2012). Ampakine CX546 increases proliferation and neuronal differentiation in subventricular zone stem/progenitor cell cultures. *Eur. J. Neurosci.* 35, 1672–1683. doi: 10.1111/j.1460-9568.2012.08072.x

Schneider, N., and Yvon, C. (2013). A review of multidomain interventions to support healthy cognitive ageing. *J. Nutr. Health Aging* 17, 252–257. doi: 10.1007/s12603-012-0402-8

Shatil, E. (2013). Does combined cognitive training and physical activity training enhance cognitive abilities more than either alone? A four-condition randomized controlled trial among healthy older adults. *Front. Aging Neurosci.* 5:8. doi: 10.3389/fnagi.2013.00008

Simon, P., Hémet, C., Ramassamy, C., and Costentin, J. (1995). Non-amphetaminic mechanism of stimulant locomotor effect of modafinil in mice. *Eur. Neuropsychopharmacol.* 5, 509–514. doi: 10.1016/0924-977x(95)00041-m

Simpson, T., Camfield, D., Pipingas, A., Macpherson, H., and Stough, C. (2012). Improved processing speed: online computer-based cognitive training in older adults. *Educ. Gerontol.* 38, 445–458. doi: 10.1080/03601277.2011.559858

Smith, P. J., Blumenthal, J. A., Hoffman, B. M., Cooper, H., Strauman, T. A., Welsh-Bohmer, K., et al. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosom. Med.* 72, 239–252. doi: 10.1097/psy.0b013e3181d14633

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia* 47, 2015–2028. doi: 10.1016/j.neuropsychologia.2009.03.004

Stern, Y., Alexander, G. E., Prohovnik, I., Stricks, L., Link, B., Lennon, M. C., et al. (1995). Relationship between lifetime occupation and parietal flow: implications for a reserve against Alzheimer's disease pathology. *Neurology* 45, 55–60. doi: 10.1212/wnl.45.1.55

Stern, Y., Habeck, C., Moeller, J., Scarmeas, N., Anderson, K. E., Hilton, H. J., et al. (2005). Brain networks associated with cognitive reserve in healthy young and old adults. *Cereb. Cortex* 15, 394–402. doi: 10.1093/cercor/bhh142

Stone, E. A., Cotecchia, S., Lin, Y., and Quartermain, D. (2002). Role of brain alpha 1B-adrenoceptors in modafinil-induced behavioral activity. *Synapse* 46, 269–270. doi: 10.1002/syn.10127

Szelag, E., and Skolimowska, J. (2012). Cognitive function in elderly can be ameliorated by training in temporal information processing. *Restor. Neurol. Neurosci.* 30, 419–434. doi: 10.3233/RNN-2012-120240

Turner, D. C., Robbins, T. W., Clark, L., Aron, A. R., Dowson, J., and Sahakian, B. J. (2003). Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacology (Berl)* 165, 260–269.

Urban, K. R., and Gao, W. J. (2014). Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain. *Front. Syst. Neurosci.* 8:38. doi: 10.3389/fnsys.2014.00038

Vallesi, A., McIntosh, A. R., and Stuss, D. T. (2011). Overrecruitment in the aging brain as a function of task demands: evidence for a compensatory view. *J. Cogn. Neurosci.* 23, 801–815. doi: 10.1162/jocn.2010.21490

van Praag, H., Kempermann, G., and Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nat. Rev. Neurosci.* 1, 191–198. doi: 10.1038/35044558

Vaughan, S., Morris, N., Shum, D., O'Dwyer, S., and Polit, D. (2012). Study protocol: a randomised controlled trial of the effects of a multi-modal exercise program on cognition and physical functioning in older women. *BMC Geriatr.* 12:60. doi: 10.1186/1471-2318-12-60

Vincent, J. L., Kahn, I., Snyder, A. Z., Raichle, M. E., and Buckner, R. L. (2008). Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. *J. Neurophysiol.* 100, 3328–3342. doi: 10.1152/jn.90355.2008

Volkow, N. D., Fowler, J. S., Logan, J., Alexoff, D., Zhu, W., Telang, F., et al. (2009). Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. *JAMA* 301, 1148–1154. doi: 10.1001/jama.2009.351

Volkow, N. D., Wang, G. J., Fowler, J. S., Gatley, S. J., Logan, J., Ding, Y. S., et al. (1998). Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. *Am. J. Psychiatry.* 155, 1325–1331.

Voss, M. W., Vivar, C., Kramer, A. F., and van Praag, H. (2013). Bridging animal and human models of exercise-induced brain plasticity. *Trends Cogn. Sci.* 17, 525–544. doi: 10.1016/j.tics.2013.08.001

Wezenberg, E., Verkes, R. J., Ruigt, G. S., Hulstijn, W., and Sabbe, B. G. (2007). Acute effects of the ampakinefarampator on memory and information processing in healthy elderly volunteers. *Neuropsychopharmacology* 32, 1272–1283. doi: 10.1038/sj.npp.1301257

Wezenberg, E., Verkes, R. J., Sabbe, B. G., Ruigt, G. S., and Hulstijn, W. (2005). Modulation of memory and visuospatial processes by biperiden and rivastigmine in elderly healthy subjects. *Psychopharmacology (Berl)* 181, 582–594. doi: 10.1007/s00213-005-0083-7

Whitlock, L. A., Collins, A. C., and Allaire, J. C. (2012). Individual differences in response to cognitive training: using a multi-modal, attentionally demanding game-based intervention for older adults. *Comput. Human Behav.* 28, 1091–1096. doi: 10.1016/j.chb.2012.01.012

Wild-Wall, N., Falkenstein, M., and Gajewski, P. D. (2012). Neural correlates of changes in a visual search task due to cognitive training in seniors. *Neural Plast.* 2012:529057. doi: 10.1155/2012/529057

Wilson, R. S., Li, Y., Aggarwal, N. T., Barnes, L. L., McCann, J. J., Gilley, D. W., et al. (2004). Education and the course of cognitive decline in Alzheimer disease. *Neurology* 63, 1198–1202. doi: 10.1212/01.wnl.0000140488.65299.53

Wittenberg, G. F. (2010). Experience, cortical remapping and recovery in brain disease. *Neurobiol. Dis.* 37, 252–258. doi: 10.1016/j.nbd.2009.09.007

Wood, S., Sage, J. R., Shuman, T., and Anagnostaras, S. G. (2013). Psychostimulants and cognition: a continuum of behavioral and cognitive activation. *Pharmacol. Rev.* 66, 193–221. doi: 10.1124/pr.112.007054

Xerri, C. (2008). Imprinting of idiosyncratic experience in cortical sensory maps: neural substrates of representational remodeling and correlative perceptual changes. *Behav. Brain Res.* 192, 26–141. doi: 10.1016/j.bbr.2008.02.038

Yerkes, R. M., and Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol. Psychol.* 18, 459–482. doi: 10.1002/cne.920180503

Yesavage, J. A., Mumenthaler, M. S., Taylor, J. L., Friedman, L., O'Hara, R., Sheikh, J., et al. (2002). Donepezil and flight simulator performance: effects on retention of complex skills. *Neurology* 59, 123–125. doi: 10.1212/wnl.59.1.123

Zelinski, E. M., Spina, L. M., Yaffe, K., Ruff, R., Kennison, R. F., Mahncke, H. W., et al. (2011). Improvement in memory with plasticity-based adaptive cognitive training: results of the 3-month follow-up. *J. Am. Geriatr. Soc.* 59, 258–265. doi: 10.1111/j.1532-5415.2010.03277.x

Zolkowska, D., Jain, R., Rothman, R. B., Partilla, J. S., Roth, B. L., Setola, V., et al. (2009). Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J. Pharmacol. Exp. Ther.* 329, 738–746. doi: 10.1124/jpet.108.146142

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 June 2014; accepted: 05 August 2014; published online: 02 September 2014.

*Citation: Pieramico V, Esposito R, Cesinaro S, Frazzini V and Sensi SL (2014) Effects of non-pharmacological or pharmacological interventions on cognition and brain plasticity of aging individuals. Front. Syst. Neurosci. **8**:153. doi: 10.3389/fnsys.2014.00153*

This article was submitted to the journal Frontiers in Systems Neuroscience.

frontiers in
SYSTEMS NEUROSCIENCE

REVIEW ARTICLE
published: 18 September 2014
doi: 10.3389/fnsys.2014.00172

Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception

Jun Il Kang[1,2], Frédéric Huppé-Gourgues[1] and Elvire Vaucher[1]*

[1] *École d'optométrie, Université de Montréal, Montréal, QC, Canada*
[2] *Département de Neuroscience, Université de Montréal, Montréal, QC, Canada*

Edited by:
Mikhail Lebedev, Duke University, USA

Reviewed by:
Michael A. Silver, University of California at Berkeley, USA
Pietro Pietrini, University of Pisa Medical School, Italy

***Correspondence:**
Elvire Vaucher, École d'optométrie, Université de Montréal, CP 6128 succursale centre-ville, Montréal QC H3C 3J7, Canada
e-mail: elvire.vaucher@umontreal.ca

The cholinergic system is a potent neuromodulatory system that plays critical roles in cortical plasticity, attention and learning. In this review, we propose that the cellular effects of acetylcholine (ACh) in the primary visual cortex during the processing of visual inputs might induce perceptual learning; i.e., long-term changes in visual perception. Specifically, the pairing of cholinergic activation with visual stimulation increases the signal-to-noise ratio, cue detection ability and long-term facilitation in the primary visual cortex. This cholinergic enhancement would increase the strength of thalamocortical afferents to facilitate the treatment of a novel stimulus while decreasing the cortico-cortical signaling to reduce recurrent or top-down modulation. This balance would be mediated by different cholinergic receptor subtypes that are located on both glutamatergic and GABAergic neurons of the different cortical layers. The mechanisms of cholinergic enhancement are closely linked to attentional processes, long-term potentiation (LTP) and modulation of the excitatory/inhibitory balance. Recently, it was found that boosting the cholinergic system during visual training robustly enhances sensory perception in a long-term manner. Our hypothesis is that repetitive pairing of cholinergic and sensory stimulation over a long period of time induces long-term changes in the processing of trained stimuli that might improve perceptual ability. Various non-invasive approaches to the activation of the cholinergic neurons have strong potential to improve visual perception.

Keywords: attention, cholinergic system, cognitive enhancement, cortical plasticity, nicotinic receptors, muscarinic receptors, perceptual learning, visual cortex

INTRODUCTION

Boosting the brain's functioning during rehabilitation paradigms might help individuals with cognitive or sensory deficits to better recover their abilities. In this review, we will examine how the cholinergic system might help in this regard by specifically focusing on visual function. Recent knowledge about the cellular and functional organization of the primary visual cortex (V1) is particularly interesting for the deciphering of the neurobiological mechanisms of perceptual learning and its modulation by the cholinergic system. V1 is the first cortical step of the integration of complex visual stimuli and is decisive in the selection of specific stimuli from the visual field. This process further orients processing in higher cognitive cortical areas involved in elaboration of fine visual conscious perception. Thus, cholinergic modulation of visual processing in V1 would have strong effects on the fine-tuning of perception and the acquisition of memory traces.

Perceptual learning is the long-term improvement of the ability to detect or discriminate specific sensory stimuli without interfering with or diminishing other skills that results from training over a sustained period of time (Fahle and Poggio, 2002; Fahle, 2009; Roelfsema et al., 2010). In vision, improvements in the discrimination of specific attributes of a stimulus, such as its orientation (Ramachandran and Braddick, 1973; Fiorentini and Berardi, 1980; Mayer, 1983), contrast (Hua et al., 2010) or vernier acuity (McKee and Westheimer, 1978), have been demonstrated using such paradigms. Increases in visual capacity should go together with increases in the numbers of neurons that encode the trained stimulus in the V1 and the expansions of the cortical maps that represent the stimulus (Kilgard and Merzenich, 1998). The signal-to-noise ratio is usually increased. The connectivity between neurons and efficiency of the neuronal transmission, i.e., the strength of the input they transmit as well as the short processing time, should also be increased. Changes in dendritic spines number, morphology and synaptic plasticity (i.e., long-lasting modifications of the strength of the post-synaptic electrical signal) have also been demonstrated during perceptual learning (Gilbert and Li, 2012). However, it should be assumed that the neurons involved in perceptual learning increase the amount of information that they carry while preserving their primary selective response properties (Gilbert et al., 2001). Perceptual learning is also facilitated either by attention (Ahissar and Hochstein, 1993) or reinforcement by reward expectation (Seitz et al., 2009); both of these process enhance neuronal transmission efficiency.

Perceptual learning or increased cortical processing of specific stimuli is generally achieved with repetitive training. It has been recently suggested that it can also be boosted by neuromodulation and extrinsic control of the cerebral neuromodulatory systems by electrical or pharmacological means. The cholinergic system, which uses acetylcholine (ACh) as a neurotransmitter, is particularly relevant because it widely innervates V1 and alters the efficiency of neurons. The injection of ACh or its analogs into V1 has been shown to increase neuronal responses and trigger synaptic plasticity (Gu, 2003) and cortical plasticity (Bear and Singer, 1986). More specifically, the administration of ACh during visual processing increases thalamocortical input while reducing intracortical recurrence (Gil et al., 1997; Disney et al., 2007; Soma et al., 2013a) and thus enhances specific stimulus processing and output. This diversity of the actions of ACh is due to the ubiquitous localization of both ionotropic nicotinic receptors (nAChRs) and metabotropic muscarinic receptors (mAChRs) in V1 (Levey et al., 1991; Disney et al., 2006; Amar et al., 2010), which are involved in the facilitation of cortical activity and synchronized cortical activity. In addition to the direct and acute effects of ACh, an increasing number of studies have recently shown that repetitive cholinergic activation of the visual cortex has also the ability to enhance visual perception. The repetitive pairing of ACh release with exposure to a visual stimulus improves several visual capacities, such as contrast sensitivity (Mayer, 1983; Hua et al., 2010), motion detection (Rokem and Silver, 2010), working memory (Furey et al., 2000; Bentley et al., 2004), texture discrimination (Beer et al., 2013) and visual acuity (Kang et al., 2014) in both humans and animals. Many animal studies have also demonstrated the involvement of the cholinergic system in perceptual learning in different sensory modalities, including olfaction (Wilson et al., 2004) and audition (Bakin and Weinberger, 1996). These improvements suggest that paired visual and cholinergic stimulation induces perceptual learning possibly via synaptic and cortical modifications linked to attention mechanisms (Herrero et al., 2008) or reward expectation (Chubykin et al., 2013) and cortical plasticity. The repetition of such pairings would result in a more efficient processing and increased automaticity of visual stimuli. This could be related to reduced strength of connectivity between attention regions and V1 (Ricciardi et al., 2013) and a role of ACh in perceptual inference and repetition suppression (Moran et al., 2013).

Our research hypothesis proposes that cholinergic effects in V1 contribute to perceptual learning and can thus be used to voluntarily develop one's brain capacity and aid the restoration of visual function. In the present review, we will discuss how ACh might improve perceptual capacities, particularly during repetitive stimulation paired with visual stimulation, which are related to its roles in the long-term enhancement of cortical responsiveness and cortical plasticity (**Figure 1**). Specifically, we will first discuss the diverse effects of ACh on V1 neuron function and connectivity and relate these effects to the background theory of the cholinergic modulation of neural mechanisms and brain function. To assess these neuronal mechanisms, we will primarily discuss studies that have been performed in rodents and non-human primates (for more information about cholinergic effects on human cognition, see Drevets et al., 2008; Bentley et al., 2011).

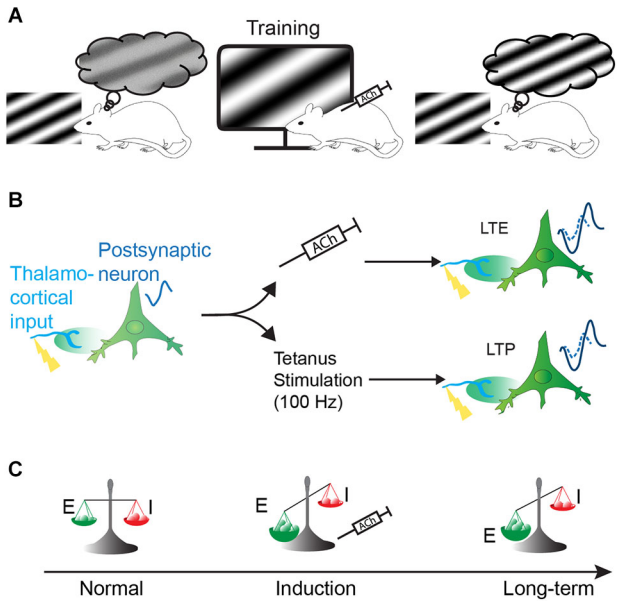

FIGURE 1 | Hypothesis of the effect of the cholinergic system on visual perception. Increase of perceptual capacity (perceptual learning) can be obtained by naturally or artificially activating the cholinergic system during sensory training. This perceptual learning might be achieved by long-term facilitation of cortical responses and/or change of the excitatory/inhibitory balance. **(A)** Representation of the improvement of visual perception in the rat by pairing the presentation of a specific sinusoidal grating coupled to cholinergic system activation (represented by injection of acetylcholine, ACh). **(B)** Long-term enhancement (LTE) of the cortical responses by ACh (upper path) share common features with classical long-term potentiation (LTP, lower path): visual stimulation of presynaptic input evokes small responses (represented by a resulting small visual evoked potential (VEP) signal waveform) in post-synaptic neurons. If paired to cholinergic activation, the presynaptic stimulation induces a long-term enhancement of neuronal responses (upper path, represented by an increased VEP signal waveform). This mechanism is similar to LTP where theta-burst stimulation (100 Hz) in lateral geniculate nucleus (LGN) induces an increase of postsynaptic potentiation in the cortex (lower path). VEP signals are imaginary waveform to compare neuronal response magnitude, as recorded in our previous experiments. **(C)** Cortical plasticity induced by ACh could also result from a change in excitatory and inhibitory balance by changing the strength of the excitatory synapse over inhibitory synapses, resulting in long-term modification of cortical responses.

ORGANIZATION OF THE CHOLINERGIC SYSTEM IN V1

Cholinergic fibers are distributed throughout the cortical layers of V1 (Lysakowski et al., 1989; Avendaño et al., 1996; Mechawar et al., 2000), which suggests that ACh might affect every step of visual processing (**Figure 2A**).

LOCAL EFFECT OF THE CHOLINERGIC FIBERS

The cholinergic system influences the local network by diffuse transmission rather than by synaptic transmission (Descarries et al., 1997; Yamasaki et al., 2010). This property is related to the fact that ACh is released from the varicosities that are distributed along the cholinergic axons and that these varicosities show only rare synaptic organizations at the ultrastructural level (Umbriaco et al., 1994; Vaucher and Hamel, 1995; Mechawar et al., 2000).

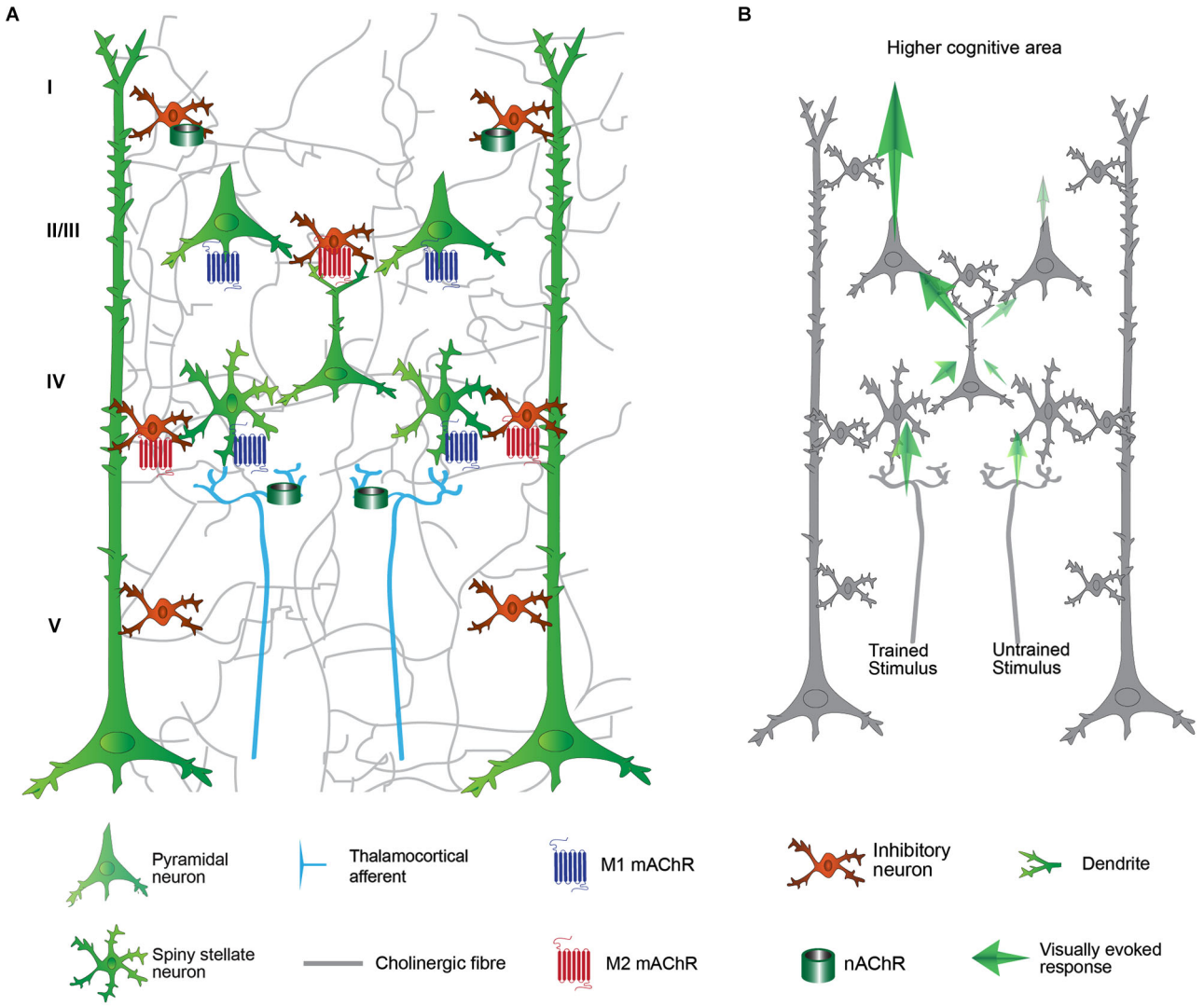

FIGURE 2 | Schematic representation of the primary visual cortex (V1) and its cholinergic modulation on cortical processing. (A) Thalamocortical afferent (light blue fibers) from LGN conveying stimulus information reach spiny stellate neuron in the layer IV. The input is transferred to the layer II/III, then layer V and to higher visual area. The cholinergic activation modulates the visual processing in virtually all the levels of V1 connectivity by nicotinic (green cylinder) and muscarinic (seven transmembrane domains molecules) receptors. **(B)** Cortical processing after VS/HDB training. The cortical processing for the trained stimulus is significantly enhanced after VS/HDB training but un-trained stimulus processing is not affected. Note that the input from the thalamus is similar but the feedforward propagation is increased. Excitatory influences are shown in blue arrows. The strength of the response enhancement is represented by the contrast of the arrow. Layer VI and horizontal connections are omitted for clarity.

However, the modulation of the cortex by ACh is not widespread and is primarily selective and adapted to the local microfunction due to the differential distribution of varicosities along the cholinergic axons (Zhang et al., 2011) and the differential distribution of the cholinergic receptor subtypes on different neuronal targets. Moreover, ACh release might be triggered by local neuronal activity to induce locally restricted rather than generalized action of the cholinergic system (Laplante et al., 2005). The variety of the cholinergic receptors and their distributions convey subtype-specific functions (Thiele, 2013; Groleau et al., 2014). In V1, AChRs exhibit differential subtype densities across the cortical layers (I-VI) on both excitatory (Gulledge et al., 2009; Thiele, 2013) and inhibitory neurons (Hashimoto et al., 1994). The distinct actions of cholinergic receptors can be related to differences in the conductances of the ionotropic receptor nAChRs for Na^+, K^+ ($\alpha_4\beta_2$) and Ca^{2+} (α_7) (Rang, 2003) and in the intracellular pathways of the different subtypes of the G-protein coupled mAChRs. Amongst the five mAChR subtypes identified, the M1, M3 and M5 mAChRs are coupled with Gq/11 proteins, which activate phospholipase C and lead to increases in intracellular Ca^{2+} and the M2 and M4 mAChRs are bound with Gi protein that inhibits adenylyl cyclase, which leads to a decrease in cAMP, the inhibition of voltage-gated Ca^{2+} channels and an increased K^+ efflux (Caulfield and Birdsall, 1998; Wess, 2003). In addition,

M1 promotes the opening of NMDARs and induces LTP in the hippocampus (Buchanan et al., 2010; Giessel and Sabatini, 2010).

CHOLINERGIC FIBERS ACTIVATION IN V1

Stimulation of the cholinergic system in V1 can be achieved via the administration of ACh analogs (e.g., carbachol), cholinergic receptor agonists (e.g., nicotine and selective mAChR drugs) or cholinesterase inhibitors or through electrical or optogenetic stimulation of the cholinergic neurons that project to V1. The cholinergic neurons that project to V1 are located in the basal forebrain (BF), particularly the ventral pallidum, substantia innominata and the horizontal limb of the diagonal band of Broca (HDB; Gaykema et al., 1990; Laplante et al., 2005). Although the nucleus basalis magnocellularis is the main cholinergic nucleus of the BF which innervates the cortical mantle, it projects only weakly to V1 (Luiten et al., 1987; Vaucher and Hamel, 1995); nevertheless, some studies report that the stimulation of this nucleus might induce functional changes in the visual cortex (Goard and Dan, 2009; Pinto et al., 2013). Moreover, although there are GABAergic neurons in the BF, many studies have confirmed that the effects of BF stimulation are identical to those of intracerebral injections of ACh agonists and are primarily mediated by the cholinergic fibers (Dauphin et al., 1991; Ma and Suga, 2005; Dringenberg et al., 2007; Kocharyan et al., 2008; Kang and Vaucher, 2009). There are also intrinsic cholinergic neurons that represent only 10–15% of the total cortical innervation (Eckenstein et al., 1988; Chédotal et al., 1994), and the involvement of these neurons in cortical processing remains unclear.

ACETYLCHOLINE MODULATES THE FLOW OF VISUAL INFORMATION IN V1

The efficiencies of the cortical inputs and outputs are altered by the different cholinergic receptors in both the glutamatergic and GABAergic systems according to the cortical layer, neuron and receptor subtype reached by ACh (**Figure 2**). V1 integrates visual information via different pathways that include the following: the feedforward thalamocortical pathways, V1 intracortical connectivities, and the feedback influence from higher cortical areas (**Figure 3**). The visual information arriving to layer IV of V1 from the lateral geniculate nucleus (LGN) is considered to be the dominant thalamocortical visual pathway. In contrast, the intracortical pathway might arise from neighboring neurons, local recurrent axons or more broadly from horizontal networks. The cholinergic system induces facilitation, suppression or does not affect the visual cells. Direct local effects of ACh might be opposed to the indirect effects of ACh due to neuronal interactions across layers. The general picture of the cholinergic influence on V1 is that the response to a stimulus is increased by cholinergic modulation in the thalamocortical pathway while the intracortical influence is suppressed. The cholinergic influence described in the following paragraph represents the acute effects in V1 that can participate in attention and trigger perceptual learning. The effects of the cholinergic system on long-range corticocortical relationships are also of interest but are beyond the scope of this review.

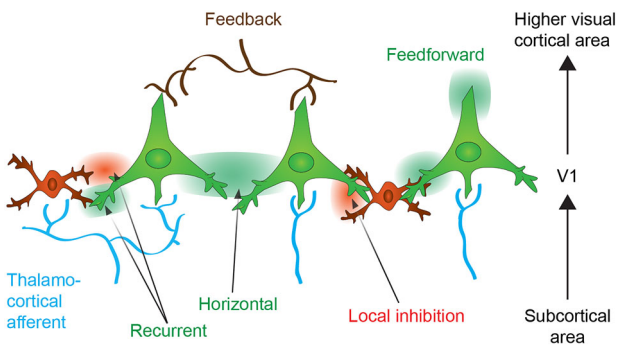

FIGURE 3 | Neuronal connectivity within the primary visual cortex (V1). Neurons from V1 receive thalamocortical (in blue) and corticocortical inputs originating from upper cortical areas (feedback control, in brown). The thalamocortical information is integrated within V1 and further transmitted to upper cortical areas (feedforward transmission). The activation of neurons might enhance activation or inhibition of neighboring neuron by the horizontal connections or through the local inhibitory interneurons. Recurrent connections auto-regulates neuronal activity (see text for more details). Excitatory effect is expressed as green color and inhibitory effect as red.

CHOLINERGIC MODULATION OF THALAMOCORTICAL INPUTS

Cortical responses to sensory stimuli transmitted by the LGN are amplified during learning and experience-dependent plasticity to emphasize relevant information (Sarter et al., 2005; Wang et al., 2013). These thalamic afferents are of prime relevance because they define the receptive fields and other properties of V1 neurons. Complex information is extracted according to its properties (e.g., orientation) via projections to different columns (in primates) or specific cells (in rodents). Cholinergic activation in this layer induces a general increase in responsiveness regardless of the features of the visual stimuli (e.g., orientation; Disney et al., 2012), which allows the cortex to respond reliably to weak stimulation (Disney et al., 2007). ACh increases the thalamocortical input through presynaptic nAChRs on the thalamocortical fibers (Gil et al., 1997; Disney et al., 2007; **Figures 2A**, **4**). The M1 mAChR also amplifies the spiny stellate cell/pyramidal cell response through a postsynaptic intracellular pathway (Gu, 2003), but inhibition through the M4 mAChR has also been observed on spiny neurons in the somatosensory cortex (Eggermann and Feldmeyer, 2009). Interestingly, the cholinergic facilitation of thalamocortical inputs in sensory cortex slices is ACh-concentration dependent. High doses of ACh enhance the thalamocortical afferents both *in vitro* and in computational models (Hasselmo, 2006; Deco and Thiele, 2011). Together, these results indicate that, under conditions of high levels of ACh release, the enhancement of the thalamocortical inputs in layer IV facilitates the transmission of sensory information and induces experience-dependent plasticity (e.g., learning).

CHOLINERGIC MODULATION OF INTRACORTICAL INTERACTIONS

In addition to the enhancement of thalamocortical inputs, ACh might modulate intracortical connectivity either by suppressing lateral inhibition (Kimura and Baughman, 1997; Metherate et al., 2005; Metherate, 2011) or suppressing the spread of the excitation

of thalamic inputs (Kimura et al., 1999; Silver et al., 2008). The presynaptic mAChRs that are located on the glutamatergic fibers induce a suppression of the intracortical neurons (Gil et al., 1997), although the inhibition of GABAergic terminals induces a disinhibition of the pyramidal cells (Ji and Dani, 2000; Christophe et al., 2002; Seeger et al., 2004; Salgado et al., 2007). Intracortical connectivity modulates the response intensity and the output of V1 neurons (**Figure 3**). The lateral connections also synchronize the firing of similar neuronal populations (Gilbert and Wiesel, 1989; Lien and Scanziani, 2013), which allows for lateral correlation between neurons with similar orientation preferences during typical perceptual learning tasks (e.g., the Vernier acuity test) (Ramalingam et al., 2013). The differential action of ACh on lateral connections might simultaneously enhance specific modules of the same orientation (lateral correlation) while depressing adjacent irrelevant modules (McGuire et al., 1991; Stettler et al., 2002). A recent study using optogenetics showed that inhibition of the intracortical excitatory neurons leads to a receptive field reduction (Li et al., 2013), and this finding is consistent with the effect of ACh release in V1 (Roberts et al., 2005; Zinke et al., 2006) and the increases in the population receptive fields of M1/M3 mAChR knock-out mice (Groleau et al., 2014). Furthermore, an ACh esterase inhibitor reduces surround suppression in a perceptual study in humans (Kosovicheva et al., 2012), which could be indicative of a weakening of lateral connections. Hasselmo (2006) proposed that high ACh levels suppress the magnitude of feedback excitation, whereas low ACh levels result in weaker afferent input to the cortex. Similarly, Deco and Thiele (2011) also proposed that high ACh levels decrease the intracortical interactions and that low ACh increase these interactions. The hypothesis of these authors was confirmed in an *in vitro* study that showed that the enhancement of the recurrent cortical activity in low-dose ACh conditions was independent of the thalamocortical input (Wester and Contreras, 2013). Together, these results suggest that during intense ACh release, the intracortical connections are inhibited, which relieves the sensory cortices from recurrent connections. However, in low concentration of ACh situations, the lateral connections might amplify the thalamocortical activity amongst similarly tuned neurons.

These effects have primarily been recorded within layer II/III; however, in layers I, V and VI, which are primarily involved in feedback mechanisms, ACh might also influence feedforward processing by interacting with neurons in layers IV and II/III (De Pasquale and Sherman, 2012). Layer I neurons are densely innervated by the cholinergic projections (Vaucher and Hamel, 1995; Mechawar et al., 2000). It has been shown that inhibitory actions mediated by AChRs can suppress layer II/III (Zinke et al., 2006; Alitto and Dan, 2012; Soma et al., 2013b) and layer V pyramidal neuron activity (Lucas-Meunier et al., 2009; Amar et al., 2010) and can also inhibit the cortical GABAergic network and thus result in the disinhibition of the majority of the cortical layers (Christophe et al., 2002). It has been observed that local ACh application primarily suppresses the activity of layer VI neurons (Disney et al., 2012), which can alter the activation of all of the layers of V1 in a linear manner via the intracortical pathway (Olsen et al., 2012) and alter the activation of the thalamocortical fibers (Cudeiro and Sillito, 2006; Sillito et al., 2006). Cholinergic action might thus disinhibit the activities of other layers by suppressing layer VI. Topical injections of ACh into layer V produce the predominant effect of facilitation of the regular and fast-spiking cells (Soma et al., 2013b), although local ACh activation seems to decrease excitatory drive through presynaptic M1 mAChRs (Kimura and Baughman, 1997) and to increase inhibitory drive through M3 mAChRs (Amar et al., 2010). Similarly, an increase in the activation of GABAergic neurons activation in layer V has been observed following repetitive BF/visual pairing (Kang et al., 2014). Layer V pyramidal neurons send dense projections to the superior colliculus and diverse thalamic nuclei that are involved in focused attention.

Finally, ACh can promote the co-activation of different cortical areas and layers which might be an efficient method for the selection of visual information via a summation of the temporally coincident presynaptic spikes (Fries et al., 2007). It has been shown that visually driven gamma power is differentially distributed across the layers of V1 (Xing et al., 2012) and that gamma oscillations can be induced by cholinergic stimulation (Rodriguez et al., 2004; Bhattacharyya et al., 2013).

In conclusion, BF stimulation that facilitates the release of ACh in multiple layers of V1 might act in diverse manners and results in the enhancement of visual stimulus-driven responses. The pre-amplified responses of layer IV are filtered out by GABAergic neurons of layer II/III to transfer task-relevant information to higher visual cortical areas. The activated synaptic connections can be modulated by layers V and VI or by the feedback mechanism of layer I. Differential responses across layers might be integrated by the synchronization of their activities in the gamma-band to facilitate visual processes.

CELLULAR EFFECTS OF ACETYLCHOLINE IN V1-RELATED ATTENTION

Most of these cellular mechanisms contribute to attentional mechanisms in V1. Attention increases the cortical response to stimuli (i.e., the signal) while lowering interference from the background (i.e., the noise). Several animal studies have described deficits of attention following cholinergic lesions or injections of cholinergic antagonists (Voytko et al., 1994; McGaughy and Sarter, 1998, 1999) and ACh has been shown to be involved in attention in V1 (Herrero et al., 2008). However, ACh release promotes rather than initiates attention. Because ACh-mediated attention and perceptual learning have crucial effects on each other, the role of ACh during visual attention is delineated in the following section to better understand how ACh enhances cortical functioning.

CHOLINERGIC INVOLVEMENT IN BOTTOM-UP AND TOP-DOWN ATTENTION

ACh has been suggested to control the balance between bottom-up and top-down processing through attentional mechanisms (Yu and Dayan, 2002, 2005; Sarter et al., 2005). This influence is mediated by pre-synaptic thalamocortical nAChRs (Gil et al., 1997; Disney et al., 2007). Attention that is prompted by the properties of a stimulus, i.e., the saliency of the stimulus relative to the background, is said to be bottom-up attention, whereas attention that is prompted by the voluntary direction of focus toward a

specific stimulus is defined as top-down attention. Although it can be difficult to separate bottom-up and top-down attentional control (Ansorge et al., 2010; Egeth et al., 2010; Eimer and Kiss, 2010; Theeuwes, 2010), some studies have shown that cholinergic activity influences bottom-up attention. The effect of ACh on bottom-up attention might occur not only in V1 but also in early processing areas such as the thalamus. For example, the direct injection of 192-IgG saporin into the BF causes a complete loss of cholinergic projections to the neocortex but causes restricted fiber lesions when injected into V1. The injection of 192-IgG saporin into the BF but not V1 affects performance in the sustained attention task (McGaughy and Sarter, 1998, 1999). In addition, compared to controls and ex-smokers, human smokers have been shown to exhibit increased subcortical activity during an attentional task (Nestor et al., 2011). These data indicate that attentional dysfunction following cholinergic lesions might be due to the disruption of detection processes that are independent of V1. However, there is no direct evidence of cholinergic enhancement effect in bottom-up attention in human studies (Rokem et al., 2010). In contrast, there is a growing body of evidence showing that ACh is involved in top-down attention. Direct effects of ACh on attention in the visual cortex have been measured (Herrero et al., 2008; Bauer et al., 2012). Specifically, Herrero et al. provided direct evidence that ACh in V1 enhances the cortical response to an attentional demand (Herrero et al., 2008). It has also been shown that lesions to the cholinergic system impair attention performance and increase neuronal activity in the PFC upon the presentation of distractors (which trigger top-down attention) (Gill et al., 2000). Taken together, these results indicate that ACh can facilitate task-relevant learning in V1 by promoting attentional states in both top-down and bottom-up manners.

CHOLINERGIC MODULATION OF RESPONSE GAIN

Response gain modulation by ACh has frequently been observed (Disney et al., 2007; Aggelopoulos et al., 2011; Bhattacharyya et al., 2013; Soma et al., 2013a) and follows the gain control model at least in terms of the contrast-response function. Increasing thalamocortical pathway input in a context-independent manner while context-dependent intracortical suppression occurs might facilitate the transmission of information related to novel stimuli. In V1, context-dependent (i.e., increases in the maximal response) and independent (i.e., increases in the baseline response) gain control due to cholinergic effects have both been observed (80% and 20%, respectively) without any laminar bias (Soma et al., 2013b). These findings could be related to the optimization of the gain of supragranular pyramidal cells controlled by ACh which could result in the detection of novel stimuli and hence perceptual learning (Moran et al., 2013). Interestingly, gain modulation was proposed as function that underlies of attentional control (Keitel et al., 2013) and network connectivity (Haider and McCormick, 2009). The high gain that results from the amplification of the responses of excited neurons is similar to attention processes (Servan-Schreiber et al., 1990; Eldar et al., 2013) and hence facilitates learning. Taken together, these results suggest that ACh might assist in visual perceptual learning via modulation of cortical responses through gain control in both stimulus-dependent and -independent manners.

CELLULAR EFFECTS OF ACETYLCHOLINE IN V1 IN RELATION TO CORTICAL PLASTICITY

Learning and perceptual learning are sustained by cortical plasticity which triggers anatomical reorganization of the cortical connectivity. The cholinergic system plays also a key role in cortical plasticity. For example, the blockade of cholinergic activation via cholinergic antagonists or cholinergic fiber lesions results in robust impairment of learning in rats (Conner et al., 2003; Dotigny et al., 2008) and ocular dominance plasticity in kittens (Bear and Singer, 1986). In acute preparations, cholinergic pairing is also involved in plasticity as observed in the cat auditory cortex; the application of ACh during acoustic processing alters the receptive fields of single neurons in a tone-specific manner (Metherate and Weinberger, 1990). The pairing of cholinergic and auditory stimulation also leads to the reorganization of the cortical map (Kilgard and Merzenich, 1998); i.e., an enlargement of the representation of the specifically trained frequency. Cholinergic pairing with sensory stimulation also induces long-lasting effects on cortical responsiveness observed in both the visual cortex (Dringenberg et al., 2007; Kang et al., 2014) and the somatosensory cortex (Verdier and Dykes, 2001). Cortical plasticity is essential for the occurrence of perceptual learning (for review see Fahle, 2009), although not systematic, cholinergic-sensory paired activation would thus facilitate the induction of perceptual learning in the sensory cortices (Reed et al., 2011).

CHOLINERGIC MODULATION OF LONG-TERM CORTICAL RESPONSIVENESS

At the neuronal level, ACh has been shown to contribute to cortical plasticity through both the acute and long-term modulation of synaptic responses (Sato et al., 1987; Soma et al., 2012). The impairment of learning by cholinergic antagonists is similar to the effect of blocking cortical plasticity mechanisms and LTP with NMDA receptor (NMDAR) antagonists (Morris et al., 1986; Artola and Singer, 1987; Cooke and Bear, 2010). In most situations, LTP in the visual cortex induced by high theta-burst stimulation (100 Hz) (Heynen and Bear, 2001; Dringenberg et al., 2007) has been found to be NMDAR-dependent. Interestingly, cholinergic system-induced cortical plasticity has also been found to be NMDAR-dependent (Verdier and Dykes, 2001; Dringenberg et al., 2007; Kang and Vaucher, 2009) but independent of theta-burst stimulation (Kirkwood et al., 1999; **Figure 1B**). Previous studies in hippocampal slices have shown that NMDAR opening during LTP induction is facilitated by mAChR activation (Buchanan et al., 2010) and administration of ACh to pyramidal neurons (Shinoe et al., 2005). Additionally, NMDAR-dependent long-term facilitation of synaptic responses is associated with ACh release in V1, and LTP is impaired in the visual cortices of mAChR knock-out mice (Origlia et al., 2006).

CHOLINERGIC MODULATION OF THE EXCITATION-INHIBITION BALANCE

Another contribution of the cholinergic system to cortical plasticity mechanisms in V1 is the alteration of the excitatory and inhibitory (E-I) balance (**Figure 1C**). The excitatory and inhibitory synaptic inputs tend to equilibrate during maturation to optimally tune the neurons according to sensory experiences

(Hensch et al., 1998; Sun et al., 2010) during the critical period; i.e., the post-natal time window during which mammals visual cortices are highly plastic that terminates with the maturation of the neurons. It has been proposed that disrupting the E-I balance can re-open the critical period after maturation (Hensch, 2004). Neuromodulation can also disrupt the E-I balance and contribute to cortical plasticity. Recent studies have also demonstrated numerous examples of cortical plasticity that are modified by the inhibitory system (Hensch, 2005). The onset of the critical period is accelerated by GABAA inhibitory receptor activation (Fagiolini and Hensch, 2000; Iwai et al., 2003). Conversely, it is also possible to re-induce plasticity after the critical period by reducing the inhibitory drive via the injection of GABAA receptor antagonists (Harauzov et al., 2010). As the inhibitory system is strongly modulated by the cholinergic system through the protein Lynx1 (Takesian and Hensch, 2013), which acts as a brake on nAChR-dependent plasticity (Morishita et al., 2010), by nAChRs (Christophe et al., 2002; Arroyo et al., 2012), or by mAChRs (Salgado et al., 2007), cholinergic activation might modulate the E-I balance and facilitate cortical plasticity in adults that would promote perceptual learning. An interaction between the cholinergic and GABAergic systems has been shown to occur following BF stimulation that increases the activation of Parvalbumin-positive (PV+) neurons through mAChRs (Dotigny et al., 2008; Alitto and Dan, 2012). Interestingly, Alitto and Dan used an optogenetic method to show that the nAChRs on vasoactive intestinal peptide-positive (VIP+) neurons and layer I neurons can inhibit excitatory and PV+ neurons (Christophe et al., 2002).

The cholinergic modulation of V1 thus promotes cortical plasticity through LTP-like long-term enhancement of synaptic responses to subsequent presentations of a visual stimulus and through control of the excitatory-inhibitory balance that regulate the strength of cortical output and internal connectivity. The cortical plasticity induced by cholinergic stimulation could transfer the acute cholinergic effect into long-term scale to produce visual precision.

REPETITIVE CHOLINERGIC STIMULATION TRIGGERS PERCEPTUAL LEARNING

In summary, acute effects of cholinergic activation might amplify the thalamocortical response that promotes the transmission of sensory inputs. Intensive release of ACh might also inhibit intracortical interactions and relieve the internal brake on processing in the sensory cortices. Simultaneously, neurons with similar tuning characteristics (e.g., orientation) are co-activated via lateral connections to enhance the transfer of visual information. This cholinergic alternation might contribute to gain control modulation in both stimulus-dependent or and -independent manners and prioritize the processing of selected visual stimuli; this process might be linked to attention and is the first step of perceptual learning. The cholinergic activation also induces the NMDAR-dependent LTP-like long-term enhancement (i.e., cortical plasticity) and relief of the brakes on plasticity by altering the E-I balance. The repetitive coupling of visual and cholinergic stimulation results in reinforcement of all of these acute mechanisms and generate gamma-band synchronization. This would result in the consolidation of the synaptic strengths of new and existing neuronal connections, facilitation of the processing of certain thalamocortical inputs while suppressing others. It has been shown that increases in the cortical responses by expanding the number of neurons to a stimulation (via increases in the strength of the connections) would improve perceptual capacity (Anton-Erxleben and Carrasco, 2013). The repetitive cholinergic-visual stimulation would also increase the efficiency and automaticity of these selected pathways. These processes contribute to perceptual learning.

REPETITIVE CHOLINERGIC STIMULATION PROMOTES LONG-TERM POTENTIATION

As mentioned above, ACh can induce NMDAR-dependent long-term modifications of postsynaptic glutamatergic neurons which are related to memory formation. The opening of the NMDAR launches a second messenger cascade and guides the expression of synaptic glutamate receptors (Regehr and Tank, 1990; Zhong et al., 2006) but also activates autoregulated kinases that confer a persistent improved response of the neuron to the stimulus. Immunohistochemistry for the c-Fos, which is an immediate early gene and also a transcription factor for synaptogenesis genes, has revealed that c-Fos is increased in layer II/III pyramidal neurons following a repetitive BF/visual stimulation (Kang et al., 2014), which may be indicative of the formation of new synapses and LTP mechanisms. Repetitive pairing of the cholinergic and visual stimulation also induces morphological reorganization, i.e., increase in the numbers of cholinergic varicosities in the proximity of the neurons that are sensitive to the orientation of the stimulus (Zhang et al., 2011). This increased number of cholinergic inputs, along with postsynaptic mechanisms, would increase and consolidate the response of the activated neurons to ameliorate its long-term efficiency. Thus repetitive cholinergic stimulation might enhance the encoding of the memory and morphological modifications.

REPETITIVE CHOLINERGIC STIMULATION PROMOTES STIMULUS SELECTION AND AMPLIFICATION

We suggest that selection of decisive inputs is controlled by the cholinergic system and contributes to the specific enhancement of a particular stimulus in perceptual learning. Modulation of the orientation selectivity of the neurons provides a great example of the possible improvement of perceptual sensitivity. Training of the rat to a preferred or a non-preferred orientation might increase the cortical response for this orientation (Cooke and Bear, 2010; **Figure 4**). These mechanisms are facilitated by repetitive cholinergic activation, which improve orientation discrimination of human or rats (Rokem and Silver, 2010; Kang et al., 2014). Repetitive cholinergic stimulation coupled with a certain orientation stimulus might favor the discrimination of this stimulus by two different cellular mechanisms (**Figure 4**). First, ACh can harmonize the activation of the whole dendritic tree of layer II/III neurons to preserve their orientation selectivity and confer responsiveness to new orientation—the dendrites of the layer II/III neurons receive inputs randomly over all of their branches, some of which are selective for the neurons'

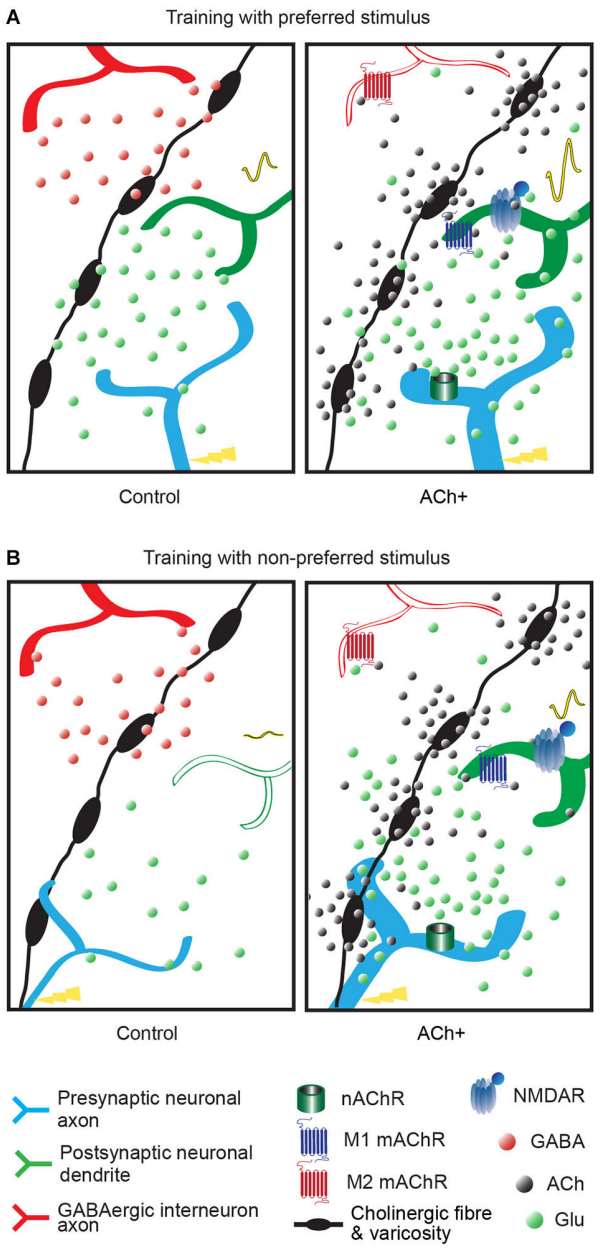

FIGURE 4 | Summary of the effect of acetylcholine on neuronal transmission of the visual inputs. The varicose cholinergic fiber (black fiber with swellings) can act on excitatory input (blue axon), neighboring GABAergic inhibitory input (red axon) and on V1 neurons (green dendrite). Excitatory/inhibitory influences are represented by red and green dots, respectively. Cholinergic activation (ACh+, right panel) is represented by black dots. The cortical response to the stimulus is represented by a VEP signal waveform which changes are elicited by increased numbers of neurons responding to the trained stimulus or increased neurons efficiency. **(A)** Response of the V1 neuron after a training with preferred stimulus coupled to cholinergic activation (right panel, ACh+) or without (left panel, control). The cortical response to this stimulus is increased (high VEP signal waveform in right panel compared to small VEP signal waveform in left panel). In presence of cholinergic activation the inhibitory influence is reduced by M2 muscarinic receptors (mAChRs), the postsynaptic excitatory influence is increased by M1 mAChRs located on the postsynaptic neuron

(*Continued*)

FIGURE 4 | Continued
and nAChRs located on the thalamocortical fiber and a long-term effect is triggered by NMDA receptor activation, compared to normal condition (control, left panel). In a normal visual process (control) local or recurrent inhibition via GABAergic interneuron (in red) blocks the development to a long-term modification. **(B)** Response of the V1 neuron after a training with non-preferred stimulus coupled to cholinergic activation (right panel, ACh+) or without (left panel, control). The neuronal response to this stimulus is increased (small VEP signal waveform in right panel compared to flat VEP signal waveform in left panel). In normal condition (control, left panel), non-preferred orientation stimulus does not evoke activation in postsynaptic neurons in V1. Weak thalamocortical innervation is suppressed by GABAergic inhibition and hence fails to transmit to postsynaptic neuron. Acetylcholine can amplify the weak presynaptic input (ACh+) by nicotinic receptors and activates postsynaptic neuron through M1 muscarinic receptor. GABAergic inhibition is suppressed by M2 muscarinic receptor and NMDA receptor opening occurs leading to long-term modification.

un-preferred orientations (Jia et al., 2010). Second, the cholinergic system can enhance orientation discrimination through its interaction with the GABAergic system which assists in the sharpening (Isaacson and Scanziani, 2011) of the convergent input in the layer II/III neurons (Nassi and Callaway, 2009) but also filters out task-relevant information during perceptual learning (Roberts and Thiele, 2008). PV+ and somatostatin-positive (SOM+) GABAergic neurons are particularly involved in orientation tuning in V1 (Atallah et al., 2012; Wilson et al., 2012). It has been shown that the specific activation of PV+ neurons in V1 improves orientation discrimination abilities in awake rats during perceptual learning (Lee et al., 2012) and repetitive coupling of ACh to visual stimulation activates the V1 GABAergic neurons (Dotigny et al., 2008; Kang et al., 2014).

Thus repetitive cholinergic pairing to sensory training enhances the cortical response to trained feature of the sensory stimulus that increases the influence of the feedforward afferent.

REPETITIVE CHOLINERGIC STIMULATION PROMOTES PERCEPTUAL LEARNING RELATED TO ATTENTION, REWARD EXPECTATION AND CONNECTIVITY

Repetitive cholinergic stimulation first promotes attentional mechanisms that are necessary to perceptual learning (Ahissar and Hochstein, 1993; Schoups et al., 2001; Li et al., 2004; Mukai et al., 2007). These attentional processes might be also related to synchronization in the gamma band (30–90 Hz) (Fries et al., 2008) induced by repetitive cholinergic stimulation which has been proposed to facilitate the transfer of the visual information to higher visual areas. ACh can also promote task-irrelevant perceptual learning that occurs in the absence of conscious effort (Skrandies and Fahle, 1994; Watanabe et al., 2002; Gutnisky et al., 2009). Compared to task-relevant learning, which utilizes focused attention as reinforcement, studies of task-irrelevant learning have suggested that reward serves as the reinforcement signal (Seitz et al., 2009; Chubykin et al., 2013). During task-irrelevant learning, the response to a feature on which attention was not directed can also be enhanced (Watanabe et al., 2001; Giordano et al., 2009; Gutnisky et al., 2009). Interestingly, rewards can affect the visual response in V1 (Shuler and Bear, 2006), and

the cholinergic system can influence reward timing expectancy (Chubykin et al., 2013). To reconcile studies showing a role of attention in perceptual learning or not, Roelfsema proposed that the attentional feedback signal related to the cholinergic system that enhances the plasticity of task-relevant features in the visual cortex also causes the inhibition of task-irrelevant features so that their plasticity is switched off (Roelfsema et al., 2010).

To a cognitive point of view, by modulating synaptic transmission in V1 and modifying the cortical dynamics, ACh can also participates in the perceptual inference to increase the strength of the representation of trained stimuli and reduce the sensory noise (Yu and Dayan, 2002) and induce sensory precision (Moran et al., 2013). It might suppress the top-down sources in the balance between top-down and bottom-up information integration in V1 (Yu and Dayan, 2005). This is in agreement with a recent study demonstrating that the cholinergic enhancement reduces the connectivity strength between cortical regions involved in attention and V1 (Ricciardi et al., 2013) and reduce the activity in frontoparietal regions (Furey et al., 2008). This suggests an increased neural efficiency in the processing of the trained stimulus that leads to an improved perceptual task performance (Ricciardi et al., 2013) linked to an automation of the cortical processing and a reduction of the attentional load required to process the trained stimulus (Furey, 2011).

Together, the findings from recent work using different techniques suggests that cholinergic pairing induces perceptual learning via different mechanisms that include the following: (1) the use of the layer II/III GABAergic system to filter the pre-amplified response from layer IV; (2) NMDAR-dependent modification at the postsynaptic level to induce long-term augmentations of individual neurons, and an increase in the numbers of cholinergic varicosities to facilitate ACh release; and (3) changes in the efficiency of the connectivity between cortical areas and bottom-up and top-down control.

CLINICAL PERSPECTIVES OF CHOLINERGIC MODULATION OF BRAIN'S FUNCTION

Similar with experimental data, some clinical studies have demonstrated that enhancing cholinergic system improves perception (Furey et al., 2000; Bentley et al., 2004; Wilson et al., 2004; Rokem and Silver, 2010; Beer et al., 2013; Ricciardi et al., 2013). Clinically, a method to enhance cholinergic function might involve the use of ACh esterase inhibitors, such as physostigmine, galantamine, rivastigmine or donepezil. Nicotine is also a well-known molecule that enhances cognitive function. These drugs are currently used to the treatment of Alzheimer's disease or diverse dementia. Orally administered nicotine or smoking improve attentional performance (Nestor et al., 2011; Newhouse et al., 2011), learning (Riekkinen and Riekkinen, 1997; Olausson et al., 2004), attention (Thiel et al., 2005; Nestor et al., 2011) and memory consolidation (Beer et al., 2013) through the activation of nAChRs. Increases in ACh action due to the administration of acetylcholinesterase inhibitors or direct mAChRs agonists alleviate cognitive deficits in Alzheimer's disease (Cummings, 2003), Parkinson's disease (Fagerström et al., 1994; Holmes et al., 2011) and schizophrenia patients (Shekhar et al., 2008). An α7 nAChR agonist is also used as a cognitive enhancer in patients with schizophrenia (Freedman, 2013) and Alzheimer's disease (Hilt et al., 2009). As shown in an fMRI study, cholinergic action potentiates communication efficiency between cortical areas (Wylie et al., 2012). The use of these drugs in cholinergically healthy subjects might also be beneficial for enhancing cognitive function (Buchanan et al., 2008; Demeter and Sarter, 2013).

Some pharmacological approaches have been developed to increase the perceptual learning in healthy humans. Performance improvements following the use of donepezil during a motion direction discrimination task have confirmed that systemic blockade of ACh esterase can induce perceptual learning (Rokem and Silver, 2010, 2013). Cholinergic amplifications paired with sensory stimulations might also be a promising approach to accelerating visual recovery following lesions to the retina or the optical nerve. If the neuronal mechanisms that occur during perceptual learning and after retinal lesions are similar (Gilbert and Li, 2012) (i.e., they both involve changes in the responsiveness of cortical neurons to inputs from outside the neurons' preferred receptive fields (Darian-Smith and Gilbert, 1994)), then ACh might also aid to boost structural and functional plasticity of the visual cortex to recover from losses of retinal input.

CONCLUSION

In this review, we proposed that the neuromodulator ACh, which is known for its involvement in attention and learning, might participate in and promote perceptual learning. We proposed that, via the inhibition of intracortical feedback, ACh can render V1 more sensitive to incoming thalamocortical information and enhance sensory performance. During visual processing, ACh acts on different layers to amplify the encoding of weak stimuli by strengthening synaptic connectivity, which leads to behavioral improvements. Furthermore, ACh might not only facilitate task-relevant perceptual learning via attention but also facilitate task-irrelevant learning via reward reinforcement. However, much remains to be uncovered regarding whether the cholinergic system has the potential to be used as a key mechanism for improving the function of the brain and speeding rehabilitation. Specifically, because perceptual learning occurs easily under conditions of attentional control, the development of a method to improve one's brain capacity through improved attention and cholinergic stimulation is very attractive.

ACKNOWLEDGMENTS

Authors acknowledge the financial support of the Canadian Institutes of Health Research (CIHR, MOP-111003), the Natural Sciences and Engineering Research council of Canada (NSERC, 238835–2011), the FRQS Vision Research Network and the Ecole d'optométrie, Université de Montréal. Authors are thankful to Micheline P. Gloin for help with the artwork.

REFERENCES

Aggelopoulos, N. C., Liebe, S., Logothetis, N. K., and Rainer, G. (2011). Cholinergic control of visual categorization in macaques. *Front. Behav. Neurosci.* 5:73. doi: 10.3389/fnbeh.2011.00073

Ahissar, M., and Hochstein, S. (1993). Attentional control of early perceptual learning. *Proc. Natl. Acad. Sci. U S A* 90, 5718–5722. doi: 10.1073/pnas.90.12.5718

Alitto, H. J., and Dan, Y. (2012). Cell-type-specific modulation of neocortical activity by basal forebrain input. *Front. Syst. Neurosci.* 6:79. doi: 10.3389/fnsys.2012.00079

Amar, M., Lucas-Meunier, E., Baux, G., and Fossier, P. (2010). Blockade of different muscarinic receptor subtypes changes the equilibrium between excitation and inhibition in rat visual cortex. *Neuroscience* 169, 1610–1620. doi: 10.1016/j.neuroscience.2010.06.019

Ansorge, U., Horstmann, G., and Scharlau, I. (2010). Top-down contingent attentional capture during feed-forward visual processing. *Acta Psychol. (Amst)* 135, 123–126. doi: 10.1016/j.actpsy.2010.05.008

Anton-Erxleben, K., and Carrasco, M. (2013). Attentional enhancement of spatial resolution: linking behavioural and neurophysiological evidence. *Nat. Rev. Neurosci.* 14, 188–200. doi: 10.1038/nrn3443

Arroyo, S., Bennett, C., Aziz, D., Brown, S. P., and Hestrin, S. (2012). Prolonged disynaptic inhibition in the cortex mediated by slow, non-α7 nicotinic excitation of a specific subset of cortical interneurons. *J. Neurosci.* 32, 3859–3864. doi: 10.1523/JNEUROSCI.0115-12.2012

Artola, A., and Singer, W. (1987). Long-term potentiation and NMDA receptors in rat visual cortex. *Nature* 330, 649–652. doi: 10.1038/330649a0

Atallah, B. V., Bruns, W., Carandini, M., and Scanziani, M. (2012). Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. *Neuron* 73, 159–170. doi: 10.1016/j.neuron.2011.12.013

Avendaño, C., Umbriaco, D., Dykes, R. W., and Descarries, L. (1996). Acetylcholine innervation of sensory and motor neocortical areas in adult cat: a choline acetyltransferase immunohistochemical study. *J. Chem. Neuroanat.* 11, 113–130. doi: 10.1016/0891-0618(96)00132-9

Bakin, J. S., and Weinberger, N. M. (1996). Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proc. Natl. Acad. Sci. U S A* 93, 11219–11224. doi: 10.1073/pnas.93.20.11219

Bauer, M., Kluge, C., Bach, D., Bradbury, D., Heinze, H. J., Dolan, R. J., et al. (2012). Cholinergic enhancement of visual attention and neural oscillations in the human brain. *Curr. Biol.* 22, 397–402. doi: 10.1016/j.cub.2012.01.022

Bear, M. F., and Singer, W. (1986). Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature* 320, 172–176. doi: 10.1038/320172a0

Beer, A. L., Vartak, D., and Greenlee, M. W. (2013). Nicotine facilitates memory consolidation in perceptual learning. *Neuropharmacology* 64, 443–451. doi: 10.1016/j.neuropharm.2012.06.019

Bentley, P., Driver, J., and Dolan, R. J. (2011). Cholinergic modulation of cognition: insights from human pharmacological functional neuroimaging. *Prog. Neurobiol.* 94, 360–388. doi: 10.1016/j.pneurobio.2011.06.002

Bentley, P., Husain, M., and Dolan, R. J. (2004). Effects of cholinergic enhancement on visual stimulation, spatial attention and spatial working memory. *Neuron* 41, 969–982. doi: 10.1016/s0896-6273(04)00145-x

Bhattacharyya, A., Veit, J., Kretz, R., Bondar, I., and Rainer, G. (2013). Basal forebrain activation controls contrast sensitivity in primary visual cortex. *BMC Neurosci.* 14:55. doi: 10.1186/1471-2202-14-55

Buchanan, R. W., Conley, R. R., Dickinson, D., Ball, M. P., Feldman, S., Gold, J. M., et al. (2008). Galantamine for the treatment of cognitive impairments in people with schizophrenia. *Am. J. Psychiatry* 165, 82–89. doi: 10.1176/appi.ajp.2007.07050724

Buchanan, K. A., Petrovic, M. M., Chamberlain, S. E., Marrion, N. V., and Mellor, J. R. (2010). Facilitation of long-term potentiation by muscarinic M(1) receptors is mediated by inhibition of SK channels. *Neuron* 68, 948–963. doi: 10.1016/j.neuron.2010.11.018

Caulfield, M. P., and Birdsall, N. J. (1998). International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50, 279–290.

Chédotal, A., Cozzari, C., Faure, M. P., Hartman, B. K., and Hamel, E. (1994). Distinct choline acetyltransferase (ChAT) and vasoactive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. *Brain Res.* 646, 181–193. doi: 10.1016/0006-8993(94)90076-0

Christophe, E., Roebuck, A., Staiger, J. F., Lavery, D. J., Charpak, S., and Audinat, E. (2002). Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. *J. Neurophysiol.* 88, 1318–1327. doi: 10.1152/jn.00199.2002

Chubykin, A. A., Roach, E. B., Bear, M. F., and Shuler, M. G. (2013). A cholinergic mechanism for reward timing within primary visual cortex. *Neuron* 77, 723–735. doi: 10.1016/j.neuron.2012.12.039

Conner, J. M., Culberson, A., Packowski, C., Chiba, A. A., and Tuszynski, M. H. (2003). Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. *Neuron* 38, 819–829. doi: 10.1016/s0896-6273(03)00288-5

Cooke, S. F., and Bear, M. F. (2010). Visual experience induces long-term potentiation in the primary visual cortex. *J. Neurosci.* 30, 16304–16313. doi: 10.1523/JNEUROSCI.4333-10.2010

Cudeiro, J., and Sillito, A. M. (2006). Looking back: corticothalamic feedback and early visual processing. *Trends Neurosci.* 29, 298–306. doi: 10.1016/j.tins.2006.05.002

Cummings, J. L. (2003). Use of cholinesterase inhibitors in clinical practice: evidence-based recommendations. *Am. J. Geriatr. Psychiatry* 11, 131–145. doi: 10.1097/00019442-200303000-00004

Darian-Smith, C., and Gilbert, C. D. (1994). Axonal sprouting accompanies functional reorganization in adult cat striate cortex. *Nature* 368, 737–740. doi: 10.1038/368737a0

Dauphin, F., Lacombe, P., Sercombe, R., Hamel, E., and Seylaz, J. (1991). Hypercapnia and stimulation of the substantia innominata increase rat frontal cortical blood flow by different cholinergic mechanisms. *Brain Res.* 553, 75–83. doi: 10.1016/0006-8993(91)90232-k

Deco, G., and Thiele, A. (2011). Cholinergic control of cortical network interactions enables feedback-mediated attentional modulation. *Eur. J. Neurosci.* 34, 146–157. doi: 10.1111/j.1460-9568.2011.07749.x

Demeter, E., and Sarter, M. (2013). Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology* 64, 294–304. doi: 10.1016/j.neuropharm.2012.06.060

De Pasquale, R., and Sherman, S. M. (2012). Modulatory effects of metabotropic glutamate receptors on local cortical circuits. *J. Neurosci.* 32, 7364–7372. doi: 10.1523/JNEUROSCI.0090-12.2012

Descarries, L., Gisiger, V., and Steriade, M. (1997). Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* 53, 603–625. doi: 10.1016/s0301-0082(97)00050-6

Disney, A. A., Aoki, C., and Hawken, M. J. (2007). Gain modulation by nicotine in macaque v1. *Neuron* 56, 701–713. doi: 10.1016/j.neuron.2007.09.034

Disney, A. A., Aoki, C., and Hawken, M. J. (2012). Cholinergic suppression of visual responses in primate V1 is mediated by GABAergic inhibition. *J. Neurophysiol.* 108, 1907–1923. doi: 10.1152/jn.00188.2012

Disney, A. A., Domakonda, K. V., and Aoki, C. (2006). Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas V1 and V2 of the macaque monkey. *J. Comp. Neurol.* 499, 49–63. doi: 10.1002/cne.21096

Dotigny, F., Ben Amor, A. Y., Burke, M., and Vaucher, E. (2008). Neuromodulatory role of acetylcholine in visually-induced cortical activation: behavioral and neuroanatomical correlates. *Neuroscience* 154, 1607–1618. doi: 10.1016/j.neuroscience.2008.04.030

Drevets, W. C., Price, J. L., and Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118. doi: 10.1007/s00429-008-0189-x

Dringenberg, H. C., Hamze, B., Wilson, A., Speechley, W., and Kuo, M. C. (2007). Heterosynaptic facilitation of in vivo thalamocortical long-term potentiation in the adult rat visual cortex by acetylcholine. *Cereb. Cortex* 17, 839–848. doi: 10.1093/cercor/bhk038

Eckenstein, F. P., Baughman, R. W., and Quinn, J. (1988). An anatomical study of cholinergic innervation in rat cerebral cortex. *Neuroscience* 25, 457–474. doi: 10.1016/0306-4522(88)90251-5

Egeth, H. E., Leonard, C. J., and Leber, A. B. (2010). Why salience is not enough: reflections on top-down selection in vision. *Acta Psychol. (Amst)* 135, 130–132; discussion 133–139. doi: 10.1016/j.actpsy.2010.05.012

Eggermann, E., and Feldmeyer, D. (2009). Cholinergic filtering in the recurrent excitatory microcircuit of cortical layer 4. *Proc. Natl. Acad. Sci. U S A* 106, 11753–11758. doi: 10.1073/pnas.0810062106

Eimer, M., and Kiss, M. (2010). The top-down control of visual selection and how it is linked to the N2pc component. *Acta Psychol. (Amst)* 135, 100–102. doi: 10.1016/j.actpsy.2010.04.010

Eldar, E., Cohen, J. D., and Niv, Y. (2013). The effects of neural gain on attention and learning. *Nat. Neurosci.* 16, 1146–1153. doi: 10.1038/nn.3428

Fagerström, K. O., Pomerleau, O., Giordani, B., and Stelson, F. (1994). Nicotine may relieve symptoms of Parkinson's disease. *Psychopharmacology (Berl)* 116, 117–119. doi: 10.1007/bf02244882

Fagiolini, M., and Hensch, T. K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 404, 183–186. doi: 10.1038/35004582

Fahle, M. (2009). Perceptual learning and sensomotor flexibility: cortical plasticity under attentional control? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 313–319. doi: 10.1098/rstb.2008.0267

Fahle, M., and Poggio, T. (2002). *Perceptual Learning.* Cambridge, Mass.: MIT Press.

Fiorentini, A., and Berardi, N. (1980). Perceptual learning specific for orientation and spatial frequency. *Nature* 287, 43–44. doi: 10.1038/287043a0

Freedman, R. (2013). α7-Nicotinic acetylcholine receptor agonists for cognitive enhancement in schizophrenia. *Annu. Rev. Med.* 65, 245–261. doi: 10.1146/annurev-med-092112-142937

Fries, P., Nikolić, D., and Singer, W. (2007). The gamma cycle. *Trends Neurosci.* 30, 309–316. doi: 10.1016/j.tins.2007.05.005

Fries, P., Womelsdorf, T., Oostenveld, R., and Desimone, R. (2008). The effects of visual stimulation and selective visual attention on rhythmic neuronal synchronization in macaque area V4. *J. Neurosci.* 28, 4823–4835. doi: 10.1523/JNEUROSCI.4499-07.2008

Furey, M. L. (2011). The prominent role of stimulus processing: cholinergic function and dysfunction in cognition. *Curr. Opin. Neurol.* 24, 364–370. doi: 10.1097/WCO.0b013e328348bda5

Furey, M. L., Pietrini, P., and Haxby, J. V. (2000). Cholinergic enhancement and increased selectivity of perceptual processing during working memory. *Science* 290, 2315–2319. doi: 10.1126/science.290.5500.2315

Furey, M. L., Ricciardi, E., Schapiro, M. B., Rapoport, S. I., and Pietrini, P. (2008). Cholinergic enhancement eliminates modulation of neural activity by task difficulty in the prefrontal cortex during working memory. *J. Cogn. Neurosci.* 20, 1342–1353. doi: 10.1162/jocn.2008.20092

Gaykema, R. P., Luiten, P. G., Nyakas, C., and Traber, J. (1990). Cortical projection patterns of the medial septum-diagonal band complex. *J. Comp. Neurol.* 293, 103–124. doi: 10.1002/cne.902930109

Giessel, A. J., and Sabatini, B. L. (2010). M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. *Neuron* 68, 936–947. doi: 10.1016/j.neuron.2010.09.004

Gil, Z., Connors, B. W., and Amitai, Y. (1997). Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19, 679–686. doi: 10.1016/s0896-6273(00)80380-3

Gilbert, C. D., and Li, W. (2012). Adult visual cortical plasticity. *Neuron* 75, 250–264. doi: 10.1016/j.neuron.2012.06.030

Gilbert, C. D., Sigman, M., and Crist, R. E. (2001). The neural basis of perceptual learning. *Neuron* 31, 681–697. doi: 10.1016/s0896-6273(01)00424-x

Gilbert, C. D., and Wiesel, T. N. (1989). Columnar specificity of intrinsic horizontal and corticocortical connections in cat visual cortex. *J. Neurosci.* 9, 2432–2442.

Gill, T. M., Sarter, M., and Givens, B. (2000). Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. *J. Neurosci.* 20, 4745–4757.

Giordano, A. M., McElree, B., and Carrasco, M. (2009). On the automaticity and flexibility of covert attention: a speed-accuracy trade-off analysis. *J. Vis.* 9, 30.1–30.10. doi: 10.1167/9.3.30

Goard, M., and Dan, Y. (2009). Basal forebrain activation enhances cortical coding of natural scenes. *Nat. Neurosci.* 12, 1444–1449. doi: 10.1038/nn.2402

Groleau, M., Nguyen, H. N., Vanni, M., Huppé-Gourgues, F., Casanova, C., and Vaucher, E. (2014). Impaired functional organization in the visual cortex of muscarinic receptors knock-out mice. *Neuroimage* 98, 233–242. doi: 10.1016/j.neuroimage.2014.05.016

Gu, Q. (2003). Contribution of acetylcholine to visual cortex plasticity. *Neurobiol. Learn. Mem.* 80, 291–301. doi: 10.1016/s1074-7427(03)00073-x

Gulledge, A. T., Bucci, D. J., Zhang, S. S., Matsui, M., and Yeh, H. H. (2009). M1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. *J. Neurosci.* 29, 9888–9902. doi: 10.1523/JNEUROSCI.1366-09.2009

Gutnisky, D. A., Hansen, B. J., Iliescu, B. F., and Dragoi, V. (2009). Attention alters visual plasticity during exposure-based learning. *Curr. Biol.* 19, 555–560. doi: 10.1016/j.cub.2009.01.063

Haider, B., and McCormick, D. A. (2009). Rapid neocortical dynamics: cellular and network mechanisms. *Neuron* 62, 171–189. doi: 10.1016/j.neuron.2009.04.008

Harauzov, A., Spolidoro, M., DiCristo, G., De Pasquale, R., Cancedda, L., Pizzorusso, T., et al. (2010). Reducing intracortical inhibition in the adult visual cortex promotes ocular dominance plasticity. *J. Neurosci.* 30, 361–371. doi: 10.1523/JNEUROSCI.2233-09.2010

Hashimoto, T., Shu, H., and Kuriyama, K. (1994). Muscarinic M1 receptor mediated inhibition of GABA release from rat cerebral cortex. *Neurochem. Int.* 24, 389–394. doi: 10.1016/0197-0186(94)90117-1

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Curr. Opin. Neurobiol.* 16, 710–715. doi: 10.1016/j.conb.2006.09.002

Hensch, T. K. (2004). Critical period regulation. *Annu. Rev. Neurosci.* 27, 549–579. doi: 10.1146/annurev.neuro.27.070203.144327

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nat. Rev. Neurosci.* 6, 877–888. doi: 10.1038/nrn1787

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S., and Kash, S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282, 1504–1508. doi: 10.1126/science.282.5393.1504

Herrero, J. L., Roberts, M. J., Delicato, L. S., Gieselmann, M. A., Dayan, P., and Thiele, A. (2008). Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454, 1110–1114. doi: 10.1038/nature07141

Heynen, A. J., and Bear, M. F. (2001). Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo. *J. Neurosci.* 21, 9801–9813.

Hilt, D., Gawryl, M., and Koenig, G. (2009). Safety, tolerability and cognitive effects of a novel α7 nicotinic receptor agonist in Alzheimer's disease patients on stable donepezil or rivastigmine therapy. *Alzheimers Dement.* 5:e32. doi: 10.1016/j.jalz.2009.07.147

Holmes, A. D., Copland, D. A., Silburn, P. A., and Chenery, H. J. (2011). Acute nicotine enhances strategy-based semantic processing in Parkinson's disease. *Int. J. Neuropsychopharmacol.* 14, 877–885. doi: 10.1017/S1461145710001665

Hua, T., Bao, P., Huang, C. B., Wang, Z., Xu, J., Zhou, Y., et al. (2010). Perceptual learning improves contrast sensitivity of V1 neurons in cats. *Curr. Biol.* 20, 887–894. doi: 10.1016/j.cub.2010.03.066

Isaacson, J. S., and Scanziani, M. (2011). How inhibition shapes cortical activity. *Neuron* 72, 231–243. doi: 10.1016/j.neuron.2011.09.027

Iwai, Y., Fagiolini, M., Obata, K., and Hensch, T. K. (2003). Rapid critical period induction by tonic inhibition in visual cortex. *J. Neurosci.* 23, 6695–6702.

Ji, D., and Dani, J. A. (2000). Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J. Neurophysiol.* 83, 2682–2690.

Jia, H., Rochefort, N. L., Chen, X., and Konnerth, A. (2010). Dendritic organization of sensory input to cortical neurons in vivo. *Nature* 464, 1307–1312. doi: 10.1038/nature08947

Kang, J. I., Groleau, M., Dotigny, F., Giguère, H., and Vaucher, E. (2014). Visual training paired with electrical stimulation of the basal forebrain improves orientation-selective visual acuity in the rat. *Brain Struct. Funct.* 219, 1493–1507. doi: 10.1007/s00429-013-0582-y

Kang, J. I., and Vaucher, E. (2009). Cholinergic pairing with visual activation results in long-term enhancement of visual evoked potentials. *PLoS One* 4:e5995. doi: 10.1371/journal.pone.0005995

Keitel, C., Andersen, S. K., Quigley, C., and Müller, M. M. (2013). Independent effects of attentional gain control and competitive interactions on visual stimulus processing. *Cereb. Cortex* 23, 940–946. doi: 10.1093/cercor/bhs084

Kilgard, M. P., and Merzenich, M. M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science* 279, 1714–1718. doi: 10.1126/science.279.5357.1714

Kimura, F., and Baughman, R. W. (1997). Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. *J. Neurophysiol.* 77, 709–716.

Kimura, F., Fukuda, M., and Tsumoto, T. (1999). Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. *Eur. J. Neurosci.* 11, 3597–3609. doi: 10.1046/j.1460-9568.1999.00779.x

Kirkwood, A., Rozas, C., Kirkwood, J., Perez, F., and Bear, M. F. (1999). Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. *J. Neurosci.* 19, 1599–1609.

Kocharyan, A., Fernandes, P., Tong, X. K., Vaucher, E., and Hamel, E. (2008). Specific subtypes of cortical GABA interneurons contribute to the neurovascular coupling response to basal forebrain stimulation. *J. Cereb. Blood Flow Metab.* 28, 221–231. doi: 10.1038/sj.jcbfm.9600558

Kosovicheva, A. A., Sheremata, S. L., Rokem, A., Landau, A. N., and Silver, M. A. (2012). Cholinergic enhancement reduces orientation-specific surround suppression but not visual crowding. *Front. Behav. Neurosci.* 6:61. doi: 10.3389/fnbeh.2012.00061

Laplante, F., Morin, Y., Quirion, R., and Vaucher, E. (2005). Acetylcholine release is elicited in the visual cortex, but not in the prefrontal cortex, by patterned visual stimulation: a dual in vivo microdialysis study with functional correlates in the rat brain. *Neuroscience* 132, 501–510. doi: 10.1016/j.neuroscience.2004.11.059

Lee, S. H., Kwan, A. C., Zhang, S., Phoumthipphavong, V., Flannery, J. G., Masmanidis, S. C., et al. (2012). Activation of specific interneurons improves V1 feature selectivity and visual perception. *Nature* 488, 379–383. doi: 10.1038/nature11312

Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., and Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* 11, 3218–3226.

Li, Y. T., Ibrahim, L. A., Liu, B. H., Zhang, L. I., and Tao, H. W. (2013). Linear transformation of thalamocortical input by intracortical excitation. *Nat. Neurosci.* 16, 1324–1330. doi: 10.1038/nn.3494

Li, W., Piëch, V., and Gilbert, C. D. (2004). Perceptual learning and top-down influences in primary visual cortex. *Nat. Neurosci.* 7, 651–657. doi: 10.1038/nn1255

Lien, A. D., and Scanziani, M. (2013). Tuned thalamic excitation is amplified by visual cortical circuits. *Nat. Neurosci.* 16, 1315–1323. doi: 10.1038/nn.3488

Lucas-Meunier, E., Monier, C., Amar, M., Baux, G., Frégnac, Y., and Fossier, P. (2009). Involvement of nicotinic and muscarinic receptors in the endogenous cholinergic modulation of the balance between excitation and inhibition in the young rat visual cortex. *Cereb. Cortex* 19, 2411–2427. doi: 10.1093/cercor/bhn258

Luiten, P. G., Gaykema, R. P., Traber, J., and Spencer, D. G. Jr. (1987). Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported phaseolus vulgaris leucoagglutinin. *Brain Res.* 413, 229–250. doi: 10.1016/0006-8993(87)91014-6

Lysakowski, A., Wainer, B. H., Bruce, G., and Hersh, L. B. (1989). An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. *Neuroscience* 28, 291–336. doi: 10.1016/0306-4522(89)90180-2

Ma, X., and Suga, N. (2005). Long-term cortical plasticity evoked by electric stimulation and acetylcholine applied to the auditory cortex. *Proc. Natl. Acad. Sci. U S A* 102, 9335–9340. doi: 10.1073/pnas.0503851102

Mayer, M. J. (1983). Practice improves adults' sensitivity to diagonals. *Vision Res.* 23, 547–550. doi: 10.1016/0042-6989(83)90130-x

McGaughy, J., and Sarter, M. (1998). Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. *Behav. Neurosci.* 112, 1519–1525. doi: 10.1037//0735-7044.112.6.1519

McGaughy, J., and Sarter, M. (1999). Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation and administration of estradiol on sustained attention performance in rats. *Behav. Neurosci.* 113, 1216–1232. doi: 10.1037/0735-7044.113.6.1216

McGuire, B. A., Gilbert, C. D., Rivlin, P. K., and Wiesel, T. N. (1991). Targets of horizontal connections in macaque primary visual cortex. *J. Comp. Neurol.* 305, 370–392. doi: 10.1002/cne.903050303

McKee, S. P., and Westheimer, G. (1978). Improvement in vernier acuity with practice. *Percept. Psychophys.* 24, 258–262. doi: 10.3758/bf03206097

Mechawar, N., Cozzari, C., and Descarries, L. (2000). Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. *J. Comp. Neurol.* 428, 305–318. doi: 10.1002/1096-9861(20001211)428:2<305::aid-cne9>3.0.co;2-y

Metherate, R. (2011). Functional connectivity and cholinergic modulation in auditory cortex. *Neurosci. Biobehav. Rev.* 35, 2058–2063. doi: 10.1016/j.neubiorev.2010.11.010

Metherate, R., Kaur, S., Kawai, H., Lazar, R., Liang, K., and Rose, H. J. (2005). Spectral integration in auditory cortex: mechanisms and modulation. *Hear. Res.* 206, 146–158. doi: 10.1016/j.heares.2005.01.014

Metherate, R., and Weinberger, N. M. (1990). Cholinergic modulation of responses to single tones produces tone-specific receptive field alterations in cat auditory cortex. *Synapse* 6, 133–145. doi: 10.1002/syn.890060204

Moran, R. J., Campo, P., Symmonds, M., Stephan, K. E., Dolan, R. J., and Friston, K. J. (2013). Free energy, precision and learning: the role of cholinergic neuromodulation. *J. Neurosci.* 33, 8227–8236. doi: 10.1523/jneurosci.4255-12.2013

Morishita, H., Miwa, J. M., Heintz, N., and Hensch, T. K. (2010). Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* 330, 1238–1240. doi: 10.1126/science.1195320

Morris, R. G., Anderson, E., Lynch, G. S., and Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319, 774–776. doi: 10.1038/319774a0

Mukai, I., Kim, D., Fukunaga, M., Japee, S., Marrett, S., and Ungerleider, L. G. (2007). Activations in visual and attention-related areas predict and correlate with the degree of perceptual learning. *J. Neurosci.* 27, 11401–11411. doi: 10.1523/jneurosci.3002-07.2007

Nassi, J. J., and Callaway, E. M. (2009). Parallel processing strategies of the primate visual system. *Nat. Rev. Neurosci.* 10, 360–372. doi: 10.1038/nrn2619

Nestor, L., Mccabe, E., Jones, J., Clancy, L., and Garavan, H. (2011). Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage* 56, 2258–2275. doi: 10.1016/j.neuroimage.2011.03.054

Newhouse, P. A., Potter, A. S., Dumas, J. A., and Thiel, C. M. (2011). Functional brain imaging of nicotinic effects on higher cognitive processes. *Biochem. Pharmacol.* 82, 943–951. doi: 10.1016/j.bcp.2011.06.008

Olausson, P., Jentsch, J. D., and Taylor, J. R. (2004). Nicotine enhances responding with conditioned reinforcement. *Psychopharmacology (Berl)* 171, 173–178. doi: 10.1007/s00213-003-1575-y

Olsen, S. R., Bortone, D. S., Adesnik, H., and Scanziani, M. (2012). Gain control by layer six in cortical circuits of vision. *Nature* 483, 47–52. doi: 10.1038/nature10835

Origlia, N., Kuczewski, N., Aztiria, E., Gautam, D., Wess, J., and Domenici, L. (2006). Muscarinic acetylcholine receptor knockout mice show distinct synaptic plasticity impairments in the visual cortex. *J. Physiol.* 577, 829–840. doi: 10.1113/jphysiol.2006.117119

Pinto, L., Goard, M. J., Estandian, D., Xu, M., Kwan, A. C., Lee, S. H., et al. (2013). Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* 16, 1857–1863. doi: 10.1038/nn.3552

Ramachandran, V. S., and Braddick, O. (1973). Orientation-specific learning in stereopsis. *Perception* 2, 371–376. doi: 10.1068/p020371

Ramalingam, N., McManus, J. N., Li, W., and Gilbert, C. D. (2013). Top-down modulation of lateral interactions in visual cortex. *J. Neurosci.* 33, 1773–1789. doi: 10.1523/jneurosci.3825-12.2013

Rang, H. P. (2003). *Pharmacology.* Edinburgh; Toronto: Churchill Livingstone.

Reed, A., Riley, J., Carraway, R., Carrasco, A., Perez, C., Jakkamsetti, V., et al. (2011). Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron* 70, 121–131. doi: 10.1016/j.neuron.2011.02.038

Regehr, W. G., and Tank, D. W. (1990). Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. *Nature* 345, 807–810. doi: 10.1038/345807a0

Ricciardi, E., Handjaras, G., Bernardi, G., Pietrini, P., and Furey, M. L. (2013). Cholinergic enhancement reduces functional connectivity and BOLD variability in visual extrastriate cortex during selective attention. *Neuropharmacology* 64, 305–313. doi: 10.1016/j.neuropharm.2012.07.003

Riekkinen, M., and Riekkinen, P. Jr. (1997). Nicotine and D-cycloserine enhance acquisition of water maze spatial navigation in aged rats. *Neuroreport* 8, 699–703. doi: 10.1097/00001756-199702100-00024

Roberts, M. J., and Thiele, A. (2008). Spatial integration and its moderation by attention and acetylcholine. *Front. Biosci.* 13, 3742–3759. doi: 10.2741/2963

Roberts, M. J., Zinke, W., Guo, K., Robertson, R., Mcdonald, J. S., and Thiele, A. (2005). Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. *J. Neurophysiol.* 93, 2062–2072. doi: 10.1152/jn.00911.2004

Rodriguez, R., Kallenbach, U., Singer, W., and Munk, M. H. (2004). Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. *J. Neurosci.* 24, 10369–10378. doi: 10.1523/jneurosci.1839-04.2004

Roelfsema, P. R., van Ooyen, A., and Watanabe, T. (2010). Perceptual learning rules based on reinforcers and attention. *Trends Cogn. Sci.* 14, 64–71. doi: 10.1016/j.tics.2009.11.005

Rokem, A., Landau, A. N., Garg, D., Prinzmetal, W., and Silver, M. A. (2010). Cholinergic enhancement increases the effects of voluntary attention but does not affect involuntary attention. *Neuropsychopharmacology* 35, 2538–2544. doi: 10.1038/npp.2010.118

Rokem, A., and Silver, M. A. (2010). Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. *Curr. Biol.* 20, 1723–1728. doi: 10.1016/j.cub.2010.08.027

Rokem, A., and Silver, M. A. (2013). The benefits of cholinergic enhancement during perceptual learning are long-lasting. *Front. Comput. Neurosci.* 7:66. doi: 10.3389/fncom.2013.00066

Salgado, H., Bellay, T., Nichols, J. A., Bose, M., Martinolich, L., Perrotti, L., et al. (2007). Muscarinic M2 and M1 receptors reduce GABA release by Ca2+ channel modulation through activation of PI3K/Ca2+ -independent and PLC/Ca2+ -dependent PKC. *J. Neurophysiol.* 98, 952–965. doi: 10.1152/jn.00060.2007

Sarter, M., Hasselmo, M. E., Bruno, J. P., and Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res. Brain Res. Rev.* 48, 98–111. doi: 10.1016/j.brainresrev.2004.08.006

Sato, H., Hata, Y., Masui, H., and Tsumoto, T. (1987). A functional role of cholinergic innervation to neurons in the cat visual cortex. *J. Neurophysiol.* 58, 765–780.

Schoups, A., Vogels, R., Qian, N., and Orban, G. (2001). Practising orientation identification improves orientation coding in V1 neurons. *Nature* 412, 549–553. doi: 10.1038/35087601

Seeger, T., Fedorova, I., Zheng, F., Miyakawa, T., Koustova, E., Gomeza, J., et al. (2004). M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory and hippocampal plasticity. *J. Neurosci.* 24, 10117–10127. doi: 10.1523/jneurosci.3581-04.2004

Seitz, A. R., Kim, D., and Watanabe, T. (2009). Rewards evoke learning of unconsciously processed visual stimuli in adult humans. *Neuron* 61, 700–707. doi: 10.1016/j.neuron.2009.01.016

Servan-Schreiber, D., Printz, H., and Cohen, J. D. (1990). A network model of catecholamine effects: gain, signal-to-noise ratio and behavior. *Science* 249, 892–895. doi: 10.1126/science.2392679

Shekhar, A., Potter, W. Z., Lightfoot, J., Lienemann, J., Dube, S., Mallinckrodt, C., et al. (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am. J. Psychiatry* 165, 1033–1039. doi: 10.1176/appi.ajp.2008.06091591

Shinoe, T., Matsui, M., Taketo, M. M., and Manabe, T. (2005). Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. *J. Neurosci.* 25, 11194–11200. doi: 10.1523/jneurosci.2338-05.2005

Shuler, M. G., and Bear, M. F. (2006). Reward timing in the primary visual cortex. *Science* 311, 1606–1609. doi: 10.1126/science.1123513

Sillito, A. M., Cudeiro, J., and Jones, H. E. (2006). Always returning: feedback and sensory processing in visual cortex and thalamus. *Trends Neurosci.* 29, 307–316. doi: 10.1016/j.tins.2006.05.001

Silver, M. A., Shenhav, A., and D'esposito, M. (2008). Cholinergic enhancement reduces spatial spread of visual responses in human early visual cortex. *Neuron* 60, 904–914. doi: 10.1016/j.neuron.2008.09.038

Skrandies, W., and Fahle, M. (1994). Neurophysiological correlates of perceptual learning in the human brain. *Brain Topogr.* 7, 163–168. doi: 10.1007/bf01186774

Soma, S., Shimegi, S., Osaki, H., and Sato, H. (2012). Cholinergic modulation of response gain in the primary visual cortex of the macaque. *J. Neurophysiol.* 107, 283–291. doi: 10.1152/jn.00330.2011

Soma, S., Shimegi, S., Suematsu, N., and Sato, H. (2013a). Cholinergic modulation of response gain in the rat primary visual cortex. *Sci. Rep.* 3:1138. doi: 10.1038/srep01138

Soma, S., Shimegi, S., Suematsu, N., Tamura, H., and Sato, H. (2013b). Modulation-specific and laminar-dependent effects of acetylcholine on visual responses in the rat primary visual cortex. *PLoS One* 8:e68430. doi: 10.1371/journal.pone.0068430

Stettler, D. D., Das, A., Bennett, J., and Gilbert, C. D. (2002). Lateral connectivity and contextual interactions in macaque primary visual cortex. *Neuron* 36, 739–750. doi: 10.1016/s0896-6273(02)01029-2

Sun, Y. J., Wu, G. K., Liu, B. H., Li, P., Zhou, M., Xiao, Z., et al. (2010). Fine-tuning of pre-balanced excitation and inhibition during auditory cortical development. *Nature* 465, 927–931. doi: 10.1038/nature09079

Takesian, A. E., and Hensch, T. K. (2013). Balancing plasticity/stability across brain development. *Prog. Brain Res.* 207, 3–34. doi: 10.1016/b978-0-444-63327-9.00001-1

Theeuwes, J. (2010). Top-down and bottom-up control of visual selection. *Acta Psychol. (Amst)* 135, 77–99. doi: 10.1016/j.actpsy.2010.02.006

Thiel, C. M., Zilles, K., and Fink, G. R. (2005). Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. *Neuropsychopharmacology* 30, 810–820. doi: 10.1038/sj.npp.1300633

Thiele, A. (2013). Muscarinic signaling in the brain. *Annu. Rev. Neurosci.* 36, 271–294. doi: 10.1146/annurev-neuro-062012-170433

Umbriaco, D., Watkins, K. C., Descarries, L., Cozzari, C., and Hartman, B. K. (1994). Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J. Comp. Neurol.* 348, 351–373. doi: 10.1002/cne.903480304

Vaucher, E., and Hamel, E. (1995). Cholinergic basal forebrain neurons project to cortical microvessels in the rat: electron microscopic study with anterogradely transported phaseolus vulgaris leucoagglutinin and choline acetyltransferase immunocytochemistry. *J. Neurosci.* 15, 7427–7441.

Verdier, D., and Dykes, R. W. (2001). Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. *Exp. Brain Res.* 137, 71–82. doi: 10.1007/s002210000646

Voytko, M. L., Olton, D. S., Richardson, R. T., Gorman, L. K., Tobin, J. R., and Price, D. L. (1994). Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J. Neurosci.* 14, 167–186.

Wang, L., Kloc, M., Gu, Y., Ge, S., and Maffei, A. (2013). Layer-specific experience-dependent rewiring of thalamocortical circuits. *J. Neurosci.* 33, 4181–4191. doi: 10.1523/JNEUROSCI.4423-12.2013

Watanabe, T., Nanez, J. E. Sr., Koyama, S., Mukai, I., Liederman, J., and Sasaki, Y. (2002). Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. *Nat. Neurosci.* 5, 1003–1009. doi: 10.1038/nn915

Watanabe, T., Nanez, J. E., and Sasaki, Y. (2001). Perceptual learning without perception. *Nature* 413, 844–848. doi: 10.1038/35101601

Wess, J. (2003). Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. *Trends Pharmacol. Sci.* 24, 414–420. doi: 10.1016/s0165-6147(03)00195-0

Wester, J. C., and Contreras, D. (2013). Differential modulation of spontaneous and evoked thalamocortical network activity by acetylcholine level in vitro. *J. Neurosci.* 33, 17951–17966. doi: 10.1523/jneurosci.1644-13.2013

Wilson, D. A., Fletcher, M. L., and Sullivan, R. M. (2004). Acetylcholine and olfactory perceptual learning. *Learn. Mem.* 11, 28–34. doi: 10.1101/lm.66404

Wilson, N. R., Runyan, C. A., Wang, F. L., and Sur, M. (2012). Division and subtraction by distinct cortical inhibitory networks in vivo. *Nature* 488, 343–348. doi: 10.1038/nature11347

Wylie, K. P., Rojas, D. C., Tanabe, J., Martin, L. F., and Tregellas, J. R. (2012). Nicotine increases brain functional network efficiency. *Neuroimage* 63, 73–80. doi: 10.1016/j.neuroimage.2012.06.079

Xing, D., Yeh, C. I., Burns, S., and Shapley, R. M. (2012). Laminar analysis of visually evoked activity in the primary visual cortex. *Proc. Natl. Acad. Sci. U S A* 109, 13871–13876. doi: 10.1073/pnas.1201478109

Yamasaki, M., Matsui, M., and Watanabe, M. (2010). Preferential localization of muscarinic M1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. *J. Neurosci.* 30, 4408–4418. doi: 10.1523/jneurosci.5719-09.2010

Yu, A. J., and Dayan, P. (2002). Acetylcholine in cortical inference. *Neural Netw.* 15, 719–730. doi: 10.1016/s0893-6080(02)00058-8

Yu, A. J., and Dayan, P. (2005). Uncertainty, neuromodulation and attention. *Neuron* 46, 681–692. doi: 10.1016/j.neuron.2005.04.026

Zhang, Z. W., Kang, J. I., and Vaucher, E. (2011). Axonal varicosity density as an index of local neuronal interactions. *PLoS One* 6:e22543. doi: 10.1371/journal.pone.0022543

Zhong, W. X., Dong, Z. F., Tian, M., Cao, J., Xu, L., and Luo, J. H. (2006). N-methyl-D-aspartate receptor-dependent long-term potentiation in CA1 region

affects synaptic expression of glutamate receptor subunits and associated proteins in the whole hippocampus. *Neuroscience* 141, 1399–1413. doi: 10.1016/j.neuroscience.2006.04.070

Zinke, W., Roberts, M. J., Guo, K., Mcdonald, J. S., Robertson, R., and Thiele, A. (2006). Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized Marmoset monkeys. *Eur. J. Neurosci.* 24, 314–328. doi: 10.1111/j.1460-9568.2006.04882.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 April 2014; accepted: 31 August 2014; published online: 18 September 2014.

Citation: Kang JI, Huppé-Gourgues F and Vaucher E (2014) Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. Front. Syst. Neurosci. ***8****:172. doi: 10.3389/fnsys.2014.00172*

This article was submitted to the journal Frontiers in Systems Neuroscience.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership