NON-INVASIVE BRAIN STIMULATION IN NEUROLOGY AND PSYCHIATRY

EDITED BY: Ignacio Obeso, Antonio Oliviero and Marjan Jahanshahi
PUBLISHED IN: Frontiers in Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-134-0
DOI 10.3389/978-2-88945-134-0

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

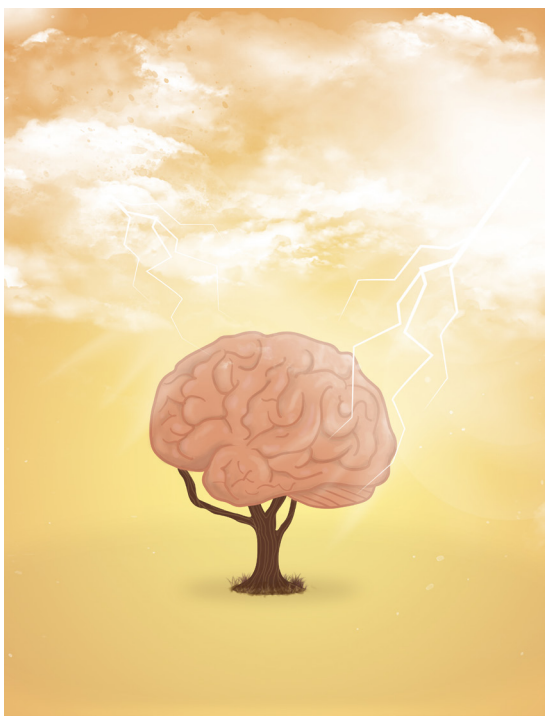
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - 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**

NON-INVASIVE BRAIN STIMULATION IN NEUROLOGY AND PSYCHIATRY

Topic Editors:
Ignacio Obeso, Centro Integral en Neurociencias A.C., HM Puerta del Sur, CEU-San Pablo University Madrid, Spain; Centro de Investigación Biomédica en Red, Enfermedades Neurodegenerativas Madrid, Spain
Antonio Oliviero, FENNSI Group, Hospital Nacional de Parapléjicos, Servicio de Salud de Castilla-La Mancha Toledo, Spain
Marjan Jahanshahi, Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology London, UK



Cover image by Fernando Del Moral

The potential efficacy of non-invasive brain stimulation procedures for the management of specific symptoms in diverse neurological and psychiatric conditions has been tested in the past decade or so. For example, repetitive transcranial magnetic stimulation (rTMS) over prefrontal areas has been extensively investigated as a treatment for patients with medication-resistant depression and has been shown to be associated with improvement of mood. Similarly, non-invasive stimulation techniques have been applied to various symptoms of Parkinson's disease such as bradykinesia and dyskinesias, with variables degrees of success reported. However, attempts to

expand previously observed clinical improvements to other neurological disorders (e.g. Tourette's syndrome, autism, epilepsy) has been controversial. In trying to bypass potential confounding elements, researchers aim to target neural populations altered in disease to either increase or decrease their corrupted baseline activity. In addition, a complementary approach is to extend stimulation protocols that results enhanced behavior in healthy participants.

This Frontiers Research Topic on non-invasive brain stimulation and enhancement of function tries to combine a series of articles from researchers who used non-invasive brain stimulation to aim improvement of either a motoric, cognitive or behavioral nature investigated behaviorally, physiologically or using brain imaging techniques in clinical populations.

Investigation of the relation between enhancement of function in healthy populations and clinical improvement in patients with neurological or psychiatric disorders needs further consideration.

Citation: Obeso, I., Oliviero, A., Jahanshahi, M., eds. (2017). Non-invasive Brain Stimulation in Neurology and Psychiatry. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-134-0

Table of Contents

COGNITIVE DOMAIN

frontiers in Neuroscience

EDITORIAL
published: 15 December 2016
doi: 10.3389/fnins.2016.00574

Editorial: Non-invasive Brain Stimulation in Neurology and Psychiatry

Ignacio Obeso[1,2]*, *Antonio Oliviero*[3] and *Marjan Jahanshahi*[4]

[1] Centro Integral en Neurociencias A.C., HM Puerta del Sur, CEU-San Pablo University, Madrid, Spain, [2] Centro de Investigación Biomédica en Red, Enfermedades Neurodegenerativas, Madrid, Spain, [3] FENNSI Group, Hospital Nacional de Parapléjicos, Servicio de Salud de Castilla-La Mancha, Toledo, Spain, [4] Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK

Keywords: neuromodulation, brain stimulation, rTMS, tDCS

Editorial on the Research Topic

Non-invasive Brain Stimulation in Neurology and Psychiatry

In recent years, greater attention has been paid to alternative treatments in neurology and psychiatry, with the main aim of restoring or "normalizing" function in aberrant brain circuits, in order to have a positive impact on the patient's quality of life. Non-invasive brain stimulation (NIBS) methods such as transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS) have been increasingly used not only in research but also in clinical settings. To date, depression is the only psychiatric disorder for which TMS has been approved and used extensively as a therapeutic approach (Padberg and George, 2009; George et al., 2013). Meanwhile, application of NIBS for other brain disorders such as tinnitus, chronic pain, migraine, dementia, Parkinson's disease (PD), and dystonia are currently in development by optimizing key parameters such as the most appropriate brain target, stimulation protocols and candidate symptoms to treat. Thus, while there has been relatively wide interest in clinical applications of NIBS, yet with refinement of techniques, future improvement of protocols and the possibility of achieving more prolonged and longer-lasting beneficial effects, we believe NIBS will potentially become an approved therapeutic approach for some disorders. The current Special Issue is a compilation of literature reviews or experimental studies using TMS or tDCS as a therapeutic tool in different neurological and psychiatric disorders.

NON-INVASIVE BRAIN STIMULATION METHODS AS THERAPEUTIC TOOLS

The 16 papers in the current Research Topic demonstrate the value of NIBS in the psychiatry and neurology domains and also in cognitive training.

Evidence reveals TMS (Dunlop et al.) and tDCS (Sauvaget et al.) as effective methods for reducing craving in people suffering from eating disorders. A comprehensive review of eating disorders (anorexia, bulimia, and binge eating) confirms the positive use of repetitive TMS (rTMS) to reduce relapse rates. The suggested brain target area is the dorsolateral prefrontal cortex (DLPFC), with incremental clinical success with 10 repeated stimulation sessions. The clinical changes are considered to be potentially associated with improved cognitive control or conflict processing (Dunlop et al.), both prefrontal cortex functions. Similar results have been shown with tDCS, although in fewer studies (Sauvaget et al.). These studies used clinical ratings by patients as measures of stimulation induced change, as they are considered to more accurately reflect the patient's experience and expectations, albeit that they are subject to the common biases of self-report measures, highlighting the need for inclusion of sham-controlled conditions to control

OPEN ACCESS

Edited and reviewed by:
Einar M. Sigurdsson, New York University, USA

***Correspondence:**
Ignacio Obeso
iobeso.hmcinac@hmhospitales.com

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

Received: *27 September 2016*
Accepted: *30 November 2016*
Published: *15 December 2016*

Citation:
Obeso I, Oliviero A and Jahanshahi M (2016) Editorial: Non-invasive Brain Stimulation in Neurology and Psychiatry. Front. Neurosci. 10:574. doi: 10.3389/fnins.2016.00574

for potential placebo effects. A validated method is to combine brain stimulation with imaging (Bestmann et al., 2004). In fact, imaging has proved essential in understanding the positive response to TMS in depression, as shown by a link between clinical improvement and changes in cingulate activity (Fox et al., 2012).

A succinct overview on use of NIBS for auditory hallucinations highlights the efficacy of rTMS and TDCS in reducing the frequency of hallucinations (Moseley et al.). Higher temporo-parietal junction activity (mainly left-sided) is a potential source of hallucinations (Homan et al., 2012), which identifies this as the target location for NIBS. Repeated sessions during 5 consecutive days of cathodal tDCS reduced the hallucinations and this improvement persisted for a 3-month period. In their review, the authors considered the value and efficacy of transcranial random noise stimulation (tRNS) or transcranial alternating current stimulation (tACS) as potential future treatments for hallucination. An additional meta-analysis on conversion disorder shows in 75/86 patients under rTMS treatment a marked improvement as measured by clinical scales (Schönfeldt-Lecuona et al.), which gives further support for NIBS tools in complex neuropsychiatric conditions.

In recent years, application of NIBS in the treatment of neurological patients has been gaining pace and the use of both TMS and tDCS in neurological conditions such as stroke (Corti et al., 2012), tinnitus (Fregni et al., 2006), and PD (Koch et al., 2009) has been evaluated (for review see Obeso et al.). Yet, proof-of-principle studies are needed in treating specific neurological symptoms and to date beneficial changes are limited to acute effects, with limited long-lasting effects. Following the NIBS research approach in depression, larger and well-controlled clinical trials (i.e., use of placebo condition and coils), with longer follow-up periods are urgently needed to confirm the value of stimulation protocols with enhanced durability of clinical benefits.

TMS is useful for differential diagnosis in tremor or stroke by using motor evoked potentials (Brum et al.). Moreover, a classical clinical use of TMS has been to measure cortico-spinal integrity through examining the functioning of the cortico-spinal tracts after stimulation of motor regions. This method is adequate for differential diagnosis based on central motor conduction time (the time taken from TMS pulse activation of the motor cortex and firing of spinal motor neurons). The use of TMS and diffusion tensor imaging showed in stroke patients a correlation between the speed of conduction in the cortico-spinal tract and the integrity of premotor and supplementary tracts (but not the motor area) (Potter-Baker et al.). Their results are of interest for understanding how stroke patients compensate by using higher-order motor control regions upon fatal loss of the principal motor cortical area. For long-term effects, rTMS for stroke treatment is becoming more and more promising as positive findings are being replicated. In the current special topic, authors report in stroke patients how consecutive rTMS session resulted in movement improvement (Di Lazzaro et al.) but also increased tactile detection (Fujimoto et al.). Sample size and gender effects need attention in stroke research as they seem to interact when using rTMS as a treatment tool (Chalah et al.; Di Lazzaro et al.). Last, patients with chronic pain not responsive to pharmacological treatment may benefit from NIBS tools over the primary motor region (DosSantos et al.), whereby distant changes in cortico-subcortical structures and neurotransmitter modulation (serotonin, GABA, glutamate) were associated to clinical improvement.

There is also a novel contribution from light therapy used as a NIBS protocol (see Johnstone et al.). The use of light stimulation has been tested on animal models of PD and AD using low-level near infrared light (NIr) therapy (Shaw et al., 2010; De Taboada et al., 2011), reported to lessen behavioral deficits in both animal models. It is noted that this procedure did not produce any beneficial effects in AD or PD (Johnstone et al.). Only a non-controlled and non-randomized clinical report showed some improvement in speech, some aspects of cognition and gait after NIr therapy in PD patients (Maloney et al., 2010), which needs to be replicated in a larger sample in a better controlled study. Thus, based on valid animal models, Nlr therapy warrants evaluation in larger samples in well-controlled studies, with other targets, and selection of intracranial or extracranial approaches based on the disease, to allow future clinical application.

New avenues of positive results are also obtained in attempts to improve cognitive functioning. AD patients showed improved working memory after tDCS and this was associated to changes in high-frequency bands (Marceglia et al.). However, the use of associated paradigms such as exercise (Morris et al., 2016) or cognitive rehabilitation (Cappon et al.) will boost the cognitive remediation and positive effects.

FUTURE WORK

There are a number of parallel issues across the therapeutic applications of NIBS that need to be addressed. The ultimate value of NIBS rests on proving it to be an efficient and long-lasting therapy that alleviates patient's specific psychiatric, neurological or cognitive symptomatology. However, the questions of *where*, *how* and *when* to stimulate are essential to be addressed in order to follow the logical steps to reach maximal NIBS efficacy for different symptoms and disorders. Although candidate cortical regions to act as targets for receiving NIBS are somewhat more clear for some neurological conditions, other neurological and psychiatric disorders still require evidence from imaging and physiological studies to identify the region or network to be targeted with NIBS. A critical factor is the inclusion of repeated stimulation sessions to achieve potentiation effects. This may be done with an initial period of daily stimulation for example 5 days of consecutive stimulation, followed by once a week booster sessions. Other procedural issues may also influence the quality and efficacy of the NIBS such as the state or subject dependency of the effects, use of neuronavigation vs. EEG localization of the target and these require due attention in future investigations. New methods to better quantify potential beneficial effects of NIBS are the use of models that account for long-term effects (Mahmud and Vassanelli). In future trials, to ensure that NIBS is cost-effective compared to standard medical therapy, there is a need to maximize the efficacy and positive

outcomes of NIBS protocols. Sham-controlled randomized trials of NIBS are essential. Moreover, if the symptoms to be treated have a high within subject variability (e.g., pain, tinnitus and psychiatric symptoms) the clinical trials required could be even more complex and expensive. Using a telemedicine approach and/or using smartphone and wearable technology, continuous patient evaluation can be easier. This will allow NIBS technologies to be tested in a more efficient way.

It is also extremely important to reconsider NIBS variability at individual level. The same target with the same NIBS protocol may produce different effects in different individuals. A personalized approach is needed to reduce this source of variability. Nowadays, many technological tools are available for evaluating central nervous system disorders. However, the general approach is to apply a single therapy or an isolated technology to find the way to help a group of patients that have a common etiology but sometimes very different nervous system pathology and clinical presentations. It is necessary to find the perfect combination of assessment methods to evaluate symptoms and their change after application of a smart mix of therapeutic options, applied at a given time and at the appropriate "doses" to face the great complexity of neurological and psychiatric problems. This may be one of the future strategies for NIBS therapies to find a place in psychiatric and neurological clinics.

Finally, there is a need for safe, efficient and cost-effective NIBS methods such as transcranial static magnetic field stimulation (tSMS) or tDCS that can be portable and usable in patients' homes, which would facilitate generalization of the treatment to the patients' daily life environment.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., and Frahm, J. (2004). Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur. J. Neurosci.* 19, 1950–1962. doi: 10.1111/j.1460-9568.2004.03277.x

Corti, M., Patten, C., and Triggs, W. (2012). Repetitive transcranial magnetic stimulation of motor cortex after stroke: a focused review. *Am. J. Phys. Med. Rehabil.* 91, 254–270. doi: 10.1097/PHM.0b013e318228bf0c

De Taboada, L., Yu, J., El-Amouri, S., Gattoni-Celli, S., Richieri, S., McCarthy, T., et al. (2011). Transcranial laser therapy attenuates amyloid-β peptide neuropathology in amyloid-β protein precursor transgenic mice. *J. Alzheimers Dis.* 23, 521–535. doi: 10.3233/JAD-2010-100894

Fox, M. D., Buckner, R. L., White, M. P., Greicius, M. D., and Pascual-Leone, A. (2012). Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. *Biol. Psychiatry* 72, 595–603. doi: 10.1016/j.biopsych.2012.04.028

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

George, M. S., Taylor, J. J., and Short, E. B. (2013). The expanding evidence base for rTMS treatment of depression. *Curr. Opin. Psychiatry* 26, 13–18. doi: 10.1097/YCO.0b013e32835ab46d

Homan, P., Kindler, J., Hauf, M., Hubl, D., and Dierks, T. (2012). Cerebral blood flow identifies responders to transcranial magnetic stimulation in auditory verbal hallucinations. *Transl. Psychiatry* 2:e189. doi: 10.1038/tp.2012.114

Koch, G., Brusa, L., Carrillo, F., Lo Gerfo, E., Torriero, S., Oliveri, M., et al. (2009). Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. *Neurology* 73, 113–119. doi: 10.1212/WNL.0b013e3181ad5387

Maloney, R., Shanks, S., and Maloney, J. (2010). The application of low-level laser therapy for the symptomatic care of late stage Parkinson's disease: a non-controlled, non-randomized study. *Am. Soc. Laser Med. Surg.* 185.

Morris, T., Gomes Osman, J., Tormos Muñoz, J. M., Costa-Miserachs, D., and Pascual Leone, A. (2016). The role of physical exercise in cognitive recovery after traumatic brain injury: a systematic review. *Restor. Neurol. Neurosci.* 34, 977–988. doi: 10.3233/rnn-160687

Padberg, F., and George, M. S. (2009). Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression. *Exp. Neurol.* 219, 2–13. doi: 10.1016/j.expneurol.2009.04.020

Shaw, V. E., Spana, S., Ashkan, K., Benabid, A.-L., Stone, J., Baker, G. E., et al. (2010). Neuroprotection of midbrain dopaminergic cells in MPTP-treated mice after near-infrared light treatment. *J. Comp. Neurol.* 518, 25–40. doi: 10.1002/cne.22207

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 Neuroscience

REVIEW
published: 09 October 2015
doi: 10.3389/fnins.2015.00349

Transcranial direct current stimulation (tDCS) in behavioral and food addiction: a systematic review of efficacy, technical, and methodological issues

Anne Sauvaget[1,2,3], Benoît Trojak[4,5], Samuel Bulteau[1,2], Susana Jiménez-Murcia[3,6], Fernando Fernández-Aranda[3,6], Ines Wolz[3,6], José M. Menchón[3,7], Sophia Achab[5], Jean-Marie Vanelle[1,2] and Marie Grall-Bronnec[1,2,8]*

[1] Addictology and Liaison Psychiatry Department, Nantes University Hospital, Nantes, France, [2] Clinical Investigation Unit 18-BALANCED "BehaviorAL AddictioNs and ComplEx Mood Disorders", University Hospital of Nantes, Nantes, France, [3] Department of Psychiatry, University Hospital of Bellvitge-IDIBELL, Barcelona, Spain, [4] Department of Psychiatry and Addictology, University Hospital of Dijon, Dijon, France, [5] Behavioral Addictions Program, NANT New Addictions New Treatments, Addiction Division, Department of Mental Health and Psychiatry, University Hospital of Geneva, Geneva, Switzerland, [6] CIBER Fisiopatología Obesidad y Nutrición, Instituto de Salud Carlos III, Barcelona, Spain, [7] CIBER Salud Mental, Instituto de Salud Carlos III, Barcelona, Spain, [8] EA 4275 "Biostatistics, Clinical Research and Subjective Measures in Health Sciences", University of Nantes, Nantes, France

OPEN ACCESS

Edited by:
Antonio Oliviero, Hospital Nacional de Parapléjicos, Spain

Reviewed by:
Andre R. Brunoni, Universidade de São Paulo, Brazil
Laura Mordillo-Mateos, Hospital Nacional de Parapléjicos, Spain

***Correspondence:**
Anne Sauvaget, Cappa Jacques Prevert, Addictology and Liaison-Psychiatry Department, Hôtel Dieu 3éme Nord, 1 Place Alexis-Ricordeau, 44000 Nantes, France
anne.sauvaget@chu-nantes.fr

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

Received: *24 July 2015*
Accepted: *14 September 2015*
Published: *09 October 2015*

Citation:
Sauvaget A, Trojak B, Bulteau S, Jiménez-Murcia S, Fernández-Aranda F, Wolz I, Menchón JM, Achab S, Vanelle J-M and Grall-Bronnec M (2015) Transcranial direct current stimulation (tDCS) in behavioral and food addiction: a systematic review of efficacy, technical, and methodological issues. Front. Neurosci. 9:349. doi: 10.3389/fnins.2015.00349

Objectives: Behavioral addictions (BA) are complex disorders for which pharmacological and psychotherapeutic treatments have shown their limits. Non-invasive brain stimulation, among which transcranial direct current stimulation (tDCS), has opened up new perspectives in addiction treatment. The purpose of this work is to conduct a critical and systematic review of tDCS efficacy, and of technical and methodological considerations in the field of BA.

Methods: A bibliographic search has been conducted on the Medline and ScienceDirect databases until December 2014, based on the following selection criteria: clinical studies on tDCS and BA (namely eating disorders, compulsive buying, Internet addiction, pathological gambling, sexual addiction, sports addiction, video games addiction). Study selection, data analysis, and reporting were conducted according to the PRISMA guidelines.

Results: Out of 402 potential articles, seven studies were selected. So far focusing essentially on abnormal eating, these studies suggest that tDCS (right prefrontal anode/left prefrontal cathode) reduces food craving induced by visual stimuli.

Conclusions: Despite methodological and technical differences between studies, the results are promising. So far, only few studies of tDCS in BA have been conducted. New research is recommended on the use of tDCS in BA, other than eating disorders.

Keywords: transcranial direct current stimulation, neuromodulation, behavioral addiction, craving, eating disorders, food craving, non-invasive brain stimulation

Introduction

Substance Use Disorder and Behavioral Addictions

Addictions are complex disorders conventionally represented by substance use disorders (SUDs). Other behaviors without any substance use share many clinical similarities, and are therefore categorized as addictions without drug use,—more commonly called behavioral addictions (BAs) (O'Brien, 2011; Potenza, 2014) -, as evidenced in the recent release of the DSM-5 (American Psychiatric Association, 2013), where gambling disorders now appear in the "substance-related and addictive disorders" category, among other SUDs. Until now, this is the only BA that the task force researchers included into the edited version of the manual. However, for many authors, BAs also encompass video games addiction, Internet addiction, sexual addiction, compulsive buying, sports addiction, and eating disorders (Gearhardt et al., 2011; Farré et al., 2015; Jiménez-Murcia et al., 2015). It has increasingly been suggested that some eating habits, such as the uncontrolled intake of high-calorie food rich in sugar and fat, can also be seen as behavioral addictions and was recently referred to as "food addiction" (Davis and Carter, 2009; Gearhardt et al., 2011; Hebebrand et al., 2014; Schulte et al., 2015).

As in any SUD, one of the key symptoms in BAs is craving, defined as a pressing, urgent, and irrepressible desire to give in to a BA, which results in most cases in a loss of control (Skinner and Aubin, 2010; O'Brien, 2011). The craving contributes to the development, continuation and relapse of an addictive behavior. Although craving is not pathognomonic of addiction, it remains a key symptom in the addictive process, to the point that it is now considered in the DSM-5 as a diagnostic criterion for substance-related and addictive disorders (American Psychiatric Association, 2013). Craving can lead to a loss of control over one's behavior. Executive functions (such as decision making and risk-taking process) and working memory impairments have been found in both SUDs and BAs (Fernández-Serrano et al., 2010; Marazziti et al., 2014). These clinical features suggest that BAs and SUDs may share similar neurophysiopathological abnormalities. Some authors support the idea of common neurochemical and genetic mechanisms involved with both substance and non-substance, addictive behaviors, linked to disturbances of the reward system, so-called "reward deficiency syndrome" (Blum et al., 2014). The central reward pathway involves the dopaminergic system such as the mesolimbic cortical ventral tegmental area and projections to the nucleus accumbens and the prefrontal cortex (Goldstein and Volkow, 2002; García-García et al., 2014). Neuroimanging studies underlined the important function of the prefrontal cortex, especially the dorsolateral prefrontal cortex (DLPFC), in both SUDs and BAs (Goudriaan et al., 2012).

The pharmacological and psychotherapeutic treatments of addictions and of the craving in particular, have shown their limits (Achab and Khazaal, 2011; Marazziti et al., 2014), which indicates the need for new treatment possibilities.

Non-invasive Brain Stimulation, a Promising Treatment for Addictions

More recently, new treatment modalities such as non-invasive brain stimulation (NIBS) have been explored in the field of addiction, such as Transcranial Direct Current Stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS) (Jansen et al., 2013; Grall-Bronnec and Sauvaget, 2014). rTMS generates a magnetic field in a coil that is placed on the scalp. The magnetic field induces an electrical current in the brain tissue beneath the coil, resulting in alterations of neural excitability (Ziad, 2002). In addition to its cortical action, TMS may act remotely on deeper structures, via brain circuits and interhemispheric connections (Fox et al., 1997). tDCS is another NIBS method capable of modulating cortical excitability (Feil and Zangen, 2010). tDCS consists in delivering a low intensity electric field (1–2 mA) through the brain between two electrodes. The current enters the brain from the anode, travels through the tissue, and exits out the cathode (Higgins and George, 2009). The anodic stimulation increases cortical excitability, whereas the cathodic stimulation reduces it. The administration of tDCS is relatively easy. Electrodes can be placed anywhere on the scalp and are held in place with an elastic headband (Higgins and George, 2009). In general, one session lasts 10–20 min. Two sessions a day can be given easily if required. Like rTMS (Keck et al., 2002; Hanlon et al., 2013), tDCS showed that it could have remote effects (Chib et al., 2013).

rTMS and tDCS, applied to the DLPFC, may transiently modify decision-making, risk-taking, and impulsivity, processes directly linked to behavioral disorders. It has thus been shown that applying tDCS on prefrontal areas modifies the decision process in sane subjects (Fecteau et al., 2007a,b; Knoch et al., 2008; Boggio et al., 2010), but also in addicted subjects (Fecteau et al., 2014). The decision-making process shares common mechanisms with the impulsive behaviors observed in addictions. By modulating it, we could decrease impulsivity in addicted patients, and, indirectly, act on the craving (Fecteau et al., 2010). Anodal tDCS over the DLPFC may enhance executive function and provide improved cognitive control, and thus reduce the probability of relapse to drug use (da Silva et al., 2013).

Finally, even if the neurophysiological effects behind the effects of tDCS on craving are not completely clarified yet, choosing the DLPFC as a stimulation area is justified by the involvement of frontal areas in the neurobiology of eating disorders, either bulimia, or anorexia nervosa (Kaye et al., 2009; van Kuyck et al., 2009; Frank et al., 2013; Friederich et al., 2013). More precisely, the DLPFC might be involved in the food restriction and cognitive control mechanisms, which are linked with the working memory (von Hausswolff-Juhlin et al., 2015).

rTMS and tDCS applied to the DLPFC may therefore indirectly modulate dopaminergic pathways (Addolorato et al., 2012) and may consequently have an impact on the symptoms of addiction (Keck et al., 2002; Feil and Zangen, 2010). Cognitive control could be improved and/or cravings could be reduced (Jansen et al., 2013). So far, tDCS have proven its efficacy to decrease craving, mainly in SUDs (Jansen et al., 2013; Naim-Feil and Zangen, 2013; Kuo et al., 2014). Moreover, reviews and

comprehensive work about tDCS in the field of psychiatry and addictions did not have considered BAs (Feil and Zangen, 2010; Kuo et al., 2014; Tortella et al., 2015).

The goal of this study is to conduct a systematic review of the efficacy, and of the technical and methodological stakes of applying tDCS to the field of BAs.

Methods

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Search Resources

Two independent reviewers conducted the literature search, including different sources such as electronic databases (PubMed and Science Direct), citations, and reference lists, as well as gray literature. In addition, the reference lists of all included studies were hand searched, limiting the search to articles published in English. To ensure the recency of articles, the search was limited from inception to December, 31st, 2014.

The search terms used were a combination of MESH terms and keywords and included "tDCS" and "addiction," "anorexia nervosia," "behavioral addiction," "bulimia nervosa," "eating disorders," "binge eating disorders," "compulsive buying/shopping," "craving," "Dorsolateral prefrontal cortex (DLPFC)," "dependence," "dopamine," "eating disorders and not otherwise specified (EDNOS)," "exercise," "food craving," "gambling disorder," "impulsivity," "Internet addiction," "pathological gambling," "risk-taking behavior," "sex addiction," and "sports addiction" in the title, abstract, or keywords.

Eligibility Criteria

Studies had to fulfill the following inclusion criteria to be included: the target problem was a BA; the intervention was performed using tDCS; the study was a clinical trial, as defined by the WHO (WHO, 2015)—including randomized controlled trials (RCTs), controlled trials, cohort studies, case-control studies and multiple base-line studies. Exclusion criteria were: clinical studies about tDCS among SUDs; review and didactic articles; physiopathological studies and case reports.

Study Selection

First, all studies were screened based on their titles and abstracts. Second, the two reviewers read the full text of all studies identified in this search process. This work was carried out independently using the same bibliographic search. In the event of a disagreement between the two reviewers, the relevant studies were discussed (see **Figure 1** for the study selection flow chart).

Data Extraction

Extracted data included clinical, methodological, and technical considerations (see **Tables 1, 2**).

Results

The initial search identified 402 independent articles. Seven articles met the criteria for inclusion. Food craving, in different clinical conditions was the only symptom to be tested. To the best of our knowledge, we found that tDCS has not yet been tested for the following BAs: compulsive buying/shopping, pathological gambling, gambling disorder, Internet addition, video game addiction, sex addiction and sports addiction.

Efficacy of tDCS in Behavioral Addictions

The main characteristics of the studies are summarized in **Table 1**.

Six out of the seven published studies (Fregni et al., 2008b; Goldman et al., 2011; Montenegro et al., 2012; Jauch-Chara et al., 2014; Kekic et al., 2014; Lapenta et al., 2014) have demonstrated the efficacy of tDCS applied to the DLPFC in reducing food craving. Khedr et al. reported an improvement in anorexic conducts (Khedr et al., 2014). Two of these studies were led by the same team (Fregni et al., 2008b; Lapenta et al., 2014) with the same design. All studies but one (Khedr et al., 2014) were blinded, randomized, and controlled. The used sample sizes vary between 7 (Khedr et al., 2014) and 23 (Fregni et al., 2008b) subjects. The participants were majoritarily women aged less than 30 years old on average, in good health, and with frequent food cravings. Only one study included overweight patients (Montenegro et al., 2012), and only one included anorexic patients (Khedr et al., 2014). In all studies but two (Montenegro et al., 2012; Khedr et al., 2014), the craving was induced visually, either with images, or with real food. The craving was induced through visual stimuli before and after stimulation in four of the seven studies (Fregni et al., 2008b; Goldman et al., 2011; Kekic et al., 2014; Lapenta et al., 2014). One of the studies repeated the induction after half of the stimulation time (Goldman et al., 2011). Three studies used exposure to real, high-calorie food, combined with one or two short movies showing high-calorie foods (Fregni et al., 2008b; Kekic et al., 2014; Lapenta et al., 2014). One study used pictures of high calorie food items to induce craving (Goldman et al., 2011). Both types of craving induction were reported to lead to increased craving. The level of food craving was usually measured before and after stimulation by means of visual analog scales (VAS) with the exception of one study which did not assess craving at all (Khedr et al., 2014). Some studies used additional measures such as eye tracking (Fregni et al., 2008b; Lapenta et al., 2014) or the Food Craving Questionnaire-State (Kekic et al., 2014). Five studies assessed actual food intake after stimulation using a bogus taste test (Fregni et al., 2008b; Goldman et al., 2011; Jauch-Chara et al., 2014; Kekic et al., 2014; Lapenta et al., 2014).

Further assessment methods were also used, either clinical with specific impulsivity scales (Kekic et al., 2014), or physiological like visual attention, measured by eye tracking (Fregni et al., 2008b), salivary cortisol levels (Kekic et al., 2014) or event-related potentials (Lapenta et al., 2014).

tDCS Technical Procedures

The results are summarized in **Table 2**.

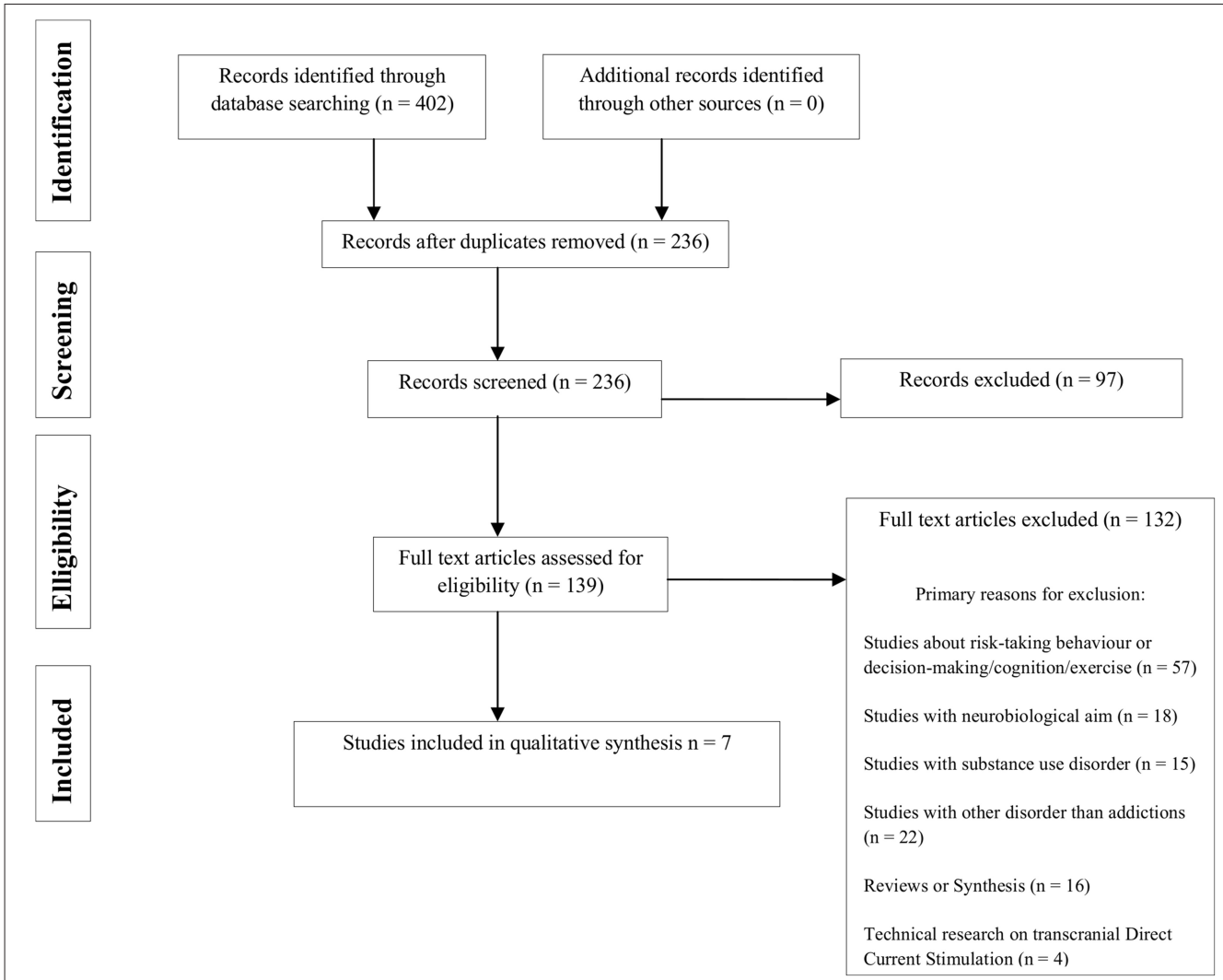


FIGURE 1 | PRISMA flow chart of literature search.

Most studies tested the effect of just one active tDCS session vs. a sham tDCS session (20 min, 2 mA) on food craving. No cortical target other than the DLPFC was tested. Electrodes were most often placed with the anode on the right and the cathode on the left, respectively on F4 and F3 according to the International 10–20 System. Three teams placed them the other way around (cathode on the right and anode on the left) (Fregni et al., 2008b; Montenegro et al., 2012; Khedr et al., 2014). Montenegro and colleagues had two comparing arms (active tDCS and placebo) (Montenegro et al., 2012), whereas Fregni et al. had three comparing arms (anode/right and cathode/left; anode/left and cathode/right; placebo) (Fregni et al., 2008b). The interval between two sessions (active and placebo) ranges from 48 h to a week, to avoid a carry-over effect. The placebo method was described more or less precisely in all studies but one (Jauch-Chara et al., 2014). Tolerance and side effects were reported in 50% of studies (Fregni et al., 2008b; Jauch-Chara et al., 2014; Kekic et al., 2014).

Discussion

General Instructions

The initial works on tDCS in BAs are recent, and started around the same period (Fregni et al., 2008b) as studies on tDCS in SUDs (Boggio et al., 2008; Fregni et al., 2008a). However, they have not generated the same intererest overtime, so that the application of tDCS in SUDs has been much more investigated than tDCS in BAs. Works on tDCS in BAs were first and only interested in eating behavior, based on the model of rTMS, which is another NIBS which efficacy in BAs was first tested in eating disorders (Grall-Bronnec and Sauvaget, 2014). Whether through rTMS or tDCS, no study has been conducted to this day on other BAs (pathological gambling, sexual addiction, sports addiction, Internet addiction, compulsive shopping) (Grall-Bronnec and Sauvaget, 2014). Furthermore, although tDCS is a more manageable and less expensive means than rTMS (Brunoni et al., 2013), we observe that fewer studies are conducted with

TABLE 1 | Clinical trials of tDCS and behavioral addictions: general and clinical characteristics.

Studies	Studied condition	*N*	Participants inclusion criteria	Mean age (years)	Proportion of females	Main exclusion criteria	Design	Objective	Main experimental conditions	Main outcome measures	Main results	Drop out (reason)
Fregni et al., 2008b	Food craving	23	Healthy subjects aged 18–55. Frequent food craving (>3 times/day) and strong urges to eat	23.7	91.3%	NS	Randomized Sham-controlled Double-blind Crossover design	Investigate the effect of tDCS on food cue-induced craving-related behavior	Exposition to food and watching a movie of food associated with strong craving	Food craving (VAS) and food consumption before and after treatment Visual attention to food using an eye tracking system	Craving for viewed foods was reduced by anode right/cathode left tDCS After sham stimulation, exposure to real food or food-related movie increased craving After anode left/cathode right tDCS, the food-related stimuli did not increase craving levels	2 (School work)
Goldman et al., 2011	Food craving	19	Healthy subjects aged 21–70 with frequent food cravings (≥3 times/week during the past month) BMI < 40	32.4	68.4%	Pregnancy History of an ED or depression Suicidality Implanted metal devices History of seizures, brain surgery	Randomized Sham-controlled Single-blind Within-subject crossover design	Investigate the effect of tDCS on food cue-induced craving and the ability to resist foods	Twenty-four images of foods (e.g., ice cream, cheese-burgers, pizza) were presented in random	Food craving and ability to resist tasting (VAS) while viewing food image	Food cravings ratings were reduced in both conditions The percent change in self-reported cravings from pre- to post-stimulation was significantly greater for real stimulation Decrease in food craving, particularly for sweets and carbohydrates No change in food consumption	1 (NS)
Montenegro et al., 2012	Hunger, satiety and desire to eat sensations	9	Overweight subjects 2–3 h fasting	24	44.4%	Cardiovascular disease Pregnancy, History of eating disorders Depression Implanted metal parts	Randomized Single-blinded Sham-Controlled Crossover design	Investigate the effect of tDCS isolated or combined with aerobic exercise on the desire to eat, hunger, and satiety	No exposition to food	Appetite sensations (VAS) evaluated at four moments: baseline; after tDCS; post-exercise; 30 min post-exercise	tDCS on left DLPFC decreased the desire to eat at baseline tDCS associated with exercise had greater suppressing effect in desire to eat compared to either tDCS or exercise alone tDCS associated with exercise decreased hunger and increased satiety immediately after exercise	0

(Continued)

TABLE 1 | Continued

Studies	Studied condition	*N*	Participants inclusion criteria	Mean age (years)	Proportion of females	Main exclusion criteria	Design	Objective	Main experimental conditions	Main outcome measures	Main results	Drop out (reason)
Kekic et al., 2014	Food craving and temporal discounting	17	Healthy women aged 18–60 with frequent food cravings (≥1 per day)	26.4	100%	SUD Major psychiatric disorder Current or past history of an eating disorder Personal or family history of seizures Implanted metal devices Pregnancy	Randomized Double-blind Sham-controlled Within-subjects crossover design	Investigate the effect of tDCS on food cravings, intertemporal choice behavior, actual food consumption and temporal discounting	Exposition to real food	VAS measuring baseline hunger FCT FCQ-S Saliva sample TD task	tDCS reduced cravings for sweet but not savory foods Participants who exhibited more reflective choice behavior were more susceptible to the anticraving effects of tDCS than those who displayed more impulsive choice behavior.	1 (Skin irritation)
Khedr et al., 2014	Urge to restrict food intake	7	Treatment-resistant AN BMI between 14 and 17.5 kg/m2	21.75	85.7%	Drugs (dopaminergic, psychotropic, antiepileptic, or hormonal drugs Estrogen) at least 2 weeks before the study Six patients had been receiving antidepressant (SSRIs) which was kept constant throughout the study	Open-label, single-arm study	Evaluate the acceptability of tDCS as a potential treatment for AN Investigate the effect of tDCS on the urge to restrict food intake and symptoms of depression associated with AN.	No exposition to food	EAT EDI BDI	Significant effect of time (pre, post, and 1 month later) on the three rating scores Significant correlation between the percent improvement of BDI and EAT and between BDI and EDI Ten daily sessions of anodal tDCS over the left DLPFC improved symptoms of both depression and AN for up to 1 month	0
Lapenta et al., 2014	Food consumption and ERP-indexed inhibitory control	9	Healthy females. Frequent (>3 times/day) and strong urges to eat	23.4 ± 2 years)	100%	Neuropsychiatric disorder History of abuse of alcohol or another drug Psychiatric medication Pregnancy Eating disorder	Randomized Double-blind Sham-controlled Crossover design	Evaluate the cognitive ERPs that are associated with the effects of DLPFC tDCS on food craving	Exposition to food and watching a movie of food associated with strong craving Go/No-go task that contained pictures of food and furniture (a control visual stimulus).	ERP during a Go/No-Go task Food craving (VAS) while exposed to real food and a movie of food Snack intake; Attentional bias for food (eye tracking)	Active DLPFC tDCS (anode right/cathode left), compared with sham stimulation, reduced the frontal N2 component and enhanced the P3a component of responses to No-go stimuli, regardless of the stimulus condition (food or furniture). Active tDCS was also associated with a reduction in caloric intake	0

(Continued)

TABLE 1 | Continued

Studies	Studied condition	*N*	Participants inclusion criteria	Mean age (years)	Proportion of females	Main exclusion criteria	Design	Objective	Main experimental conditions	Main outcome measures	Main results	Drop out (reason)
Jauch-Chara et al., 2014	Food intake	14	Healthy young normal-weight men with BMI from 20 to 25. Low cognitive restraint, low disinhibition, and normal susceptibility to hunger scores	24.8	0%	Any medication Acute and chronic medical diseases Alcohol or drug abuse, Smoking Participation in competitive sports Disturbances in sleep continuity	Randomized Sham-controlled Single-blind Code-based, Counter-balanced Crossover design	Investigate the effect of repetitive tDCS to the right DLPFC on food intake	Exposition to food and consomption	Subjective appetite (ratings and VAS) Food intake behavior from a standardized *ad libitum* buffet	tDCS reduced food consumption in humans	0

AN, Anorexia Nervosia; BDI, Beck Depression Inventory; BMI (in kg/m2), Body Mass Index; DLPFC, Dorsolateral Prefrontal Cortex; EAT, Eating Attitude Test; EDI, Eating Disorder Inventory; ERPs, event-related potentials; FCQ-S, Food Craving Questionnaire-State; FCT, Food Challenge Task; NS, Not Specified; SUD, Substance Use Disorders; TD task, temporal discounting task; SSRIs, serotonin reuptake inhibitors; tDCS, transcranial direct current stimulation; VAS, Visual Analog Scales.

tDCS in the field of BAs and SUD compared to rTMS (Grall-Bronnec and Sauvaget, 2014). The later development of tDCS could explain the smaller number of studies.

tDCS is found effective in reducing craving in BAs in controlled studies comparing stimulation vs. placebo, until now for food craving. These results point in the same direction as those of tDCS for SUDs, which have been consolidated by a recent meta-analysis arguing that applying NIBS to the DLPFC decreases craving levels in substance dependence (Jansen et al., 2013), without any significant difference between rTMS and tDCS. However, the efficacy of tDCS must be discussed in light of methodological and technical considerations. All possible biases have been discussed (see **Table 3**).

Methodological Issues

Characteristics of the Participants

Health status of the participants

Patient inclusion criteria can be relatively confusing: indeed, most participants are defined as "healthy" subjects, whereas the study aims at investigating the effect of brain stimulation on food craving, which is a clearly defined disorder from a psychopathological standpoint. The frequency of food craving is relatively low in tDCS studies. It varies, depending on the studies, from 1/day (Kekic et al., 2014) to 3/day (Fregni et al., 2008b; Goldman et al., 2011; Lapenta et al., 2014). Moreover, most patients included in studies on tDCS have a normal weight, apart from one study in obese patients (Montenegro et al., 2012) and another in patients suffering from anorexia nervosa (Khedr et al., 2014). Only Jauch-Chara et al.'s study can be considered as conducted in a physiological condition, since the included subjects had a normal body mass index and no daily food craving (Jauch-Chara et al., 2014). In fact, the studies were more interested in the process of food craving than in full-syndrome eating disorders. Patient morphology could be an important criterion to take into account in configurating tDCS. This precise point is developed in the "Technical Issues" Section.

Age

Participants are rather young (<40 years old). The age of the studied population is important to interpret results since the clinical expression of craving is likely to evolve with age. Age is also a factor in the variation of cortical excitability (Feil and Zangen, 2010; Clark and Taylor, 2011).

Gender

Apart from Jauch-Chara et al.'s study (2014) in which all subjects are male, the other studies mainly included women, either on purpose (Kekic et al., 2014; Lapenta et al., 2014) or because they were predominant (Fregni et al., 2008b; Goldman et al., 2011; Montenegro et al., 2012; Khedr et al., 2014). A higher prevalence of food craving in women than in men explains the sex-ratio imbalance between patients included in these studies (Mitchison and Hay, 2014). Moreover, the fluctuation of eating behavior throughout the menstrual cycle could affect the result of the studies (Lester et al., 2003). The sexual hormonal variations could also affect the functional cerebral asymmetries (Neufang et al., 2009). The right hemispheric predominance in spatial

TABLE 2 | Clinical trials of tDCS and behavioral addictions: technical characteristics.

Studies	Procedure of tDCS number of sessions	Duration (min/session)	Anode	Cathode	Current density (A/m2)	Sham conditions	Tolerance/Adverse effects
Fregni et al., 2008b	Three types of bilateral stimulation of DLPFC (48 h ii)—1 session	–	–	–	–	–	Mild and similar in the three conditions of stimulation Most frequent adverse effects were scalp burning, headache, local itching, burning sensation, and somnolence
	Active anode left/cathode right tDCS	20	F3	F4	2 mA	–	
	Active anode right/cathode left tDCS	20	F4	F3	2 mA	–	
	Sham tDCS	20	–	–	–	Electrodes were placed at the same positions as in active stimulation The stimulator was turned off after 30 s of stimulation	
Goldman et al., 2011	Two types of bilateral stimulation of DLPFC (48–72 h ii)—1 session	–	–	–	–	–	NS
	Active anode right Cathode left tDCS	20	F4	F3	2 mA		
	Sham tDCS with the same electrode placement	20	F4	F3	–	The tDCS device was turned up to 2 mA for 30 s, then slowly ramped-down to 0 mA over the period of 1 min, and finally turned off for the duration of the 20 min session Participants were asked to guess whether they received real or sham stimulation at each session, as well as how confident they were in their guess	
Montenegro et al., 2012	Two types of unilateral stimulation over DLPFC (48–120 h ii)—1 session	–	–	–	–	–	NS
	Anodal unilateral stimulation on left DLPFC, alone or combined with isocaloric exercise bouts	20	F3	Fp2	2 mA		
	Sham tDCS with the same electrode placement, alone, or combined with isocaloric exercise bouts	20	F3	Fp2	–	The stimulator was turned off after 30 s	
Kekic et al., 2014	Two types of bilateral stimulation of DLPFC (≥48 h) ii)—1 session	–	–	–	–	–	One participant withdrew from the study after the first appointment due to skin irritation at the site of stimulation Slight headache following active tDCS Participants reported experiencing minimal Discomfort
	Anode right/cathode left	20	F4	F3	2 mA	–	
	Sham tDCS with the same electrode placement	20	F4	F3	–	The stimulation automatically turned off after 30 s	

(Continued)

TABLE 2 | Continued

Studies	Procedure of tDCS number of sessions	Duration (min/session)	Anode	Cathode	Current density (A/m2)	Sham conditions	Tolerance/Adverse effects
Khedr et al., 2014	One type o bilateral stimulation of anodal tDCS, over the left DLPFC (Reference electrode over the contralateral arm)—10 sessions (5 sessions/week)	25	6 cm anterior to the left (M I)	–	2 mA	No sham condition	NS
Lapenta et al., 2014	Two types of bilateral stimulation of DLPFC (1 week ii)—1 session	–	–	–	–	–	NS
	Active tDCS, anode right/cathode left	20	F4	F3	2 mA		
	Sham tDCS with the same electrode placement	20	F4	F3	–	The stimulation automatically turned off after 30 s	
Jauch-Chara et al., 2014	Two types of bilateral stimulation of DLPFC (2 weeks ii)—8 daily sessions						All sensations were transient and ranged from mild to moderate: skin redness ($n = 9$), tingling ($n = 4$), itching ($n = 7$), and feelings of skin burning ($n = 2$).
	Active tDCS, anode right/cathode left	20	5 cm method	Over the left forehead	1 mA		
	Sham tDCS	20	NS	NS	–	NS	

DLPFC, Dorso Lateral Prefrontal Cortex; F3, 10–20 EEG system; F4, 10-20 EEG system; ii, intersession interval; M I, primary motor cortex; NS, Not Specified; tDCS, transcranial Direct Current Stimulation.

TABLE 3 | Main sources of bias in the studies of tDCS in behavioral addictions.

SELECTION BIAS
Method of recruiting subjects (healthy participant, non-healthy participant, with or without treatment participants).
Duration and severity of the addiction or related disorder.
Stage of treatment prior to tDCS (detoxification or continuation of substance use).
OBSERVATION BIAS
Over or underestimating the intensity of craving.
Placebo effect of tDCS itself.
Placebo effect of therapeutic trials carried out in the field of addiction and related disorders.
Order of the placebo session and active session in a crossover study.
Insufficient number of pulses and number of sessions.
Attrition bias (drop out).
CONFOUNDING BIAS
Sociodemographic characteristics: age, gender, ethnicity.
Hormonal status.
Volume of gray matter.
Psychiatric and somatic comorbidities.
Handedness.
Psychotropic treatments (in particular, continuation of anti-craving drugs during the trial).
Duration of the session, which may overlap with the duration required for the craving to subside naturally.
Cumulative and persistent effects of tDCS when the interval between two sessions is very short.
Sample size.
Ability of the treatment-seeking participants to use relapse prevention techniques during cue-induced craving procedure.

All these biases are discussed in Sections Methodological Issues and Technical Issues.

attention, which seems linked to gender, would disappear under the effect of left anodal tDCS (de Tommaso et al., 2014). Finally, a recent study showed that electric transmission of tDCS is different between men and women, mainly for bone density reasons (Russell et al., 2014).

Handedness

None of the studies analyzed in this review evoked the importance of this parameter in the interpretation of results, conversely to other NIBS works, whether on rTMS (Van den Eynde et al., 2012) or tDCS (Kasuga et al., 2015). Yet, the effects of tDCS could differ according to the handedness of stimulated subjects (Kasuga et al., 2015). The problem of hemispheric dominance remains complex, since the left hemisphere could be the dominant hemisphere in 95–99% of right-handed subjects, and in 70% of left-handed subjects (Corballis, 2014). Moreover, laterality has clinical relevance since left-handers are more at risk of developing addictive disorders (Sperling et al., 2000). Evaluating the hemispheric dominance thanks to a specific questionnaire focused on laterality (Oldfield, 1971) in patients included in studies involving NIBS would allow gathering new data on brain functioning.

Main exclusion criteria

The exclusion criteria are generally mentioned and detailed. Although contra-indications are usually exclusion criteria, in accordance with the literature (Brunoni et al., 2011), the psychiatric and somatic comorbidities can be confounding factors in evaluating the efficacy of tDCS. For example, in eating disorders, the conjoint improvement of depressive symptoms and obsessive-compulsive symptoms on the one hand, and of binge-eating and purging conducts on the other hand, could simply be due to a common physiopathological process rather than to a specific effect of tDCS on the addictive symptoms only (Khedr et al., 2014; Shiozawa et al., 2014). Finally, the use of medication, particularly psychotropic and anticraving drugs, should be considered, as they could interfere with the assessment of craving, but also with the efficacy of tDCS, through their action on cortical excitability. Selective serotonin reuptake inhibitors could indeed aid tDCS response (Nitsche et al., 2009).

Sample size

Among all examined studies, the sample size is always very small, ranging between 7 (Khedr et al., 2014) and 23 subjects (Fregni et al., 2008b). This is probably why all of them, except for two studies (Montenegro et al., 2012; Khedr et al., 2014), adopted a crossover design, to increase the statistical power of their work.

Cortical Excitability

tDCS modifies cortical excitability (Nitsche et al., 2008). The efficacy of tDCS thus depends on numerous factors, which have an influence on cortical excitability, such as age, gender, hormonal status, anxiety levels, lack of sleep, and the use of psychotropic drugs (either legal or illegal). Cortical excitability would also vary according to ethnic origins (Yi et al., 2014). The results of the studies should thus be discussed according to these parameters.

Design

All studies but one (Khedr et al., 2014) were designed following the rules of RCTs, which facilitates comparisons. Although food craving is the main evaluation criterion, some authors have underlined the importance of considering other target symptoms such as impulsivity (Kekic et al., 2014). In their studies, patients with "more reflective choice behavior" are more sensitive to the anti-craving effects of the tDCS than patients with "more impulsive choice behavior." These results show that the craving involves multiple dimensions that interact with each other, and that can also be modified by tDCS.

The craving induction procedure differs between studies. Although the induction medium (either real or virtual) is most of the time visual, this may vary. Addressing other sense organs like olfaction may increase the external validity of craving induction methods. Although craving is not necessarily related to physiological hunger, food intake before the experimental session may be an important interfering factor. Most studies tried to control this variable, by asking participants to refrain from food intake for a period of time before the session, which varied between 2 and 6 h depending on the study design (Fregni et al., 2008b; Goldman et al., 2011; Montenegro et al., 2012; Jauch-Chara et al., 2014; Lapenta et al., 2014). Some also used a 24 h dietary recall protocol to assess previous food intake (Goldman et al., 2011; Montenegro et al., 2012). One study controlled food intake only by a VAS on hunger (Kekic et al., 2014), and

another study did not control this factor at all (Khedr et al., 2014).

Technical Issues

General Considerations

The tDCS procedure is generally well-described and detailed, which allows for a better comparison between studies. Teams that consecutively conduct several studies tend to replicate the same protocol (Fregni et al., 2008b; Lapenta et al., 2014).

Stimulation Site

Only the DLPFC drew the researchers' attention, most often in the following setting: anode on the right DLPFC (excitation) and cathode on the left DLPFC (inhibition). The results of Lapenta et al.'s team suggest that stimulating the DLPFC could facilitate the inhibitory response and modify the connections between the cortical and subcortical structures (Lapenta et al., 2014).

The positive results both in overeating with the "anode on the right DLPFC and cathode on the left DLPFC" scheme and in food restriction with the "anode on the left DLPFC and cathode on the right DLPFC" scheme argue in favor of a different hemispheric functioning in eating disorders. In the case of overeating and obesity, increasing the activity of the right DLPFC and inhibiting the left DLPFC would help reducing the induced food craving. This could decrease appetite and restore food control mechanisms, in line with the "right brain hypothesis for obesity" theory (Alonso-Alonso and Pascual-Leone, 2007; Alonso-Alonso, 2013). In the case of food restriction, the hypothesis of an imbalanced interhemispheric balance (with hyperactive right frontal regions) combined with anorexia nervosa (Hecht, 2010) has been partly confirmed by Khedr's work (Khedr et al., 2014). The possible predominance of the right hemisphere in the genesis of an eating disorder has also been evoked in a post-lesional context (Uher and Treasure, 2005). These results are consistent with the works on rTMS that put forward the respective roles of the right and left DLPFC in the control of craving. Whereas, the left DLPFC seems to have a role in the control of craving (Hayashi et al., 2013), the right DLPFC seems to play a part in the inhibitory control of emotional impulses (Pripfl et al., 2013).

However, the studies published to this day do not all have the same methodology, clinical populations or objectives. The results they put forward are still at a very preliminary stage and do not allow concluding on a hemispheric specialization in eating disorders and in BAs generally speaking. Besides switching electrodes between the two hemispheres to test the two conditions (excitatory or inhibitory) (McClelland et al., 2013), it would be interesting to stimulate other cortical regions, such as the parietal cortex, which is a cerebral region that seems to be involved in body image (Gaudio and Quattrocchi, 2012).

The choice of the stimulation site must also take into account the neural loops involved in the studied BA. It seems more pertinent to choose the stimulation site based on the neural loops involved in the studied behavioral addiction, rather than sticking to a given cortical region.

General Design of the Sessions and tDCS Parameters

Apart from two studies (Jauch-Chara et al., 2014; Khedr et al., 2014), the included studies only tested the effects of one tDCS session. It can be assumed that repeating sessions could increase and sustain efficacy on craving and other eating disorder symptoms, as reported in studies on the treatment of depressive disorders (Shiozawa et al., 2014) and SUDs (Feil and Zangen, 2010; Tortella et al., 2015).

tDCS is generally described as easier to implement than rTMS. However, this should not overshadow the importance of some tDCS parameters, which might have an influence on the results (Horvath et al., 2014).

Indeed, beside the optimal position of the electrodes, current intensity, and stimulation duration, other parameters should be taken into account, such as hair thickness, sweat (Horvath et al., 2014), but also electrode size (Russell et al., 2014; Nasseri et al., 2015), with individual differences (Russell et al., 2013). The location of the reference electrode may also have an impact on tDCS effects (Nasseri et al., 2015). The electric current in tDCS is not relayed in the same way by the different anatomical structures it passes through (Shahid et al., 2014). For example, regarding adipose tissue, Truong et al. have showed that, based on the MRI analysis of five patients' adipose tissue, that tissue could influence the intensity of tDCS electric current in the brain (Truong et al., 2013). Furthermore, patients suffering from eating disorders could present changes in gray matter and its thickness (Frank et al., 2013). Consequently, the transmission of tDCS electric current could be altered, and the results could differ from the intended target.

Sham Procedure

The placebo conditions, as described more or less precisely in all studies are similar, and follow a validated method (Gandiga et al., 2006). The placebo tDCS method seems more reliable and easier to implement than the rTMS placebo method, thus limiting the interpretation biases (Grall-Bronnec and Sauvaget, 2014). However, some authors have evidenced that sham tDCS was not as reliable as it seemed (Horvath et al., 2014).

Safety and Tolerance

When reported (Fregni et al., 2008; Jauch-Chara et al., 2014), the side effects were similar to those found in literature (Brunoni et al., 2011). Indeed, tDCS is generally known as a safe technique with mild and transient adverse effects (Brunoni et al., 2011). Even though seizures induced by tDCS are very rare (Ekici, 2015), subjects suffering from substance use addiction present an increased risk of seizures, especially during the alcohol or benzodiazepine withdrawal periods (Leach et al., 2012). The tDCS techniques could be tolerated better by patients suffering from BAs, as they are less likely to have seizures than patients with a SUD.

Conclusion and Future Perspectives

The application field of tDCS in BAs is for now restricted to the study of food craving, mainly in so-called "sane" participants, i.e., who do not fulfill the diagnostic criteria of characterized eating

disorders. These studies show that stimulating the right DLPFC and inhibiting the left DLPFC reduces the induced food craving.

Therefore, there is a clinical interest in having a symptomatic treatment of craving, by considering tDCS as a complementary therapy to the standard treatment of eating disorder. On a neuroscientific level, tDCS could reduce inter-hemispheric imbalance, since data report overactivity in the frontal area of the right hemisphere in anorexia nervosa, as ventured by Hecht in 2010 (Hecht, 2010), and recently comforted by Khedr et al.'s work (2014). Also, other therapeutic effects could be observed with tDCS, especially on food restriction (Jauch-Chara et al., 2014).

The rationale of expanding tDCS work to other behavioral addictions is justified by its positive effect in sane subjects on their decision-making process (improved) and on their risk-taking (reduced) (Fecteau et al., 2007a,b), both strongly linked to addictive issues. Indeed, the recent works on tDCS in the field of cognition and impulsivity (Feil and Zangen, 2010; Elmasry et al., 2015) demonstrate promising therapeutic prospects for tDCS.

The tDCS techniques offer many undeniable advantages in treating BAs: they are non-invasive, well-tolerated, implemented through a portable, and compact device, and relatively cheap compared to other techniques (such as rTMS). Thus, tDCS could be implemented in outpatient structures specialized in addictions.

Several research avenues must be explored, in line with the research conducted with rTMS. The effects of tDCS in other BAs could be explored, like pathological gambling, sports addiction, sexual addictions, or video games. It would also be particularly interesting to evaluate the effects of tDCS in the longer term, whether on craving or on other BA symptoms, such as maintained abstinence.

Finally, combining neuroimaging and electrophysiology studies (Val-Laillet et al., 2015; Wolz et al., 2015) to tDCS studies should be considered, to understand better the pathophysiological mechanisms involved in BAs, and allow for a better identification of targets and stimulation parameters.

In summary, the main goals of tDCS application in BAs are all at once therapeutic, by modulating craving, impulsivity, executive functions and physiopathological, by enhancing the knowledge of neurophysiological basis of BAs.

Acknowledgments

We would like to thank warmly A. F. Goalic for her assistance in the literature search and Hélène Henderson for language editing. This manuscript was partially supported by grants from Instituto Salud Carlos III (PI14/290 and CIBERobn) and Fondo Europeo de Desarrollo Regional (FEDER), but also PROMOSAM (PSI2014-56303-REDT), Spain. IW was supported by a predoctoral grant of AGAUR (2014FI_B 00372). CIBERobn and CIBERsam are both initiatives of ISCIII.

References

Achab, S., and Khazaal, Y. (2011). Psychopharmacological treatment in pathological gambling: a critical review. *Curr. Pharm. Des.* 17, 1389–1395. doi: 10.2174/138161211796150774

Addolorato, G., Leggio, L., Hopf, F. W., Diana, M., and Bonci, A. (2012). Novel therapeu-tic strategies for alcohol and drug addiction: focus on GABA, ion channels andtranscranial magnetic stimulation. *Neuropsychopharmacology* 37, 163–177. doi: 10.1038/npp.2011.216

Alonso-Alonso, M. (2013). Translating tDCS into the field of obesity: mechanism-driven approaches. *Front. Hum. Neurosci.* 7:512. doi: 10.3389/fnhum.2013.00512

Alonso-Alonso, M., and Pascual-Leone, A. (2007). The right brain hypothesis for obesity. *JAMA* 297, 1819–1822. doi: 10.1001/jama.297.16.1819

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders, 5th Edn.* Washington, DC: Association AP, 947 p.

Blum, K., Febo, M., McLaughlin, T., Cronjé, F. J., Han, D., and Gold, S. M. (2014). Hatching the behavioral addiction egg: Reward Deficiency Solution System (RDSS)™ as a function of dopaminergic neurogenetics and brain functional connectivity linking all addictions under a common rubric. *J. Behav. Addict.* 3, 149–156. doi: 10.1556/JBA.3.2014.019

Boggio, P. S., Campanhã, C., Valasek, C. A., Fecteau, S., Pascual-Leone, A., and Fregni, F. (2010). Modulation of decision-making in a gambling task in older adults with transcranial direct current stimulation. *Eur. J. Neurosci.* 31, 593–597. doi: 10.1111/j.1460-9568.2010.07080.x

Boggio, P. S., Sultani, N., Fecteau, S., Merabet, L., Mecca, T., Pascual-Leone, A., et al. (2008). Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: a double-blind, sham-controlled study. *Drug Alcohol Depend.* 92, 55–60. doi: 10.1016/j.drugalcdep.2007.06.011

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., and Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychopharmacol.* 14, 1133–1145. doi: 10.1017/S1461145710001690

Brunoni, A. R., Boggio, P. S., Ferrucci, R., Priori, A., and Fregni, F. (2013). Transcranial direct current stimulation: challenges, opportunities, and impact on psychiatry and neurorehabilitation. *Front. Psychiatry* 4:19. doi: 10.3389/fpsyt.2013.00019

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

Clark, B. C., and Taylor, J. L. (2011). Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. *Curr. Aging Sci.* 4, 192–199. doi: 10.2174/1874609811104030192

Corballis, M. C. (2014). Left brain, right brain: facts and fantasies. *PLoS Biol.* 12:e1001767. doi: 10.1371/journal.pbio.1001767

da Silva, M. C., Conti, C. L., Klauss, J., Alves, L. G., do Nascimento Cavalcante, H. M., Fregni, F., et al. (2013). Behavioral effects of transcranial direct current stimulation (tDCS) induced dorsolateral prefrontal cortex plasticity in alcohol dependence. *J. Physiol. Paris* 107, 493–502. doi: 10.1016/j.jphysparis.2013.07.003

Davis, C., and Carter, J. C. (2009). Compulsive overeating as an addiction disorder. A review of theory and evidence. *Appetite* 53, 1–8. doi: 10.1016/j.appet.2009.05.018

de Tommaso, M., Invitto, S., Ricci, K., Lucchese, V., Delussi, M., Quattromini, P., et al. (2014). Effects of anodal TDCS stimulation of left parietal cortex on visual spatial attention tasks in men and women across menstrual cycle. *Neurosci. Lett.* 574, 21–25. doi: 10.1016/j.neulet.2014.05.014

Ekici, B. (2015). Transcranial direct current stimulation-induced seizure: analysis of a case. *Clin. EEG Neurosci.* 46, 169. doi: 10.1177/1550059414540647

Elmasry, J., Loo, C., and Martin, D. (2015). A systematic review of transcranial electrical stimulation combined with cognitive training. *Restor. Neurol. Neurosci.* 33, 263–278. doi: 10.3233/RNN-140473

Farré, J. M., Fernández-Aranda, F., Granero, R., Aragay, N., Mallorquí-Bague, N., Ferrer, V., et al. (2015). Sex addiction and gambling disorder: similarities and differences. *Compr. Psychiatry* 56, 59–68. doi: 10.1016/j.comppsych.2014.10.002

Fecteau, S., Agosta, S., Hone-Blanchet, A., Fregni, F., Boggio, P., Ciraulo, D., et al. (2014). Modulation of smoking and decision-making behaviors with transcranial direct current stimulation in tobacco smokers: a preliminary study. *Drug Alcohol Depend.* 140, 78–84. doi: 10.1016/j.drugalcdep.2014.03.036

Fecteau, S., Fregni, F., Boggio, P. S., Camprodon, J. A., and Pascual-Leone, A. (2010). Neuromodulation of decision-making in the addictive brain. *Subst. Use Misuse* 45, 1766–1786. doi: 10.3109/10826084.2010.482434

Fecteau, S., Knoch, D., Fregni, F., Sultani, N., Boggio, P., and Pascual-Leone, A. (2007a). Diminishing risk-taking behavior by modulating activity in the prefrontal cortex: a direct current stimulation study. *J. Neurosci.* 27, 12500–12505. doi: 10.1523/JNEUROSCI.3283-07.2007

Fecteau, S., Pascual-Leone, A., Zald, D. H., Liguori, P., Théoret, H., Boggio, P. S., et al. (2007b). Activation of prefrontal cortex by transcranial direct current stimulation reduces appetite for risk during ambiguous decision making. *J. Neurosci.* 27, 6212–6218. doi: 10.1523/JNEUROSCI.0314-07.2007

Feil, J., and Zangen, A. (2010). Brain stimulation in the study and treatment of addiction. *Neurosci. Biobehav. Rev.* 34, 559–574. doi: 10.1016/j.neubiorev.2009.11.006

Fernández-Serrano, M. J., Pérez-García, M., Perales, J. C., and Verdejo-García, A. (2010). Prevalence of executive dysfunction in cocaine, heroin and alcohol usersenrolled in therapeutic communities. *Eur. J. Pharmacol.* 626, 104–112 doi: 10.1016/j.ejphar.2009.10.019

Fox, P., Ingham, R., George, M. S., Mayberg, H., Ingham, J., Roby, J., et al. (1997). Imaging human intra-cerebral connectivity by PET during *TMS Neuroreport* 8, 2787–2791. doi: 10.1097/00001756-199708180-00027

Frank, G. K., Shott, M. E., Hagman, J. O., and Mittal, V. A. (2013). Alterations in brain structures related to taste reward circuitry in ill and recovered anorexia nervosa and in bulimia nervosa. *Am. J. Psychiatry* 170, 1152–1160. doi: 10.1176/appi.ajp.2013.12101294

Fregni, F., Liguori, P., Fecteau, S., Nitsche, M. A., Pascual-Leone, A., and Boggio, P. S. (2008a). Cortical stimulation of the prefrontal cortex with transcranial direct current stimulation reduces cue-provoked smoking craving: a randomized, sham-controlled study. *J. Clin. Psychiatry* 69, 32–40. doi: 10.4088/JCP.v69n0105

Fregni, F., Orsati, F., Pedrosa, W., Fecteau, S., Tome, F. A. M., Nitsche, M. A., et al. (2008b). Transcranial direct current stimulation of the prefrontal cortex modulates the desire for specific foods. *Appetite* 51, 34–41. doi: 10.1016/j.appet.2007.09.016

Friederich, H. C., Wu, M., Simon, J. J., and Herzog, W. (2013). Neurocircuit function in eating disorders. *Int. J. Eat. Disord.* 46, 425–432. doi: 10.1002/eat.22099

Gandiga, P. C., Hummel, F. C., and Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin. Neurophysiol.* 117, 845–850. doi: 10.1016/j.clinph.2005.12.003

García-García, I., Horstmann, A., Jurado, M. A., Garolera, M., Chaudhry, S. J., Margulies, D. S., et al. (2014). Reward processing in obesity, substance addiction and non-substance addiction. *Obes. Rev.* 15, 853–869. doi: 10.1111/obr.12221

Gaudio, S., and Quattrocchi, C. C. (2012). Neural basis of a multidimensional model of body image distortion in anorexia nervosa. *Neurosci. Biobehav. Rev.* 36, 1839–1847. doi: 10.1016/j.neubiorev.2012.05.003

Gearhardt, A. N., White, M. A., and Potenza, M. N. (2011). Binge eating disorder and food addiction. *Curr. Drug Abuse Rev.* 4, 201–207. doi: 10.2174/1874473711104030201

Goldman, R. L., Borckardt, J. J., Frohman, H. A., O'Neil, P. M., Madan, A., Campbell, L. K., et al. (2011). Prefrontal cortex transcranial direct current stimulation (tDCS) temporarily reduces food cravings and increases the self-reported ability to resist food in adults with frequent food craving. *Appetite* 56, 741–746. doi: 10.1016/j.appet.2011.02.013

Goldstein, R. Z., and Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am. J. Psychiatry* 159, 1642–1652. doi: 10.1176/appi.ajp.159.10.1642

Goudriaan, A. E., de Ruiter, M. B., van den Brink, W., Oosterlaan, J., and Veltman, D. J. (2012). Brain activation patterns associated with cue reactivity and craving in abstinent problem gamblers, heavy smokers and healthy controls: an fMRI study. *Addict. Biol.* 15, 491–503. doi: 10.1111/j.1369-1600.2010.00242.x

Grall-Bronnec, M., and Sauvaget, A. (2014). The use of repetitive transcranial magnetic stimulation for modulating craving and addictive behaviours: a critical literature review of efficacy, technical and methodological considerations. *Neurosci. Biobehav. Rev.* 47C, 592–613. doi: 10.1016/j.neubiorev.2014.10.013

Hanlon, C. A., Canterberry, M., Taylor, J. J., DeVries, W., Li, X., Brown, T. R., et al. (2013). Probing the frontostriatal loops involved in executive and limbic processing via interleaved TMS and functional MRI at two prefrontal locations: a pilot study. *PLoS ONE* 8:e67917. doi: 10.1371/journal.pone.0067917

Hayashi, T., Ko, J. H., Strafella, A. P., and Dagher, A. (2013). Dorsolateral prefrontal and orbitofrontal cortex interactions during self-control of cigarette craving. *Proc. Natl. Acad. Sci. U.S.A.* 110, 4422–4427. doi: 10.1073/pnas.1212185110

Hebebrand, J., Albayrak, Ö., Adan, R., Antel, J., Dieguez, C., de Jong, J., et al. (2014). "Eating addiction", rather than "food addiction," better captures addictive-like eating behavior. *Neurosci. Biobehav. Rev.* 47, 295–306. doi: 10.1016/j.neubiorev.2014.08.016

Hecht, D. (2010). Transcranial direct current stimulation in the treatment of anorexia. *Med. Hypotheses* 74, 1044–1047. doi: 10.1016/j.mehy.2009.12.032

Higgins, E. S., and George, M. S. (2009). *Brain Stimulation Therapies for Clinicians. 1st Edn.* Arlington, TX: APPI.

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

Jansen, J. M., Daams, J. G., Koeter, M. W. J., Veltman, D. J., van den Brink, W., and Goudriaan, A. E. (2013). Effects of non-invasive neurostimulation on craving: a meta-analysis. *Neurosci. Biobehav. Rev.* 37, 2472–2480. doi: 10.1016/j.neubiorev.2013.07.009

Jauch-Chara, K., Kistenmacher, A., Herzog, N., Schwarz, M., Schweiger, U., and Oltmanns, K. M. (2014). Repetitive electric brain stimulation reduces food intake in humans. *Am. J. Clin. Nutr.* 100, 1003–1009. doi: 10.3945/ajcn.113.075481

Jiménez-Murcia, S., Granero, R., Moragas, L., Steiger, H., Israel, M., Aymamí, N., et al. (2015). Differences and similarities between bulimia nervosa, compulsive buying and gambling disorder. *Eur. Eat. Disord. Rev.* 23, 111–118.

Kasuga, S., Matsushika, Y., Kasashima-Shindo, Y., Kamatani, D., Fujiwara, T., Liu, M., et al. (2015). Transcranial direct current stimulation enhances mu rhythm desynchronization during motor imagery that depends on handedness. *Laterality* 20, 453–468. doi: 10.1080/1357650X.2014.998679

Kaye, W. H., Fudge, J. L., and Paulus, M. (2009). New insights into symptoms and neurocircuit function of anorexia nervosa. *Nat. Rev. Neurosci.* 10, 573–584. doi: 10.1038/nrn2682

Keck, M. E., Welt, T., Müller, M. B., Erhardt, A., Ohl, F., Toschi, N., et al. (2002). Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. *Neuropharmacology* 43, 101–109. doi: 10.1016/S0028-3908(02)00069-2

Kekic, M., McClelland, J., Campbell, I., Nestler, S., Rubia, K., David, A. S., et al. (2014). The effects of prefrontal cortex transcranial direct current stimulation (tDCS) on food craving and temporal discounting in women with frequent food cravings. *Appetite* 78, 55–62. doi: 10.1016/j.appet.2014.03.010

Khedr, E. M., Elfetoh, N. A., Ali, A. M., and Noamany, M. (2014). Anodal transcranial direct current stimulation over the dorsolateral prefrontal cortex improves anorexia nervosa: a pilot study. *Restor. Neurol. Neurosci.* 32, 789–797. doi: 10.3233/RNN-140392

Knoch, D., Nitsche, M. A., Fischbacher, U., Eisenegger, C., Pascual-Leone, A., and Fehr, E. (2008). Studying the neurobiology of social interaction with transcranial direct current stimulation–the example of punishing unfairness. *Cereb. Cortex* 18, 1987–1990. doi: 10.1093/cercor/bhm237

Kuo, M.-F., Paulus, W., and Nitsche, M. A. (2014). Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 85(Pt 3), 948–960. doi: 10.1016/j.neuroimage.2013.05.117

Lapenta, O. M., Sierve, K., de Macedo, E. C., Fregni, F., and Boggio, P. S. (2014). Transcranial direct current stimulation modulates ERP-indexed inhibitory control and reduces food consumption. *Appetite* 83, 42–48. doi: 10.1016/j.appet.2014.08.005

Leach, J. P., Mohanraj, R., and Borland, W. (2012). Alcohol and drugs in epilepsy: pathophysiology, presentation, possibilities, and prevention. *Epilepsia* 53(Suppl. 4), 48–57. doi: 10.1111/j.1528-1167.2012.03613.x

Lester, N. A., Keel, P. K., and Lipson, S. F. (2003). Symptom fluctuation in bulimia nervosa: relation to menstrual-cycle phase and cortisol levels. *Psychol. Med.* 33, 51–60. doi: 10.1017/S0033291702006815

Marazziti, D., Presta, S., Baroni, S., Silvestri, S., and Dell'Osso, L. (2014). Behavioral addictions: a novel challenge for psychopharmacology. *CNS Spectr.* 19, 486–495. doi: 10.1017/S1092852913001041

McClelland, J., Bozhilova, N., Campbell, I., and Schmidt, U. (2013). A systematic review of the effects of neuromodulation on eating and body weight: evidence from human and animal studies. *Eur. Eat. Disord. Rev.* 21, 436–455. doi: 10.1002/erv.2256

Mitchison, D., and Hay, P. J. (2014). The epidemiology of eating disorders: genetic, environmental, and societal factors. *Clin. Epidemiol.* 6, 89–97. doi: 10.2147/CLEP.S40841

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J. Clin. Epidemiol.* 62, 1006–1012. doi: 10.1016/j.jclinepi.2009.06.005

Montenegro, R. A., Okano, A. H., Cunha, F. A., Gurgel, J. L., Fontes, E. B., and Farinatti, P. T. V. (2012). Prefrontal cortex transcranial direct current stimulation associated with aerobic exercise change aspects of appetite sensation in overweight adults. *Appetite* 58, 333–338. doi: 10.1016/j.appet.2011.11.008

Naim-Feil, J., and Zangen, A. (2013). Addiction. *Handb. Clin. Neurol.* 116, 613–630. doi: 10.1016/B978-0-444-53497-2.00049-8

Nasseri, P., Nitsche, M. A., and Ekhtiari, H. (2015). A framework for categorizing electrode montages in transcranial direct current stimulation. *Front. Hum. Neurosci.* 9:54. doi: 10.3389/fnhum.2015.00054

Neufang, S., Specht, K., Hausmann, M., Güntürkün, O., Herpertz-Dahlmann, B., Fink, G. R., et al. (2009). Sex differences and the impact of steroid hormones on the developing human brain. *Cereb. Cortex* 19, 464–473. doi: 10.1093/cercor/bhn100

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., 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

O'Brien, C. (2011). Addiction and dependence in DSM-V. *Addiction* 106, 866–867. doi: 10.1111/j.1360-0443.2010.03144.x

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

Potenza, M. N. (2014). Non-substance addictive behaviors in the context of DSM-5. *Addict. Behav.* 39, 1–2. doi: 10.1016/j.addbeh.2013.09.004

Pripfl, J., Neumann, R., Köhler, 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

Russell, M., Goodman, T., Wang, Q., Groshong, B., and Lyeth, B. G. (2014). Gender differences in current received during transcranial electrical stimulation. *Front. Psychiatry* 5:104. doi: 10.3389/fpsyt.2014.00104

Russell, M. J., Goodman, T., Pierson, R., Shepherd, S., Wang, Q., Groshong, B., et al. (2013). Individual differences in transcranial electrical stimulation current density. *J. Biomed. Res.* 27, 495–508. doi: 10.7555/JBR.27.20130074

Schulte, E. M., Avena, N. M., and Gearhardt, A. N. (2015). Which foods may be addictive? The roles of processing, fat content, and glycemic load. *PLoS ONE* 10:e0117959. doi: 10.1371/journal.pone.0117959

Shahid, S., Wen, P., and Ahfock, T. (2014). Assessment of electric field distribution in anisotropic cortical and subcortical regions under the influence of tDCS. *Bioelectromagnetics* 35, 41–57. doi: 10.1002/bem.21814

Shiozawa, P., Fregni, F., Benseñor, I. M., Lotufo, P. A., Berlim, M. T., Daskalakis, J. Z., et al. (2014). Transcranial direct current stimulation for major depression: an updated systematic review and meta-analysis. *Int. J. Neuropsychopharmacol.* 17, 1443–1452. doi: 10.1017/S1461145714000418

Skinner, M. D., and Aubin, H.-J. (2010). Craving's place in addiction theory: contributions of the major models. *Neurosci. Biobehav. Rev.* 34, 606–623. doi: 10.1016/j.neubiorev.2009.11.024

Sperling, W., Frank, H., Martus, P., Mader, R., Barocka, A., Walter, H., et al. (2000). The concept of abnormal hemispheric organization in addiction research. *Alcohol Alcohol.* 35, 394–399. doi: 10.1093/alcalc/35.4.394

Tortella, G., Casati, R., Aparicio, L. V. M., Mantovani, A., Senço, N., D'Urso, G., et al. (2015). Transcranial direct current stimulation in psychiatric disorders. *World J. Psychiatry* 5, 88–102. doi: 10.5498/wjp.v5.i1.88

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

Uher, R., and Treasure, J. (2005). Brain lesions and eating disorders. *J. Neurol. Neurosurg. Psychiatr.* 76, 852–857. doi: 10.1136/jnnp.2004.048819

Val-Laillet, D., Aarts, E., Weber, B., Ferrari, M., Quaresima, V., Stoeckel, L., et al. (2015). Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. *Neuroimage Clin.* 8, 1–31. doi: 10.1016/j.nicl.2015.03.016

Van den Eynde, F., Broadbent, H., Guillaume, S., Claudino, A., Campbell, I. C., and Schmidt, U. (2012). Handedness, repetitive transcranial magnetic stimulation and bulimic disorders. *Eur. Psychiatry* 27, 290–293. doi: 10.1016/j.eurpsy.2010.08.015

van Kuyck, K., Gérard, N., van Laere, K., Casteels, C., Pieters, G., Gabriëls, L. et al. (2009). Towards a neurocircuitry in anorexia nervosa: evidence from functional neuroimaging studies. *J. Psychiatr. Res.* 43, 1133–1145. doi: 10.1016/j.jpsychires.2009.04.005

von Hausswolff-Juhlin, Y., Brooks, S. J., and Larsson, M. (2015). The neurobiology of eating disorders–a clinical perspective. *Acta Psychiatr. Scand.* 131, 244–255. doi: 10.1111/acps.12335

WHO. (2015). *WHO*. Available online at: http://www.who.int/topics/research/en/ (Retrieved May 24, 2015).

Wolz, I., Fagundo, A. B., Treasure, J., and Fernández-Aranda, F. (2015). The processing of food stimuli in abnormal eating: a systematic review of electrophysiology. *Eur. Eat. Disord. Rev.* 23, 251–261. doi: 10.1002/erv.2366

Yi, X., Fisher, K. M., Lai, M., Mansoor, K., Bicker, R., and Baker, S. N. (2014). Differences between Han Chinese and Caucasians in transcranial magnetic stimulation parameters. *Exp. Brain Res.* 232, 545–553. doi: 10.1007/s00221-013-3763-2

Ziad, N. (2002). *Handbook of Transcranial Magnetic Stimulation*. New York, NY: Oxford University Press Inc.

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 Neuroscience

REVIEW
published: 19 January 2016
doi: 10.3389/fnins.2015.00515

Non-invasive Brain Stimulation and Auditory Verbal Hallucinations: New Techniques and Future Directions

Peter Moseley[1], Ben Alderson-Day[2], Amanda Ellison[2], Renaud Jardri[3] and Charles Fernyhough[2]*

[1] School of Psychology, University of Central Lancashire, Preston, UK, [2] Science Laboratories, Department of Psychology, Durham University, Durham, UK, [3] Centre National de la Recherche Scientifique UMR-9193, SCA-Lab & CHU Lille, Fontan Hospital, CURE Platform, Lille University, Lille, France

Auditory verbal hallucinations (AVHs) are the experience of hearing a voice in the absence of any speaker. Results from recent attempts to treat AVHs with neurostimulation (rTMS or tDCS) to the left temporoparietal junction have not been conclusive, but suggest that it may be a promising treatment option for some individuals. Some evidence suggests that the therapeutic effect of neurostimulation on AVHs may result from modulation of cortical areas involved in the ability to monitor the source of self-generated information. Here, we provide a brief overview of cognitive models and neurostimulation paradigms associated with treatment of AVHs, and discuss techniques that could be explored in the future to improve the efficacy of treatment, including alternating current and random noise stimulation. Technical issues surrounding the use of neurostimulation as a treatment option are discussed (including methods to localize the targeted cortical area, and the state-dependent effects of brain stimulation), as are issues surrounding the acceptability of neurostimulation for adolescent populations and individuals who experience qualitatively different types of AVH.

Keywords: hallucinations, neurostimulation, neuronavigation, state dependency, transcranial random noise stimulation (tRNS), transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), transcranial magnetic stimulation (TMS)

OPEN ACCESS

Edited by:
Antonio Oliviero, Hospital Nacional de Paraplejicos, Spain

Reviewed by:
Samir Kumar-Singh, University of Antwerp, Belgium
Luis Caballero, HM Hospitales Madrid, Spain

****Correspondence:***
Peter Moseley
pmoseley@uclan.ac.uk

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

Received: *07 August 2015*
Accepted: *22 December 2015*
Published: *19 January 2016*

Citation:
Moseley P, Alderson-Day B, Ellison A, Jardri R and Fernyhough C (2016) Non-invasive Brain Stimulation and Auditory Verbal Hallucinations: New Techniques and Future Directions. Front. Neurosci. 9:515. doi: 10.3389/fnins.2015.00515

INTRODUCTION

Auditory verbal hallucinations (AVHs) are the experience of hearing a voice in the absence of any speaker (Aleman and Larøi, 2008). They are commonly associated with a diagnosis of schizophrenia, but also occur in other psychiatric diagnoses such as bipolar disorder and post-traumatic stress disorder (Larøi et al., 2012), as well as in individuals with no psychiatric diagnosis (Beavan et al., 2011; Johns et al., 2014). Evidence from cognitive neuroscience suggests that AVHs are accompanied by high levels of activation in, among other areas, the superior temporal gyrus, particularly in the left hemisphere (Allen et al., 2008; Jardri et al., 2011). Recent attempts to provide novel treatment options for individuals experiencing AVHs have accordingly attempted to use neurostimulation techniques to selectively decrease activity in temporal cortical regions, with a moderate degree of success (Hoffman et al., 2005, 2013; Slotema et al., 2013).

AVHs have been theoretically linked to atypical functioning of inner speech processes, with the most prominent model suggesting that atypical self-monitoring or reality monitoring may lead to a lack of agency over self-generated language processes (Frith, 1992; Jones and Fernyhough, 2007). Evidence from cognitive psychology suggests that individuals with a diagnosis of schizophrenia

who experience AVHs, compared to individuals with the same diagnosis who do not experience AVHs, and to healthy controls, are more likely to misattribute self-generated speech in source memory tasks (Stephane et al., 2010) or signal detection tasks (Bentall and Slade, 1985; Brookwell et al., 2013). This is consistent with fMRI research showing that superior temporal cortical regions show high levels of activation both during AVHs (Allen et al., 2008; Jardri et al., 2011) and purposely generated inner speech (Simons et al., 2010). Furthermore, evidence from EEG studies suggests that self- and non-self-generated vocalizations are processed differently in the auditory cortex of healthy, non-hallucinating individuals, as indexed by the N1 event-related potential. This difference was not evident in a sample of individuals with a diagnosis of schizophrenia (Ford et al., 2001; Ford and Mathalon, 2005). These findings have been interpreted as evidence for atypical functioning of forward model mechanisms that usually predict the sensory consequences of self-generated actions. This "efference copy" mechanism acts to attenuate cortical activity in sensory regions resulting from the action, contributing to those actions being experienced as self- or non-self-generated. As such, it has previously been suggested that targeting the left temporoparietal junction (TPJ) or posterior superior temporal gyrus (STG) with neurostimulation may have therapeutic potential because it affects cortical regions involved in the prediction/subsequent sensory attenuation of self-generated actions, such as inner speech (Moseley et al., 2013).

This paper aims to provide a short overview of contemporary research into the efficacy of neurostimulation as a treatment option for AVHs, but also to build upon previous reviews (e.g., Montagne-Larmurier et al., 2011; Moseley et al., 2013) by discussing a number of avenues for future research. In particular, the therapeutic potential of two recently developed techniques, transcranial alternating current stimulation (tACS), and transcranial random noise stimulation (tRNS), are discussed, and it is also suggested that an important line of research may be to maximize efficacy of treatment by utilizing the state dependency of the effects of neurostimulation (i.e., to harness the possibility that neurostimulation may have different effects on cortical excitability levels depending on the state of the brain when it is applied). Furthermore, we discuss a number of technical issues surrounding the use of neurostimulation techniques, such as the most efficient methods for localizing stimulation, and issues surrounding the acceptability and tolerability of neurostimulation in adolescent patients, and for different subtypes of AVH.

TMS AND tDCS AS TREATMENT OPTIONS FOR AUDITORY VERBAL HALLUCINATIONS

Repetitive transcranial magnetic stimulation (rTMS), a non-invasive brain stimulation technique that uses a rapidly changing magnetic field to induce an electrical current in selective cortical regions (Hallett, 2007), has recently shown promise as a treatment option for various neurological disorders such as post-stroke neglect (Cazzoli et al., 2010) or aphasia (Naeser et al., 2010), and psychiatric disorders such as depression (George et al., 1995, 2010). The rationale underlying treatment is that, dependent on the frequency of the repetitive pulses, activity in specific brain regions (or networks of regions) which may be associated with certain disorders can be increased or decreased (Maeda et al., 2000). First tested as a treatment option for AVHs by Hoffman et al. (1999, 2005), low frequency (1 Hz) rTMS over the left temporoparietal junction (TPJ) was employed in a sample of 50 patients with a diagnosis of schizophrenia who hallucinated, in order to reduce cortical activity in this area. Patients received active stimulation or sham stimulation (a control condition in which the participant is led to believe they are receiving TMS, but no stimulation is applied) each day for 15 min, for a total of 9 days, in a parallel design. Using patient-generated narrative reports to create an "Hallucination Change Score" and self-report clinical scales measuring hallucination frequency, vividness, loudness, and attentional salience, it was demonstrated that active rTMS significantly reduced scores, compared to the sham condition. 51.9% of participants in the active condition were classified as "responders" to the treatment (showing a decrease of $\geq$ 5 on the Hallucination Change Score), compared to 17.4% in the sham condition.

This initial finding has subsequently been replicated in a number of studies (e.g., Lee et al., 2005; Vercammen et al., 2009; Hoffman et al., 2013), although there are also numerous studies that have not shown a significant effect of low frequency rTMS to the left TPJ on AVH frequency (e.g., McIntosh et al., 2004; de Jesus et al., 2011). Notably, in two of the largest single trials of rTMS efficacy for treating AVHs, Slotema et al. (2011) found no effect of active rTMS, compared to sham rTMS, whilst Koops et al. (2016) found no evidence of efficacy of theta-burst rTMS (consisting of a pattern of pulses thought to have a stronger inhibitory effect) in reduction of AVH frequency, compared to sham. Nevertheless, meta-analyses of studies that have tested therapeutic efficacy of low frequency rTMS on AVHs indicate that it may be effective, with a moderate effect size (Demeulemeester et al., 2012; Slotema et al., 2012, 2013). Given that these meta-analyses suggest an overall effect size of approximately 0.4 on AVH frequency, it is possible that, despite being one of the larger published trials in this area, Slotema et al.'s negative finding may reflect a lack of statistical power.

Studies that have used fMRI to investigate the effects of low frequency rTMS to the left TPJ have shown that a reduction in activity in the left STG is associated with a reduction in AVHs (although there was also a decrease in activity in the left inferior frontal gyrus and anterior cingulate cortex in the active stimulation condition, compared to the sham condition) (Kindler et al., 2013). High levels of activity in the left STG also appears to be a marker for a response to rTMS treatment for AVHs (Homan et al., 2012). Although it seems to be a promising treatment option, further refinement of the technique is needed to establish efficacy; for example, differences in the sham condition and localization techniques used may partially explain inconsistent findings in the literature (see Section Issues with Localization of Targeted Regions).

More recently, transcranial direct current stimulation (tDCS) has also been tested for therapeutic efficacy with AVHs. tDCS involves passing a weak electrical current between two electrodes placed on the scalp, which, dependent on the direction of current flow, depolarizes, or hyperpolarises neuronal membrane potentials. This increases the cortical excitability underneath the anodal electrode and decreases cortical excitability underneath the cathodal electrode (Nitsche and Paulus, 2000; Nitsche et al., 2008). Importantly, the effects of tDCS on cortical excitability may last longer than the period of stimulation, probably mediated by GABAergic and glutamatergic mechanisms (Stagg and Nitsche, 2011). The first use of tDCS as a treatment for AVH was presented in a case report by Homan et al. (2011), in which cathodal stimulation over a posterior STG region was combined with the anodal electrode placed over the right supraorbital cortex. Homan and colleagues reported improvements in hallucination symptoms and reductions in cerebral blood flow in left frontal and temporal regions in a man with persistent, treatment-resistant AVH following 10 days of 1 mA tDCS sessions.

Following this, Brunelin et al. (2012) tested the efficacy of cathodal tDCS (at a strength of 2 mA) to the left TPJ in reducing the frequency of AVHs. Thirty patients with a diagnosis of schizophrenia who hallucinated received tDCS twice a day for five consecutive days, with half receiving active stimulation and half receiving sham stimulation in a parallel design. The cathodal electrode was positioned over the left TPJ, and the anodal electrode over the left dorsolateral prefrontal cortex. Results indicated that active stimulation was associated with a significant decrease in self-reported AVH severity, which was maintained over a 3 month period. There have been comparatively fewer sham-controlled studies utilizing tDCS than rTMS (and therefore not a sufficient number for meta-analysis), and results have been somewhat equivocal, with one study replicating Brunelin et al.'s finding (Mondino et al., 2015) and one study showing no effect of active tDCS, compared to a sham condition (Fitzgerald et al., 2014). If effective, though, tDCS is more tolerable, simpler to apply, and cheaper than rTMS, and so further investigation is needed to test efficacy in larger samples.

The majority of studies testing the efficacy of neurostimulation techniques for AVHs have assessed severity of AVHs in individuals with a diagnosis of schizophrenia or schizoaffective disorder using relatively simple questionnaire measures, most commonly the Auditory Hallucinations Rating Scale (AHRS). The AHRS is a seven-item scale that assesses number of voices experienced, as well as voice frequency, loudness, vividness, attentional salience, length, and distress caused, and has shown acceptable levels of internal consistency, test-retest reliability, and inter-rater reliability (Hoffman et al., 2005). Patients typically complete the AHRS before the first treatment session, after 5–10 sessions of treatment, and, in some studies, up to 3 months later (e.g., Brunelin et al., 2012). Of course, quantifying the success or failure of treatment using this relatively simple measure may exclude observation of other interesting changes that may be of clinical relevance (see Thomas, 2015, for a similar critique of trials of cognitive behavioral therapy for psychosis). As will be argued below, careful attention to phenomenological properties of AVHs will be an important step in fully understanding any therapeutic effect of neurostimulation.

ALTERNATIVE NEUROSTIMULATION TECHNIQUES

Transcranial Alternating Current Stimulation (tACS)

The recently-developed technique of tACS uses a sine-wave electric field to affect oscillatory activity in stimulated regions. tACS works on a similar premise to tDCS, by changing the membrane voltages of underlying neurons, hence depolarizing or hyperpolarizing neurons in specific cortical regions. Unlike tDCS, the sine-wave field, theoretically at least, leads to entrainment of a pattern of oscillatory activity at the frequency of stimulation. Research using this technique is still in its early stages, although studies using electroencephalography (EEG) and tACS have suggested that stimulating in the alpha frequency band (8–12 Hz) can lead to enhancement of oscillatory activity at that frequency (Zaehle et al., 2010; Helfrich et al., 2014). Initial research also suggests that frequency-specific stimulation has the potential to affect cognitive task performance. For example, based on previous literature implicating theta frequency oscillations (4–7 Hz) in dorsolateral prefrontal cortex during working memory tasks, Meiron and Lavidor (2014) showed that stimulation at a frequency in the theta band improved performance on an n-back task.

The use of tACS also has the potential for tailoring the frequency of stimulation based on individual oscillatory activity using EEG. This was recently demonstrated by Vosskuhl et al. (2015), who used EEG directly before task performance to determine individual theta frequency, then stimulated at a slightly lower frequency in an attempt to modulate the ratio between theta and gamma (>30 Hz) oscillations in prefrontal cortex. Using this methodology, they showed an improvement in short-term memory performance during stimulation. These studies demonstrate the potential of tACS to affect complex network dynamics by subtly altering ongoing oscillations. It has therefore been suggested that tACS may be a promising therapeutic technique if utilized to alter atypical patterns of oscillatory activity in psychiatric disorders.

Atypical cortical oscillatory activity in the beta (12–30 Hz) and gamma frequencies have been linked to schizophrenia (Uhlhaas and Singer, 2010). Synchronous neural activity is thought to be one way in which disparate neural assemblies communicate and are identified as part of the same functional network (Singer, 1999; Ford et al., 2007a), and as such are likely to play a key role in sensorimotor predictive mechanisms that operate across different brain regions (which, as discussed earlier, are implicated in the genesis of auditory verbal hallucinations). Using EEG, Ford et al. (2007b) have shown lower levels of temporal coherence (a measure of neural synchrony across time) directly before speech in patients with a diagnosis of schizophrenia, which was also associated specifically with the reported severity of auditory hallucinations. Furthermore, in a separate study, Ford et al. (2008) showed that gamma synchrony

before a simple motor action was both associated with the size of the subsequent somatosensory event related potential (ERP), and deficient in patients with a diagnosis of schizophrenia. A later study using electrocorticography also indicated that pre-speech gamma synchrony between Broca's area and auditory cortex was associated with the size of subsequent event related potentials (Chen et al., 2011). Attenuation of the N1 ERP has been hypothesized to reflect the functioning of a forward model system which normally predicts the sensory consequences of self-generated actions, and lack of N1 attenuation in response to self-generated actions have previously been associated with schizophrenia (Ford and Mathalon, 2005).

A handful of studies have highlighted either state or trait relations between AVH and neural oscillations. Using symptom-capture measures in which MEG was used whilst participants experienced AVHs, van Lutterveld et al. (2012) observed that a decrease in beta power in the left STG and MTG was associated with hallucination onset (van Lutterveld et al., 2012). An earlier EEG study by Sritharan et al. (2005) also indicated that occurrence of AVH was linked to an increase in alpha coherence (i.e., synchronization) between the left and right auditory cortices. The tendency to experience AVH, meanwhile, has been correlated with auditory steady-state power in the gamma range for left auditory cortex (Spencer et al., 2009) and gamma synchronization between the auditory cortices (Mulert et al., 2011) in people with schizophrenia. As is often the case, however, it is not clear whether differences in power and coherence observed in these studies reflect a cause or effect of the hallucinatory experience.

The literature surrounding the use of tACS as a treatment option for psychiatric disorders is sparse, but Fröhlich et al. (2015) suggest that it should be tested in clinical trials to reduce symptoms known to be associated with atypical oscillatory activity. One avenue of inquiry could be to investigate the therapeutic potential of tACS to entrain or enhance oscillatory activity in patients with auditory verbal hallucinations. For example, stimulating with scalp electrodes placed over inferior frontal and superior temporal areas may be capable of enhancing gamma synchrony between these areas, which, as described above, could improve functioning of forward model systems which ultimately contribute to experiencing (inner) speech as self-generated. Further, comparing the effects of stimulating at different frequencies (i.e., beta and gamma band) could provide information relating to the causal role oscillations in different frequency bands may play in the genesis of AVH.

Drawing on the described research, a number of testable hypotheses can be made regarding the effect of modulating oscillatory activity in patients experiencing AVHs. Firstly, it would be predicted that stimulating frontal and temporal regions in the gamma frequency band would entrain oscillatory activity, decreasing the difference in gamma synchrony between patient and control samples. Secondly, it would be predicted that gamma entrainment would lead to increased sensory attenuation of the N1 ERP in response to self-generated speech. Thirdly, this should be associated with a reduction in the frequency of AVHs.

There are, though, a number of possible issues with using tACS therapeutically. As yet, the length of any after-effects of tACS are unknown. Helfrich et al. (2014) used tACS and EEG simultaneously, showing that stimulation in the alpha frequency range entrained oscillations to the precise frequency of stimulation, but that this effect did not last past the cessation of stimulation. This implies that tACS may not be ideal as a therapeutic tool; however, this study did not use daily stimulation sessions, so it is unclear whether lasting effects would be possible if tACS was used over a 10 day period, as is typical of therapeutic trials using neurostimulation. Future studies should monitor after-effects of tACS using EEG, when used over repeated sessions. This information will be crucial before tACS is tested in a clinical context.

A further question mark over the use of tACS relates to findings indicating that effects may be highly dependent on the state of the brain before stimulation. Feurra et al. (2011) showed that motor cortex excitability (as measured by motor evoked potentials) was increased during beta frequency tACS, inferring that beta oscillations play a causal role in corticospinal excitability. Further work showed that this effect was abolished if the participant was engaged in motor imagery during stimulation; in these conditions, theta frequency stimulation was the only frequency under which motor cortical excitability was increased (Feurra et al., 2013). This is potentially important in the application of tACS to auditory cortical regions, the effects of which could plausibly be modulated by the use of auditory mental imagery and inner speech (both of which may be linked to the occurrence of AVH). The issue of state-dependency is returned to in Section State Dependent Effects of Neurostimulation, below.

Transcranial Random Noise Stimulation (tRNS)

tRNS is a variant of tACS which also uses a constantly changing current. Whilst tACS stimulates at a set frequency, aiming to entrain oscillatory activity, tRNS stimulates at a randomly changing frequency, usually between 0.1–640 Hz. It has been suggested that tRNS at higher frequencies (>100 Hz) may induce larger excitability changes than stimulating with a direct current (as in tDCS), because the sodium channels of underlying neurons are repeatedly opened by stimulation, and because neuronal homeostatic mechanisms are prevented (i.e., underlying neurons cannot adjust to the constantly randomly changing electrical field; Fertonani et al., 2011). Terney et al. (2008) were the first to demonstrate that tRNS, applied to the motor cortex, increased cortical excitability (as measured by motor evoked potential) and improved performance on a serial reaction time task (associated with implicit motor learning). Fertonani et al. (2011) have also demonstrated that, applied over primary visual cortex, tRNS can improve perceptual learning (as measured by performance on an orientation discrimination task) at a greater rate than tDCS or sham stimulation, whilst tRNS to primary auditory cortex is capable of affecting the auditory steady-state response (Van Doren et al., 2014). Interestingly, Fertonani et al.'s findings suggested a stronger effect when the frequencies were restricted to between 100–640 Hz (compared to < 100 Hz). The authors interpreted this as supporting the argument that a higher rate of repetitive stimulation may lead to a "temporal summation" effect not observable with constant stimulation such as with tDCS

(that is, the higher the frequency of stimulation, the more times neurons are stimulated in a short space of time, which may have a summative effect on excitability). Initial findings therefore seem to indicate that tRNS may have a larger effect than tDCS.

As a relatively new technique, there are few reports of therapeutic use of tRNS in neurological and psychiatric disorders. Vanneste et al. (2013) tested the efficacy of tRNS in treating tinnitus, comparing the effects to those of tDCS and tACS over bilateral auditory cortices. The results suggested that tRNS shows promise as a therapeutic technique, yielding larger effect sizes than the other stimulation conditions. Palm et al. (2013) reported a single case in which tRNS with a DC-offset was used over left dorsolateral prefrontal cortex (anode) and right orbitofrontal cortex (cathode) to treat negative symptoms in a 29-year old man with schizophrenia. Moderate improvements were observed in the target symptoms such as emotional withdrawal, along with some amelioration of depression and anxiety. Moreover, the treatment was deemed acceptable and incurred no side effects.

Of more relevance to the treatment of AVHs, in a case study design, Haesebaert et al. (2014) used tRNS offset by 1 mA to test efficacy and safety in the treatment of schizophrenia (including measures of hallucination frequency). The same frontotemporal electrode montage used in previous tDCS studies [see Section Transcranial Alternating Current Stimulation (tACS)] was used, with the anodal electrode placed over left prefrontal cortex and the cathodal electrode placed over the left TPJ. Although only a case study (with no control condition), Haesebaert et al. (2014) showed a decrease in positive and negative symptoms following stimulation, and demonstrated that the technique seems safe and tolerable for the patient. Indeed, two studies have reported that the tactile sensations underneath the electrodes are perceived less with tRNS than with tDCS (Ambrus et al., 2010; Fertonani et al., 2011), suggesting that this may be a preferable technique from the patient's point of view, as well as potentially enabling a more comparable sham condition. Future research should therefore test the efficacy of tRNS applied to TPJ/STG in affecting cognitive mechanisms associated with AVHs, as well as testing its therapeutic efficacy in randomized controlled trials.

TECHNICAL ISSUES

Issues with Localization of Targeted Regions

Neurostimulation treatment for AVH, applied over the left TPJ, has conventionally used the 10–20 international system, designed for EEG electrode placement, targeting the point midway between the T3 and P3 electrodes. However, one problem with the T3-P3 localization method is that it does not take into account inter-individual anatomical and functional variations, which could be one reason why neurostimulation treatment is not effective for some patients. A more pragmatic approach using an individualized strategy, using neuroimaging data to guide the treatment (neuronavigation), may be able to overcome this issue.

An illustration of how neuronavigation of the TMS coil may lead to therapeutic success in the field of AVH was first provided in a number of case reports. Langguth et al. (2006) used positron emission tomography (PET) with a patient with a diagnosis of schizophrenia, targeting the point of maximal activity in the left temporal cortex with low frequency (1 Hz) rTMS over a number of days, which was followed by a reduction in AVH frequency. Similarly, an fMRI capture of AVH in a child with early-onset schizophrenia (Jardri et al., 2007) and somatosensory hallucinations in an adult schizo-affective patient (Jardri et al., 2008) indicated that neuronavigation may be a useful strategy to localize stimulation.

Later studies have compared groups of hallucinating individuals using either 10–20 based localization methods or neuronavigation methods. In an open-label trial using fMRI whilst patients reported AVHs, rTMS sessions were performed over the individual locations of activation peaks (Sommer et al., 2007). Seven patients received fMRI-guided rTMS, compared to 6 patients treated with T3-P3 rTMS. Although, there was a significant reduction in AVH frequency over the whole sample, no advantage was identified for the neuronavigated group (which may have been linked to the lack of statistical power when comparing small samples). However, a follow-up study with 62 patients, which was split into 3 experimental arms (rTMS targeted at the area of maximal fMRI activity during AVH, rTMS directed at the left TPJ using the 10–20 system, and sham treatment), also found no difference between the localization methods (Slotema et al., 2011). On the contrary, Klirova et al. (2013) reported clinical superiority of neuronavigated rTMS over standard positioning and sham rTMS, although in a smaller sample of 15.

These findings make it difficult to draw conclusions on the effect of fMRI/PET guidance. The general linear model analysis of fMRI used in the described studies has not been shown to provide reliable results at the individual level (Foucher, 2013), and it is possible that the approach used might have been sub-optimal, especially considering recent models suggesting atypical network activity is more important in AVH genesis than any one region (Wolf et al., 2011; Ćurčić-Blake et al., 2015). In a case report providing preliminary evidence for a network approach, Jardri et al. (2009) showed that it may be possible to combine activation maps with fiber bundle tractography between activated functional regions to determine the optimal stimulation target. One of the strengths of this approach is the reference to a functional conceptual framework rather that a "lesional" one; the brain target can be defined as the best point in the network to stimulate, rather than simply expecting a change in activity in one brain region. Indeed, Kindler et al. (2013), using fMRI, showed widespread changes in superior temporal and inferior frontal regions after rTMS treatment, demonstrating wider effects on a network of regions involved in hallucinatory experiences. This functional approach is in accordance with findings regarding the propagation of the effects of rTMS in the entire functional network of a stimulated region (Siebner et al., 2003), and a randomized controlled trial is currently underway to validate such an approach (https://clinicaltrials.gov/ct2/show/NCT01373866).

State Dependent Effects of Neurostimulation

The efficacy of tDCS, tACS, and tRNS in changing behavioral outcomes has been shown over the last decade to be variable at best, and has recently been criticized as non-replicable (Horvath et al., 2015). Taking tDCS as the most widely used example, the concept of increasing or decreasing cortical excitability via anodal and cathodal stimulation is only truly valid when discussing the motor system, in which the efficacy and mechanism of tDCS was originally elucidated (Nitsche et al., 2008). The reason for this is somewhat obvious; it is easy to examine the excitability and the functional effects of this excitability using subsequent TMS and electromyography recording. There are, however, at least two problems with such a theoretical model being extended to other brain regions: firstly, motor cortex excitability may have no bearing on excitability in other regions of the cortex, particularly secondary cortex (Stewart et al., 2001), and secondly, such a model only takes into account tDCS application to resting state neurons in a neurotypical system.

These issues collectively may explain the heterogeneity in findings relating to anodal and cathodal tDCS effects on behavior in which the anodal/facilitatory, cathodal/inhibitory dichotomy often breaks down (Jacobson et al., 2012). It may be that anodal tDCS is only effective when a task is very familiar (Dockery et al., 2009) or that cathodal tDCS will only negate practice effects but not impair the processing of the task *per se* (Ball et al., 2013). Even in the motor system, voluntary motor contraction can reverse the effects of anodal and cathodal stimulation over M1 (Thirugnanasambandam et al., 2011). As mentioned above, even motor imagery will change the excitability of M1 neurons such that beta frequency tACS no longer facilitates MEPs when imagery is employed, whereas theta frequency tACS will (Feurra et al., 2013). A concurrent combination of excitability increasing events such as fast motor practice and anodal tDCS, which have the same neuronal effect, actually hinders neuroplasticity due to a non-additive mechanism in which the signal-to-noise ratio is already saturated (Bortoletto et al., 2015). In higher level cognition, it is possible to demonstrate a neutralization of the effect of anodal tDCS over left dorsolateral prefrontal cortex in executive function (Gill et al., 2015), and for complex tasks, it is not uncommon to have similar behavioral effects manifested by both anodal and cathodal tDCS albeit via different mechanisms (Miniussi et al., 2013; Knight et al., 2015).

These findings illuminate the partnership that exists between the task demands and its contingent neuronal excitability, and the effect that neurostimulation has on this network. The behavioral consequences of neurostimulation cannot be interpreted without taking these issues into account. This point is critical in the case of tDCS since it is a neuromodulatory technique, and as such can only influence the excitability of neurons, meaning that its effects are dependent on the baseline state of activity. This is in contrast to TMS which will induce action potentials in the underlying tissue (Paulus, 2011; though see below).

In addition to the more transient task related factors already discussed, the state (excitability) of the neurons to be stimulated can be modulated by steady state factors such as age or pathology. There exists recent evidence for the complex interaction between tDCS and the level of excitation in the system which is modulated by the task to affect the final behavioral outcome. Aging has been shown to change the brain both structurally and functionally leading to characteristic changes in behavior (such as visuospatial processing in which pseudoneglect, prevalent in younger adults, disappears in older samples; Benwell et al., 2015). This has been linked to changes in the dominance of hemispheres over the lifespan. However, Learmonth et al. (2015) could find no evidence that age-related changes in excitability modulated tDCS effect. Rather, the effect of tDCS in their lateralized visual detection task was state-dependent in relation to task performance at baseline, with only poor task performers being impaired by anodal tDCS over the left posterior parietal cortex. This would seem to suggest that the task's modulatory effects on neuronal excitability and its interaction with the modulatory effect of tDCS is the key, and adds further context to the contention that baseline performance in addition to practice effects (Dockery et al., 2009; Ball et al., 2013) have a role to play. To further complicate matters, there would seem to be a non-linear interaction between tDCS intensity and baseline performance (Benwell et al., 2015). Further careful work must be done to untangle and further define these relationships, which may be important in clinical applications of tDCS.

The mechanism by which TMS affects the population of neurons under the stimulating coil has also become clearer in recent years. There is now robust evidence from a variety of measures that a TMS pulse will induce an electrical current that will preferentially activate the least active cohort of neurons (Silvanto et al., 2007). First demonstrated using an adaptation paradigm for a variety of visual stimuli, the principle has since been generalized across different stimulation paradigms (suprathreshold and subthreshold TMS, as well as theta burst TMS), different visual areas of the brain, and different paradigms (priming, color, movement, and orientation contingent color) using both psychophysical measures and subjective report (Silvanto et al., 2008).

When considering the use of neurostimulation as a treatment option in AVHs, therefore, the current knowledge concerning factors that may influence their effect across cognitive domains must be integrated. In the case of AVHs, a reduction in activity of left STG is associated with a reduction in frequency (Kindler et al., 2013), perhaps providing evidence that the effect of cathodal tDCS to the left TPJ is consistent with the effects of tDCS applied over motor cortex. However, if applied concurrently with a task that would drive neuronal excitability in one controllable way or another, it may be possible to maximize the clinical effect by defining the underlying state for each patient. Supporting this point, there is evidence that rTMS has greater efficacy in patients with high levels of activity in the left STG pre-treatment (Homan et al., 2012).

It would therefore seem that a further elucidation of these aspects of state dependency of neurostimulation in relation to AVHs would allow us to better tailor a clinical intervention using non-invasive brain stimulation and create a predictive model for its efficacy across the clinical sample.

ACCEPTABILITY ISSUES

In general the acceptability and side-effect profile of contemporary neurostimulation techniques for AVH treatment is thought to be good (Sommer et al., 2012), particularly in comparison to use of antipsychotic medication. Although many more trials have been conducted using TMS, single-case reports and group studies suggest that tDCS and tRNS are also acceptable to patients (Homan et al., 2011; Brunelin et al., 2012; Palm et al., 2013).

Nevertheless, the use of neurostimulation techniques will only be appropriate as a treatment option for some people with AVH and not others. The recommendation of neurostimulation (specifically TMS) as a treatment for AVH has been criticized in the past for lacking a strong evidence base, and it has been suggested that such techniques may ignore important psychological and social factors that may be better explored via psychotherapy (e.g., Corstens et al., 2013). Nevertheless, for some people reduction in AVH frequency and persistence will be a specific treatment goal, and neurostimulation may prove to be a viable option.

More broadly, rTMS or tDCS do not appear to be related to any long-term adverse effects if applied within commonly used parameters (relating to frequency, output strength, and stimulation duration in rTMS, and current strength, electrode size, and stimulation duration in tDCS; Brunoni et al., 2012). Hoffman et al. (2005), in a study using rTMS to treat AVHs, reported a higher occurrence of headaches in active rTMS compared to the sham condition, although other adverse effects did not occur more in one condition than the other. Recently developed techniques discussed above (tACS and tRNS) are likely to have similar acceptability to the patient as tDCS, since they use similar equipment. There is some evidence to suggest that the tactile effects of tRNS on the scalp are less perceptible than tDCS (Ambrus et al., 2010; Fertonani et al., 2011), indicating that tRNS may be more tolerable to the participant than tDCS; however, no large-scale study has yet compared the tolerability of tDCS, tACS, and tRNS in a clinical sample. Nevertheless, these three electrical stimulation methods are all likely to have higher tolerability than rTMS, which elicits a larger tactile sensation on the scalp (although is still only mildly uncomfortable for the participant).

Use in Adolescent Populations

Neurostimulation may be a promising therapeutic option in adolescent populations because it might help to avoid the adverse developmental consequences of anti-psychotic medication, and frequent suboptimal clinical response (Croarkin et al., 2011). However, in the absence of clear guidelines on the use of non-invasive brain stimulation during developmental periods, the major concern relates to safety. The occurrence of seizures (i.e., one of the most serious TMS-related adverse effects) has been extremely rare in adult populations and none were reported in two large meta-analyses conducted in 850 and 1034 children/adolescents, respectively (Gilbert et al., 2004; Quintana, 2005). TMS-related seizures are more common in high frequency ($>$ 5 Hz) stimulation procedures, whilst treatment of AVHs usually uses low frequency (1 Hz) stimulation (Gilbert, 2008), further lessening the risk of seizure. Furthermore, when observed, transitory neurophysiological changes were not associated with a significant increase in spike-wave discharges in a population of brain-damaged children (Gilbert et al., 2004). rTMS was not found to be associated with cochlear damage or hearing-loss in children or adolescents who received neurostimulation treatment (Collado-Corona et al., 2001). Finally, using a self-report acceptability questionnaire, Garvey et al. (2001) found that for 38 children and adolescents receiving this treatment, the TMS tolerability ranged between a long drive and an appointment to the dentist.

Whilst pilot studies investigating the therapeutic efficacy of rTMS in disorders such as depression, attention-deficit/hyperactivity or autism have been conducted (Croarkin et al., 2011), little is known about the efficacy of rTMS on early-onset AVH. A number of single case-reports have described clinical improvements in the severity of AVH in patients with childhood-onset schizophrenia after low-frequency rTMS (Walter et al., 2001; Fitzgerald et al., 2006; Jardri et al., 2007), and more recently a case-series highlighted the potential beneficial effects of low frequency rTMS on alleviating early-onset refractory hallucinations (Jardri et al., 2012). This case-series provided the first evidence for a significant improvement in the severity of AVH and global functioning after 10 sessions of 1 Hz rTMS over the left TPJ in a group of 10 adolescents with childhood-onset schizophrenia. The therapeutic effect was sustained at the 1-month follow-up and no specific adverse effects were observed. Implementing larger controlled trials is now required to (1) validate 1 Hz rTMS against sham in this population; (2) determine optimized stimulation parameters in developmental periods; and (3) evaluate the long-term duration of the therapeutic effect on early-onset AVH.

Treatment of Subtypes of AVH

There is a growing awareness that AVHs are a heterogeneous phenomenon (Nayani and David, 1996; Jones, 2010; McCarthy-Jones et al., 2014; Woods et al., 2015). Given the variety in underlying cognitive and neural processes likely to be involved in qualitatively distinct AVH subtypes, therapeutic interventions need to be appropriately targeted at relevant underlying processes (Smailes et al., 2015). In this section, we consider the potential applicability of neurostimulation to three common subtypes of AVH: inner speech, memory and hypervigilance hallucinations.

As outlined above, inner speech hallucinations are proposed to arise as a result of a misattribution of an utterance generated in inner speech to an external agent. Targeting selected areas of the fronto-temporal network therefore presents promising opportunities for therapeutic management; as described in Section TMS and tDCS as Treatment Options for Auditory Verbal Hallucinations, it has previously been suggested that stimulating the left TPJ may affect cortical areas involved in self-monitoring, specifically affecting a network of regions involved in inner speech production (Moseley et al., 2013). This is supported by a range of evidence suggesting that superior temporal and temporoparietal regions are involved in discriminating between self- and non-self-generated actions (Blanke et al., 2002; Wang et al., 2011; Moseley et al., 2014) as well-being active during inner speech production (Simons et al., 2010; Alderson-Day and

Fernyhough, 2015; Alderson-Day et al., 2016). If the mechanism of action of left TPJ stimulation is indeed via modulation of activity in a self-monitoring network, it is possible that treatment using neurostimulation would be most efficacious for patients experiencing AVHs that are explicable by an inner speech model.

Memory-based AVH are proposed to occur when typical processes of memory retrieval lead to the aberrant generation of an intrusive verbal cognition. The content of such cognitions may relate to the content of what was said during a traumatic event (Jones, 2010). In one model (Waters et al., 2006), the occurrence of the intrusion cognition, coupled with a lack of the contextual information that would usually lead to the cognition being identified as a memory, results in the cognition being attributed to an external source. Existence of memory-based AVHs is supported by cluster analysis of data relating to phenomenological properties of AVHs (McCarthy-Jones et al., 2012) indicating that these AVHs may be distinct from those related to inner speech.

It is unclear whether neurostimulation would be an effective therapeutic option for memory-based AVHs. Evidence from fMRI has shown that some AVHs may be preceded by activation in left parahippocampal regions (Diederen et al., 2010), which the authors interpret as evidence that areas involved in memory recollection may dysfunctionally trigger language related regions, resulting in AVH. Although subcortical regions such as parahippocampal cortex cannot easily be targeted by transcranial techniques such as rTMS or tDCS, their interaction with temporal language regions may be affected by stimulation of the left TPJ. Furthermore, stimulation of prefrontal regions (as is common in tDCS montages) may be able to modulate top-down control involved in metacognitive processes, which may relate to the intrusiveness with which resurfacing memories are experienced (Jones and Fernyhough, 2006; Fleming and Dolan, 2012). Some evidence for this comes from the literature on post-traumatic stress disorder, in which a number of studies have shown efficacy of high frequency (20 Hz) rTMS to the left or right dorsolateral prefrontal cortex in the reduction of PTSD symptoms (Boggio et al., 2010). Interestingly, a recent paper has suggested that some AVHs associated with schizophrenia and with PTSD may share common phenomenological and aetiological mechanisms (McCarthy-Jones and Longden, 2015). Nevertheless, a more in-depth understanding of memory-based AVH, both phenomenologically and at a neural level, is required before confident predictions can be made about neurostimulation efficacy.

A third subtype of AVH, termed hypervigilance AVH (Dodgson and Gordon, 2009) may differ in its cognitive and neural substrates from both inner speech and memory-based AVH. These are defined as the perception of a threat-related utterance in the context of a noisy environment. Hypervigilance AVH appear to result from top-down biases toward the perception of certain emotionally salient stimuli, and have recently been accounted for within a predictive processing framework (Wilkinson, 2014). Although, little is known about the neural basis of hypervigilance AVH, it might be predicted that cortical areas involved in attentional biases, particularly in the auditory modality, would be involved in these AVHs. An extensive body of research using dichotic listening paradigms has linked AVHs to biased attentional processes (Hugdahl et al., 2008, 2012), with some evidence suggesting that interhemispheric synchrony (between left and right auditory cortices) may be atypical in individuals that hallucinate (Mulert et al., 2011; Steinmann et al., 2014). This may be related to the ability to exert cognitive control over attentional processes, which could feasibly be related to hypervigilance AVH. If so, neurostimulation may be best targeted to normalize activity in bilateral auditory cortical regions (using anodal and cathodal stimulation), or to enhance neural synchrony between these regions using gamma-frequency tACS. Alternatively, it is possible that these AVHs may be more amenable to psychological therapies which aim to alter patient's appraisal of the experiences (Smailes et al., 2015).

Overall, a deeper understanding of the cognitive and neural mechanisms of different subtypes of AVH is needed before confident predictions can be made about neurostimulation efficacy. To date, neuroimaging studies of AVHs simply tend to compare hallucinating and non-hallucinating patients (usually with a diagnosis of schizophrenia) with healthy controls, but a more fruitful approach may be to divide samples into groups based on phenomenological variables relating to inner speech, memory, and hypervigilance processes. In this way, treatment options could be targeted with higher success rates, and in particular, treatment using techniques such as rTMS, tDCS, tRNS, or tACS might be applied to different regions, dependent on the likelihood of efficacy. It is likely that the heterogeneity of current findings regarding efficacy of neurostimulation treatment is, at least partially, because some types of AVH are more likely than others to be affected by stimulation of the left TPJ.

SUMMARY

Here, we have outlined a number of future avenues for research into the use of neurostimulation techniques as a treatment option for AVHs. To summarize, whilst studies testing the efficacy of rTMS and tDCS indicate that they may be effective at reducing AVH frequency, new techniques such as tACS and tRNS should be tested, both in clinical trials and in relation to their effect on self-monitoring and inner speech processes in healthy populations. This paper has argued that, due to it's effects on cortical oscillatory activity, tACS may be capable of affecting network communication between frontal and temporal regions, thought to be involved in predictive models which relate to self-monitoring. tRNS, meanwhile, may be a more effective option than tDCS, potentially over-riding homeostatic mechanisms that may lessen the effect of tDCS on excitability.

There are also outstanding questions relating to the best approaches to localizing the target of stimulation. The evidence so far does not strongly support efficacy of neuronavigated rTMS compared to the T3-P3 method, but further research taking into account more complex inter-individual differences in structural and functional connectivity may increase efficacy. An important future direction for research will also be to explore the best way to harness state dependent effects of neurostimulation, which may have the potential to further increase the effectiveness

of treatment. There are also issues relating to acceptability and utility in adolescent samples, or individuals experiencing qualitatively different types of AVH, which will be important to address in future research.

FUNDING

This work was supported by the Wellcome Trust (grant numbers: WT098455MA & WT108720).

REFERENCES

Alderson-Day, B., and Fernyhough, C. (2015). Inner speech: development, cognitive functions, phenomenology, and neurobiology. *Psychol. Bull.* 141, 931–965. doi: 10.1037/bul0000021

Alderson-Day, B., Weis, S., McCarthy-Jones, S., Moseley, P., Smailes, D., and Fernyhough, C. (2016). The brain's conversation with itself: neural substrates of dialogic inner speech. *Soc. Cogn. Affect. Neurosci.* 11, 110–120. doi: 10.1093/scan/nsv094

Aleman, A., and Larøi, F. (2008). *Hallucinations: The Science of Idiosyncratic Perception*. Washington, DC: APA Books.

Allen, P., Larøi, F., McGuire, P. K., and Aleman, A. (2008). The hallucinating brain: a review of structural and functional neuroimaging studies of hallucinations. *Neurosci. Biobehav. Rev.* 32, 175–191. doi: 10.1016/j.neubiorev.2007.07.012

Ambrus, G. G., Paulus, W., and Antal, A. (2010). Cutaneous perception thresholds of electrical stimulation methods: comparison of tDCS and tRNS. *Clin. Neurophysiol.* 121, 1908–1914. doi: 10.1016/j.clinph.2010.04.020

Ball, K., Lane, A. R., Smith, D. T., and Ellison, A. (2013). Site-dependent effects of tDCS uncover dissociations in the communication network underlying the processing of visual search. *Brain Stimul.* 6, 959–965. doi: 10.1016/j.brs.2013.06.001

Beavan, V., Read, J., and Cartwright, C. (2011). The prevalence of voice-hearers in the general population: a literature review. *J. Ment. Health* 20, 281–292. doi: 10.3109/09638237.2011.562262

Bentall, R. P., and Slade, P. D. (1985). Reality testing and auditory hallucinations - a signal detection analysis. *Br. J. Clin. Psychol.* 24, 159–169. doi: 10.1111/j.2044-8260.1985.tb01331.x

Benwell, C. S. Y., Learmonth, G., Miniussi, C., Harvey, M., and Thut, G. (2015). Non-linear effects of transcranial direct current stimulation as a function of individual baseline performance: evidence from biparietal tDCS influence on lateralized attentional bias. *Cortex* 69, 152–165. doi: 10.1016/j.cortex.2015.05.007

Blanke, O., Ortigue, S., Landis, T., and Seeck, M. (2002). Neuropsychology: stimulating illusory own-body perceptions. *Nature* 419, 269–270. doi: 10.1038/419269a

Boggio, P. S., Rocha, M., Oliveira, M. O., Fecteau, S., Cohen, R. B., Campanhã, C., et al. (2010). Noninvasive brain stimulation with high-frequency and low-intensity repetitive transcranial magnetic stimulation treatment for posttraumatic stress disorder. *J. Clin. Psychiatry* 71, 992–999. doi: 10.4088/JCP.08m04638blu

Bortoletto, M., Pellicciari, M. C., Rodella, C., and Miniussi, C. (2015). The interaction with task-induced activity is more important than polarization: a tDCS study. *Brain Stimul.* 8, 269–276. doi: 10.1016/j.brs.2014.11.006

Brookwell, M. L., Bentall, R. P., and Varese, F. (2013). Externalizing biases and hallucinations in source-monitoring, self-monitoring and signal detection studies: a meta-analytic review. *Psychol. Med.* 43, 2465–2475. doi: 10.1017/S0033291712002760

Brunelin, J., Mondino, M., Gassab, L., Haesebaert, F., Gaha, L., Suaud-Chagny, M. F., et al. (2012). Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *Am. J. Psychiatry* 169, 719–724. doi: 10.1176/appi.ajp.2012.11071091

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., et al. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Cazzoli, D., Müri, R. M., Hess, C. W., and Nyffeler, T. (2010). Treatment of hemispatial neglect by means of rTMS-a review. *Restor. Neurol. Neurosci.* 28, 499–510. doi: 10.3233/RNN-2010-0560

Ćurčić-Blake, B., Nanetti, L., van der Meer, L., Cerliani, L., Renken, R., Pijnenborg, G. M., et al. (2015). Not on speaking terms: hallucinations and structural network disconnectivity in schizophrenia. *Brain Struct. Funct.* 220, 407–418. doi: 10.1007/s00429-013-0663-y

Chen, C. M. A., Mathalon, D. H., Roach, B. J., Cavus, I., Spencer, D. D., and Ford, J. M. (2011). The corollary discharge in humans is related to synchronous neural oscillations. *J. Cogn. Neurosci.* 23, 2892–2904. doi: 10.1162/jocn.2010.21589

Collado-Corona, M. A., Mora-Magana, I., Cordero, G. L., Toral-Martinon, R., Shkurovich-Zaslavsky, M., Ruiz-Garcia, M., et al. (2001). Transcranial magnetic stimulation and acoustic trauma or hearing loss in children. *Neurol. Res.* 23, 343–346. doi: 10.1179/016164101101198532

Corstens, D., Longden, E., Rydinger, B., Bentall, R., and van Os, J. (2013). Treatment of hallucinations: a comment. *Psychosis* 5, 98–102. doi: 10.1080/17522439.2012.740069

Croarkin, P. E., Wall, C. A., and Lee, J. (2011). Applications of transcranial magnetic stimulation (TMS) in child and adolescent psychiatry. *Int. Rev. Psychiatry* 23, 445–453. doi: 10.3109/09540261.2011.623688

de Jesus, D. R., Gil, A., Barbosa, L., Lobato, M. I., Magalhães, P., Favalli, G., et al. (2011). A pilot double-blind sham-controlled trial of repetitive transcranial magnetic stimulation for patients with refractory schizophrenia treated with clozapine. *Psychiatry Res.* 188, 203–207. doi: 10.1016/j.psychres.2010.11.022

Demeulemeester, A., Amad, A., Bubrovszky, M., Pins, D., Thomas, P., and Jardri, R. (2012). What is the real effect of 1-Hz repetitive transcranial magnetic stimulation on hallucinations? Controlling for publication bias in neuromodulation trials. *Biol. Psychiatry* 71, e15–e16. doi: 10.1016/j.biopsych.2011.10.010

Diederen, K. M., Neggers, S. F., Daalman, K., Blom, J. D., Goekoop, R., Kahn, R. S., et al. (2010). Deactivation of the parahippocampal gyrus preceding auditory hallucinations in schizophrenia. *Am. J. Psychiatry* 167, 427–435. doi: 10.1176/appi.ajp.2009.09040456

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

Dodgson, G., and Gordon, S. (2009). Avoiding false negatives: are some auditory hallucinations an evolved design flaw? *Behav. Cogn. Psychother.* 37, 325–334. doi: 10.1017/S1352465809005244

Fertonani, A., Pirulli, C., and Miniussi, C. (2011). Random noise stimulation improves neuroplasticity in perceptual learning. *J. Neurosci.* 31, 15416–15423. doi: 10.1523/JNEUROSCI.2002-11.2011

Feurra, 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

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

Fitzgerald, P. B., Benitez, J., Daskalakis, J. Z., De Castella, A., and Kulkarni, J. (2006). The treatment of recurring auditory hallucinations in schizophrenia with rTMS. *World J. Biol. Psychiatry* 7, 119–122. doi: 10.1080/15622970500474705

Fitzgerald, P. B., McQueen, S., Daskalakis, Z. J., and Hoy, K. E. (2014). A negative pilot study of daily bimodal transcranial direct current stimulation in schizophrenia. *Brain Stimul.* 7, 813–816. doi: 10.1016/j.brs.2014.08.002

Fleming, S. M., and Dolan, R. J. (2012). The neural basis of metacognitive ability. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 1338–1349. doi: 10.1098/rstb.2011.0417

Ford, J. M., Krystal, J. H., and Mathalon, D. H. (2007a). Neural synchrony in schizophrenia: from networks to new treatments. *Schizophr. Bull.* 33, 848–852. doi: 10.1093/schbul/sbm062

Ford, J. M., and Mathalon, D. H. (2005). Corollary discharge dysfunction in schizophrenia: can it explain auditory hallucinations? *Int. J. Psychophysiol.* 58, 179–189. doi: 10.1016/j.ijpsycho.2005.01.014

Ford, J. M., Mathalon, D. H., Kalba, S., Whitfield, S., Faustman, W. O., and Roth, W. T. (2001). Cortical responsiveness during talking and listening in schizophrenia: an event-related brain potential study. *Biol. Psychiatry* 50, 540–549. doi: 10.1016/S0006-3223(01)01166-0

Ford, J. M., Roach, B. J., Faustman, W. O., and Mathalon, D. H. (2007b). Synch before you speak: auditory hallucinations in schizophrenia. *Am. J. Psychiatry* 164, 458–466. doi: 10.1176/ajp.2007.164.3.458

Ford, J. M., Roach, B. J., Faustman, W. O., and Mathalon, D. H. (2008). Out-of-synch and out-of-sorts: dysfunction of motor-sensory communication in schizophrenia. *Biol. Psychiatry* 63, 736–743. doi: 10.1016/j.biopsych.2007.09.013

Foucher, J. R. (2013)."Perspectives in brain imaging and computer-assisted technologies for the treatment of hallucinations," in *The Neuroscience of Hallucinations,* eds R. Jardri, A. Cachia, P. Thomas, D. Pins (New York, NY: Springer), 529–547.

Frith, C. D. (1992). *The Cognitive Neuropsychology of Schizophrenia*. Hove: Lawrence Erlbaum Associates.

Fröhlich, F., Sellers, K. K., and Cordle, A. L. (2015). Targeting the neurophysiology of cognitive systems with transcranial alternating current stimulation. *Expert Rev. Neurother.* 15, 145–167. doi: 10.1586/14737175.2015.992782

Garvey, M. A., Kaczynski, K. J., Becker, D. A., and Bartko, J. J. (2001). Subjective reactions of children to single-pulse transcranial magnetic stimulation. *J. Child Neurol.* 16, 891–894. doi: 10.1177/088307380101601205

George, M. S., Lisanby, S. H., Avery, D., McDonald, W. M., Durkalski, V., Pavlicova, M., et al. (2010). Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. *Arch. Gen. Psychiatry* 67, 507–516. doi: 10.1001/archgenpsychiatry.2010.46

George, M. S., Wassermann, E. M., Williams, W. A., Callahan, A., Ketter, T. A., Basser, P., et al. (1995). Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *Neuroreport* 6, 1853–1856. doi: 10.1097/00001756-199510020-00008

Gilbert, D. L. (2008)."Design and analysis of motor-evoked potential data in pediatric neurobehavioral disorder investigations," in *The Oxford Handbook of Transcranial Stimulation*, eds E. M. Wassermann, C. M. Epstein, U. Ziemann, V. Walsh, T. Paus, S.H. Lisanby (New York, NY: Oxford University Press), 389–400.

Gilbert, D. L., Garvey, M. A., Bansal, A. S., Lipps, T., Zhang, J., and Wassermann, E. M. (2004). Should transcranial magnetic stimulation research in children be considered minimal risk? *Clin. Neurophysiol.* 115, 1730–1739. doi: 10.1016/j.clinph.2003.10.037

Gill, J., Shah-Basak, P. P., and Hamilton, R. (2015). It's the thought that counts: examining the task-dependent effects of transcranial direct current stimulation on executive function. *Brain Stimul.* 8, 253–259. doi: 10.1016/j.brs.2014.10.018

Haesebaert, F., Mondino, M., Saoud, M., Poulet, E., and Brunelin, J. (2014). Efficacy and safety of fronto-temporal transcranial random noise stimulation (tRNS) in drug-free patients with schizophrenia: a case study. *Schizophr. Res.* 159, 251–252. doi: 10.1016/j.schres.2014.07.043

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* 55, 187–199. doi: 10.1016/j.neuron.2007.06.026

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

Hoffman, R. E., Boutros, N. N., Berman, R. M., Roessler, E., Belger, A., Krystal, J. H., et al. (1999). Transcranial magnetic stimulation of left temporoparietal cortex in three patients reporting hallucinated "voices". *Biol. Psychiatry* 46, 130–132. doi: 10.1016/S0006-3223(98)00358-8

Hoffman, R. E., Gueorguieva, R., Hawkins, K. A., Varanko, M., Boutros, N. N., Wu, Y. T., et al. (2005). Temporoparietal transcranial magnetic stimulation for auditory hallucinations: safety, efficacy and moderators in a fifty patient sample. *Biol. Psychiatry* 58, 97–104. doi: 10.1016/j.biopsych.2005.03.041

Hoffman, R. E., Wu, K., Pittman, B., Cahill, J. D., Hawkins, K. A., Fernandez, T., et al. (2013). Transcranial magnetic stimulation of Wernicke's and right homologous sites to curtail "voices": a randomized trial. *Biol. Psychiatry* 73, 1008–1014. doi: 10.1016/j.biopsych.2013.01.016

Homan, P., Kindler, J., Federspiel, A., Flury, R., Hubl, D., Hauf, M., et al. (2011). Muting the voice: a case of arterial spin labeling-monitored transcranial direct current stimulation treatment of auditory verbal hallucinations. *Am. J. Psychiatry* 168, 853–854. doi: 10.1176/appi.ajp.2011.11030496

Homan, P., Kindler, J., Hauf, M., Hubl, D., and Dierks, T. (2012). Cerebral blood flow identifies responders to transcranial magnetic stimulation in auditory verbal hallucinations. *Transl. Psychiatry* 2:e189. doi: 10.1038/tp.2012.114

Horvath, J. C., Forte, J. D., and Carter, O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: a systematic review. *Neuropsychologia* 66, 213–236. doi: 10.1016/j.neuropsychologia.2014.11.021

Hugdahl, K., Løberg, E.-M., Falkenberg, L. E., Johnsen, E., Kompus, K., Kroken, R. A., et al. (2012). Auditory verbal hallucinations in schizophrenia as aberrant lateralized speech perception: evidence from dichotic listening. *Schizophr. Res.* 140, 59–64. doi: 10.1016/j.schres.2012.06.019

Hugdahl, K., Løberg, E. M., Specht, K., Steen, V. M., van Wageningen, H., and Jørgensen, H. A. (2008). Auditory hallucinations in schizophrenia: the role of cognitive, brain structural and genetic disturbances in the left temporal lobe. *Front. Hum. Neurosci.* 1:6. doi: 10.3389/neuro.09.006.2007

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

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

Jardri, R., Lucas, B., Delevoye-Turrell, Y., Delmaire, C., Delion, P., Thomas, P., et al. (2007). An 11-year-old boy with drug-resistant schizophrenia treated with temporo-parietal rTMS. *Mol. Psychiatry* 12, 320. doi: 10.1038/sj.mp.4001968

Jardri, R., Pins, D., Bubrovszky, M., Lucas, B., Lethuc, V., Delmaire, C., et al. (2009). Neural functional organization of hallucinations in schizophrenia: multisensory dissolution of pathological emergence in consciousness. *Conscious. Cogn.* 18, 449–457. doi: 10.1016/j.concog.2008.12.009

Jardri, R., Pins, D., and Thomas, P. (2008). A case of fMRI-guided rTMS treatment of coenesthetic hallucinations. *Am. J. Psychiatry* 165, 1490–1491. doi: 10.1176/appi.ajp.2008.08040504

Jardri, R., Pouchet, A., Pins, D., and Thomas, P. (2011). Cortical activations during auditory verbal hallucinations in schizophrenia: a coordinate-based meta-analysis. *Am. J. Psychiatry* 168, 73–81. doi: 10.1176/appi.ajp.2010.09101522

Johns, L., Kompus, K., Connell, M., Humpston, C., Lincoln, T., Longden, E., et al. (2014). Auditory verbal hallucinations in persons with and without a need for care. *Schizophr. Bull.* 40, S255–S264. doi: 10.1093/schbul/sbu005

Jones, S. R. (2010). Do we need multiple models of auditory verbal hallucinations? Examining the phenomenological fit of cognitive and neurological models. *Schizophr. Bull.* 36, 566–575. doi: 10.1093/schbul/sbn129

Jones, S. R., and Fernyhough, C. (2006). The roles of thought suppression and metacognitive beliefs in proneness to auditory verbal hallucinations in a non-clinical sample. *Pers. Individ. Dif.* 41, 1421–1432. doi: 10.1016/j.paid.2006.06.003

Jones, S. R., and Fernyhough, C. (2007). Thought as action: inner speech, self-monitoring, and auditory verbal hallucinations. *Conscious. Cogn.* 16, 391–399. doi: 10.1016/j.concog.2005.12.003

Kindler, J., Homan, P., Jann, K., Federspiel, A., Flury, R., Hauf, M., et al. (2013). Reduced neuronal activity in language-related regions after transcranial magnetic stimulation therapy for auditory verbal hallucinations. *Biol. Psychiatry* 73, 518–524. doi: 10.1016/j.biopsych.2012.06.019

Klirova, M., Horacek, J., Novak, T., Cermak, J., Spaniel, F., Skrdlantova, L., et al. (2013). Individualized rTMS neuronavigated according to regional brain metabolism (18FDG PET) has better treatment effects on auditory hallucinations than standard positioning of rTMS: a double-blind, sham-controlled study. *Eur. Arch. Psychiatry Clin. Neurosci.* 263, 475–484. doi: 10.1007/s00406-012-0368-x

Knight, H. C., Smith, D. T., and Ellison, A. (2015). "The role of the left dorsolateral prefrontal cortex in attentional bias: a tDCS study," in *Paper presented at European Convention of Visual Perception*, (Liverpool).

Koops, S., van Dellen, E., Schutte, M. J. L., Nieuwdorp, W., Neggers, S. F. W., and Sommer, I. E. C. (2016). Theta burst transcranial magnetic stimulation for auditory verbal hallucinations: negative findings from a double-blind randomized trial. *Schizophr. Bull.* 42, 250–257. doi: 10.1093/schbul/sbv100

Langguth, B., Zowe, M., Spiessl, H., Hajak, G., Marienhagen, J., and Eichhammer, P. (2006). Neuronavigated transcranial magnetic stimulation and auditory hallucinations in a schizophrenic patient: monitoring of neurobiological effects. *Schizophr. Res.* 84, 185–186. doi: 10.1016/j.schres.2006.02.019

Larøi, F., Sommer, I. E., Blom, J. D., Fernyhough, C., and ffytche, D. H., Hugdahl, K., et al. (2012). The characteristic features of auditory verbal hallucinations in clinical and nonclinical groups: state-of-the-art overview and future directions. *Schizophr. Bull.* 38, 724–733. doi: 10.1093/schbul/sbs061

Learmonth, G., Thut, G., Benwell, C. S., and Harvey, M. (2015). The implications of state-dependent tDCS effects in aging: behavioural response is determined by baseline performance. *Neuropsychologia* 74, 108–119. doi: 10.1016/j.neuropsychologia.2015.01.037

Lee, S. H., Kim, W., Chung, Y. C., Jung, K. H., Bahk, W. M., Jun, T. Y., et al. (2005). A double blind study showing that two weeks of daily repetitive TMS over the left or right temporoparietal cortex reduces symptoms in patients with schizophrenia who are having treatment-refractory auditory hallucinations. *Neurosci. Lett.* 376, 177–181. doi: 10.1016/j.neulet.2004.11.048

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., and Pascual-Leone, A. (2000). Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. *Clin. Neurophysiol.* 111, 800–805. doi: 10.1016/S1388-2457(99)00323-5

McCarthy-Jones, S., and Longden, E. (2015). Auditory verbal hallucinations in schizophrenia and post-traumatic stress disorder: common phenomenology, common cause, common interventions? *Front. Psychol.* 6:1071. doi: 10.3389/fpsyg.2015.01071

McCarthy-Jones, S. R., Thomas, N., Strauss, C., Dodgson, G., Jones, N., Woods, A., et al. (2014). Better than mermaids and stray dogs? Subtyping auditory verbal hallucinations and its implications for research and practice. *Schizophr. Bull.* 40, S275–S284. doi: 10.1093/schbul/sbu018

McCarthy-Jones, S., Trauer, T., Mackinnon, A., Sims, E., Thomas, N., and Copolov, D. L. (2012). A new phenomenological survey of auditory hallucinations: evidence for subtypes and implications for theory and practice. *Schizophr. Bull.* 40, 231–235. doi: 10.1093/schbul/sbs156

McIntosh, A. M., Semple, D., Tasker, K., Harrison, L. K., Owens, D. G. C., Johnstone, E. C., et al. (2004). Transcranial magnetic stimulation for auditory hallucinations in schizophrenia. *Psychiatry Res.* 127, 9–17. doi: 10.1016/j.psychres.2004.03.005

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

Miniussi, C., Harris, J. A., and Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neurosci. Biobehav. Rev.* 37, 1702–1712. doi: 10.1016/j.neubiorev.2013.06.014

Mondino, M., Haesebaert, F., Poulet, E., Suaud-Chagny, M.-F., and Brunelin, J. (2015). Fronto-temporal transcranial direct current stimulation (tDCS) reduces source-monitoring deficits and auditory hallucinations in patients with schizophrenia. *Schizophr. Res.* 161, 515-516. doi: 10.1016/j.schres.2014.10.054

Montagne-Larmurier, A., Etard, O., Maïza, O., and Dollfus, S. (2011). Repetitive transcranial magnetic stimulation in the treatment of auditory hallucinations in schizophrenic patients. *Curr. Opin. Psychiatry* 24, 533–540. doi: 10.1097/yco.0b013e32834bd26e

Moseley, P., Fernyhough, C., and Ellison, A. (2013). Auditory verbal hallucinations as atypical inner speech monitoring, and the potential of neurostimulation as a treatment option. *Neurosci. Biobehav. Rev.* 37, 2794–2805. doi: 10.1016/j.neubiorev.2013.10.001

Moseley, P., Fernyhough, C., and Ellison, A. (2014). The role of the superior temporal lobe in auditory false perceptions: a transcranial direct current stimulation study. *Neuropsychologia* 62C, 202–208. doi: 10.1016/j.neuropsychologia.2014.07.032

Mulert, C., Kirsch, V., Pascual-Marqui, R., McCarley, R. W., and Spencer, K. M. (2011). Long-range synchrony of gamma oscillations and auditory hallucination symptoms in schizophrenia. *Int. J. Psychophysiol.* 79, 55–63. doi: 10.1016/j.ijpsycho.2010.08.004

Naeser, M. A., Martin, P. I., Treglia, E., Ho, M., Kaplan, E., Bashir, S., et al. (2010). Research with rTMS in the treatment of aphasia. *Restor. Neurol. Neurosci.* 28, 511–529. doi: 10.3233/RNN-2010-0559

Nayani, T. H., and David, A. S. (1996). The auditory hallucination: a phenomenological survey. *Psychol. Med.* 26, 177–189. doi: 10.1017/S003329170003381X

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

Palm, U., Hasan, A., Keeser, D., Falkai, P., and Padberg, F. (2013). Transcranial random noise stimulation for the treatment of negative symptoms in schizophrenia. *Schizophr. Res.* 146, 372-373. doi: 10.1016/j.schres.2013.03.003

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

Quintana, H. (2005). Transcranial magnetic stimulation in persons younger than the age of 18. *J. ECT* 21, 88–95. doi: 10.1097/01.yct.0000162556.02720.58

Siebner, H. R., Filipovic, S. R., Rowe, J. B., Cordivari, C., Gerschlager, W., Rothwell, J. C., et al. (2003). Patients with focal arm dystonia have increased sensitivity to slow-frequency repetitive TMS of the dorsal premotor cortex. *Brain* 126, 2710–2725. doi: 10.1093/brain/awg282

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

Simons, C. J. P., Tracy, D. K., Sanghera, K. K., O'Daly, O., Gilleen, J., Dominguez, M., et al. (2010). Functional magnetic resonance imaging of inner speech in schizophrenia. *Biol. Psychiatry* 67, 232–237. doi: 10.1016/j.biopsych.2009.09.007

Singer, W. (1999). Neuronal synchrony: a versatile code for the definition of relations? *Neuron*, 24, 49–65, 111–125.

Slotema, C. W., Aleman, A., Daskalakis, Z. J., and Sommer, I. E. (2012). Meta-analysis of repetitive transcranial magnetic stimulation in the treatment of auditory verbal hallucinations: update and effects after one month. *Schizophr. Res.* 142, 40–45. doi: 10.1016/j.schres.2012.08.025

Slotema, C. W., Blom, J. D., de Weijer, A. D., Diederen, K. M., Goekoop, R., Looijestijn, J., et al. (2011). Can low-frequency repetitive transcranial magnetic stimulation really relieve medication-resistant auditory verbal hallucinations? Negative results from a large randomized controlled trial. *Biol. Psychiatry* 69, 450–456. doi: 10.1016/j.biopsych.2010.09.051

Slotema, C. W., Blom, J. D., van Lutterveld, R., Hoek, H. W., and Sommer, I. E. (2013). Review of the efficacy of transcranial magnetic stimulation for auditory verbal hallucinations. *Biol. Psychiatry* 76, 101–110. doi: 10.1016/j.biopsych.2013.09.038

Smailes, D., Alderson-Day, B., Fernyhough, C., McCarthy-Jones, S., and Dodgson, G. (2015). Tailoring cognitive behavioural therapy to subtypes of voice-hearing. *Front. Psychol.* 6:1933. doi: 10.3389/fpsyg.2015.01933

Sommer, I. E. C., Slotema, C. W., Daskalakis, Z. J., Derks, E. M., Blom, J. D., and van der Gaag, M. (2012). The treatment of hallucinations in schizophrenia spectrum disorders. *Schizophr. Bull.* 38, 704–714. doi: 10.1093/schbul/sbs034

Sommer, I. E., de Weijer, A. D., Daalman, K., Neggers, S. F., Somers, M., Kahn, R. S., et al. (2007). Can fMRI-guidance improve the efficacy of rTMS treatment for auditory verbal hallucinations? *Schizophr. Res.* 93, 406–408. doi: 10.1016/j.schres.2007.03.020

Spencer, K. M., Niznikiewicz, M. A., Nestor, P. G., Shenton, M. E., and McCarley, R. W. (2009). Left auditory cortex gamma synchronization and auditory hallucination symptoms in schizophrenia. *BMC Neurosci.* 10:85. doi: 10.1186/1471-2202-10-85

Sritharan, A., Line, P., Sergejew, A., Silberstein, R., Egan, G., and Copolov, D. (2005). EEG coherence measures during auditory

hallucinations in schizophrenia. *Psychiatry Res.* 136, 189–200. doi: 10.1016/j.psychres.2005.05.010

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

Steinmann, S., Leicht, G., and Mulert, C. (2014). Interhemispheric auditory connectivity: structure and function related to auditory verbal hallucinations. *Front. Hum. Neurosci.* 8:55. doi: 10.3389/fnhum.2014.00055

Stephane, M., Kuskowski, M., McClannahan, K., Surerus, C., and Nelson, K. (2010). Evaluation of speech misattribution bias in schizophrenia. *Psychol. Med.* 40, 741–748. doi: 10.1017/S003329170999081X

Stewart, L. M., Walsh, V., and Rothwell, J. C. (2001). Motor and phosphene thresholds: a transcranial magnetic stimulation correlation study. *Neuropsychologia* 39, 415–419. doi: 10.1016/S0028-3932(00)00130-5

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

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

Thomas, N. (2015). What's really wrong with cognitive behavioral therapy for psychosis? *Front. Psychol.* 6:323. doi: 10.3389/fpsyg.2015.00323

Uhlhaas, P. J., and Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat. Rev. Neurosci.* 11, 100–113. doi: 10.1038/nrn2774

Van Doren, J., Langguth, B., and Schecklmann, M. (2014). Electroencephalographic effects of transcranial random noise stimulation in the auditory cortex. *Brain Stimul.* 7, 807–812. doi: 10.1016/j.brs.2014.08.007

van Lutterveld, R., Hillebrand, A., Diederen, K. M. J., Daalman, K., Kahn, R. S., Stam, C. J., et al. (2012). Oscillatory cortical network involved in auditory verbal hallucinations in schizophrenia. *PLoS ONE* 7:e41149. doi: 10.1371/journal.pone.0041149

Vanneste, S., Fregni, F., and De Ridder, D. (2013). Head-to-head comparison of transcranial random noise stimulation, transcranial AC stimulation, and transcranial DC stimulation for tinnitus. *Front. Psychiatry* 4:158. doi: 10.3389/fpsyt.2013.00158

Vercammen, A., Knegtering, H., Bruggeman, R., Westenbroek, H. M., Jenner, J. A., Slooff, C. J., et al. (2009). Effects of bilateral repetitive transcranial magnetic stimulation on treatment resistant auditory-verbal hallucinations in schizophrenia: a randomized controlled trial. *Schizophr. Res.* 114, 172–179. doi: 10.1016/j.schres.2009.07.013

Vosskuhl, J., Huster, R. J., and Herrmann, C. S. (2015). Increase in short-term memory capacity induced by down-regulating individual theta frequency via transcranial alternating current stimulation. *Front. Hum. Neurosci.* 9:257. doi: 10.3389/fnhum.2015.00257

Walter, G., Tormos, J. M., Israel, J. A., and Pascual-Leone, A. (2001). Transcranial magnetic stimulation in young persons: a review of known cases. *J. Child Adolesc. Psychopharmacol.* 11, 69–75. doi: 10.1089/104454601750143483

Wang, L., Metzak, P. D., and Woodward, T. S. (2011). Aberrant connectivity during self–other source monitoring in schizophrenia. *Schizophr. Res.* 125, 136–142. doi: 10.1016/j.schres.2010.11.012

Waters, F., Badcock, J., Michie, P., and Maybery, M. (2006). Auditory hallucinations in schizophrenia: intrusive thoughts and forgotten memories. *Cogn. Neuropsychiatry* 11, 65–83 doi: 10.1080/13546800444000191

Wilkinson, S. (2014). Accounting for the variety and phenomenology of auditory verbal hallucinations within a predictive processing framework. *Conscious. Cogn.* 30, 142–155. doi: 10.1016/j.concog.2014.09.002

Wolf, N. D., Sambataro, F., Vasic, N., Frasch, K., Schmid, M., Schönfeldt-Lecuona, C., et al. (2011). Dysconnectivity of multiple resting-state networks in patients with schizophrenia who have persistent auditory verbal hallucinations. *J. Psychiatry Neurosci.* 36, 366–374. doi: 10.1503/jpn.110008

Woods, A., Jones, N., Alderson-Day, B., Callard, F., and Fernyhough, C. (2015). Experiences of hearing voices: analysis of a novel phenomenological survey. *Lancet Psychiatry* 2, 323–331. doi: 10.1016/S2215-0366(15)00006-1

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 Neuroscience

REVIEW
published: 31 March 2016
doi: 10.3389/fnins.2016.00140

Non-Invasive Brain Stimulation in Conversion (Functional) Weakness and Paralysis: A Systematic Review and Future Perspectives

Carlos Schönfeldt-Lecuona[1], Jean-Pascal Lefaucheur[2,3], Peter Lepping[4,5,6], Joachim Liepert[7], Bernhard J. Connemann[1], Alexander Sartorius[8], Dennis A. Nowak[9,10] and Maximilian Gahr[1]*

[1] Department of Psychiatry and Psychotherapy III, University of Ulm, Ulm, Germany, [2] Department of Physiology, Henri Mondor Hospital, Assistance Publique – Hôpitaux de Paris, Créteil, France, [3] EA 4391, Nerve Excitability and Therapeutic Team, Faculty of Medicine, Paris Est Créteil University, Créteil, France, [4] Department of Psychiatry, Betsi Cadwaladr University Health Board, Wrexham, UK, [5] Centre for Mental Health and Society, Bangor University, Wrexham, UK, [6] Department of Psychiatry, Mysore Medical College and Research Institute, Mysore, India, [7] Department of Neurorehabilitation, Kliniken Schmieder, Allensbach, Germany, [8] Department of Psychiatry and Psychotherapy, Medical Faculty Mannheim, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany, [9] Department of Neurology, Helios-Klinik Kipfenberg, Kipfenberg, Germany, [10] Department of Neurology, University Hospital Marburg, Marburg, Germany

OPEN ACCESS

Edited by:
Ignacio Obeso, Centro Integral Neurociencias A.C. (CINAC), Hospitales Madrid and CEU-San Pablo University, Spain

Reviewed by:
Isabel Pareés, Hospital Clínico San Carlos, Spain
Maja Kojovic, University Clinical Center Ljubljana, Slovenia

***Correspondence:**
Carlos Schönfeldt-Lecuona
carlos.schoenfeldt@uni-ulm.de

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

Received: *13 January 2016*
Accepted: *18 March 2016*
Published: *31 March 2016*

Citation:
Schönfeldt-Lecuona C, Lefaucheur J-P, Lepping P, Liepert J, Connemann BJ, Sartorius A, Nowak DA and Gahr M (2016) Non-Invasive Brain Stimulation in Conversion (Functional) Weakness and Paralysis: A Systematic Review and Future Perspectives. Front. Neurosci. 10:140. doi: 10.3389/fnins.2016.00140

Conversion (functional) limb weakness or paralysis (FW) can be a debilitating condition, and often causes significant distress or impairment in social, occupational, or other important areas of functioning. Most treatment concepts are multi-disciplinary, containing a behavioral approach combined with a motor learning program. Non-invasive brain stimulation (NIBS) methods, such as electroconvulsive therapy (ECT), and transcranial magnetic stimulation (TMS) have been used in the past few decades to treat FW. In order to identify all published studies that used NIBS methods such as ECT, TMS and transcranial direct current stimulation (tDCS) for treating FW patients a systematic review of the literature was conducted in PubMed and Web of Science. In a second step, narratives were used to retrospectively determine nominal CGI-I (Clinical Global Impression scale–Improvement) scores to describe approximate changes of FW symptoms. We identified two articles (case reports) with ECT used for treatment of FW, five with TMS with a total of 86 patients, and none with tDCS. In 75 out of 86 patients treated with repetitive (r)TMS a nominal CGI-I score could be estimated, showing a satisfactory short-term improvement. Fifty-four out of seventy-five identified patients (72%) had a CGI-I score of 1 (very much improved), 13 (17%) a score of 2 (much improved), 5 (7%) a score of 3 (minimally improved), and 3 (5%) remained unchanged (CGI-I = 4). In no case did patients worsen after rTMS treatment, and no severe adverse effects were reported. At follow-up, symptom improvement was not quantifiable in terms of CGI-I for the majority of the cases. Patients treated with ECT showed a satisfactory short-term response (CGI-I = 2), but deterioration of FW symptoms at follow-up. Despite the predominantly positive results presented in the identified studies and satisfactory levels of efficacy measured with retrospectively calculated nominal CGI-I scores, any

assumption of a beneficial effect of NIBS in FW has to be seen with caution, as only few articles could be retrieved and their quality was mostly poor. This article elucidates how NIBS might help in FW and gives recommendations for future study designs using NIBS in this condition.

Keywords: hysterical paralysis, hysterical neuroses, medically unexplained motor symptoms, functional neurological disorder, functional lesion, psychogenic movement disorders, magnetic stimulation, electroshock

INTRODUCTION

Conversion Disorder is a frequent condition. It is classed under "dissociative and conversion disorders" in the international WHO-classification (WHO ICD-10, 1991) and "Functional Neurological Symptom Disorder (FNS)" in DSM-5 (DSM-5, 2013). The precise prevalence of the disorder is unknown. The reported incidence is between 4 and 12 cases per 100,000 habitants/year (DSM-5, 2013). In the largest prospective cohort study, conversion disorder accounted for 5.6% of 3781 Scottish patients referred from primary care to a National Health Service neurology clinic (Stone et al., 2009).

Conversion (functional) weakness or paralysis (FW) [DSM-5 300.11/ICD-10 F44.4], a subgroup of FNS that affect limbs, can be very incapacitating and causes significant distress or impairment in social, occupational, or other important areas of functioning (**Table 1** for DSM-5 criteria). In FW, symptoms either cannot be explained by a neurological condition (or other general medical condition), or clinical findings are inconsistent with recognized neurological or medical disease (DSM-5, 2013). Therefore, in the literature, such disorders have been referred to as "psychogenic," "hysterical," "non-organic". or rather unfortunately, "pseudo-neurological" (Nowak and Fink, 2009). The underlying etiological mechanisms involved remain unclear. Psychological factors were required in DSM-IV (former criterion B: "Psychological factors are judged to be associated with the symptom or deficit because the initiation or exacerbation of the symptom or deficit is preceded by conflicts or other stressors"; Carson et al., 2012). This criterion has been removed in DSM-5. Although conflicts and stressors may influence patients' vulnerability there is increasing evidence for a neurobiological component in the etiology of FW (Liepert et al., 2008, 2009, 2011). Over the last decade, neuroimaging findings examining differential brain activity in FW have started to support a neuro-biological hypothesis (Marshall et al., 1997; Spence et al., 2000; Vuilleumier et al., 2001; Vuilleumier, 2005; Burgmer et al., 2006; Stone et al., 2007; de Lange et al., 2008; Cojan et al., 2009; van Beilen et al., 2011; Ludwig et al., 2015), for review (Nowak and Fink, 2009). Even if the disorder is sometimes not easy to differentiate from simulation or malingering in a phenomenological way, FW is different from a neurobiological point of view and shares similarities with hypnotically induced paralysis (Bell et al., 2011; Ludwig et al., 2015).

FW affecting limbs may be transient but can persist. The socio-economic disease-burden is significant because of direct treatment costs and the consequences of an often-permanent loss of limb function leading to incapacity-related benefits (Carson et al., 2011). In the past years, various treatment strategies have been tested in FW related symptoms, including different forms of physiotherapy (for review Nielsen et al., 2013), pharmacotherapy (Rampello et al., 1996; Voon and Lang, 2005), behavioral therapy (Shapiro and Teasell, 2004), and hypnotherapy (Moene et al., 2002). The reported symptom recovery is very heterogeneous and varies depending on the treatment strategy and study. A large amount of new studies reported marked short-term improvements, mostly in the region of a 60–70% symptom reduction (Nielsen et al., 2013). However, long-term outcome, especially in patients with a long duration of illness at presentation is invariably poor (Feinstein et al., 2001). Factors related to patient beliefs and disease concepts often generate difficulties in the treatment of FW. UK neurologists

TABLE 1 | DSM-5 diagnostic criteria for Conversion Disorder (Functional Neurological Symptom Disorder/FNS).

A. One or more symptoms or altered voluntary motor or sensory function.

B. Clinical findings provide evidence of incompatibility between the symptom and recognized neurological or medical condition.

C. The symptom or deficit is not better explained by another medical or mental disorder.

D. The symptom or deficit causes clinically significant distress or impairment in social, occupational, or other important areas of functioning or warrants medical evaluation.

Coding note: The ICD-9-CM code for conversion disorder is 300.11, which is assigned regardless of the symptom type. The ICD-10-CM codes depends on the symptom type (see below).

Specify symptom type:

- (F44.4) With weakness or paralysis
- (F44.4) With abnormal movement (e.g., tremor, dystonic movement, myoclonus, gait disorder)
- (F44.4) With swallowing symptoms
- (F44.4) With speech symptoms (e.g., dysphonia, slurred speech)
- (F44.5) With attacks or seizures
- (F44.6) With anesthesia or sensory loss
- (F44.6) With special sensory symptoms
- (F44.7) With mixed symptoms

Specify if:

Acute episode: Symptoms present for <6 months.

Persistent: Symptoms occurring for 6 months or more.

Specify if:

With psychological stressor *(specify stressor)*.

Without psychological stressor.

describe patients with FNS as being "the most difficult to help" (Carson et al., 2004). Although there is no agreement on the most effective therapy for FW, most treatment concepts contain at least two components: a behavioral approach and a motor learning program using a multidisciplinary team (Nielsen et al., 2013).

Non-invasive brain stimulation (NIBS) methods, such as electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), and transcranial direct current stimulation (tDCS), have been used in the past decades to treat various mental disorders and may show beneficial effects in FW symptoms:

(1) ECT, which was experimentally developed in the late 1930s (Cerletti, 1940) was the first NIBS method to become established within the framework of psychiatry. Based on an electrically induced generalized seizure ECT is used for the treatment of various mental disorders including affective and schizophrenia spectrum disorders, and is considered the most effective treatment in major depression (Taylor, 2008).
(2) TMS is a non-convulsive NIBS method, which was initially developed for diagnostic purposes in order to measure motor latencies in the 1980s (Barker et al., 1985), and rapidly expanded in its repetitive form (rTMS) to a treatment strategy in the early 1990s. In 2010, the American FDA approved it for the treatment of therapy-resistant major depression in adults, although the clinical relevance of its efficacy remains doubtful (Schönfeldt-Lecuona et al., 2010; Lepping et al., 2014). The American Psychiatric Association (APA), the Canadian Network for Mood and Anxiety Treatments (CANMAT), and the World Federation of Societies of Biological Psychiatry (WFSBP) have accepted it as a treatment option for depression. It has been tested experimentally in other neuropsychiatric conditions (Lefaucheur et al., 2014).
(3) tDCS is based on a homogeneous electrical field at direct current (DC) intensities of around 1 mA applied transcranially to accessible cortical areas (Nitsche and Paulus, 2011). tDCS induces long-lasting cortical changes and thus can be used to manipulate brain excitability via membrane polarization. The induced after-effects depend on polarity, duration and intensity of the stimulation (Paulus, 2011). tDCS is still an experimental treatment method in psychiatry but has demonstrated potential therapeutic efficacy in different conditions (Koops et al., 2015; Meron et al., 2015; Saba et al., 2015).

The exact mechanism of action of any of these NIBS methods on cortical networks is not yet comprehensively understood. However, it is known, that ECT facilitates the release of brain derived neurotrophic factor (BDNF) (Polyakova et al., 2015). It causes enlargement of hippocampal (and other) regions, possibly through boosting neurogenesis (Nordanskog et al., 2014). rTMS and tDCS have been shown to induce long-lasting changes in cortical excitability in directly stimulated cortical areas (Siebner and Rothwell, 2003; Powell et al., 2014; Romero Lauro et al., 2014) and in deeper interconnected brain areas (Strafella et al., 2003, 2004; Pogarell et al., 2007).

Measuring motor evoked potentials (MEP) using TMS was postulated for the first time to be advantageous in the management of FW patients by Jellinek et al. (1992). Using a figure-8 coil placed over the vertex, they performed MEPs of the first dorsal interosseus muscle for diagnostic purposes in a 25-year-old man with an acute functional flaccid paraplegia. MEPs of the paralyzed limb were within the normal range. One week after diagnostic TMS he experienced a full recovery. The authors associated the MEP-related muscular activation of the limbs with his recovery and argued that the patient's observation of the brisk (involuntary) limb contraction due to the cortical activation facilitated the successful symptom management. Schönfeldt-Lecuona et al. performed the first therapeutic rTMS trial in FW in 2003 in a patient suffering a right upper limb paralysis leading to a full and sustained recovery (Schönfeldt-Lecuona et al., 2003).

Our systematic review of the literature was conducted to identify all published studies that used NIBS methods for treating FW patients, and to discuss the potential of NIBS in this disorder. To achieve this we reviewed all published studies and reports (articles, published congress abstracts) of the use of TMS [in every modality: single-pulse(sp)TMS, rTMS including theta-burst protocol], tDCS and ECT in the treatment of FW affecting limbs.

METHODS

Search Strategy and Selection Criteria for the Systematic Review

A literature search was performed using PubMed and Web of Science databases with the below-elucidated search strategy. The literature search includes reports published until the 15 of December 2015. We defined search terms for the here explored forms of FW and NIBS methods. The following search terms were used for FW: "conversion disorder," "motor conversion disorder," "conversion weakness," "conversion paralysis," "dissociative weakness," "dissociative paralysis," "dissociative motor symptoms," "dissociative ∗ plegia," "psychogenic disorder," "psychogenic weakness," "psychogenic paralysis," "hysterical weakness," "hysterical paralysis," "hysterical conversion," "hysterical ∗ plegia," "non-organic disorder," "non-organic weakness," "non-organic paralysis," "non-organic ∗ plegia," "functional disorder," "functional weakness," "functional paralysis," "functional ∗ plegia," "functional neurological symptoms," and "medically unexplained neurological symptoms," [*plegia, meaning all forms: mono-, hemi-, para-, tetra-, quadriplegia]. The following search terms were used for the different types of NIBS methods explained above: "stimulation," "stimulation therapy," "transcranial magnetic stimulation," "TMS," "rTMS," "theta-burst*," "transcranial direct current stimulation," "tDCS," "electroconvulsive,*" "electroshock," and "ECT."

In a first step, the number of search hits related to each of the mentioned search terms for FW was retrieved (**Figure 1**). In a second step, each of the mentioned search terms related to FW was linked to all of the mentioned search terms ("AND") related to the different types of NIBS methods (combined search),

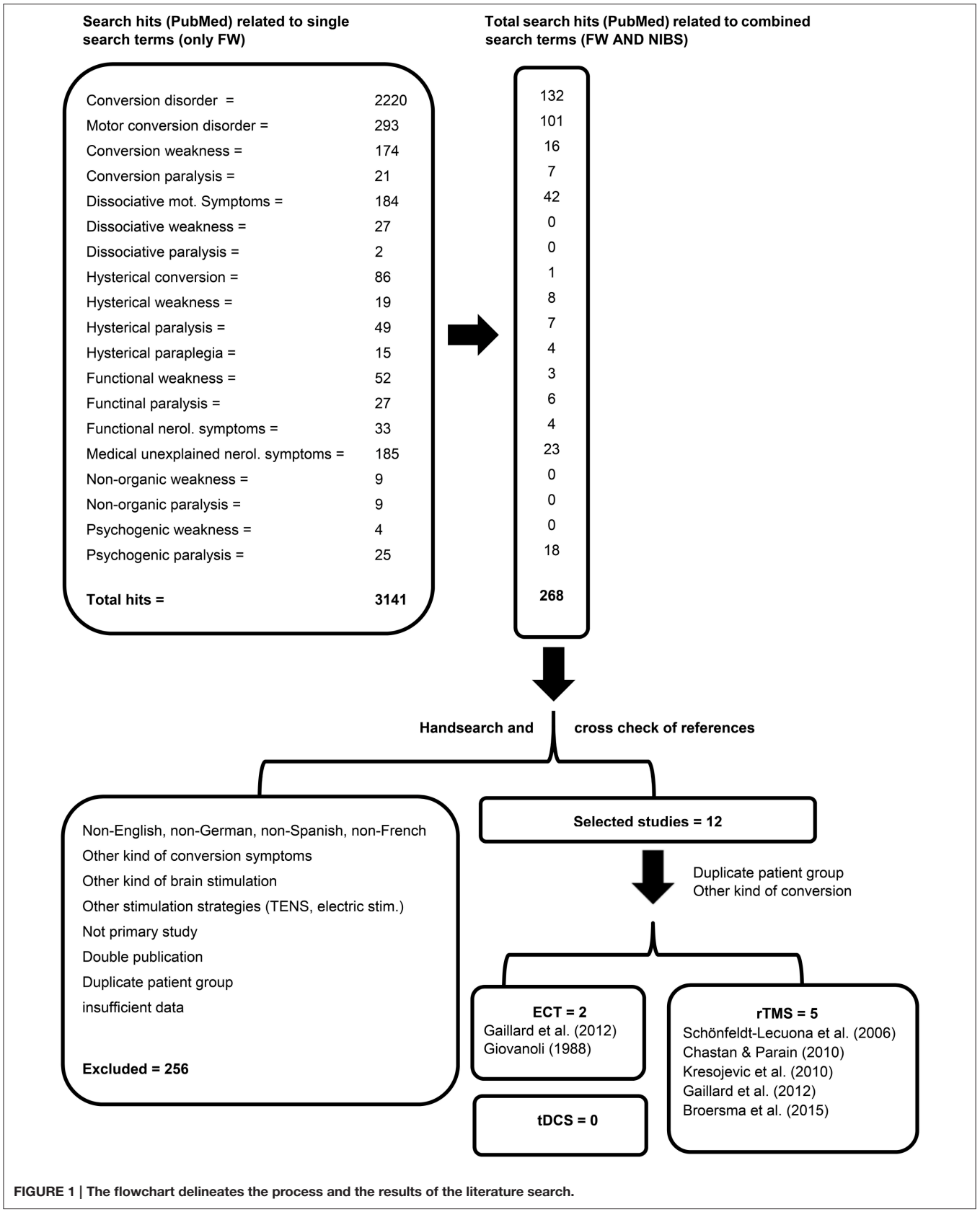


FIGURE 1 | The flowchart delineates the process and the results of the literature search.

and the respective search hits were checked. Titles and abstracts related to the retrieved hits identified with the combined search were then checked manually by two examiners independently (CSL and MG, see below). In order to detect published conference and meeting abstracts edited in supplements not available in PubMed, a second independent search was carried out in Web of Science with the above mentioned search terms and then cross-checked. Because of space limitations, only the PubMed search results are shown in the **Figure 1**.

Inclusion criteria:

- Therapeutic trials only
- Patients exclusively suffering from FW as described above
- FW patients treated with TMS (in all variants: spTMS, rTMS including theta-burst TMS), tDCS or ECT
- Any kind of study design: randomized-controlled trials (RCT), non-RCT, open-label, naturalistic designs; all population sizes reported were allowed (full study, case series or case report)

Exclusion criteria:

- Non-English, non-German, non-Spanish, non-French language studies
- Conversion symptoms other than functional weakness or functional paralysis [non-FW as described here (DSM-5 300.11/ICD-10 F44.4), with or without sensory loss]
- Non therapeutic trial
- Non primary study, duplication, duplicated publication of data, duplicate patient group
- Insufficient data to evaluate treatment strategy and symptom outcome
- Disorders of consciousness presented as coma, vegetative state, minimally conscious state, stupor or catatonia.

Titles and abstracts of the articles retrieved from the combined search were checked for the presence of data of relevant topics (see above). Only articles addressing weakness or paralysis affecting limbs (often also accompanied by loss or reduction of sensory feeling) were considered. Two examiners (CSL and MG) then searched the retrieved titles and abstracts by hand and independently. After retrieval of abstracts fulfilling inclusion-criteria, full text versions of all identified articles were obtained. A cross check of the references from retrieved articles was performed to identify related publications not listed in the examined databases. Data were extracted independently by the two authors (CSL and MG). The two data bases were compared manually and then examined again by both reviewers. Discrepancies were corrected by reference to the original papers.

Retrospective Reconstruction of the Clinical Global Impression - Improvement (CGI-I) Score

From all selected articles, the manuscript content was checked for clinical descriptions of symptom severity before and after treatment. The narratives were then used to determine an approximate change of FW symptoms, using the principles of the CGI-I scale for a nominal CGI-I score. Narratives were checked independently by two examiners (CSL and MG) and a nominal CGI-I score was established. In case of discrepancies, a consensus decision was reached between the two examiners. The CGI-I score is a 7-point scale which is commonly used to describe changes of a patient's clinical overall improvement related to a specific treatment. It was developed for use in NIMH (National Institute of Mental Health)-sponsored clinical trials to provide a brief, stand-alone assessment of the clinician's view of the patient's global functioning prior to and after initiating a study (Busner and Targum, 2007). CGI-I comprises the following categories: 1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse. If single data were available a nominal CGI-I was estimated for each patient reported (case reports and case series). In case of studies not reporting single data, a nominal CGI-I was calculated for the group of patients treated with a certain NIBS method.

RESULTS

On December 15, 2015, our literature search resulted in the following numbers of hits related to the different search terms: "conversion disorder" $n = 2220$, "motor conversion disorder" $n = 293$, "conversion weakness" $n = 174$, "conversion paralysis" $n = 21$, "dissociative motor symptoms" $n = 184$, "dissociative weakness" $n = 27$, "dissociative paralysis" $n = 2$, "hysterical conversion" $n = 86$, "hysterical weakness" $n = 19$, "hysterical paralysis" $n = 49$, "hysterical paraplegia" $n = 15$, "functional weakness" $n = 52$, "functional paralysis" $n = 27$, "functional neurological symptoms" $n = 33$, "medically unexplained neurological symptoms" $n = 185$, "non-organic weakness" $n = 9$, "non-organic paralysis" $n = 9$, "psychogenic weakness" $n = 4$, "psychogenic paralysis" $n = 25$. All in all, the literature search retrieved 3141 hits. The combined term search led to the results shown in the **Figure 1**. The search performed in Web of Science allowed the identification of one meeting abstract (Kresojevic et al., 2010) that was not identified using the PubMed database. We could not retrieve any other relevant publications using Web of Science, which were not identified using PubMed. Two articles reported the same patients and therefore had to be excluded (Schönfeldt-Lecuona et al., 2003; Broersma et al., 2013).

Electroconvulsive Therapy in FW

We identified $n = 2$ articles in which ECT was performed in FW (**Table 2**). In both peer-reviewed articles, a single case was reported (Giovanoli, 1988; Gaillard et al., 2012).

Case Description

Giovanoli (1988) presented a 61-year old man with a complete right hand paralysis after superficial laceration of the middle finger, 11 months duration prior to ECT. Bilateral ECT (Medcraft B-24) was performed on an outpatient basis 3-times per week for 2 weeks, then twice weekly for 6 weeks (ECT parameters not available). Within the first 10 ECT sessions, a progressive change in color and a decrease in swelling were observed; after the 10th ECT session, approximation of thumb and index finger was possible; after the 19th ECT edema had disappeared and the hand exhibited a full range of motion. One week after completion of

TABLE 2 | Electroconvulsive Therapy (ECT) and Transcranial Magnetic Stimulation (TMS) in functional weakness or paralysis (FW).

Author (year) Nibs method-study type	Patients and clinical presentation	Stimulation protocol and technical data	Symptom development and efficacy (CGI-I)	Follow-up and other important issues
Giovanoli, 1988 (ECT)-*Case report*	Fw pat (male, 61 years), right hand paralysis, 11 month prior to ect, after superficial laceration of middle finger	Ect on outpatient basis. Narcosis with thiopental sodium (50 mg), atropine (4 mg), and succinylcholine (10 mg). Bilateral ect (medcraft b-24), 3x/weekly for 2 weeks, then 2x/weekly for 6 weeks	Progressive improvement from the first ect in color and skin tone. 1 week after completion fine motor function of fingers restored (CCI rating), CGI-I = 2	After 6 month, and after 1 year patient was not using the hand any more but it was normal in appearance (CCI rating), CGI-I = 3
Gaillard et al., 2012 (ECT)-*Case report*	Fw pat (male 33 years), quadriplegia, 3 years prior to ECT	Initially 2–3 ects per week, modality (ns). Somewhat later 1 ect per week; than once a fortnight (in order to train motor skills and maintain mobility). Ect was performed at increasingly intensity until a maximum of 1152 mc in order to reach a seizure of at least 30–40 s	Until the 9th ect the progression in muscular activity allowed the patient to perform movements with increasingly complexity. He gained progressively more function and was able to eat without help, and to manage all activities of daily life in the perimeter of his room with only little help. Up to the 25th ect he was able to walk without help (CCI rating), CGI-I = 2	Relapse occurred after a while (ns), with great symptom fluctuation, dependent on the momentary circumstances, but muscular activity remained better than on admission (CCI rating), CGI-I = 3
Schönfeldt-Lecuona et al., 2006 (TMS)-*Case series, open-label*	3 FW pat. (1 male) + 1 malingerer. Age mv = 38 years; symptom duration: 5 weeks to 5 years	F8c, Dantec MacPro X 100, M1 stimulation, 4000 pulses/d, rTMS at 15Hz (2se train, ITI 8 sec), 5 times a week (working days); I = 110% MT for the first 2 weeks, then 90% MT for 4 to 12 weeks	All FW improved markedly (CCI rating) FW-Pat Nr. 1 CGI-I = 2 FW-Pat Nr. 2 CGI-I = 1 FW-Pat Nr. 3 CGI-I = 2	Improvement sustained at 6 and 12 months (CCI rating) FW-Pat Nr. 1 CGI-I = 1 FW-Pat Nr. 2 CGI-I = 1 FW-Pat Nr. 1 CGI-I = 2
Chastan and Parain, 2010 (TMS)-*Open-label, retrospective symptom assessment*	70 FW pat., age mv = 24.7 years (8–79); acute FW in 55 pat. (median duration 4 days); chronic FW in 15 pat. (median duration 240 days)	Cc, M1 stimulation, 30 pulses every 4 or 5 sec; 1 or 2 session in only 1 day, I = 100% maximal stimulator output	Immediately or within hours after rTMS effective in 89% of FW; ineffective in 11% (CCI rating) n = 53 pat. (75.7%) CGI-I = 1 n=9 pat. (12.8%) CGI-I = 2 n=5 pat. (7.2%) CGI-I = 3 n = 3 pat. (4.3%) CGI-I = 4	Effect sustained for the majority after 5 to 6 months. Recurrence of FW in 8 pat. In those pat., repeated rTMS was effective in 6 (CCI rating)
Kresojevic et al., 2010 (TMS)-*Case series*	1 FW pat. (male 24 years), "hemiparesis that compromised his walk." Duration of symptoms (ns) 1 PMD pat. (not entered in the evaluation)	Cc, vertex stimulation, single rTMS session with 12 single pulses at initially 30% maximal stimulator output intensity and increasing I in 10% steps up to 80% of maximal stimulator output	"Immediate response, the pat. was able to walk again independently" (CCI rating) CGI-I = 2	Recurrence of mild symptoms after 6 months (partial deterioration), but mild walk difficulties did not influence his daily activities (CCI rating) CGI-I = 3
Gaillard et al., 2012 (TMS)-*Case report*	1 FW pat. male (33 years), quadriplegia, 6 months prior to rTMS	Coil type and I ns, rTMS at 1 Hz Fr. M1 stimulation, right and left over the arm-hand area, and right and left over legs" cortical motor area, 1000 pulses over each region (total = 4000 pulses per day), 5 times a week (working days, over a period of 8 weeks), after that, twice a week	Progressive amelioration: he was able to walk again, (rater impression, CGI-I = 1.5). Further deterioration led to a new rTMS treatment causing again symptom amelioration (CGI-I = 2.5), he was mobile only with a wheelchair. A third deterioration led to a new rTMS (CCI rating), CGII = 2	At follow-up recurrence of FW occurred (ns); he developed a phlebitis, pulmonary embolus and pressure soars, was referred for ECT (CCI rating), CGI-I = 4
Broersma et al., 2015 (TMS)-*placebo-controlled cross-over, single blinded*	11 FW pat. (4 male, 34-65 years), at least a flaccid hand paralysis; symptom duration: 4 weeks to 25 years	F8c, Magstim rapid2, contra-lateral M1 stimulation, 9000 pulses/d, rTMS at 15Hz (2setrain, ITI 4 sec), 5 times a week (working days) for 2 weeks; I = 80% MT (11 pat. received active, 8 pat. received placebo rTMS. Placebo rTMS with an electromagnetic device (REMP) placed in front of the magnetic coil at otherwise identical parameters	Primary outcome measure: muscle strength as measured by dynamometry; secondary outcome measure: subjective change in muscle strength; active rTMS induced a significantly larger median increase in objectively measured muscle strength (24%) compared to sham rTMS (6%); subjective ratings showed no statistical difference between treatments; no CCI rating	No follow-up data available.

pat., patient; F8c, figure-8-coil; Cc, Circular coil; mv, mean value; M1, motor cortex; MT, motor threshold intensity; Fr., Frequency; I, Intensity; d, day; s, seconds; (ns), no specified; PMD, psychogenic movement disorder; CCI, clinician's clinical impression; CGI-I, Clinical global impression scale-improvement item: 1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse.

ECT fine motor function of the patient's fingers was restored (he was able to button his shirt, tie his shoes and write). At 6 months follow-up and after 1 year, the patient did not use his hand during the examination but it was completely normal in appearance. No specific ECT parameters were stated. CGI-I after ECT was rated: 2 (much improved). The long-term CGI-I was rated: 3 (minimally improved).

Gaillard et al. (2012) presented a 33-year old man with fluctuating quadriplegia, developed 3 years prior to ECT. In total, 35 ECT sessions were performed, initially 2–3 ECT treatments per week. Subsequently, ECTs were performed less frequently (first once per week; then once a fortnight) in order to train motor skills and maintain mobility. Until the ninth ECT the progress in muscular activity allowed the patient to perform movements with increasing complexity. Progress continued with the patient gaining progressively more function, being able to eat without help, and managing all activities of daily living in the perimeter of his room with little help. Until the 25th ECT he was able to walk without help. Relapse occurred after a while, with great symptom fluctuation, dependent on circumstances, but muscular activity remained better than at admission. ECT was performed with increasing intensity until a maximum of 1152 mC in order to reach a seizure of at least 30–40 s. CGI-I after ECT was rated: 2 (much improved). The long term nominal CGI-I was rated: 3 (minimally improved).

Repetitive Transcranial Magnetic Stimulation (All Variants)

We identified five articles in that TMS/rTMS was performed in FW affecting limb(s) (**Table 2** for characteristics) No articles were identified reporting theta-burst TMS for the treatment of FW. Four articles were published in peer-review journals (Schönfeldt-Lecuona et al., 2006; Chastan and Parain, 2010; Gaillard et al., 2012; Broersma et al., 2015), and a fifth article was retrieved from a conference abstract (Kresojevic et al., 2010). Three articles reported single patients (case reports or case series); only two articles included a larger sample [$n = 70$ in Chastan et al. (Chastan and Parain, 2010) and $n = 11$ in Broersma et al. (2015)].

Case Description

The study by Schönfeldt-Lecuona et al. (2006) had a prospective design and a clearly defined stimulation protocol based on a biological and functional-anatomical etiological hypothesis (see Discussion). This open-label, non-placebo controlled trial reported four patients (3 FW, 1 malingerer). Patients received 2 weeks (5-sessions/week) of rTMS in supra-threshold intensity [110% resting motor threshold (MT)] over the contra-lateral motor cortex to the paralyzed limb with a focal figure-8 coil and 4000 stimuli per day. Thereafter, once the patient started to independently perform own movements of the fingers, sub-threshold rTMS (90% MT) was continued for 4–12 weeks with otherwise the same parameters depending on clinical needs. In all three FW patients rTMS caused a marked amelioration of symptoms over time that was sustained at 1-year follow-up. The estimated nominal group-CGI-I after rTMS was rated (for the 3 FW patients): 2 (much improved). The long term CGI-I was sustained after 1 year at the same level (**Table 2** for case related retrospective calculated CGI-I score).

Gaillard et al. (2012) described the case of a 33-year old man who had developed a quadriplegia (and anasthesia, December 2004) 6 months before admission. rTMS was performed at 1 Hz frequency (intensity and coil type not stated) targeting the motor cortex, right and left, over the arm-hand area, and right and left over the legs' cortical motor area (1000 pulses over each region) with a total of 4000 pulses per day. Initially, treatment was applied five times a week for 8 weeks, and thereafter twice in a week. The authors reported marked, progressive symptom amelioration, so that he was able to walk again. A further deterioration led to a new rTMS treatment leading again to symptom amelioration (not otherwise specified), but the patient was then mobile only with a wheelchair. A third deterioration led to phlebitis, pulmonary embolism and pressure soars, and ECT was performed (the ECT performed in this case was illustrated above). CGI-I after the first rTMS series was rated: 2 (much improved). The long term CGI-I was rated: 4 (unchanged in relation to rTMS beginning).

Kresojevic et al. (2010) presented two cases treated with rTMS. One of them (24-year old man) was suffering from a FW (hemiparesis). This patient was treated in a single session with 12 single pulses using a round coil (at initially 30% maximal stimulator output intensity and increasing intensity in 10% steps up to a maximum of 80% stimulator output) over the vertex. The response to rTMS was stated as immediate ("the patient was able to walk again independently"). At 6-months follow-up, a partial deterioration occurred, but he was still able to walk and minor walking difficulties did not influence his daily activities. The patients' CGI-I after TMS was rated: 2 (much improved). The long term CGI-I after 6 months follow-up was rated: 3 (minimally improved).

Chastan et al. (Chastan and Parain, 2010) presented a retrospective analysis of medical records of 70 FW patients (26 male), who had received TMS. Fifty-seven percent of the patients had paraplegia, 37% had a monoplegia, 3% had a tetraplegia, and 3% a hemiplegia. The stimulation protocol was variable. The TMS was principally used for routine diagnostic purposes in each patient. An average of 30 pulses were delivered at about 0.2–0.25 Hz with a circular coil and an intensity of 100% of maximum stimulator output over the motor cortex ("opposite the correspondence paralysis or on both sides for bilateral paralysis," not otherwise specified). Another session of 30 pulses was sometimes added a few minutes later in case of incomplete improvement. TMS was very effective in 62 patients, with a dramatic improvement in nine, a total recovery in 53 (immediately in 43 patients, within minutes or hours in eight patients, within days in two patients), mild improvement in five, and no effect in three patients. Acute onset of FW was associated with a better outcome (but not age, gender or co-morbid psychiatric disorder). CGI-I after TMS was rated for each reported patient group: 1 (very much improved) for the majority of the FW patients ($n = 53$; 76%); 2 (much improved) for nine patients (13%); 3 (minimally improved) for five patients (7%); and 4 (unchanged) for three patients (4%). Five to six months after TMS, recurrence of FW occurred in eight patients, six of whom were re-stimulated and responded to

TMS. There was not sufficient information to calculate long-term CGI-I.

Broersma et al. (2015) presented the first study using a placebo-controlled cross-over design and reported 11 patients with FW with at least a flaccid hand paralysis treated with rTMS. Based on the stimulation parameters proposed by Schönfeldt-Lecuona et al. (2006), active rTMS was delivered with a figure-of-8 coil at 15 Hz over the motor cortex contralateral to hand paralysis (targeting being guided under neuro-navigation in seven patients) during 30 min once a day, for a total of 10 working days within 2 weeks (**Table 2** for detailed parameters). The placebo condition consisted of small electrical currents applied with a real electromagnetic device (REMP) placed in front of the magnetic coil at otherwise identical parameters. In the study design, the authors attempted to exclude any other additional therapeutic influences that could result from suggestion or afferent feedback due to rTMS-related supra-threshold muscle contraction. To achieve this goal the authors performed the active condition at an intensity of 80% of MT, and the communication with the patients was limited as much as possible. The stimulation condition switched between active and sham after the first 2 weeks of stimulation with a wash-out phase of at least 2 months between both conditions. Because of dropouts, 11 patients received active rTMS and only eight patients received sham rTMS. The primary outcome measure was an objective change in muscle strength as measured by dynamometry after treatment. The secondary outcome measure was the subjective change in muscle strength after treatment. In patients who received both treatments, active rTMS induced a significantly larger median increase in objectively measured muscle strength (24%) compared to sham rTMS (6%). Eight out of 11 patients receiving active rTMS showed an improvement of at least 20% of muscle strength. However, subjective ratings showed no statistical difference between treatments, i.e., patients did not really perceive these objectively measured motor improvements. As the patients' muscle strength improved, the authors suggested that rTMS alone could potentially improve muscle weakness in FW. However, patients did not report subjective improved functioning of the affected hand, which Broersma et al. interpreted as an indication that decreased muscle strength is not the core symptom in FW. They thus propose that rTMS should be applied as add-on therapy to behavioral approaches in FW. There was not sufficient information to calculate nominal CGI-I scores.

Nominal CGI-I Score Reconstruction

For patients treated with rTMS we retrieved sufficient information from the physician-estimated functional changes reported in the manuscripts by Schönfeldt-Lecuona et al. (2006) ($n = 3$), Chastan et al. ($n = 70$) (Chastan and Parain, 2010), Kresojevic et al. (2010) ($n = 1$), and Gaillard et al. (2012) ($n = 1$) that allowed the assessment of 75 FW patients. In the study by Broersma et al. (2015) the main outcome parameter was muscular strength changes assessed by dynamometer. The authors reported that patients were assessed neurologically for sensory deficits, coordination, reflexes and muscle strength at the beginning and end of rTMS treatment. However, the narratives provided in the paper did not allow an estimation of nominal CGI-I scores. Therefore, these patients ($n = 11$) were excluded from the analysis. For patients treated with ECT we retrieved information from the narratives in the articles by Giovanoli (1988) and Gaillard et al. (2012) that allowed us to assess the two patients reported.

For the patients treated with rTMS the estimated scores showed a satisfactory improvement at the short-term: nominal CGI-I scores were 1 (very much improvement) in 54 of 75 patients (72%) and 2 (much improvement) in 13 patients (17%). Only five of the treated patients (7%) improved minimally (CGI-I = 3), and 3 (5%) remained unchanged (CGI-I = 4). Overall, about 88% of these patients improved markedly (very much or much improvement) after stimulations. In no case did patients worsen in relation to rTMS treatment, and no serious adverse event was reported. A long-term CGI-I could not be estimated for the largest study by Chastan et al. ($n = 70$) (Chastan and Parain, 2010). FW symptoms recurred in eight patients 5–6 month after rTMS. In 62 patients treatment seem to have caused some amelioration compared to baseline (not stated). In $n = 3$ cases by Schönfeldt-Lecuona et al. follow-up CGI-I showed a sustained amelioration (Schönfeldt-Lecuona et al., 2006), while in the case by Gaillard the estimated long-term CGI-I was rated 4 (Gaillard et al., 2012). Patients treated with ECT showed a satisfactory response at short-term follow-up as well (ranging CGI-I = 2), but a deterioration of FW symptoms at long-term follow-up (ranging CGI-I = 3 in both cases; **Table 2**).

DISCUSSION

Discussion of Literature Search Results

We concentrated our search exclusively on limb weakness and limb paralysis, since other forms of FNS (such as impaired coordination or balance, dystonia, tremor, myoclonus, fainting, tics, hemiballismus, chorea, parkinsonism, bizarre gait, astasia, abasia, aphonia, swallowing difficulty, urinary retention, loss of touch sensation, double vision, blindness, and deafness) might have a different neurobiological etiology and probably other functional-anatomical correlates (Ejareh Dar and Kanaan, 2016). For this reason, we speculate that differential effects of NIBS methods might come into play when treating different forms of FNS.

The literature search identified two case reports with ECT as treatment for FW (Giovanoli, 1988; Gaillard et al., 2012), five articles with TMS (Schönfeldt-Lecuona et al., 2006; Chastan and Parain, 2010; Kresojevic et al., 2010; Gaillard et al., 2012; Broersma et al., 2015), and none for tDCS, with a total of 86 patients. All identified cases and studies reported a short-term symptom improvement. However, any assumption of a beneficial effect of NIBS in FW has to be seen with caution, as the supporting literature is very sparse and the quality of the small number of identified articles was poor. Major concerns when examining the efficacy of NIBS in FW include the heterogeneity of studies with regard to design and stimulation parameters (paragraph below for more information), the absence of randomized controlled conditions in all but one trial, and the fact that the current literature does not allow a meta-analysis of

outcome data. Most of the included studies were case reports or case series (5 out of 7).

Study Designs, Parameters, and Outcomes

For TMS, only one study by Broersma et al. used a prospective, placebo-controlled, cross-over design with an objectively measured outcome using a dynamometer (Broersma et al., 2015). All other identified trials had no sham condition and only used an unstructured physician oriented clinical impression as outcome measure. The study with the hitherto largest sample of 70 patients by Chastan et al. (Chastan and Parain, 2010) was based on a retrospective sample analysis. Moreover, patients were mostly stimulated for diagnostic purposes and about 60% of the patients were children or adolescents. In all studies, FW symptoms and illness duration of the reported patients were heterogeneous and data were insufficient for a retrospective re-analysis, which would have allowed symptom clustering and meta-analysis. In the five identified articles (Schönfeldt-Lecuona et al., 2006; Chastan and Parain, 2010; Kresojevic et al., 2010; Gaillard et al., 2012; Broersma et al., 2015) no detailed information was presented regarding the way patients were informed and the treatments explained. The magnitude of the effect of the explanatory model could therefore not be estimated. The therapeutic effect of the active rTMS in Broersma et al. (2015) was smaller than the one reported by others; the mean increase of muscular strength was only about 20% (dynamometer), but there was no subjective amelioration of symptoms. The stimulation intensity in that study was deliberately kept at 80% MT, and therefore did not trigger any muscle contractions. This may indicate the importance of the patient becoming aware of movement and intact motor pathways as part of subjective symptom improvement. Placebo effects are likely to be involved in the mechanism of action, since in the study of Broersma, six out of nine patients showed a slight improvement after sham rTMS.

The stimulation protocol and parameters used differed considerably between studies. While most studies used low-frequency stimulation (1 Hz or less), Schönfeldt-Lecuona et al. (2006) and Broersma et al. (2015) delivered rTMS with 15 Hz, considered for rTMS to be high-frequency. Most therapeutic rTMS were performed in a single session, but the studies by Schönfeldt-Lecuona et al. and Broersma et al. applied a longer protocol (over weeks).

Estimated Functional Improvement

Despite some limitations, we retrospectively managed to judge the efficacy of the investigated stimulation methods (rTMS and ECT) for treating FW patients using the principles of the CGI-I scale. In total, we identified 88 patients with FW affecting limbs that received either rTMS or ECT). For the cases in which a nominal CGI-I could be retrospectively estimated ($n = 77$) about 90% of them improved markedly (very much or much improvement) after stimulations. Only a minority of the treated patients improved minimally or remained unchanged. In no case did patients worsen significantly after treatment and no serious adverse events were reported. At follow-up, symptom improvement was not quantifiable in terms of CGI-I for the majority of the cases (**Table 2** for detailed information).

ECT vs. rTMS

To our knowledge only two cases of ECT treatment in FW of limbs have been published (Giovanoli, 1988; Gaillard et al., 2012) since this technique was established in psychiatry many decades ago. Both published cases reported dramatic improvements of limb movement related to the ECT, thus causing a great improvement of activity of daily living. Besides the known favorable effects on brain function in major depression, no specific mechanism of action has been elucidated for ECT in relation to FW symptoms. One may speculate that the possible mechanism for short term gains is the reduction of stress due to the amelioration of psychological precipitating factors and an improvement in mood after ECT. A major role of a placebo effect in both described cases accounting for the symptom improvement cannot be ruled out. On the other hand, in both cases improvement was not sustained over time, and both patients had a partial relapse after a while. None of the cases postulated or tried a continuation or maintenance ECT, which is recommended for the treatment of major depression when acute ECT effects do not persist (Petrides et al., 2011). A disadvantage of the ECT might be the economical aspect compared with other NIBS methods; the costs of the general anesthesia and the required specialized personal are included. Given the risks of the general anesthesia and (reversible) post-treatment cognitive disturbances, restriction of ECT to the severest and treatment-resistant FW cases should be considered.

Most of the included articles were related to *TMS/rTMS* ($n = 5$). rTMS may be the NIBS method that is most appropriate for the use in limb FW for different reasons: (1) rTMS can acutely provoke a muscular contraction or transient movements without needing patients cooperation (or intention) to move. (2) rTMS is relatively easy to apply in FW. This is in contrast to stimulations outside the motor cortex for other indications, in which localization strategies for coil positioning are needed (Schönfeldt-Lecuona et al., 2005). The magnetic pulses applied using intensities above MT (supra-threshold stimulation) will trigger a visually noticeable muscle contraction. Because motor pathways are intact in FW, this technique allows targeting the desired motor area with sufficient precision (Herwig et al., 2001, 2003). (3) Longer lasting rTMS causes plasticity changes in brain areas directly under the magnetic coil (Karabanov et al., 2015), but also trans-synaptic changes in areas far from the stimulation site (Strafella et al., 2003, 2004; Pogarell et al., 2007). (4) rTMS is mostly well tolerated, and has no adverse effects if performed within safety limits (Rossi et al., 2009). rTMS is considered not to be painful (depending on the intensity, frequency and train length of trains applied). (5) rTMS treatment is currently performed by physicians, but can also be performed by trained allied medical professionals (nurses, technicians, psychologists). It does not require any anesthetic, and can be performed in an outpatient setting. (6) The costs per session are lower than ECT, and rTMS devises are common nowadays in neurology and psychiatry departments, and in rehabilitation clinics in many high-income countries.

Why Might rTMS Work?

Psychological Aspects

A crucial effect of supra-threshold TMS/rTMS in contrast to other NIBS methods is the patient's conscious perception of the externally triggered movements of their paralyzed limb. This phenomenon is experienced by all patients treated with rTMS, since prior to every therapeutic trial, single-pulse TMS with different intensities will be applied over the motor cortex in order to establish individual MT. In contrast, tDCS can modulate the excitability of cortical networks but does not directly produce action potentials on stimulated networks, and is therefore unable to trigger muscle contractions. Using ECT in FW, limb movements are actually provoked through the induced seizures, but the patient is not capable of noticing them because ECT is performed under general anesthesia. The patient's awareness of the muscle contractions due to TMS may help through a psychological mechanism: depending on the information received, patients become aware of normal function of neuromuscular structures. In addition, TMS triggering of muscle contraction might make patients aware of the possibility of regaining function. All identified studies showed an excellent response to TMS, except the one by Broersma et al. It was in that study that sub-threshold intensity rTMS was used, which does no provoke a muscular contraction. In addition, rTMS bears a high technical and methodological complexity in terms of technical approaches and calibrating steps that have to be performed prior to the therapeutic application, especially when MRI-guided localization techniques for coil positioning are used. Thus, TMS may generate a placebo effect, which in turn helps the patient to recover function immediately after stimulation. The response to rTMS may also be influenced by the information received and by the style which was used to inform the patients about the treatment strategy and purpose.

Neurophysiological Aspects

An increasing body of literature data suggest that focal functional abnormalities in central networks that control motor cortex activity may play a role in the etiology of FW (Geraldes et al., 2008; Liepert et al., 2008, 2009, 2011). The most convincing hypotheses to explain FW affecting limbs include (i) deficient processing of either motor intention or disruption between motor intention and motor execution or (ii) an overactive self-monitoring with enhanced limbic neural activity, which interferes with movement planning, and initiation within frontal regions and thereby disrupting motor execution (Voon et al., 2011). Studies using functional-imaging methods in patients with FW demonstrated enhanced neural activity within the anterior cingulate area or orbito-frontal cortex and reduced neural activity within prefrontal motor areas during movement execution of the paralyzed limb (Marshall et al., 1997; Spence et al., 2000; Stone et al., 2007). These abnormal activation patterns have been interpreted to reflect an active, but unconscious inhibition of movement planning and execution. Focused rTMS protocols with appropriate stimulation parameters might be able to reverse cortical dysfunction and restore activity in suppressed cortical motor areas (Schönfeldt-Lecuona et al., 2003, 2006; Chastan and Parain, 2010; Nielsen et al., 2013). The stimulation site for rTMS in FW is usually obvious with the primary motor cortex being the most plausible candidate region. However, the most challenging issue is still the choice of stimulation protocol (frequency, intensity, inter-train intervals, duration, and number of daily sessions) that can provoke a lasting positive change in cortical network activity. With regard to the question whether complementary stimulations in other cortical regions than the primary motor cortex could enhance therapeutic efficacy of rTMS in FW, no studies could be found.

Neuromodulatory Aspects

Single-pulse TMS with short protocols (e.g., performed in only one session for measuring MEPs for diagnostic purposes) might not be causing a durable change in cortical activity. Long-lasting changes and thus changes in cortical neuro-plasticity might only be induced performing longer protocols (e.g., for one or more weeks) using high-frequency (>1 Hz) or low-frequency (≤1 Hz), thus leading to long-term potentiation- or long-term depression-like changes respectively (Pascual-Leone et al., 1994; Chen et al., 1997; Fitzgerald et al., 2006). In most identified trials using TMS in FW, cortical stimulations were performed using repetitive spTMS with frequencies ≤1 Hz for a very short time (<2 or 3 min; Chastan and Parain, 2010; Kresojevic et al., 2010; Gaillard et al., 2012). Therefore, no long-lasting cortical effects could be expected, nor any stable changes in motor function due to the used protocols. The immediate and mostly sustained positive responses to the stimulations must therefore have other reasons. Only few studies used rTMS protocols that might potentially cause plasticity changes. We need to stress that not all of these hypotheses of the mechanisms of action of rTMS have been translated into proven clinically relevant changes, and more research is needed to be sure if they have any clinically meaningful effect (McWhirter et al., 2015). Furthermore, given that rTMS has existed as a technique since the 1990s, the number of trials published in this field is amazingly low. Publication bias could be a partial explanation for this, as may be the paucity of clinicians considering rTMS in rehabilitation neurology. However, first rTMS therapeutic trials have been performed to relieve other forms of FNS such as dystonia, myoclonus, tremor, parkinsonism, stereotypies, non-epileptic seizures, functional aphonia, or sensory or visual loss (Chastan et al., 2009, 2011; Dafotakis et al., 2011; Saha et al., 2011; Garcin et al., 2013; Parain and Chastan, 2014; Shah et al., 2015), mostly yielding to symptom amelioration.

RECOMMENDATION IN EVIDENCE-BASED GUIDELINES AND FUTURE STUDY DESIGNS

In 2014 evidence-based guidelines on rTMS were published and included recommendations for "Motor Conversion Disorders" in general (Lefaucheur et al., 2014). The degree of recommendation on the efficacy of rTMS for motor conversion disorders was: "No recommendation for low or high frequency rTMS of M1 or delivered at the vertex, using a focal or a non-focal coil" (Lefaucheur et al., 2014). The Cochrane library has published

recommendations for TMS in the treatment of schizophrenia, depression, obsessive-compulsive disorder, amyotrophic lateral sclerosis, epilepsy and post-traumatic stress disorder (http://onlinelibrary.wiley.com/cochranelibrary/), but did not address the topic of FW. Regarding ECT or tDCS, no sources were identified that reported evidence-based recommendations for its use in FW or more generally in FNS. Consistent with previous recommendations for the publication of case reports or case series (Lepping et al., 2007), and to allow clinically meaningful analyses from case series, we recommend that the following information should be included in any publication:

(1) Regarding devices and targeting procedure: the type of coil, the type of stimulator, the type of pulse waveform, the definition of the target and of its localization method, including the type of navigation system (if used), and the orientation and angle of the coil. Sham rTMS should be performed using original sham coils. Other alternatives should be accurately described and the rationale for the chosen technique should be highlighted.
(2) Regarding stimulation parameters: the intensity of the stimulation according to MT (resting/active MT) or maximal stimulator output, the frequency and duration of rTMS trains, the duration of the inter-train interval, the number of trains applied, the total number of rTMS pulses per session, the duration of each session, the number of sessions, the duration of the interval between the sessions, and the total duration of the treatment. The rationale for the chosen treatment protocol should be stated.
(3) Regarding ratings for motor symptoms and quality of life: the outcome assessment should be at least performed using CGI-I scores. Limb muscular strength should be assessed for each muscular group (force rated from 0 = complete paralysis to 5 = normal strength) at the beginning of treatment and at follow-up (if possible 6 and 12 months after cessation of treatment). Objective assessment of muscle force using dynamometers and of quality of life, using validated questionnaires are desirable. Raters should be blinded to the stimulation condition. In addition to objective ratings, the assessments of the treatment efficacy should include subjective ratings of symptom severity, as there may be a disparity between the patient's and the doctor's rating.
(4) Regarding the explanatory information for patients, the information received by the patient about the rTMS procedure and its expected positive or adverse effects should be outlined. The given information should be objective. Explanatory information for patients are not yet standardized and from a therapeutic perspective, its effect magnitude on clinical symptoms is unknown.
(5) Regarding a control condition, placebo-controlled study designs would be highly desirable. However, investigators should be aware that patients who are not treatment-naïve would easily detect the difference between the two conditions (particularly due to the perceptible scalp sensations by active stimulation). Therefore, except when using special placebo coils that provoke scalp effects similar to an active stimulation [as in Broersma et al. (2015) and Rossi et al. (2007)], we suggest that future studies should either be designed as parallel-arm studies (avoiding sham detection in a cross-over design) or as head-to-head studies, comparing active rTMS with usual therapeutic management of FW.

CONCLUSION

The results of our systematic review provide preliminary evidence that NIBS methods, especially motor cortex rTMS, may be beneficial in the treatment of conversion weakness and paralysis. Most included rTMS studies reported acute beneficial effects on limb function despite heterogeneous protocols. In particular, the crucial influence of an externally triggered muscular contraction should be emphasized. Further rTMS trials should include a control condition, a greater number of sessions, and longer stimulation protocols with proven lasting effects on cortical excitability. However, although advances have been made in the last few years both in diagnostic methods and in the groundwork for a neurobiological model of FW, no definitive rationale for stimulation parameters and for the optimal setting is available. Therefore, further basic research in this area is needed (Aybek et al., 2008). Probably due to practical aspects the future of ECT in this area is expected to be less promising than rTMS. Despite this practical advantage, it remains to be demonstrated that rTMS can have a real therapeutic benefit in the long term, and any impact on the neural mechanisms of FW beyond merely inducing psychological or non-specific placebo effects. Our systematic review contributes to the current knowledge of rTMS application in the treatment of FW, updating the reviews previously published by Pollak et al. (2014) and Parain and Chastan (2014). In summary, the available evidence to date suggests that the application of NIBS in FW is feasible and beneficial. However, due to the small number of published cases in open-label studies, this conclusion should be considered with caution.

AUTHOR CONTRIBUTIONS

CS, MG, PL made substantial contributions to conception and design of the review, CS, J-PL, MG performed the literature-search, analyzed the data, and wrote the manuscript. DN, AS, JL participated in drafting the manuscript, wrote the manuscript, and revisited it critically. BC made substantial contributions to the conception of the review, and revisited the manuscript critically. All authors gave the final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

Aybek, S., Kanaan, R. A., and David, A. S. (2008). The neuropsychiatry of conversion disorder. *Curr. Opin. Psychiatry* 21, 275–280. doi: 10.1097/YCO.0b013e3282f94242

Barker, A. T., Jalinous, R., and Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* 1, 1106–1107. doi: 10.1016/S0140-6736(85)92413-4

Bell, V., Oakley, D. A., Halligan, P. W., and Deeley, Q. (2011). Dissociation in hysteria and hypnosis: evidence from cognitive neuroscience. *J. Neurol. Neurosurg. Psychiatr.* 82, 332–339. doi: 10.1136/jnnp.2009.199158

Broersma, M., Koops, E. A., Vroomen, P. C., Van der Hoeven, J. H., Aleman, A., Leenders, K. L., et al. (2015). Can repetitive transcranial magnetic stimulation increase muscle strength in functional neurological paresis? A proof-of-principle study. *Eur. J. Neurol.* 22, 866–873. doi: 10.1111/ene.12684

Broersma, M., Kremer, B., Van der Hoeven, J. H., Vroomen, P., Maurits, N. M., and van Beilen, M. (2013). Effects of rTMS in conversion paralysis. *Soc. Proc. Clin. Neurphysiol.* 124:e119. doi: 10.1016/j.clinph.2013.04.193

Burgmer, M., Konrad, C., Jansen, A., Kugel, H., Sommer, J., Heindel, W., et al. (2006). Abnormal brain activation during movement observation in patients with conversion paralysis. *Neuroimage* 29, 1336–1343. doi: 10.1016/j.neuroimage.2005.08.033

Busner, J., and Targum, S. D. (2007). The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edgmont).* 4, 28–37.

Carson, A., Stone, J., Hibberd, C., Murray, G., Duncan, R., Coleman, R., et al. (2011). Disability, distress and unemployment in neurology outpatients with symptoms 'unexplained by organic disease.' *J. Neurol. Neurosurg. Psychiatr.* 8, 810–813. doi: 10.1136/jnnp.2010.220640

Carson, A. J., Brown, R., David, A. S., Duncan, R., Edwards, M. J., Goldstein, L. H., et al. (2012). Functional (conversion) neurological symptoms: research since the millennium. *J. Neurol. Neurosurg. Psychiatr.* 83, 842–850. doi: 10.1136/jnnp-2011-301860

Carson, A. J., Stone, J., Warlow, C., and Sharpe, M. (2004). Patients whom neurologists find difficult to help. *J. Neurol. Neurosurg. Psychiatr.* 75, 1776–1778. doi: 10.1136/jnnp.2003.032169

Cerletti, U. (1940). L'Elettroshock. *Rivista Sperimentale di Freniatria e Medicina Legale delle Alienazioni Mentali* 64, 209–310.

Chastan, N., Maltete, D., Derrey, S., Guillin, O., Lefaurcheur, R., Lebas, A., et al. (2011). Psychogenic movement disorders and recovery after motor cortex transcranial magnetic stimulation. *Parkinsonism Relat. Disord. 18(Suppl.* 2), 139. doi: 10.1016/S1353-8020(11)70618-6

Chastan, N., and Parain, D. (2010). Psychogenic paralysis and recovery after motor cortex transcranial magnetic stimulation. *Mov. Disord.* 25, 1501–1504. doi: 10.1002/mds.23187

Chastan, N., Parain, D., Verin, E., Weber, J., Faure, M. A., and Marie, J. P. (2009). Psychogenic aphonia: spectacular recovery after motor cortex transcranial magnetic stimulation. *J. Neurol. Neurosurg. Psychiatr.* 80, 94. doi: 10.1136/jnnp.2008.154302

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

Cojan, Y., Waber, L., Schwartz, S., Rossier, L., Forster, A., and Vuilleumier, P. (2009). The brain under self-control: modulation of inhibitory and monitoring cortical networks during hypnotic paralysis. *Neuron* 62, 862–875. doi: 10.1016/j.neuron.2009.05.021

Dafotakis, M., Ameli, M., Vitinius, F., Weber, R., Albus, C., Fink, G. R., et al. (2011). [Transcranial magnetic stimulation for psychogenic tremor - a pilot study]. *Fortschr. Neurol. Psychiatr.* 79, 226–233. doi: 10.1055/s-0029-1246094

de Lange, F. P., Roelofs, K., and Toni, I. (2008). Motor imagery: a window into the mechanisms and alterations of the motor system. *Cortex* 44, 494–506. doi: 10.1016/j.cortex.2007.09.002

DSM-5 (2013). *Diagnostic and Statistical Manual of Mental Disorders, 5th Edn*. Arlington, VA: American Psychiatric Association.

Ejareh Dar, M., and Kanaan, R. A. (2016). Uncovering the etiology of conversion disorder: insights from functional neuroimaging. *Neuropsychiatr. Dis. Treat.* 12, 143–153. doi: 10.2147/NDT.S65880

Feinstein, A., Stergiopoulos, V., Fine, J., and Lang, A. E. (2001). Psychiatric outcome in patients with a psychogenic movement disorder: a prospective study. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 14, 169–176.

Fitzgerald, P. B., Fountain, S., and Daskalakis, Z. J. (2006). A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin. Neurophysiol.* 117, 2584–2596. doi: 10.1016/j.clinph.2006.06.712

Gaillard, A., Gaillard, R., Mouaffak, F., Radtchenko, A., and Lôo, H. (2012). [Case report: electroconvulsive therapy in a 33-year-old man with hysterical quadriplegia]. *Encephale* 38, 104–109. doi: 10.1016/j.encep.2011.06.005

Garcin, B., Roze, E., Mesrati, F., Cognat, E., Fournier, E., Vidailhet, M., et al. (2013). Transcranial magnetic stimulation as an efficient treatment for psychogenic movement disorders. *J. Neurol. Neurosurg. Psychiatr.* 84, 1043–1046. doi: 10.1136/jnnp-2012-304062

Geraldes, R., Coelho, M., Rosa, M. M., Severino, L., Castro, J., and de Carvalho, M. (2008). Abnormal transcranial magnetic stimulation in a patient with presumed psychogenic paralysis. *J. Neurol. Neurosurg. Psychiatr.* 79, 1412–1413. doi: 10.1136/jnnp.2008.154583

Giovanoli, E. J. (1988). ECT in a patient with conversion disorder. *Convuls. Ther.* 4, 236–242.

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

Herwig, U., Schönfeldt-Lecuona, C., Wunderlich, A. P., von Tiesenhausen, C., Thielscher, A., Walter, H., et al. (2001). The navigation of transcranial magnetic stimulation. *Psychiatry Res.* 108, 123–131. doi: 10.1016/S0925-4927(01)00121-4

Jellinek, D. A., Bradford, R., Bailey, I., and Symon, L. (1992). The role of motor evoked potentials in the management of hysterical paraplegia: case report. *Paraplegia* 30, 300–302. doi: 10.1038/sc.1992.73

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

Koops, S., van den Brink, H., and Sommer, I. E. (2015). Transcranial direct current stimulation as a treatment for auditory hallucinations. *Front. Psychol.* 6:244. doi: 10.3389/fpsyg.2015.00244

Kresojevic, N., Petrovic, I., Tomic, A., Svetel, M., Radovanovic, S., and Kostic, V. (2010). "Transcranial magnetic stimulation in therapy of psychogenic neurological symptoms: two case reports," in *Movement Disorders, Vol. 25: Meeting Abstract: 101; Supplement: 220 at Conference: 14th International Congress of Parkinsons Disease and Movement Disorders* (Buenos Aires).

Lefaucheur, J.-P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., et al. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin. Neurophysiol.* 125, 2150–2206. doi: 10.1016/j.clinph.2014.05.021

Lepping, P., Russell, I., and Freudenmann, R. W. (2007). Antipsychotic treatment of primary delusional parasitosis: systematic review. *Br. J. Psychiatry* 191, 198–205. doi: 10.1192/bjp.bp.106.029660

Lepping, P., Schönfeldt-Lecuona, C., Sambhi, R. S., Lanka, S. V., Lane, S., Whittington, R., et al. (2014). A systematic review of the clinical relevance of repetitive transcranial magnetic stimulation. *Acta Psychiatr. Scand.* 130, 326–341. doi: 10.1111/acps.12276

Liepert, J., Hassa, T., Tüscher, O., and Schmidt, R. (2008). Electrophysiological correlates of motor conversion disorder. *Mov. Disord.* 23, 2171–2176. doi: 10.1002/mds.21994

Liepert, J., Hassa, T., Tüscher, O., and Schmidt, R. (2009). Abnormal motor excitability in patients with psychogenic paresis. A TMS study. *J. Neurol.* 256, 121–126. doi: 10.1007/s00415-009-0090-4

Liepert, J., Hassa, T., Tüscher, O., and Schmidt, R. (2011). Motor excitability during movement imagination and movement observation in psychogenic lower limb paresis. *J. Psychosom. Res.* 70, 59–65. doi: 10.1016/j.jpsychores.2010.06.004

Ludwig, V. U., Seitz, J., Schönfeldt-Lecuona, C., Höse, A., Abler, B., Hole, G., et al. (2015). The neural correlates of movement intentions: a pilot study comparing hypnotic and simulated paralysis. *Conscious. Cogn.* 35, 158–170. doi: 10.1016/j.concog.2015.05.010

Marshall, J. C., Halligan, P. W., Fink, G. R., Wade, D. T., and Frackowiak, R. S. (1997). The functional anatomy of a hysterical paralysis. *Cognition* 64, B1–B8. doi: 10.1016/S0010-0277(97)00020-6

McWhirter, L., Carson, A., and Stone, J. (2015). The body electric: a long view of electrical therapy for functional neurological disorders. *Brain 138(Pt* 4), 1113–1120. doi: 10.1093/brain/awv009

Meron, D., Hedger, N., Garner, M., and Baldwin, D. S. (2015). Transcranial direct current stimulation (tDCS) in the treatment of depression: systematic review and meta-analysis of efficacy and tolerability. *Neurosci. Biobehav. Rev.* 57, 46–62. doi: 10.1016/j.neubiorev.2015.07.012

Moene, F. C., Spinhoven, P., Hoogduin, K. A., and van Dyck, R. (2002). A randomised controlled clinical trial on the additional effect of hypnosis in a comprehensive treatment programme for in-patients with conversion disorder of the motor type. *Psychother. Psychosom.* 71, 66–76. doi: 10.1159/000049348

Nielsen, G., Stone, J., and Edwards, M. J. (2013). Physiotherapy for functional (psychogenic) motor symptoms: a systematic review. *J. Psychosom. Res.* 75, 93–102. doi: 10.1016/j.jpsychores.2013.05.006

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

Nordanskog, P., Larsson, M. R., Larsson, E. M., and Johanson, A. (2014). Hippocampal volume in relation to clinical and cognitive outcome after electroconvulsive therapy in depression. *Acta Psychiatr. Scand.* 129, 303–311. doi: 10.1111/acps.12150

Nowak, D. A., and Fink, G. R. (2009). Psychogenic movement disorders: aetiology, phenomenology, neuroanatomical correlates and therapeutic approaches. *Neuroimage* 47, 1015–1025. doi: 10.1016/j.neuroimage.2009.04.082

Parain, D., and Chastan, N. (2014). Large-field repetitive transcranial magnetic stimulation with circular coil in the treatment of functional neurological symptoms. *Neurophysiol. Clin.* 44, 425–431. doi: 10.1016/j.neucli.2014.04.004

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(Pt* 4), 847–858.

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

Petrides, G., Tobias, K. G., Kellner, C. H., and Rudorfer, M. V. (2011). Continuation and maintenance electroconvulsive therapy for mood disorders: review of the literature. *Neuropsychobiology* 64, 129–140. doi: 10.1159/000328943

Pogarell, O., Koch, W., Pöpperl, G., Tatsch, K., Jakob, F., Mulert, C., et al. (2007). Acute prefrontal rTMS increases striatal dopamine to a similar degree as D-amphetamine. *Psychiatry Res.* 156, 251–255. doi: 10.1016/j.pscychresns.2007.05.002

Pollak, T. A., Nicholson, T. R., Edwards, M. J., and David, A. S. (2014). A systematic review of transcranial magnetic stimulation in the treatment of functional (conversion) neurological symptoms. *J. Neurol. Neurosurg. Psychiatr.* 85, 191–197. doi: 10.1136/jnnp-2012-304181

Polyakova, M., Schroeter, M. L., Elzinga, B. M., Holiga, S., Schoenknecht, P., de Kloet, E. R., et al. (2015). Brain-derived neurotrophic factor and antidepressive effect of electroconvulsive therapy: systematic review and meta-analyses of the preclinical and clinical literature. *PLoS ONE* 10:e0141564. doi: 10.1371/journal.pone.0141564

Powell, T. Y., Boonstra, T. W., Martin, D. M., Loo, C. K., and Breakspear, M. (2014). Modulation of cortical activity by transcranial direct current stimulation in patients with affective disorder. *PLoS ONE* 9:e98503. doi: 10.1371/journal.pone.0098503

Rampello, L., Raffaele, R., Nicoletti, G., Le Pira, F., Malaguarnera, M., and Drago, F. (1996). Hysterical neurosis of the conversion type: therapeutic activity of neuroleptics with different hyperprolactinemic potency. *Neuropsychobiology* 33, 186–188. doi: 10.1159/000119275

Romero Lauro, L. J., Rosanova, M., Mattavelli, G., Convento, S., Pisoni, A., Opitz, A., et al. (2014). TDCS increases cortical excitability: direct evidence from TMS-EEG. *Cortex* 58, 99–111. doi: 10.1016/j.cortex.2014.05.003

Rossi, S., Ferro, M., Cincotta, M., Ulivelli, M., Bartalini, S., Miniussi, C., et al. (2007). A real electro-magnetic placebo (REMP) device for sham transcranial magnetic stimulation (TMS). *Clin. Neurophysiol.* 118, 709–716. doi: 10.1016/j.clinph.2006.11.005

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

Saba, G., Moukheiber, A., and Pelissolo, A. (2015). Transcranial cortical stimulation in the treatment of obsessive-compulsive disorders: efficacy studies. *Curr. Psychiatry Rep.* 17, 36. doi: 10.1007/s11920-015-0571-3

Saha, B. B., Zurowski, M., and Chen, R. (2011). "Failure of motor cortex repetitive transcranial magnetic stimulation (rTMS) combined with suggestion in the treatment of chronic psychogenic movement disorders (PMDs): a pilot study," in *15th International Congress of Parkinson's Disease and Movement Disorders* (Toronto, ON).

Schönfeldt-Lecuona, C., Cárdenas-Morales, L., Freudenmann, R. W., Kammer, T., and Herwig, U. (2010). Transcranial magnetic stimulation in depression–lessons from the multicentre trials. *Restor. Neurol. Neurosci.* 28, 569–576. doi: 10.3233/RNN-2010-0561

Schönfeldt-Lecuona, C., Connemann, B. J., Spitzer, M., and Herwig, U. (2003). Transcranial magnetic stimulation in the reversal of motor conversion disorder. *Psychother. Psychosom.* 72, 286–288. doi: 10.1159/000071900

Schönfeldt-Lecuona, C., Connemann, B. J., Viviani, R., Spitzer, M., and Herwig, U. (2006). Transcranial magnetic stimulation in motor conversion disorder: a short case series. *J. Clin. Neurophysiol.* 23, 472–475. doi: 10.1097/01.wnp.0000219004.69158.1e

Schönfeldt-Lecuona, C., Thielscher, A., Freudenmann, R. W., Kron, M., Spitzer, M., and Herwig, U. (2005). Accuracy of stereotaxic positioning of transcranial magnetic stimulation. *Brain Topogr.* 17, 253–259. doi: 10.1007/s10548-005-6033-1

Shah, B. B., Chen, R., Zurowski, M., Kalia, L. V., Gunraj, C., and Lang, A. E. (2015). Repetitive transcranial magnetic stimulation plus standardized suggestion of benefit for functional movement disorders: an open label case series. *Parkinsonism Relat. Disord.* 21, 407–412. doi: 10.1016/j.parkreldis.2015.01.013

Shapiro, A. P., and Teasell, R. W. (2004). Behavioural interventions in the rehabilitation of acute v. *chronic non-organic (conversion/factitious) motor disorders. Br. J. Psychiatry* 185, 140–146. doi: 10.1192/bjp.185.2.140

Siebner, H. R., and Rothwell, J. (2003). Transcranial magnetic stimulation: new insights into representational cortical plasticity. *Exp. Brain Res.* 148, 1–16. doi: 10.1007/s00221-002-1234-2

Spence, S. A., Crimlisk, H. L., Cope, H., Ron, M. A., and Grasby, P. M. (2000). Discrete neurophysiological correlates in prefrontal cortex during hysterical and feigned disorder of movement. *Lancet* 355, 1243–1244. doi: 10.1016/S0140-6736(00)02096-1

Stone, J., Carson, A., Duncan, R., Coleman, R., Roberts, R., Warlow, C., et al. (2009). Symptoms 'unexplained by organic disease' in 1144 new neurology out-patients: how often does the diagnosis change at follow-up? *Brain 132(Pt* 10), 2878–2888. doi: 10.1093/brain/awp220

Stone, J., Zeman, A., Simonotto, E., Meyer, M., Azuma, R., Flett, S., et al. (2007). FMRI in patients with motor conversion symptoms and controls with simulated weakness. *Psychosom. Med.* 69, 961–969. doi: 10.1097/PSY.0b013e31815b6c14

Strafella, A. P., Paus, T., Fraraccio, M., and Dagher, A. (2003). Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. *Brain 126(Pt* 12), 2609–2615. doi: 10.1093/brain/awg268

Strafella, A. P., Vanderwerf, Y., and Sadikot, A. F. (2004). Transcranial magnetic stimulation of the human motor cortex influences the neuronal activity of subthalamic nucleus. *Eur. J. Neurosci.* 20, 2245–2249. doi: 10.1111/j.1460-9568.2004.03669.x

Taylor, S. M. (2008). Electroconvulsive therapy, brain-derived neurotrophic factor, and possible neurorestorative benefit of the clinical application of electroconvulsive therapy. *J. ECT* 24, 160–165. doi: 10.1097/YCT.0b013e3181571ad0

van Beilen, M., de Jong, B. M., Gieteling, E. W., Renken, R., and Leenders, K. L. (2011). Abnormal parietal function in conversion paresis. *PLoS ONE* 6:e25918. doi: 10.1371/journal.pone.0025918

Voon, V., Brezing, C., Gallea, C., and Hallett, M. (2011). Aberrant supplementary motor complex and limbic activity during motor preparation in motor conversion disorder. *Mov. Disord.* 26, 2396–2403. doi: 10.1002/mds.23890

Voon, V., and Lang, A. E. (2005). Antidepressant treatment outcomes of psychogenic movement disorder. *J. Clin. Psychiatry* 66, 1529–1534. doi: 10.4088/JCP.v66n1206

Vuilleumier, P. (2005). Hysterical conversion and brain function. *Prog. Brain Res.* 150, 309–329. doi: 10.1016/S0079-6123(05)50023-2

Vuilleumier, P., Chicherio, C., Assal, F., Schwartz, S., Slosman, D., and Landis, T. (2001). Functional neuroanatomical correlates of hysterical sensorimotor loss. *Brain 124(Pt* 6), 1077–1090. doi: 10.1093/brain/124.6.1077

WHO ICD-10 (1991). *Mental and Behavioral Disorders. Clinical Descriptions and Diagnostic Guidelines*. Geneva: World Health Organization; Tenth Revision of the International Classification of Diseases.

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 Neuroscience

REVIEW
published: 16 February 2016
doi: 10.3389/fnins.2016.00030

Targeting Neural Endophenotypes of Eating Disorders with Non-invasive Brain Stimulation

Katharine A. Dunlop[1,2], *Blake Woodside*[1,3,4,5] *and Jonathan Downar*[1,2,3,4,6*]

[1] *Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada,* [2] *MRI-Guided rTMS Clinic, University Health Network, Toronto, ON, Canada,* [3] *Department of Psychiatry, University Health Network, Toronto, ON, Canada,* [4] *Department of Psychiatry, University of Toronto, Toronto, ON, Canada,* [5] *Eating Disorders Program, University Health Network, Toronto, ON, Canada,* [6] *Toronto Western Research Institute, University Health Network, Toronto, ON, Canada*

The term "eating disorders" (ED) encompasses a wide variety of disordered eating and compensatory behaviors, and so the term is associated with considerable clinical and phenotypic heterogeneity. This heterogeneity makes optimizing treatment techniques difficult. One class of treatments is non-invasive brain stimulation (NIBS). NIBS, including repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), are accessible forms of neuromodulation that alter the cortical excitability of a target brain region. It is crucial for NIBS to be successful that the target is well selected for the patient population in question. Targets may best be selected by stepping back from conventional DSM-5 diagnostic criteria to identify neural substrates of more basic phenotypes, including behavior related to rewards and punishment, cognitive control, and social processes. These phenotypic dimensions have been recently laid out by the Research Domain Criteria (RDoC) initiative. Consequently, this review is intended to identify potential dimensions as outlined by the RDoC and the underlying behavioral and neurobiological targets associated with ED. This review will also identify candidate targets for NIBS based on these dimensions and review the available literature on rTMS and tDCS in ED. This review systematically reviews abnormal neural circuitry in ED within the RDoC framework, and also systematically reviews the available literature investigating NIBS as a treatment for ED.

Keywords: Eating Disorders (ED), RDoC, rTMS, tDCS, fMRI

OPEN ACCESS

Edited by:
Ignacio Obeso,
Hospital Universitario Puerta del Sur - HM Hospitales, Spain

Reviewed by:
Bernd Weber,
Rheinische-Friedrich-Wilhelms Universität, Germany
Anne Sauvaget,
University Hospital of Nantes, France

***Correspondence:**
Jonathan Downar
jonathan.downar@uhn.ca

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

Received: *17 November 2015*
Accepted: *25 January 2016*
Published: *16 February 2016*

Citation:
Dunlop KA, Woodside B and Downar J (2016) Targeting Neural Endophenotypes of Eating Disorders with Non-invasive Brain Stimulation. Front. Neurosci. 10:30. doi: 10.3389/fnins.2016.00030

INTRODUCTION

The term "eating disorders" (ED) encompasses a wide variety of disordered eating and compensatory behaviors that inappropriately alter the patient's body shape or weight, or the subjective experience of one's own body shape or weight. According to recent studies, the lifetime prevalence of EDs is 5.7% for females, and 1.2% in males (Golden et al., 2003; Hudson et al., 2007; Smink et al., 2014). The lifetime prevalence of the top three EDs according to the DSM-5 diagnostic criteria is 2.3, 1.7, and 0.8% for adolescent binge eating disorder (BED), anorexia nervosa (AN), and bulimia nervosa (BN), respectively (Golden et al., 2003; Hudson et al., 2007; Smink et al., 2014). BED is associated with recurrent episodes of binging, typically during negative affect (Leehr et al., 2015), and with the absence of inappropriate compensatory behaviors to avoid weight gain. Both AN and BN are associated with disturbances in the subjective experience of one's own body shape or weight; this phenotype is also known as body

dysmorphia. BN is also defined by recurrent episodes of binge eating, with inappropriate compensatory behaviors to avoid weight gain; such behaviors include vomiting, excessive exercise, laxative misuse or diuretic misuse. In contrast, AN is defined by the persistent restriction of food, an intense fear of gaining weight, and a significantly low body weight for one's developmental stage. AN has two subtypes, restricting (ANR) and binge-eating/purging (ANBP), with the latter distinguished from the former by the presence of binges and/or purges.

Despite a low lifetime prevalence rate relative to other psychiatric disorders, EDs carry a significant burden of illness, both socially and individually. Treatment capacity in specialized ED programs is presently inadequate to meet demand (Hart et al., 2011), and for patients who do manage to access specialized programs, economic difficulties and high costs often hamper treatment adherence (Gatt et al., 2014). EDs are also associated with a high mortality rate; for one, approximately 10% of AN sufferers will die within 10 years of disease onset (Sullivan, 1995). According to a recent meta-analysis, the overall standard mortality ratio (SNR) for AN is 5.86, higher than schizophrenia (2.8), bipolar disorder (2.1), and major depression (1.6) (Arcelus et al., 2011). Conventional ED treatments, including pharmacotherapy, and in- and out-patient behavioral therapies, are associated with suboptimal recovery rates (∼50% for AN; ∼45% for BN; ∼50–70% for BED), high relapse rates (ranging from 9 to 65%), and high chronicity (∼20% will develop a chronic disorder; Olmsted et al., 2005; Mitchell et al., 2007; Shapiro et al., 2007; Carter et al., 2012; Hay et al., 2012; Hilbert et al., 2012; Amianto et al., 2015). ANBP, in particular, has the poorest prognosis of the eating disorders (Steinhausen and Weber, 2009). EDs are also highly co-morbid with other psychiatric disorders, such as major depression and obsessive-compulsive disorder, whose presence negatively impacts treatment outcomes (Godart et al., 2003; Crane et al., 2007; Mischoulon et al., 2011). Thus, new treatment approaches are urgently needed, especially for the substantial proportion of ED patients who are unresponsive to conventional treatment strategies.

Neuromodulation technologies are beginning to emerge as a promising new treatment option for treatment resistant ED patients. The potential usefulness of these techniques was recently illustrated in a pilot study using subgenual cingulate deep brain stimulation (DBS) to achieve symptomatic improvements in severe and treatment-refractory AN (Lipsman et al., 2013). Although potentially powerful, DBS remains for the moment a fairly invasive treatment, and is available only to small volumes of patients in specialist neurosurgical centers. A more accessible alternative is non-invasive brain stimulation (NIBS), including techniques such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). rTMS uses rapid pulses of an electromagnetic field to elicit action potentials in the target area of cortex. tDCS uses a weaker intensity electrical stimulus, delivered by scalp electrodes, to modulate cortical excitability in the underlying regions. Both NIBS strategies attempt to alter the cortical excitability of a target brain region to normalize particular disorder-specific phenotypes. Cortical targets are typically selected based on abnormal structural or functional attributes in the disorder relative to healthy controls. Appropriate cortical targeting using NIBS is critical for optimal treatment efficacy (Fox et al., 2013). Therefore, a proper understanding of the neural substrates, as well as the cognitive and behavioral phenotypes accompanying these substrates, is crucial for optimizing future treatments.

Two major issues associated with NIBS as a treatment for ED are the tremendous heterogeneity in the cognitive and behavioral phenotypes of patients within this illness category, and the dynamic course of the illness, in which patients can switch from one ED diagnosis to another over time (Garfinkel et al., 1995; Keel and Mitchell, 1997; Lilenfeld et al., 1998; Sullivan et al., 1998; Strober et al., 2000; Bulik et al., 2005; Milos et al., 2005). This variability within a single diagnosis and this malleability of symptoms is a likely contributor to the limited clinical efficacy of NIBS (and in conventional treatments more generally) for ED.

Two possible solutions to address this heterogeneity are genomic methods, such as phenotypic linkage analyses, as well as neuroimaging methods. Such tools stratify patients on underlying behavioral, genetic, and neuropathological dimensions rather than self-reported symptoms alone. Therefore, these tools may be useful to identify the underlying behavioral and neuropathological endophenotypes related to more basic dimensions of behavior, independent of DSM-5 diagnoses. Such analyses and resulting endophenotypes can also be related to the behavioral and circuit-based dimensions of the recently described Research Domain Criteria (Insel et al., 2010) (RDoC). The RDoC is a recent strategy aimed at integrating basic neuroscientific knowledge with clinical diagnoses by first describing fundamental behaviors, described below, as dimensions. These dimensions are then used to describe the pathological behaviors of psychiatric disorders. By using the RDoC schema in combination with neuroimaging and phenotypic linkage methods, we may be able to identify sufficient stimulatory targets addressing specific phenotypes such as restrictive behavior or binging, regardless of DSM-5 diagnosis. For NIBS treatments, diagnostic systems must be capable of parsing this heterogeneity using endophenotypes so we may select the optimal stimulation target for a particular behavioral marker, or neural substrate.

Here, we will review NIBS as a treatment for the three most prevalent forms of ED: AN, BN, and BED. First, we will posit candidate dimensions as outlined by the RDoC and their underlying behavioral and neurobiological targets associated with ED as potential candidates for NIBS. Second, we will review the available literature on rTMS and tDCS as possible treatments for ED. Lastly, we will discuss the current limitations of the NIBS-ED field, and opportunities of future study and development.

GOING BEYOND THE DSM-5 DIAGNOSIS: HOW CAN WE MAXIMIZE EFFICACY?

As discussed above, one of the major obstacles in ED diagnosis and treatment is the heterogeneity within each diagnostic category; conversely, comparisons of clinical and psychological features across patients suggest that there is significant overlap

between ED diagnoses (Garfinkel et al., 1995; Lilenfeld et al., 1998; Sullivan et al., 1998; Strober et al., 2000). Compounding this problem is the evolution of the illness, such that patients may transition from one diagnostic category to another over time (Bulik et al., 2005). For example, it is estimated that approximately 50% of patients initially diagnosed with ANR will develop binge/purge behaviors, and approximately 30% of BN patients have a history of AN (Keel and Mitchell, 1997). In another study following DSM-IV-diagnosed AN and BN, only one third of subjects retained their original diagnosis after 30 months (Milos et al., 2005). To improve diagnostic consistency and treatment efficacy may require us to identify more stable, more granular, and more biologically based subgroups, or endophenotypes, within the ED population.

Some classification efforts have focused on a single DSM diagnosis. AN has been subdivided into 3 stable classes based on co-occurring symptoms: fat-phobic ANR, fat-phobic ANBP, and non-fat-phobic ANR (Wildes et al., 2013). BN has been subdivided based on personality attributes (affective-perfectionistic, impulsive and low-comorbid psychopathology clusters Wonderlich et al., 2005) and based on presenting symptoms (binging, purging, and bingeing-purging, Striegel-Moore et al., 2005).

A number of latent class (LCA) and latent profile analyses (LPA) have been performed on symptomatic and personality factors to stratify endophenotypes spanning AN and BN. One symptom-based LCA found optimal fitting for a 4-group classification. ANR, ANBP/BN, ANR without OCD, and BN with only vomiting as purging were the four groups identified (Keel et al., 2004). Another symptom-based LPA identified 4 ED classes: binging with multiple types of compensatory behavior; binging with only vomiting as compensatory behavior; binging without purging; and low/normal weight with excessive exercise (Eddy et al., 2009).

As evidenced above, there now exist a variety of different proposals for how best to subcategorize ED patients, within and across DSM-5 diagnoses. How, therefore, can we converge upon a system that offers maximum clinical usefulness? One potentially fruitful method would be to better characterize the heterogeneity among ED patients in biological terms, using techniques such as positron emission tomography (PET), electroencephalography (EEG) and magnetic resonance imaging (MRI) to identify distinct neurobiological substrates underlying the different subgroups within ED. Clinical endophenotypes could then be tied to neurobiological substrates, which could in turn serve as targets for individually- or phenotypically-tailored treatment strategies.

Such an approach would also allow us to describe illnesses in dimensional rather than categorical terms. For example, the influential RDoC framework (Insel et al., 2010) includes dimensional constructs such as positive valence, negative valence, cognitive systems, social processes, and arousal and regulatory systems (for a review of how RDoC dimensions relate to ED neurobiology, see Wildes and Marcus, 2015, **Table 1**). Many endophenotypes, previously identified as symptom clusters in the ED population, can be framed parsimoniously as the result of pathology affecting these dimensions, either singly or in combination (**Figure 1**). An "RDoC formulation" of our ED endophenotypes carries the advantage of pointing toward specific cognitive processes, neural pathways, neurotransmitter systems, molecular targets, or genes that might be targeted for therapeutic effect. For the purposes of this review, we will confine our discussion to potential novel uses of NIBS to target specific neural pathways that are associated with RDoC constructs, as they relate to specific endophenotypes within the ED population.

TABLE 1 | Overview of the 5 Research Domain Criteria domains as adapted from Insel et al. (2010) and Morris and Cuthbert (2012).

RDoC Domain	Construct
Negative valence systems	Active threat/Fear
	Potential threat/Anxiety
	Sustained threat
	Loss
	Frustrative nonreward
Positive valence systems	Approach motivation
	Responsiveness to reward
	Reward learning
	Habit
Cognitive systems	Attention
	Perception
	Working/Declarative memory
	Language
	Cognitive/Effortful control
Social processes	Imitation/Theory of mind
	Social dominance
	Facial expression identification
	Attachment/Separation
	Self-Representation
Arousal/Regulatory systems	Arousal
	Circadian rhythms
	Sleep and wakefulness

RDOC DOMAINS AS ED ENDOPHENOTYPES AND NIBS TARGETS

For the following section, a systematic review was completed using PubMed (NIH, http://www.ncbi.nlm.nih.gov/pubmed), with searches containing the following terms: first, clinical terms for the three ED diagnoses in this review (bulimia nervosa, anorexia nervosa, and binge eating disorder), and second, RDoC related terms as discussed in a recent review on RDoC cognitive systems (Morris and Cuthbert, 2012).

Negative Valence Systems

Negative valence systems are activated in response to aversive stimuli, and include fear, anxiety and loss-related behaviors. In a recent meta-analysis investigating neural activations for negative and positive affect, negative valence was associated with greater activation in the amygdala and anterior insula (Lindquist et al., 2015). The lateral orbitofrontal cortex (OFC) is also associated with negative valence, particularly during the anticipation and receipt of punishment (Ursu et al., 2008).

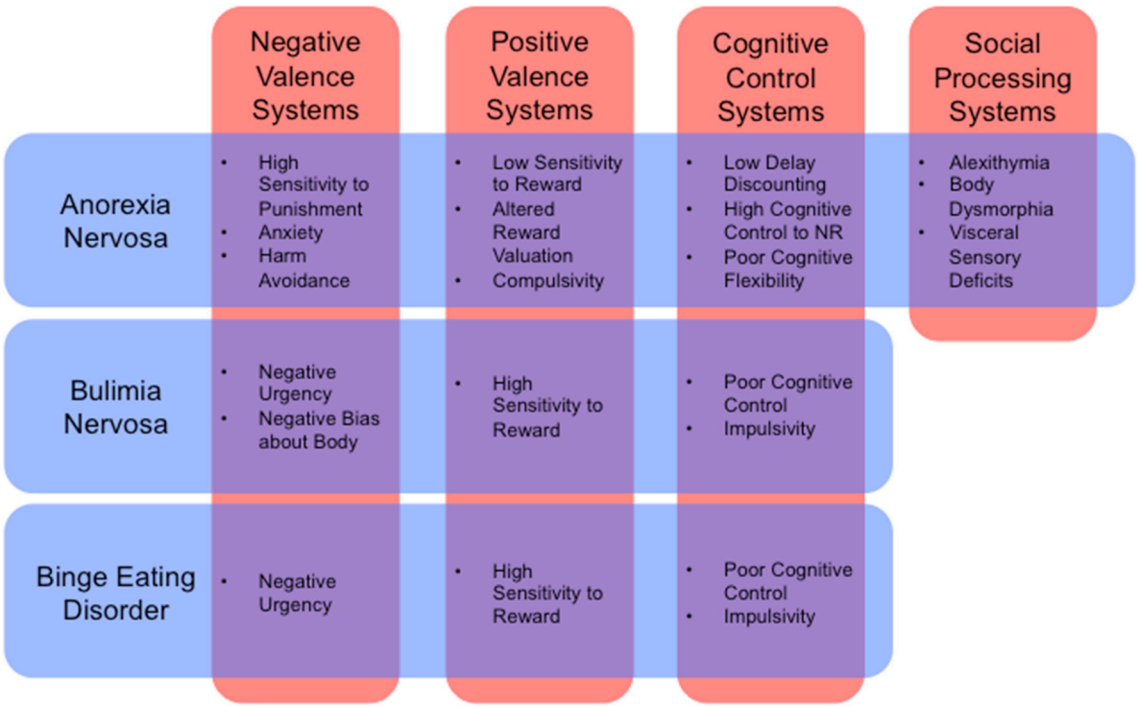


FIGURE 1 | Cognitive and behavioral phenotypes by RDoC dimension (Insel et al., 2010) for anorexia nervosa, bulimia nervosa, and binge eating disorder (Schebendach et al., 2007, 2013; Fernández-Aranda et al., 2008; Zastrow et al., 2009; Harrison et al., 2010; Klein et al., 2010; Miyake et al., 2010; Manwaring et al., 2011; Bohon and Stice, 2012; Hoffman et al., 2012; Steinglass et al., 2012; Chan et al., 2013; Giel et al., 2013; Strigo et al., 2013; Wu et al., 2013; Glashouwer et al., 2014; Kullmann et al., 2014; Mole et al., 2015; Berg et al., 2015; Racine et al., 2015; Tapajóz P de Sampaio et al., 2015). NR, Natural Rewards.

A number of studies support the role of negative valence systems in ED, mainly in behaviors associated with negative affect, sensitivity to punishment, anxiety, harm avoidance, and response to the receipt of punishment (**Figure 1**). For example, behavioral measures of negative affect and negative urgency are the two most predictive features before a binge episode in both BED and BN (Bohon and Stice, 2012; Berg et al., 2015; Leehr et al., 2015; Racine et al., 2015). On functional neuroimaging, BN patient reported negative affect is related to neural responsivity during the anticipation of a food reward in both the striatum and insula (Bohon and Stice, 2012). This relation suggests that negative affect and food-reward are inappropriately coupled in this disorder. More generally, BN patients also have higher neural responses to negative body image descriptors (Miyake et al., 2010), in areas associated with the regulation and inhibition of fear and emotional processing circuits, including the dorsomedial prefrontal cortex (DMPFC) (Kühn et al., 2011; Åhs et al., 2015). These findings shed light on the role of negative attentional bias in the psychopathology of bulimic-type disorders.

Restrictive subtypes of ED also show hypersensitivity on measures related to negative valence systems. Behaviorally, exaggerated harm avoidance and sensitivity to punishment are typically associated with forms of AN (Harrison et al., 2010). Similarly, on fMRI, AN patients display increased neural activation in right anterior insula and DLPFC during pain anticipation, and exaggerated responses to punishment (pain and monetary losses) in the DLPFC, and the anterior, mid-, and motor cingulate (Bischoff-Grethe et al., 2013; Strigo et al., 2013; Bar et al., 2015). Cowdrey and colleagues also found an exaggerated response to an aversive taste and sight of food in the insula, striatum and ACC (Cowdrey et al., 2011). Trait-anxiety is also a common feature of AN, and is associated with the exaggerated activity of fear-related circuits to food and body-related cues. Regions of exaggerated response to symptom-provoking stimuli include the amygdala, hippocampus, insula, ACC, and medial PFC (Ellison et al., 1998; Frank et al., 2002, 2012b; Seeger et al., 2002; Uher et al., 2004; Friederich et al., 2010; Vocks et al., 2010). Finally, at the receptor level, PET imaging reveals increased striatal dopamine binding potential and altered cingulate serotonergic (increased 5-HT1A, but decreased 5-HT2A) binding potential is associated with harm avoidance in AN (Bailer et al., 2004, 2007; Frank et al., 2005).

Summary of Potential Negative Valence Targets

Both bulimic and restrictive-type EDs display some form of negative valence abnormality on behavioral and neuroimaging modalities (**Figure 2**). In ED with a binging component, it appears that negative affect and food-reward responsivity are intimately coupled via the exaggerated response of the amygdala, insula and DMPFC. Restriction-related EDs display a similar pattern in the amygdala, right anterior insula, DLPFC and mPFC accompanying aspects of harm avoidance and receipt

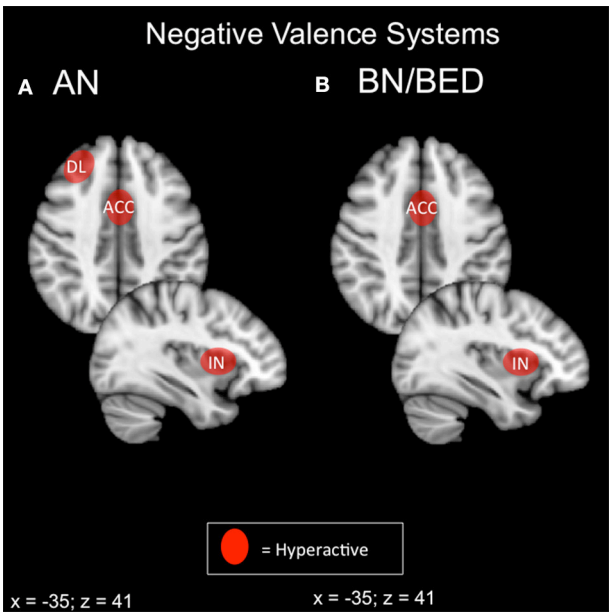


FIGURE 2 | Candidate NIBS targets that address abnormal phenotypes related to the RDoC negative valence dimension. (A) Candidate negative valence NIBS targets for anorexia nervosa (AN) (Ellison et al., 1998; Frank et al., 2002, 2012b; Seeger et al., 2002; Uher et al., 2004; Friederich et al., 2010; Vocks et al., 2010; Cowdrey et al., 2011; Bischoff-Grethe et al., 2013; Strigo et al., 2013; Bär et al., 2015). The dorsolateral prefrontal cortex (DL) is abnormally hyperactive for pain anticipation and the receipt of punishment. The anterior cingulate cortex (ACC) is hyperactive for aversive food stimuli, the receipt of punishment, and anxiety. Finally, the anterior insula (IN) is abnormally hyperactive during anxiety and the anticipation of pain. **(B)** Candidate negative valence NIBS targets for bulimia nervosa (BN) and binge eating disorder (BED). The ACC is abnormally activated for negative words about the body (Miyake et al., 2010), while the insula is hyperactive during negative affect (Bohon and Stice, 2012).

of punishment. Frontal regions, particularly the medial PFC and DMPFC, are thought to inhibit activity of the basolateral amygdala (BLA) (Cho et al., 2013; Felix-Ortiz et al., 2015).

Hyperactivation of the DMPFC, DLPFC, and anterior insula during negative valence paradigms has two possible interpretations. First, these areas may be inhibiting BLA activity, but insufficiently, in which case excitatory stimulation may be beneficial. Alternatively, these areas may actually be inappropriately driving BLA activity in a top-down fashion, in which case inhibitory stimulation would be preferable. A key study illustrated these opposite mechanisms in emotion regulation in healthy controls vs. MDD patients (Johnstone et al., 2007): during emotional reappraisal, limbic frontal regions suppressed amygdala activity in controls, but counterproductively *increased* amygdala activity in MDD.

For NIBS interventions, direct suppression of the amygdala is challenging due to its deep location; strategies aimed at damping negative valence systems will therefore likely target in prefrontal cortex and insula. Excitatory prefrontal NIBS has been recently shown to attenuate amygdala-dependent negative processing in healthy controls (Baeken et al., 2010; Guhn et al., 2014), and this strategy may be best in "bottom-up" pathology, where emotional reappraisal/self-regulation systems are underactive rather than pathologically hyperactive (i.e., in BN and BED). Conversely, where negative valence systems are driven by "top-down" pathology, and self-regulation is if anything excessive, inhibitory stimulation may be preferable. Inhibitory NIBS of the DMPFC and lateral OFC both show promise in obsessive-compulsive disorder (Mantovani et al., 2010; Nauczyciel and Drapier, 2012; Dunlop K. et al., 2015), and these strategies may be better suited to AN-R, particularly in cases with comorbid OCD.

Positive Valence Systems

Positive valence systems encompass neural circuits related to motivation, reward seeking, and habit formation behaviors. According to a recent meta-analysis in healthy controls, positive stimuli are associated with activity in the VMPFC and ACC (Lindquist et al., 2015). All three major EDs, AN, BN, and BED, have been previously shown to be altered in this dimension (**Figure 1**).

From a behavioral perspective, AN patients show diminished sensitivity to conventional reward, as evident on psychometric measures (Harrison et al., 2010; Glashouwer et al., 2014) and delay discounting tasks (Steinglass et al., 2012). From a neurobiological perspective, ANR patients likewise display a blunted neural response to food reward in the insula and striatum (Wagner et al., 2008), decreased response to food images in the insula (Holsen et al., 2012; Oberndorfer et al., 2013b), and altered striatal activation during a reward-learning paradigm (Wagner et al., 2007). In a recent fMRI study of delay discounting in AN patients and healthy controls, AN patients had a marked preference for delayed rewards, associated with lower activation in the striatum and dorsal ACC during decision-making; these behavioral and neural abnormalities normalized to control levels after treatment (Decker et al., 2014). However, another study found that weight restoration did not affect choice behavior on a delay discounting task (Ritschel et al., 2015), suggesting that a preference for delayed over immediate rewards may be an endophenotypic feature in low-BMI individuals. In either case, the identified striatal and prefrontal regions are all involved in the motivational aspect of reward and food-reward processing.

There is also evidence that reward evaluation is altered in AN, in which secondary (contextual) rewards such as exercise and dietary restriction carry higher reward value relative to food or other primary rewards (Schebendach et al., 2007; Klein et al., 2010). The so-called "reward contamination theory" of AN posits a pathological re-configuration of the patient's reward system through stress-induced activation of the mesolimbic dopamine system, via ventral tegmental area opioid receptors. In this framework, AN behaviors essentially represent a maladaptive, but well-entrenched type of habit-formation (Keating et al., 2012; Walsh, 2013).

The findings that support this theory suggest that there is altered motivational salience for disease-related stimuli. For example, AN patients tend to rate physical exercise as "pleasant," more so than food (Giel et al., 2013). In fact, food-reward in AN activates a weight-gain fear response (i.e., negative valence systems) in the amygdala and extrastriate body rather than positive valence systems from the striatum, orbitofrontal cortex,

and ACC (Vocks et al., 2011). The DLPFC is also hyperactive in response to images of food and the anticipation of reward, suggesting the presence of enhanced cognitive control over food cues and reward (Ehrlich et al., 2015; Sanders et al., 2015). ANR patients also have a high prevalence of comorbid OCD (Torresan et al., 2013). The level of compulsivity predicts the reactivity of the superior frontal gyrus, ACC and striatum and deactivation of the PFC to images of high-calorie foods (Rothemund et al., 2011), and lowered right DLPFC activity is seen in response to obsessive-compulsive symptom provocation in AN (Suda et al., 2014). Thus, hypofunctioning of primary reward systems (and potentially, hyperfunctioning of secondary/contextual reward systems) may be important target processes in ANR.

In contrast disorders in the BN/BED spectrum are often associated with elevated primary reward valuation and reward sensitivity. These are typically associated with a higher willingness to work for a food reward (Schebendach et al., 2013), as well as higher impulsivity (Manwaring et al., 2011; Chan et al., 2013; Mole et al., 2015) At the neural level, BN and BED patients show increased activity for reward receipt in areas including the medial OFC, ventral striatum and insula (Schienle et al., 2009; Frank et al., 2011, 2012a; Radeloff et al., 2012; Weygandt et al., 2012; Oberndorfer et al., 2013a). BED patients display hyperactivations in the ventral striatum and inferior frontal gyrus during reward anticipation, and reduced medial PFC activity during a monetary incentive delay task (Balodis et al., 2014, 2013a). On PET imaging, areas like the insula, PFC and ventral striatum, associated with reward-motivation and food-reward processing, have altered serotonergic and dopaminergic binding in BN (Broft et al., 2012; Galusca et al., 2014). An important associated feature may also be deficient behavioral self-regulation and impulsivity. BN patients also show reduced activation in anticipation of a food reward is seen in ACC and right anterior insula; lower ACC activity predicts how much the patient will overeat (Frank et al., 2006; Bohon and Stice, 2011). Parallels have been drawn between the neural substrates of BN/BED and addiction, due to the similar alterations to motivation and reward-related circuitry on fMRI and task-based paradigms between the two psychopathologies (Filbey et al., 2012).

Summary of Potential Positive Valence Targets

In terms of positive valence systems, it appears that both restrictive and binging phenotypes of ED display alterations in incentive salience that is potentially modulated by the opioid system (Keating et al., 2012; Giuliano and Cottone, 2015; **Figure 3**). In the case of ANR, conventional primary rewards appear to be devalued in favor of pathological secondary or contextual rewards, such as starvation and excessive exercise. A broader preference for long-term/contextual over immediate primary rewards is also apparent in choice behavior during delay discounting. Neurally, the primary reward systems of the ventral striatum and ventromedial prefrontal cortex appear hypoactive, while contextual or secondary reward systems operating through lateral orbitofrontal and lateral temporal regions appear hyperactive. Hyperactivity in lateral orbitofrontal pathways is also strongly associated with OCD, and with compulsivity in general (Ahmari et al., 2013; Beucke et al., 2013). This finding would be consistent with the broader phenotype of ANR. Neurally-based strategies in ANR might therefore include enhancing primary reward value via medial prefrontal-striatal pathways, or attenuating secondary reward value via lateral prefrontal-striatal pathways. For instances where conventional rewards are less valued than maladaptive ones (restrictive, fat-phobic ED), inhibitory NIBS over lateral networks for maladaptive secondary rewards, and excitatory NIBS over medial networks for conventional rewards, may be a possible therapeutic protocol to realign incentive-salience mechanisms to normal, adaptive functioning.

In the case of binge/purge-related EDs, repeated exposures to the transient reward value of food intake (or the transient anti-anxiety effect of purging) would cause these behaviors to acquire pathologically high incentive value (especially in the presence of negative urgency), via neural mechanisms that parallel those of addiction. Effective strategies would therefore parallel those for substance addiction: enhancing cognitive/impulse control over urges to binge and purge, or suppressing urge intensity.

NIBS strategies for enhancing cognitive control involve excitatory stimulation of the nodes of the salience network, including the DLPFC, dACC, and insula (Dunlop et al., accepted). Each of these targets have demonstrated efficacy in substance dependence (Mishra et al., 2010; De Ridder et al., 2011; Meng et al., 2014), with effects apparently mediated by enhanced control rather than reduced urge. Recently, excitatory rTMS over the dACC has been reported to reduce symptoms in treatment-resistant binge/purge ED, via enhanced integrity of frontostriatal circuits in the salience network (Dunlop J. et al., 2015).

NIBS may also be capable of suppressing urge, by targeting frontopolar and ventromedial sites. In one preclinical rTMS study, substance use disorder patients underwent inhibitory rTMS over the ventral frontal pole during a task evoked a cue-related craving response. A single session of inhibitory rTMS reduced the severity of craving in this group relative to sham, and stimulation proved capable of engaging core reward nodes in the ventral striatum, as well as the associated ventromedial prefrontal regions (Hanlon et al., 2013, 2015). Urge suppression via inhibitory ventromedial prefrontal stimulation has yet to be attempted in ED, but would be a reasonable strategy to complement excitatory salience-network stimulation in binge/purge-related ED populations.

Cognitive Systems

The cognitive systems dimension refers to processes responsible for cognitive processing, including attention, perception, memory, language, and cognitive control. In healthy control studies, these behaviors are associated with activity in the DMPFC, DLPFC, and anterior insula (Albares et al., 2014; Cho et al., 2014; Luo et al., 2014; Reineberg et al., 2015). These networks tend to be associated with the central executive and salience resting-state networks (Reineberg et al., 2015), responsible for response selection and inhibition.

Abnormal cognitive control mechanisms are evident in most ED populations (**Figure 1**). On the one hand, BN and BED-type diagnoses tend to display reduced capacity for impulse and

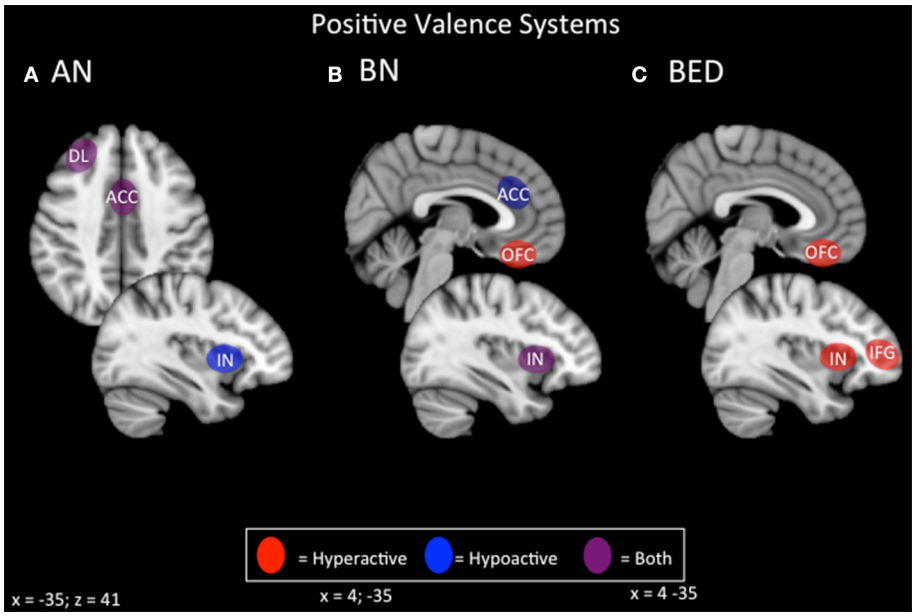


FIGURE 3 | Candidate NIBS targets that address abnormal phenotypes related to the RDoC positive valence dimension. (A) Candidate positive valence NIBS targets for anorexia nervosa (AN) (Wagner et al., 2007, 2008; Rothemund et al., 2011; Vocks et al., 2011; Holsen et al., 2012; Oberndorfer et al., 2013b; Torresan et al., 2013; Decker et al., 2014; Suda et al., 2014; Ehrlich et al., 2015; Sanders et al., 2015). The dorsolateral prefrontal cortex (DL) is both hyperactive when the participant views images of food, but hypoactive during symptom, particularly OCD-related, provocation. The anterior cingulate cortex (ACC) is also differentially activated; it is hyperactive when the participant views images of food, but hypoactive when the participant delays a reward. Also, the insula (IN) is hypoactive when the participant views images of food. **(B)** Candidate positive valence NIBS targets for bulimia nervosa (BN) (Frank et al., 2006, 2011; Bohon and Stice, 2011; Broft et al., 2012; Radeloff et al., 2012; Weygandt et al., 2012; Oberndorfer et al., 2013a; Galusca et al., 2014). The ACC is hypoactive during reward anticipation, and this hypoactivity predicts later overeating. The orbitofrontal cortex (OFC) is hyperactive during the receipt of a reward. The IN is both hyperactive during the receipt of a reward, but hypoactive during reward anticipation. **(C)** Candidate positive valence NIBS targets for binge eating disorder (BED) (Schienle et al., 2009; Frank et al., 2012a; Weygandt et al., 2012; Balodis et al., 2013a, 2014). Both the OFC and the IN are abnormally hyperactive during the receipt of a reward, while the inferior frontal gyrus (IFG) is hyperactive during reward anticipation.

cognitive control. This is particularly evident for disease-relevant stimuli (Wu et al., 2013), but is also apparent for positive and negative emotional valence images (Tapajóz P de Sampaio et al., 2015), suggesting a broader endophenotype of deficient cognitive and behavioral control. In fact, binge episodes are partially defined by the individual's loss of control during eating, and impulse control disorders (ICD) are common comorbidities (Fernández-Aranda et al., 2008). Purging behaviors are also associated with higher levels of impulsivity, and different forms of purging may represent separate manifestations of compulsivity and impulsivity (Hoffman et al., 2012).

On the other hand, restrictive-type EDs tend to show a different profile of cognitive control abnormalities. Cognitive control capacity may appear above normal levels in certain domains, such as temporal discounting (Steinglass et al., 2012). However, cognitive control may be abnormal in certain specific domains related to the illness; for example, for negative valence images (Tapajóz P de Sampaio et al., 2015), food stimuli (Oberndorfer et al., 2013b; Sanders et al., 2015), or body-image-related stimuli (Lee et al., 2014). AN patients also have altered cognitive control depending on the reward valence of the object, as the impulse control networks are overly activated for physical exercise relative to food images in a go/no-go task (Kullmann et al., 2014). AN patients also show a reduced ability to switch to an optimal decision-making strategy, called cognitive flexibility (Zastrow et al., 2009).

From a neural perspective, impulsive-type deficits on response control tasks are related to lower frontostriatal activations. BED patients show reduced activity in the inferior frontal gyrus, ventromedial PFC and insula during the Stroop task, and this diminished activity is associated with poor dietary restraint (Balodis et al., 2013b). BED prefrontal hypoactivity has also been correlated with psychometric measures of attentional impulsiveness and a disease-relevant go/no-go task (Hege et al., 2014). BN patients show hypoactivity in frontostriatal circuitry during cognitive control tasks like the Simon Spatial Incompatibility task; affected areas include the inferior frontal gyrus, striatum, ACC, OFC, DLPFC, and middle frontal gyrus (Marsh et al., 2009, 2011; Celone et al., 2011). On the go/no-go task, adolescent BN and ANBP patients display hyperactivations in the ACC and right DLPFC, albeit without impaired task performance relative to controls (Lock et al., 2011).

AN patients also show hypoactivity in frontostriatal circuits from the medial PFC on a response inhibition task related to cognitive control deficits (Oberndorfer et al., 2011; Wierenga et al., 2014), but hyperconnectivity to a response inhibition task that used exercise-related stimuli as its cue (Kullmann et al., 2014). Additionally, AN patients also display poorer performance

on cognitive flexibility tasks, and this performance is reflected by lower activity in frontostriatal circuits through the thalamus, ventral striatum, ACC, middle frontal gyrus, and ventrolateral PFC (Zastrow et al., 2009; Sato et al., 2013; Garrett et al., 2014; Wildes et al., 2014; Lao-Kaim et al., 2015). On resting-state fMRI, higher thalamo-cortical functional connectivity through the DLPFC and anterior PFC is associated with poorer performance on the Stroop task and working memory (Biezonski et al., 2015). Thus, domain-specific abnormalities of cognitive control are evident at both the behavioral and the neural level in AN.

Summary of Potential Cognitive Control Targets

Both restricting- and binge/purge-type EDs show deficits on tasks related to cognitive control, including behavioral inhibition, working memory, selective attention, and cognitive flexibility (**Figures 1**, **4**). Generally, BED displays poorer response inhibition and lower activity in the inferior frontal gyrus and ventromedial PFC, both of which are accessible via excitatory forms of NIBS. BN and ANBP display lower activity in the inferior frontal gyrus, ACC, OFC, and DLPFC; all but the OFC are easily accessible for excitatory NIBS. As noted earlier, excitatory NIBS of salience-network nodes in DLPFC, DMPFC, and anterior insula appears to improve cognitive control and impulsivity even in healthy controls (Cho et al., 2014, 2010; Meng et al., 2014). Enhanced cognitive control, via improved frontostriatal connectivity through these salience-network nodes, may mediate recently reported improvements in binge and purge behaviors with excitatory DMPFC-rTMS (Dunlop J. et al., 2015). Similar effects via similar mechanisms should be expected for excitatory rTMS targeting DLPFC and anterior insula.

For AN, neural correlates of cognitive control show considerable variability depending on the task and valence of stimuli. On the one hand, AN patients in some studies show broad deficits of cognitive control and flexibility, and hypoactivity of the frontostriatal circuitry, during many tasks related to cognitive control; hence, excitatory NIBS might be beneficial if combined with cognitive tasks during stimulation. On the other hand, patients sometimes show the reverse pattern of hyperconnectivity and excessive cognitive control/compulsivity in these same circuits, within illness-specific domains; excitatory stimulation may therefore be unhelpful, or could potentially exacerbate illness. In keeping with this concern, high-frequency DMPFC-rTMS was recently reported to exert a paradoxical *inhibitory* effect on frontostriatal connectivity in a subpopulation of ED patients with high baseline connectivity; these patients showed symptomatic worsening rather than improvement (Dunlop J. et al., 2015). Thus, targeting cognitive control in AN-R may require a more nuanced approach than is the case for binge-purge symptoms.

Social Processing Systems

Social processing systems refer to circuits involved in social communication, and the perception and understanding of oneself and others. Targets identified in healthy controls include the insula, responsible for interoception (Craig, 2002); the temporoparietral junction, for theory of mind-related processing (Saxe and Kanwisher, 2003); and higher-order visual processing regions, for processing one's own and others' faces (Hummel et al., 2013).

This dimension has received less attention in the ED literature relative to positive/negative valence systems and cognitive control (**Figure 1**). However, it may have relevance in AN patients, who show higher levels of alexithymia, deficits in visceral sensory perception or "interoception" (Craig, 2002; Strigo et al., 2013), and distorted perceptions of body shapes (Suchan et al., 2013). AN patients with higher levels of alexithymia show lower ACC, PCC, and right temporoparietal junction (TPJ) activation during social decision-making tasks (Miyake et al., 2009, 2012; McAdams and Krawczyk, 2011). More specifically, ANR patients display altered anterior and dorsal mid-insula activations based on the modality of interoception they are attending to (Kerr et al., 2015). On resting-state fMRI, AN patients also display increased functional connectivity from the anterior insula to the default mode network associated with self-reported problems with interoceptive awareness, suggesting a heightened level of cognitive control toward interoceptive processes (Boehm et al., 2014). AN patients also have altered neural responses to visually-presented body shapes, particularly in areas associated with visual processing and reward: the ventral striatum, extrastriate body area (EBA), DLPFC, parietal regions, medial PFC, and fusiform gyrus (Cowdrey et al., 2012; Spangler and Allen, 2012; Castellini et al., 2013; Fladung et al., 2013; Suchan et al., 2013; Suda et al., 2013; Fonville et al., 2014). Finally, two recent studies have also identified areas of abnormal activation in response to benevolent and malevolent social relationships. During benevolent social relations, AN patients tend to display reductions in DMPFC, possibility related to lowered reward valence for social reward and interaction (McAdams et al., 2015; Via et al., 2015).

In summary, AN patients may show deficits across multiple domains related to self-perception (alexithymia, interoception, and body shape perception) and social function (interpersonal interaction, theory of mind; **Figures 1**, **5**). The latter function has been successfully enhanced with excitatory DMPFC-rTMS in autism-spectrum disorder (Enticott et al., 2011, 2014). During social interactions, AN patients likewise tend to display DMPFC hypoactivity during social interaction, and so excitatory stimulation over this region may worth exploring. For self-perception, relevant targets include anterior insula (alexithymia), posterior insula (interoception), TPJ and EBA (social cue perception, body shape perception). NIBS has successfully targeted each of these regions in other applications (Ciampi de Andrade et al., 2012; Dinur-Klein et al., 2014; Donaldson et al., 2015). Excitatory stimulation of the insula and TPJ may be worth exploring for alexithymia and deficits in interoception. Conversely, inhibitory stimulation of the TPJ and EBA may be worth exploring for aberrant self- and body perception.

NIBS TECHNIQUES AS THERAPEUTIC INTERVENTIONS IN ED

For the following section, a systematic review was completed using PubMed (NIH, http://www.ncbi.nlm.nih.gov/pubmed),

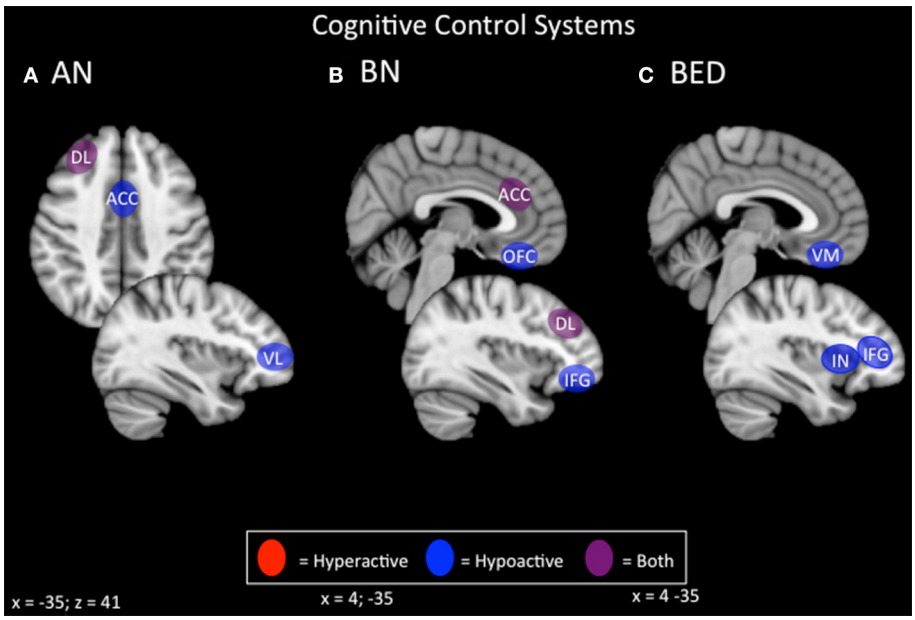


FIGURE 4 | Candidate NIBS targets that address abnormal phenotypes related to the RDoC cognitive control dimension. (A) Candidate cognitive control NIBS targets for anorexia nervosa (AN) (Oberndorfer et al., 2011; Sato et al., 2013; Garrett et al., 2014; Wierenga et al., 2014; Wildes et al., 2014; Biezonski et al., 2015; Lao-Kaim et al., 2015). The dorsolateral prefrontal cortex (DL) is both hyperactive during interference control tasks (such as the Stroop task), and for working memory, but hypoactive during cognitive flexibility and set-shifting tasks. The anterior cingulate cortex (ACC) is hypoactive during response inhibition tasks, while the ventrolateral prefrontal cortex (VL) is hypoactive during cognitive flexibility tasks. **(B)** Candidate cognitive control NIBS targets for bulimia nervosa (BN) (Marsh et al., 2009; Rossi and Hallett, 2009; Celone et al., 2011; Lock et al., 2011). Both the ACC and the DL are hyperactive during response inhibition tasks, but hypoactive during interference control tasks, while both the orbitofrontal cortex (OFC) and inferior frontal gyrus (IFG) are hypoactive during inference control tasks. **(C)** Candidate cognitive control NIBS targets for binge eating disorder (BED) (Balodis et al., 2013b; Hege et al., 2014). The ventromedial prefrontal cortex (VM), insula (IN), and IFG are abnormally hypoactive during interference control, poor dietary restraint, impulsivity, and response inhibition.

with searches containing the following terms: first, clinical terms for the three ED diagnoses in this review and related phenotypes (BN, AN, BED, binging, purging, excessive exercise), and second, NIBS related terms (rTMS, TMS, tDCS).

NIBS Overview: rTMS and tDCS

rTMS applies powerful, focused magnetic field pulses over the scalp to elicit action potentials in the underlying region of cortex. Typically, treatment sessions occur once daily, for a total of 20–30 daily sessions (Carpenter et al., 2012; Solvason et al., 2014). rTMS mechanisms are thought to involve synaptic plasticity via long-term potentiation or depression, with the direction of effect dependent on the stimulation intensity, duration, and pattern (Pascual-Leone et al., 1998; Maeda et al., 2000). Higher frequency stimulation (5–20 Hz) is usually considered to be excitatory, while low frequency (<1 Hz) stimulation is considered inhibitory (Pascual-Leone et al., 1994; Chen et al., 1997). More recently, however, considerable heterogeneity on electrophysiological, neuroimaging, and clinical measures has been found for most if not all patterns of rTMS (Maeda et al., 2000; Eldaief et al., 2011; Dunlop J. et al., 2015; Dunlop K. et al., 2015; Nettekoven et al., 2015).

tDCS, on the other hand, uses a constant, low amplitude current to modulate cortical excitability, rather than eliciting action potentials directly. As with rTMS, sessions typically occur daily, for a total of 10–30 sessions (Meron et al., 2015). While the mechanisms of tDCS are still debated, it is likely that modulated cortical excitability also elicits subtle effects on synaptic plasticity via long-term potentiation and depression (Brunoni et al., 2012). Anodal stimulation is considered excitatory, and cathodal stimulation inhibitory. However, as with rTMS, both types of tDCS display considerable inter-individual variability in their effects (Wiethoff et al., 2014). Newer variants such as transcranial alternating current stimulation (tACS), may exert more consistent, frequency-specific effects (Voss et al., 2014); however, their therapeutic potential is poorly understood at present.

NIBS as a Treatment for BED and Food Craving

To date, the majority of published NIBS-ED studies have focused on female patients with abnormally high food craving or urge to eat, as opposed to a specific formal DSM-5 diagnosis (**Tables 2**, **3**; McClelland et al., 2013; Grall-Bronnec and Sauvaget, 2014; Val-Laillet et al., 2015). These preclinical studies typically involve a single session of stimulation, with subjectively rated cue-induced craving as the primary outcome. With rTMS, two studies reported contradictory results for 10 Hz stimulation of the left DLPFC rTMS: one study ($n = 28$) found decreased craving after active vs. sham stimulation (Uher et al., 2005), while the other

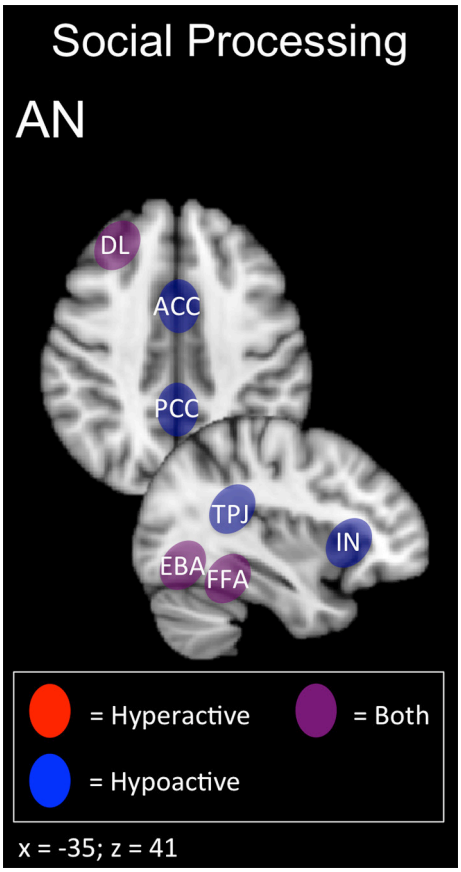


FIGURE 5 | Candidate NIBS targets that address abnormal phenotypes related to the RDoC social processing dimension in anorexia nervosa (AN) (Cowdrey et al., 2012; Miyake et al., 2012; Spangler and Allen, 2012; Castellini et al., 2013; Fladung et al., 2013; Suda et al., 2013; Boehm et al., 2014; Fonville et al., 2014; Kerr et al., 2015; McAdams et al., 2015; Via et al., 2015). The anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), and temporoparietal junction (TPJ) are abnormally hypoactive during deficits in social decision-making and alexithymia, while low insula (IN) activity is related to deficits in interoceptive awareness. The dorsolateral prefrontal cortex (DL) is both abnormally hyperactive when the participant views oversized images of themselves, but hypoactive when viewing images depicting body-checking behavior. The fusiform face area (FFA) is both abnormally hyperactive when the participant views highly emotional facial expressions, but hypoactive when viewing distorted body shapes and images depicting body-checking behavior. The extrastriate body area (EBA) is both abnormally hyperactive when the participant views images of their own body, but hypoactive when those images are distorted.

($n = 10$) found that active stimulation was no better than sham in terms of cue-induced craving control (Barth et al., 2011). The studies differed in stimulation parameters, however, and enrolled only healthy participants who self-reported having strong food cravings, but did not carry a formal ED diagnosis. Hence, it may be difficult to extrapolate these findings to the effects of a full therapeutic course of 20–30 sessions in patients with pathological deficits of self-control and a formal ED diagnosis.

There is also a growing body of literature investigating DLPFC-tDCS as a method to reduce craving and food intake. In four published studies recruiting individuals with strong food cravings, a single session of anodal right DLPFC/cathodal left DLPFC tDCS was able to reduce cue-induced craving, reduce food intake, and improve the participants' ability to resist food relative to sham-tDCS (Fregni et al., 2008; Goldman et al., 2011; Kekic et al., 2014; Lapenta et al., 2014). Future work involving tDCS should employ multiple sessions as opposed to a single session in a randomized, sham-controlled setting, as a treatment for the inappropriate eating patterns associated with BED. Studies in populations carrying a formal ED diagnosis, with significant functional impairment and distress, are also needed.

NIBS as a Treatment for BN

The earliest publication of rTMS as a potential treatment for BN is a case report of a patient diagnosed with comorbid depression and BN who achieved an unexpected remission of binge and purge symptoms and depressive improvements after 10 sessions of 20 Hz rTMS over the left DLPFC (Hausmann et al., 2004; McClelland et al., 2013; **Table 2**). Follow-up studies involving high frequency left DLPFC rTMS have been mixed: one group found that a single session reduced the urge to eat, the number of binges 24 h post-rTMS, and salivary cortisol levels (Van den Eynde et al., 2010; Claudino et al., 2011), while another study found no difference between active- and sham-rTMS after 15 sessions of 20 Hz rTMS over the left DLPFC (Walpoth et al., 2008). A more recent study applied a single session of excitatory left DLPFC-rTMS in 8 female patients with BN, and reported reduced subjective ratings of craving post-rTMS, along with lower cerebral oxygenation in the DLPFC on near-infrared spectroscopy (Sutoh et al., 2016). These findings hint at the potential promise of DLPFC-rTMS for BN, which would be in keeping with the much more extensive literature demonstrating that this intervention enhances cognitive control in healthy subjects (Cho et al., 2010), and patient populations (Van den Eynde et al., 2010), with therapeutic effects in mechanistically related disorders such as addiction (Gorelick et al., 2014).

More recently, our group has shifted the rTMS stimulation target from the DLPFC to the DMPFC, as a potential treatment for major depression (Downar et al., 2014; Salomons et al., 2014; Bakker et al., 2015). As with first case report of DLPFC-rTMS for BN, we too found an unexpected remission of chronic treatment refractory binge and purge symptoms in an MDD patient with comorbid BN, following 20 sessions of 10 Hz DMPFC-rTMS. The onset of effect was rapid, occurring in the first week of treatment, and was maintained for 9 weeks post-treatment (Downar et al., 2012). In a follow-up, open-label series of 10 Hz DMPFC-rTMS in 28 ED patients with binge/purge behaviors, we noted $\geq$ 50% symptom reduction in 57%. On resting-state fMRI, we found increased resting-state functional connectivity in fronto-striatal salience network circuits (through DMPFC, anterior insula, and ventral striatum) specifically in the treatment responders but not non-responders (Dunlop J. et al., 2015), consistent with similar findings for DMPFC-rTMS in MDD and obsessive-compulsive disorder (Salomons et al., 2014; Dunlop K. et al., 2015). These findings suggest that DMPFC-rTMS may improve bulimic symptoms through an improvement of top-down cognitive control over urges, via frontostriatal circuits through salience-network nodes. Future work should include a

TABLE 2 | Overview of the available ED-rTMS literature.

Study	Subjects	Study design	Stimulation Site	rTMS Technique	Duty cycle (on/off s)	Total pulses/session	Stimulation Intensity	Number of sessions	Primary outcome	Findings
A										
Uher et al., 2005	*n* =28, F, FC	RCT	L-DLPFC, 5cm rule	10 Hz	5.55 s	1000	110% MT	1	Cue-induced urge to eat	Food craving increased after sham stimulation
Barth et al., 2011	*n* = 10, F, FC	RCT	L-PFC	10 Hz	10.20 s	3000	100% RMT	1	Inhibition of cue-induced cravings	rTMS no better than sham
B										
Van den Eynde et al., 2013	*n* = 10, AN	RCT	L-DLPFC, 5 cm rule	10 Hz rTMS	5.55 s	1000	110% MT	1	EDE-Q	Reduced levels of feeling full, fat, anxiety
C										
Van den Eynde et al., 2010	*n* = 38, bulimic-type ED	RCT	L-DLPFC, 5 cm rule	10 Hz	5.55 s	1000	110% MT	1	Binge-eating episodes, urge to eat	Decreased urge to eat and binges 24 h post-stimulation
Claudino et al., 2011	*n* = 22, bulimic-type ED	RCT	L-DLPFC	10 Hz	5.55 s	1000	110% MT	1	Salivary cortisol concentration	Lower cortisol post-rTMS compared to sham
Walpoth et al., 2008	*n* = 16, BN	RCT	L-DLPFC	20 Hz	10.60 s	2000	120% MT	15 sessions, daily	Binge/purge status	No significant difference between active and sham-rTMS
Hausmann et al., 2004	*n* = 1, MDD-BN	Case report	L-DLPFC	20 Hz	10.60 s	2000	80% MT	10 sessions, daily	Binge/purge Diary	Remission of binge/purges, HDRS response
Downar et al., 2012	*n* = 1, MDD-BN	Case report	B-DMPFC	10 Hz	5.10 s	6000	120% RMT	20 sessions, daily	Binge/purge status	Remission of binge/purges, HDRS response
Dunlop J. et al., 2015	*n* = 28, bulimic-type ED	Open-label	B-DMPFC	10 Hz	5.10 s	6000	120% RMT	20–30 sessions, daily	Binge/purge status	16 of 28 achieved >50% reduction of binges and purges 4-weeks post-rTMS

A, rTMS studies related to food addiction and urges to eat; B and C, refer to rTMS studies related to AN and BN, respectively.

AN, anorexia nervosa; B-DMPFC, bilateral dorsomedial prefrontal cortex; BN, bulimia nervosa; ED, eating disorder; EDE-Q, eating disorder examination questionnaire; F, female; FC, food craving; HDRS, Hamilton Depression Rating Scale; L-DLPFC, left dorsolateral prefrontal cortex; L-PFC, left prefrontal cortex; MT, motor threshold; RCT, randomized control trial; RMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation.

TABLE 3 | Overview of the available ED-tDCS literature.

Study	Subjects	Study Design	Anodal Site	Cathodal Site	Stimulation Intensity	Number of sessions	Primary Outcome	Findings
A								
Fregni et al., 2008	$n = 23$, HC with urges to eat	Sham-controlled, crossover	R-DLPFC	L-DLPFC	2 mA, 20 min	1	Craving VAS, food consumption	Reduced craving in active tDCS, less consumption
Goldman et al., 2011	n = 19, HC with urges to eat	Sham-controlled, crossover	R-DLPFC	L-DLPFC	2 mA, 20 min	1	Craving VAS, Resist food	Reduced craving, increased ability to resist food
Kekic et al., 2014	$n = 20$, HC with urges to eat	Sham-controlled, crossover	R-DLPFC	L-DLPFC	2 mA, 20 min	1	Craving VAS	Reduced craving for sweet foods
Lapenta et al., 2014	$n = 9$, HC with urges to eat	Sham-controlled, crossover	R-DLPFC	L-DLPFC	2 mA, 20 min	1	Cue-induced food craving	Reduced food intake
B								
Khedr et al., 2014	$n = 7$, AN	Open-Label	L-DLPFC	N/A	2 mA, 25 min	10, daily	EDI and EAT	Significant effect of time on EAT and EDI

A, tDCS studies related to food addiction and urges to eat; B, tDCS studies related to AN.
AN, anorexia nervosa; HC, healthy controls; EAT, eating attitudes test; EDI, eating disorder inventory; L-DLPFC, left dorsolateral prefrontal cortex; R-DLPFC, right dorsolateral prefrontal cortex; tDCS, transcranial direct current stimulation; VAS, visual analog scale.

sham-controlled arm, along with behavioral measures to better characterize the cognitive domains mediating the therapeutic effects of DMPFC-rTMS in BN.

NIBS as a Treatment for AN

To date, there are few published sham-controlled trials on tDCS and rTMS as treatments for AN (Bainbridge and Brown, 2014; McClelland et al., 2013). One preclinical study in a small sample of AN patients ($n = 10$) applied a single session of 10 Hz left DLPFC-rTMS, with patients reporting less anxiety and less feeling full and feeling fat (**Table 2**; Van den Eynde et al., 2013). An open-label case series in 5 AN patients applied 20 sessions of excitatory DLPFC-rTMS, reporting improvements in anxiety, feeling fat/full and urge to restrict/exercise over the course of treatment, enduring to 6 months; however, these effects had waned by 12-months post-treatment (McClelland et al., 2016). Another open-label series in 7 AN patients applied 10 sessions of anodal left DLPFC tDCS (**Table 3**), reporting improvements on the Eating Disorders Inventory (EDI) and the Eating Attitude Test (EAT) (Khedr et al., 2014). Although, these early publications are promising, further preliminary work in larger groups, with a longer course and sham control, must be performed to determine whether rTMS and tDCS are efficacious treatments for AN.

CONSIDERATIONS FOR FUTURE STUDIES

Patient Selection

In an attempt to limit heterogeneity, inclusion criteria for NIBS studies in ED patients are often based on DSM-5 diagnostic categories. However, as noted above, DSM-5 diagnoses still encompass substantial heterogeneity, and may conflate neurobiologically distinct endophenotypes. Future studies enrolling ED patients for NIBS trials should make efforts to at least characterize the underlying phenotypes within the clinical populations they are treating, and ideally should target a specific endophenotype associated with a specific neural substrate. Such studies should also measure behavioral or biological markers of this endophenotype to assess whether the target process was successfully engaged, and whether the engaged process did indeed mediate any observed symptomatic improvements.

Intervention Parameters

Several treatment parameters are important to consider when designing NIBS studies in ED. First, treatment parameters (protocol, total number of sessions, and number of sessions per day) needs to be selected, keeping in mind both patient convenience and therapeutic efficacy. In the older MDD-NIBS literature, 20–30 sessions of once daily rTMS is the standard protocol, with sessions lasting up to 45–60 min. However, such schedules are onerous for patients and limit overall clinic capacity. More recent studies have begun to explore much briefer protocols, such as 1–3 min theta-burst stimulation (Li et al., 2014), which have been reported to achieve equivalent or superior outcomes (Bakker et al., 2015). Other protocols, such as quadripulse stimulation (QPS), have been reported to achieve much more consistent effects across individuals (Huang et al., 2005; Tsutsumi et al., 2014). Still other recent MDD trials have delivered multiple sessions per day (up to five sessions a day), to complete the full course in 4–10 days rather than the usual 4–6 weeks (Holtzheimer et al., 2010; Baeken et al., 2014). Future ED rTMS trials should make use of these innovations to reduce patient burden, increase capacity or consistency, and accelerate the pace of improvement.

Concurrent Tasks or Therapies

Another consideration for NIBS trials for ED is whether stimulation should be applied concurrently with psychotherapy or a specific cognitive/behavioral task, as opposed to simply

during rest. This is especially the case if NIBS protocols are designed based on RDoC dimensions, and targets cortical regions based on abnormal activation on certain tasks. As discussed above, many areas, including the ACC/mPFC, DLPFC, insula, inferior frontal gyrus, and ventrolateral PFC are hyperactive to some tasks, but hypoactive in others. With stimulation during rest, it is difficult to assess or constrain the activation state of the underlying cortical target. Having the patient perform illness-specific cognitive task has now been shown to enhance (or reduce) the therapeutic effects of rTMS across several different indications. For example, reading trauma-related scripts during rTMS enhanced efficacy for PTSD (Isserles et al., 2013); undergoing rTMS in the presence of substance cues enhances efficacy in addiction (Dinur-Klein et al., 2014). Analogous approaches may be helpful in ED.

Treatment Target

A final consideration for ED-NIBS concerns the feasibility of the proposed target. Although, targets such as DLPFC, DMPFC, OFC, and TPJ have now been targeted in a variety of studies, others such as ventromedial prefrontal cortex or anterior insula may be difficult to reach without specially designed coils, and without also stimulating overlying structures. More feasibility studies are needed to assess how well that these areas can be engaged with rTMS and tDCS (Chib et al., 2013).

Another consideration during target selection is determining the appropriate stimulation intensity in the case of rTMS. For example, treatment intensity is determined by measuring the resting motor threshold of region of cortex directly posterior to stimulation site; in these cases, resting motor threshold is determined by the activation of the thumb or big toe for DLPFC and DMPFC, respectively (Schutter and van Honk, 2006; Hallett, 2007). It is therefore unclear for novel stimulatory sites what would be the most appropriate and reliable sites to determine optimal stimulation intensity. Studies using finite element modeling may also be helpful for optimizing stimulator placement and intensity (Nitsche et al., 2012).

The effects of rTMS also dramatically decrease the farther the site is from the scalp surface (Kozel et al., 2000), and so it is likely that stimulation intensity will have to be quite large for deep targets such as anterior insula or VMPFC. If this is the case, it is likely that pain tolerability will be a factor. In addition, trigeminal nerve pain, scalp pain, and headaches are common adverse effects associated with rTMS (Machii et al., 2006; Rossi and Hallett, 2009). Tolerability will need to be maintained when stimulating these novel targets, particularly in scalp regions with trigeminal innervation, such as the frontopolar, orbitofrontal, or temporopolar regions. This may be challenging for more intense rTMS protocols, although helmet-shaped "deep TMS" coil geometries may be somewhat helpful in allowing deep stimulation of these regions while maintaining tolerability (Roth et al., 2007). Certain targets (e.g., OFC, frontopolar cortex) may be more amenable to tDCS, which is relatively painless compared to rTMS. Another non-invasive technique worthy of future investigation is cutaneous non-invasive vagus nerve stimulation, which is also delivered via external electrodes. Its more invasive counterpart, surgically-implanted vagus nerve stimulation has recently shown some efficacy for medication-resistant depression (Ben-Menachem et al., 2015; Grimonprez et al., 2015).

Finally, stimulating multiple targets in a single session might be the optimal way to address all the abnormal behavioral dimensions in a given ED patient. Different ED symptom dimensions map to different cortical targets, and so confining stimulation to a single target may be insufficient to address multi-dimensional ED pathology. For example, in BN, excitatory stimulation of the DMPFC/insula combined with inhibitory stimulation of the VMPFC may be a more optimal strategy for enhancing cognitive control while reducing urge intensity. "Deep TMS" coils have been designed to stimulate multiple targets simultaneously (Dinur-Klein et al., 2014), and multi-channel coils allow different protocols at different targets simultaneously (Roth et al., 2014). However, the therapeutic effects of sequential vs. simultaneous stimulation have not yet been compared directly. Further research should be done to describe the safety, tolerability, clinical efficacy, and neural mechanisms of stimulating multiple targets, either sequentially or simultaneously.

CONCLUSION

Neuroimaging, psychometric, and behavioral findings are converging upon a new approach to classifying psychiatric disorders, including EDs, in terms of endophenotypes or symptom dimensions. New proposed frameworks, such as the RDoC, seek to describe EDs in terms of dysfunction in specific underlying brain functions such as cognitive control, positive and negative valence, and social/self-related cognition. These functions in turn are gradually being linked to specific neurobiological processes, described at multiple levels spanning clinical symptomatology, behavioral task performance, neuroimaging studies of macro-scale network function, and cellular, molecular, and genetic mechanisms. With the advent of anatomically focal NIBS interventions, a "neuroanatomical formulation" of ED pathology may become relevant not only for basic science, but for clinical care.

At present, neuroanatomical, endophenotypic, and RDoC formulations of ED pathology must be considered tentative and preliminary. However, from available literature, it does appear that some of the tremendous and dynamic heterogeneity of symptoms in the ED population can be understood parsimoniously in terms of dysfunction in a few key cognitive systems and their associated neural circuits. For example, in BN and BED, binge and purge behaviors may acquire pathologically strong incentive salience by mechanisms similar to addiction; impaired cognitive control in turn renders binge/purge urges hard to resist, particularly during negative affect. NIBS strategies designed for addiction (e.g., enhancing cognitive control via salience-network stimulation and damping urge intensity via ventromedial stimulation) may be helpful in this setting. In ANR, this strategy may be less helpful; instead, targeting pathologically overactive negative-valence systems may address the excessive valuation of secondary over primary rewards, and the underlying compulsivity. NIBS strategies developed for OCD (such as inhibitory stimulation of the OFC and

DMPFC) may be more helpful in this setting. Ancillary NIBS strategies for AN may also target distortions of body image, alexithymia, and deficits of interoception via insular, TPJ, and EBA stimulation. However, it must be acknowledged that nearly all of these approaches are at present theoretically based, and lacking even in preclinical support. The field is urgently in need of future studies in clinical populations, with adequate sample sizes and sham controls, and using endophenotypic markers to validate or refute the proposed mechanisms of action for NIBS in EDs.

To conclude, patients with EDs stand to benefit tremendously from ongoing progress in three areas: symptom characterization, diagnostic formulation, and targeted intervention. Recent initiatives will allow us to make better sense of the heterogeneity of ED pathology, both across individuals and within individuals over time. As we improve our abilities to identify robust symptom clusters, link those clusters to neural substrates, and target those substrates with NIBS interventions, treatment outcomes will improve. These advances need not occur at the expense of existing and well-validated treatment strategies involving medications, psychotherapy, and behavior modification. Rather, they will likely work in a synergistic fashion to complement and facilitate our existing treatment strategies: enhancing the cognitive control that is a prerequisitive for successful cognitive-behavioral treatments in BN, or reducing the compulsivity and rigidity that hampers behavior modification in AN. Given the considerable patient burden and chronicity of EDs, these advances in treatment options will be a welcome change for patients, families and clinicians alike.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

JD has received research support from the Canadian Institutes of Health Research, the National Institutes of Health, the Klarman Family Foundation, the Edgestone Family Foundation, the Toronto General and Western Hospital Foundation, and in-kind support from MagVenture for investigator-initiated studies. BW has received research support from the Ontario Mental Health Foundation, the Price Foundation, The Klarman Foundation, the Canadian Institute for Health Research, and the National Institute for Mental Health. KD has received research support from the Canadian Institutes of Health Research, Vanier Canada Scholarship Program.

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

Åhs, F., Kragel, P. A., Zielinski, D. J., Brady, R., and LaBar, K. S. (2015). Medial prefrontal pathways for the contextual regulation of extinguished fear in humans. *Neuroimage* 122, 262–271. doi: 10.1016/j.neuroimage.2015.07.051

Albares, M., Lio, G., Criaud, M., Anton, J.-L., Desmurget, M., and Boulinguez, P. (2014). The dorsal medial frontal cortex mediates automatic motor inhibition in uncertain contexts: evidence from combined fMRI and EEG studies. *Hum. Brain Mapp.* 35, 5517–5531. doi: 10.1002/hbm.22567

Amianto, F., Ottone, L., Abbate-Daga, G., and Fassino, S. (2015). Binge-eating disorder diagnosis and treatment: a recap in front of DSM-5. *BMC Psychiatry* 15:6. doi: 10.1186/s12888-015-0445-6

Arcelus, J., Mitchell, A. J., Wales, J., and Nielsen, S. (2011). Mortality rates in patients with anorexia nervosa and other eating disorders. A meta-analysis of 36 studies. *Arch. Gen. Psychiatry* 68, 724–731. doi: 10.1001/archgenpsychiatry.2011.74

Baeken, C., De Raedt, R., Van Schuerbeek, P., Vanderhasselt, M. A., De Mey, J., Bossuyt, A., et al. (2010). Right prefrontal HF-rTMS attenuates right amygdala processing of negatively valenced emotional stimuli in healthy females. *Behav. Brain Res.* 214, 450–455. doi: 10.1016/j.bbr.2010.06.029

Baeken, C., Marinazzo, D., Wu, G.-R., Van Schuerbeek, P., De Mey, J., Marchetti, I., et al. (2014). Accelerated HF-rTMS in treatment-resistant unipolar depression: Insights from subgenual anterior cingulate functional connectivity. *World J. Biol. Psychiatry*, 15, 286–297. doi: 10.3109/15622975.2013.872295

Bailer, U. F., Frank, G. K., Henry, S. E., Price, J. C., Meltzer, C. C., Mathis, C. A., et al. (2007). Exaggerated 5-HT1A but Normal 5-HT2A Receptor Activity in Individuals Ill with Anorexia Nervosa. *Biol. Psychiatry* 61, 1090–1099. doi: 10.1016/j.biopsych.2006.07.018

Bailer, U., Price, J., Meltzer, C. C., Mathis, C. A., Frank, G. K., Weissfeld, L., et al. (2004). Altered 5-HT(2A) receptor binding after recovery from bulimia-type anorexia nervosa: relationships to harm avoidance and drive for thinness. *Neuropsychology* 29, 1143–1155. doi: 10.1038/sj.npp.1300430

Bainbridge, K., and Brown, A. (2014). rTMS as a treatment for anorexia nervosa. *Brain Stimul.* 7, 149–150. doi: 10.1016/j.brs.2013.09.010

Bakker, N., Shahab, S., Giacobbe, P., Blumberger, D. M., Daskalakis, Z. J., Kennedy, S. H., et al. (2015). rTMS of the dorsomedial prefrontal cortex for major depression: safety, tolerability, effectiveness, and outcome predictors for 10 Hz versus intermittent theta-burst stimulation. *Brain Stimul.* 8, 205–215. doi: 10.1016/j.brs.2014.11.002

Balodis, I. M., Grilo, C. M., Kober, H., Worhunsky, P. D., White, M. A., Stevens, M. C., et al. (2014). A pilot study linking reduced fronto-Striatal recruitment during reward processing to persistent bingeing following treatment for binge-eating disorder. *Int. J. Eat. Disord.* 47, 376–384. doi: 10.1002/eat.22204

Balodis, I. M., Kober, H., Worhunsky, P. D., White, M. A., Stevens, M. C., Pearlson, G. D., et al. (2013a). Monetary reward processing in obese individuals with and without binge eating disorder. *Biol. Psychiatry* 73, 877–886. doi: 10.1016/j.biopsych.2013.01.014

Balodis, I. M., Molina, N. D., Kober, H., Worhunsky, P. D., White, M. A., Sinha, R., et al. (2013b). Divergent neural substrates of inhibitory control in binge eating disorder relative to other manifestations of obesity. *Obesity* 21, 367–377. doi: 10.1002/oby.20068

Bär, K.-J., de la Cruz, F., Berger, S., Schultz, C. C., and Wagner, G. (2015). Structural and functional differences in the cingulate cortex relate to disease severity in anorexia nervosa. *J. Psychiatry Neurosci.* 40, 269–279. doi: 10.1503/jpn.140193

Bar, K.-J., de la Cruz, F., Berger, S., Schultz, C., and Wagner, G. (2015). Structural and functional differences in the cingulate cortex relate to disease severity in anorexia nervosa. *J. Psychiatry Neurosci.* 40, 269–279. doi: 10.1503/jpn.140193

Barth, K. S., Rydin-Gray, S., Kose, S., Borckardt, J. J., O'Neil, P. M., Shaw, D., et al. (2011). Food cravings and the effects of left prefrontal repetitive transcranial magnetic stimulation using an improved sham condition. *Front. Psychiatry* 2:9. doi: 10.3389/fpsyt.2011.00009

Ben-Menachem, E., Revesz, D., Simon, B. J., and Silberstein, S. (2015). Surgically implanted and non-invasive vagus nerve stimulation: a review of efficacy, safety and tolerability. *Eur. J. Neurol.* 22, 1260–1268. doi: 10.1111/ene.12629

Berg, K. C., Crosby, R. D., Cao, L., Crow, S. J., Engel, S. G., Wonderlich, S., et al. (2015). Negative affect prior to and following overeating-only, loss of control eating-only, and binge eating episodes in obese adults. *Int. J. Eat. Disord.* 48, 641–653. doi: 10.1002/eat.22401

Beucke, J. C., Sepulcre, J., Talukdar, T., Linnman, C., Zschenderlein, K., Endrass, T., et al. (2013). Abnormally high degree connectivity of orbitofrontal cortex in obsessive-compulsive disorder. *JAMA Psychiatry* 70, 619–629. doi: 10.1001/jamapsychiatry.2013.173

Biezonski, D., Cha, J., Steinglass, J., and Posner, J. (2015). Evidence for thalamocortical circuit abnormalities and associated cognitive dysfunctions in underweight individuals with anorexia nervosa. *Neuropsychopharmacology*. doi: 10.1038/npp.2015.314. [Epub ahead of print].

Bischoff-Grethe, A., McCurdy, D., Grenesko-Stevens, E., Irvine, L. E. Z., Wagner, A., Yau, W.-Y. W., et al. (2013). Altered brain response to reward and punishment in adolescents with Anorexia nervosa. *Psychiatry Res.* 214, 331–340. doi: 10.1016/j.pscychresns.2013.07.004

Boehm, I., Geisler, D., King, J. A., Ritschel, F., Seidel, M., Deza Araujo, Y., et al. (2014). Increased resting state functional connectivity in the fronto-parietal and default mode network in anorexia nervosa. *Front. Behav. Neurosci.* 8:346. doi: 10.3389/fnbeh.2014.00346

Bohon, C., and Stice, E. (2011). Reward abnormalities among women with full and subthreshold bulimia nervosa: a functional magnetic resonance imaging study. *Int. J. Eat. Disord.* 44, 585–595. doi: 10.1002/eat.20869

Bohon, C., and Stice, E. (2012). Negative affect and neural response to palatable food intake in bulimia nervosa. *Appetite* 58, 964–970. doi: 10.1016/j.appet.2012.02.051

Broft, A., Shingleton, R., Kaufman, J., Liu, F., Kumar, D., Slifstein, M., et al. (2012). Striatal dopamine in bulimia nervosa: a PET imaging study. *Int. J. Eat. Disord.* 45, 648–656. doi: 10.1002/eat.20984

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., et al. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Bulik, C. M., Bacanu, S.-A., Klump, K. L., Fichter, M. M., Halmi, K. A., Keel, P., et al. (2005). Selection of eating-disorder phenotypes for linkage analysis. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 139B, 81–87. doi: 10.1002/ajmg.b.30227

Carpenter, L. L., Janicak, P. G., Aaronson, S. T., Boyadjis, T., Brock, D. G., Cook, I. A., et al. (2012). Transcranial magnetic stimulation (TMS) for major depression: a multisite, naturalistic, observational study of acute treatment outcomes in clinical practice. *Depress. Anxiety* 29, 587–596. doi: 10.1002/da.21969

Carter, J. C., Mercer-Lynn, K. B., Norwood, S. J., Bewell-Weiss, C. V., Crosby, R. D., Woodside, D. B., et al. (2012). A prospective study of predictors of relapse in anorexia nervosa: implications for relapse prevention. *Psychiatry Res.* 200, 518–523. doi: 10.1016/j.psychres.2012.04.037

Castellini, G., Polito, C., Bolognesi, E., D'Argenio, A., Ginestroni, A., Mascalchi, M., et al. (2013). Looking at my body. similarities and differences between anorexia nervosa patients and controls in body image visual processing. *Eur. Psychiatry* 28, 427–435. doi: 10.1016/j.eurpsy.2012.06.006

Celone, K. A., Thompson-Brenner, H., Ross, R. S., Pratt, E. M., and Stern, C. E. (2011). An fMRI investigation of the fronto-striatal learning system in women who exhibit eating disorder behaviors. *Neuroimage* 56, 1749–1757. doi: 10.1016/j.neuroimage.2011.03.026

Chan, T. W. S., Ahn, W.-Y., Bates, J. E., Busemeyer, J. R., Guillaume, S., Redgrave, G. W., et al. (2013). Differential impairments underlying decision making in anorexia nervosa and bulimia nervosa: a cognitive modeling analysis. *Int. J. Eat. Disord.*, 47, 157–167. doi: 10.1002/eat.22223

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

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

Cho, J.-H., Deisseroth, K., and Bolshakov, V. Y. (2013). Synaptic encoding of fear extinction in mPFC-amygdala circuits. *Neuron* 80, 1491–1507. doi: 10.1016/j.neuron.2013.09.025

Cho, S. S., Ko, J. H., Pellecchia, G., Van Eimeren, T., Cilia, R., and Strafella, A. P. (2010). Continuous theta burst stimulation of right dorsolateral prefrontal cortex induces changes in impulsivity level. *Brain Stimul.* 3, 170–176. doi: 10.1016/j.brs.2009.10.002

Cho, S. S., Kosimori, Y., Aminian, K., Obeso, I., Rusjan, P., Lang, A., et al. (2014). Investing in the future: stimulation of the medial prefrontal cortex reduces discounting of delayed rewards. *Neuropsychopharmacology.* 40, 546–553. doi: 10.1038/npp.2014.211

Ciampi de Andrade, D., Galhardoni, R., Pinto, L. F., Lancelotti, R., Rosi, J., Marcolin, M. A., et al. (2012). Into the island: a new technique of non-invasive cortical stimulation of the insula. *Neurophysiol. Clin.* 42, 363–368. doi: 10.1016/j.neucli.2012.08.003

Claudino, A., M., Van den Eynde, F., Stahl, D., Dew, T., Andiappan, M., Kalthoff, J., et al. (2011). Repetitive transcranial magnetic stimulation reduces cortisol concentrations in bulimic disorders. *Psychol. Med.* 41, 1329–1336. doi: 10.1017/S0033291710001881

Cowdrey, F. A., Harmer, C. J., Park, R. J., and McCabe, C. (2012). Neural responses to emotional faces in women recovered from anorexia nervosa. *Psychiatry Res.* 201, 190–195. doi: 10.1016/j.pscychresns.2011.08.009

Cowdrey, F. A., Park, R. J., Harmer, C. J., and McCabe, C. (2011). Increased neural processing of rewarding and aversive food stimuli in recovered anorexia nervosa. *Biol. Psychiatry* 70, 736–743. doi: 10.1016/j.biopsych.2011.05.028

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Crane, A., Roberts, M. E., and Treasure, J. (2007). Are obsessive−compulsive personality traits associated with a poor outcome in anorexia nervosa? A systematic review of randomized controlled trials and naturalistic. *Int. J. Eat. Disord.* 40, 581–588. doi: 10.1002/eat

Decker, J. H., Figner, B., and Steinglass, J. E. (2014). On weight and waiting: delay discounting in anorexia nervosa pretreatment and posttreatment. *Biol. Psychiatry*, 78, 606–614. doi: 10.1016/j.biopsych.2014.12.016

De Ridder, D., Vanneste, S., Kovacs, S., Sunaert, S., and Dom, G. (2011). Transient alcohol craving suppression by rTMS of dorsal anterior cingulate: an fMRI and LORETA EEG study. *Neurosci. Lett.* 496, 5–10. doi: 10.1016/j.neulet.2011.03.074

Dinur-Klein, L., Dannon, P., Hadar, A., Rosenberg, O., Roth, Y., Kotler, M., et al. (2014). Smoking cessation induced by deep repetitive transcranial magnetic stimulation of the prefrontal and insular cortices: a prospective, randomized controlled trial. *Biol. Psychiatry* 76, 742–749. doi: 10.1016/j.biopsych.2014.05.020

Donaldson, P. H., Rinehart, N. J., and Enticott, P. G. (2015). Noninvasive stimulation of the temporoparietal junction: a systematic review. *Neurosci. Biobehav. Rev.* 55, 547–572. doi: 10.1016/j.neubiorev.2015.05.017

Downar, J., Geraci, J., Salomons, T. V., Dunlop, K., Wheeler, S., McAndrews, M. P., et al. (2014). Anhedonia and reward-circuit connectivity distinguish nonresponders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. *Biol. Psychiatry* 76, 176–185. doi: 10.1016/j.biopsych.2013.10.026

Downar, J., Sankar, A., Giacobbe, P., Woodside, B., and Colton, P. (2012). Unanticipated rapid remission of refractory bulimia nervosa, during high-dose repetitive transcranial magnetic stimulation of the dorsomedial prefrontal cortex: a case report. *Front. Psychiatry* 3:30. doi: 10.3389/fpsyt.2012.00030

Dunlop, J., Woodside, B., Lam, E., Olmsted, M., Colton, P., Giacobbe, P., et al. (2015). Increases in frontostriatal connectivity are associated with response to dorsomedial repetitive transcranial magnetic stimulation in refractory binge/purge behaviors. *Neuroimage Clin.* 8, 611–618. doi: 10.1016/j.nicl.2015.06.008

Dunlop, K., Hanlon, C. A., and Downar, J. (accepted). Non-invasive brain stimulation treatments for addiction and major depression. *Ann. N.Y. Acad. Sci. Addict. Rev.*

Dunlop, K., Woodside, B., Olmsted, M., Colton, P., Giacobbe, P., and Downar, J. (2015). Reductions in cortico-striatal hyperconnectivity accompany successful treatment of obsessive-compulsive disorder with dorsomedial prefrontal rTMS. *Neuropsychopharmacology*. doi: 10.1038/npp.2015.292. [Epub ahead of print].

Eddy, K. T., Crosby, R. D., Keel, P. K., Wonderlich, S. A., le Grange, D., Hill, L., et al. (2009). Empirical identification and validation of eating disorder phenotypes in a multisite clinical sample. *J. Nerv. Ment. Dis.* 197, 41–49. doi: 10.1097/NMD.0b013e3181927389

Ehrlich, S., Geisler, D., Ritschel, F., King, J., Seidel, M., Boehm, I., et al. (2015). Elevated cognitive control over reward processing in recovered female patients with anorexia nervosa. *J. Psychiatry Neurosci.* 40, 307–315. doi: 10.1503/jpn.140249

Eldaief, M. C., Halko, M. A., Buckner, R. L., and Pascual-Leone, A. (2011). Transcranial magnetic stimulation modulates the brain's intrinsic activity in a frequency-dependent manner. *Proc. Natl. Acad. Sci. U.S.A.* 108, 21229–21234. doi: 10.1073/pnas.1113103109

Ellison, Z., Foong, J., Howard, R., Bullmore, E., Williams, S., and Treasure, J. (1998). Functional anatomy of calorie fear in anorexia nervosa. *Lancet* 352, 1192.

Enticott, P. G., Fitzgibbon, B. M., Kennedy, H. A., Arnold, S. L., Elliot, D., Peachey, A., et al. (2014). A double-blind, randomized trial of deep repetitive transcranial magnetic stimulation (rTMS) for autism spectrum disorder. *Brain Stimul.* 7, 206–211. doi: 10.1016/j.brs.2013.10.004

Enticott, P. G., Kennedy, H. A., Zangen, A., and Fitzgerald, P. B. (2011). Deep repetitive transcranial magnetic stimulation associated with improved social functioning in a young woman with an autism spectrum disorder. *J. ECT* 27, 41–43. doi: 10.1097/YCT.0b013e3181f07948

Felix-Ortiz, A. C., Burgos-Robles, A., Bhagat, N. D., Leppla, C. A., and Tye, K. M. (2015). Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. *Neuroscience*. doi: 10.1016/j.neuroscience.2015.07.041. [Epub ahead of print].

Fernández-Aranda, F., Pinheiro, A. P., Thornton, L. M., Berrettini, W. H., Crow, S., Fichter, M. M., et al. (2008). Impulse control disorders in women with eating disorders. *Psychiatry Res.* 157, 147–157. doi: 10.1016/j.psychres.2007.02.011

Filbey, F. M., Myers, U. S., and DeWitt, S. (2012). Reward circuit function in high BMI individuals with compulsive overeating: similarities with addiction. *Neuroimage* 63, 1800–1806. doi: 10.1016/j.neuroimage.2012.08.073

Fladung, A.-K., Schulze, U. M. E., Schöll, F., Bauer, K., and Grön, G. (2013). Role of the ventral striatum in developing anorexia nervosa. *Transl. Psychiatry* 3, e315. doi: 10.1038/tp.2013.88

Fonville, L., Giampietro, V., Surguladze, S., Williams, S., and Tchanturia, K. (2014). Increased BOLD signal in the fusiform gyrus during implicit emotion processing in anorexia nervosa. *Neuroimage Clin.* 4, 266–273. doi: 10.1016/j.nicl.2013.12.002

Fox, M. D., Liu, H., and Pascual-Leone, A. (2013). Identification of reproducible individualized targets for treatment of depression with TMS based on intrinsic connectivity. *Neuroimage* 66, 151–160. doi: 10.1016/j.neuroimage.2012.10.082

Frank, G. K., Bailer, U. F., Henry, S. E., Drevets, W., Meltzer, C. C., Price, J. C., et al. (2005). Increased dopamine D2/D3 receptor binding after recovery from anorexia nervosa measured by positron emission tomography and [11C]raclopride. *Biol. Psychiatry* 58, 908–912. doi: 10.1016/j.biopsych.2005.05.003

Frank, G. K., Kaye, W. H., Meltzer, C. C., Price, J. C., Greer, P., McConaha, C., et al. (2002). Reduced 5-HT2A receptor binding after recovery from anorexia nervosa. *Biol. Psychiatry* 52, 896–906. doi: 10.1016/S0006-3223(02)01378-1

Frank, G. K., Wagner, A., Achenbach, S., McConaha, C., Skovira, K., Aizenstein, H., et al. (2006). Altered brain activity in women recovered from bulimic-type eating disorders after a glucose challenge: a pilot study. *Int. J. Eat. Disord.* 39, 76–79. doi: 10.1002/eat.20210

Frank, G. K. W., Reynolds, J. R., Shott, M. E., Jappe, L., Yang, T. T., Tregellas, J. R., et al. (2012a). Anorexia nervosa and obesity are associated with opposite brain reward response. *Neuropsychopharmacology* 37, 2031–2046. doi: 10.1038/npp.2012.51

Frank, G. K. W., Reynolds, J. R., Shott, M. E., and O'Reilly, R. C. (2011). Altered temporal difference learning in bulimia nervosa. *Biol. Psychiatry* 70, 728–735. doi: 10.1016/j.biopsych.2011.05.011

Frank, G. K. W., Roblek, T., Shott, M. E., Jappe, L. M., Rollin, M. D. H., Hagman, J. O., et al. (2012b). Heightened fear of uncertainty in anorexia and bulimia nervosa. *Int. J. Eat. Disord.* 45, 227–232. doi: 10.1002/eat.20929

Fregni, F., Orsati, F., Pedrosa, W., Fecteau, S., Tome, F. A. M., Nitsche, M. A., et al. (2008). Transcranial direct current stimulation of the prefrontal cortex modulates the desire for specific foods. *Appetite* 51, 34–41. doi: 10.1016/j.appet.2007.09.016

Friederich, H.-C., Brooks, S., Uher, R., Campbell, I. C., Giampietro, V., Brammer, M., et al. (2010). Neural correlates of body dissatisfaction in anorexia nervosa. *Neuropsychologia* 48, 2878–2885. doi: 10.1016/j.neuropsychologia.2010.04.036

Galusca, B., Sigaud, T., Costes, N., Redoute, J., Massoubre, C., and Estour, B. (2014). Wide impairment of cerebral serotoninergic activity but inter-individual heterogeneity in bulimia nervosa patients: a pilot [(18)F]MPPF/PET study. *World J. Biol. Psychiatry* 15, 599–608. doi: 10.3109/15622975.2014.942358

Garfinkel, P. E., Lin, E., Goering, P., Spegg, C., Goldbloom, D. S., Kennedy, S., et al. (1995). Bulimia nervosa in a Canadian community sample: prevalence and comparison of subgroups. *Am. J. Psychiatry* 152, 1052–1058.

Garrett, A., Lock, J., Datta, N., Beenhaker, J., Kesler, S. R., and Reiss, A. L. (2014). Predicting clinical outcome using brain activation associated with set-shifting and central coherence skills in anorexia nervosa. *J. Psychiatr. Res.* 57, 26–33. doi: 10.1016/j.jpsychires.2014.06.013

Gatt, L., Jan, S., Mondraty, N., Horsfield, S., Russell, J., Laba, T., et al. (2014). The household economic burden of eating disorders and adherence to treatment in Australia. *BMC Psychiatry* 29:338. doi: 10.1186/s12888-014-0338-0

Giel, K. E., Kullmann, S., Preißl, H., Bischoff, S. C., Thiel, A., Schmidt, U., et al. (2013). Understanding the reward system functioning in anorexia nervosa: crucial role of physical activity. *Biol. Psychol.* 94, 575–581. doi: 10.1016/j.biopsycho.2013.10.004

Giuliano, C., and Cottone, P. (2015). The role of the opioid system in binge eating disorder. *CNS Spectr.* 20, 537–545. doi: 10.1017/S1092852915000668

Glashouwer, K. A., Bloot, L., Veenstra, E. M., Franken, I. H. A., and de Jong, P. J. (2014). Heightened sensitivity to punishment and reward in anorexia nervosa. *Appetite* 75, 97–102. doi: 10.1016/j.appet.2013.12.019

Godart, N. T., Flament, M. F., Curt, F., Perdereau, F., Lang, F., Venisse, J., et al. (2003). Anxiety disorders in subjects seeking treatment for eating disorders: a DSM-IV controlled study. *Psychiatry Res.* 117, 245–258. doi: 10.1016/S0165-1781(03)00038-6

Golden, N. H., Katzman, D. K., Kreipe, R. E., Stevens, S. L., Sawyer, S. M., Rees, J., et al. (2003). Eating disorders in adolescents: position paper of the society for adolescent medicine. *J. Adolesc. Heal.* 33, 496–503. doi: 10.1016/j.jadohealth.2003.08.004

Goldman, R. L., Borckardt, J. J., Frohman, H. A., O'Neil, P. M., Madan, A., Campbell, L. K., et al. (2011). Prefrontal cortex transcranial direct current stimulation (tDCS) temporarily reduces food cravings and increases the self-reported ability to resist food in adults with frequent food craving. *Appetite* 56, 741–746. doi: 10.1016/j.appet.2011.02.013

Gorelick, D. A., Zangen, A., and George, M. S. (2014). Transcranial magnetic stimulation in the treatment of substance addiction. *Ann. N.Y. Acad. Sci.* 1327, 79–93. doi: 10.1111/nyas.12479

Grall-Bronnec, M., and Sauvaget, A. (2014). The use of repetitive transcranial magnetic stimulation for modulating craving and addictive behaviours: a critical literature review of efficacy, technical and methodological considerations. *Neurosci. Biobehav. Rev.* 47C, 592–613. doi: 10.1016/j.neubiorev.2014.10.013

Grimonprez, A., Raedt, R., Baeken, C., Boon, P., and Vonck, K. (2015). The antidepressant mechanism of action of vagus nerve stimulation: evidence from preclinical studies. *Neurosci. Biobehav. Rev.* 56, 26–34. doi: 10.1016/j.neubiorev.2015.06.019

Guhn, A., Dresler, T., Andreatta, M., Müller, L. D., Hahn, T., Tupak, S. V., et al. (2014). Medial prefrontal cortex stimulation modulates the processing of conditioned fear. *Front. Behav. Neurosci.* 8:44. doi: 10.3389/fnbeh.2014.00044

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* 55, 187–199. doi: 10.1016/j.neuron.2007.06.026

Hanlon, C. A., Canterberry, M., Taylor, J., Devries, W., and George, M. (2013). "Functional and structural integrity of frontal-striatal circuits in cocaine users: an interleaved TMS-BOLD & DTI study," in *Annual Meeting of the Society for Biological Psychiatry*, *Vol. 256* (San Francisco, CA).

Hanlon, C. A., Dowdle, L. T., Austelle, C. W., Devries, W., Mithoefer, O., Badran, B. W., et al. (2015). What goes up, can come down: Novel brain stimulation paradigms may attenuate craving and craving-related neural circuitry in substance dependent individuals. *Brain Res.* 1628(Pt A), 199–209. doi: 10.1016/j.brainres.2015.02.053

Harrison, A., O'Brien, N., Lopez, C., and Treasure, J. (2010). Sensitivity to reward and punishment in eating disorders. *Psychiatry Res.* 177, 1–11. doi: 10.1016/j.psychres.2009.06.010

Hart, L. M., Granillo, M. T., Jorm, A. F., and Paxton, S. J. (2011). Unmet need for treatment in the eating disorders: a systematic review of eating disorder specific treatment seeking among community cases. *Clin. Psychol. Rev.* 31, 727–735. doi: 10.1016/j.cpr.2011.03.004

Hausmann, A., Mangweth, B., Walpoth, M., Hoertnagel, C., Kramer-Reinstadler, K., Rupp, C. I., et al. (2004). Repetitive transcranial magnetic stimulation (rTMS) in the double-blind treatment of a depressed patient suffering from bulimia nervosa: a case report. *Int. J. Neuropsychopharmacol.* 7, 371–373. doi: 10.1017/S1461145704004420

Hay, P. J., Touyz, S., and Sud, R. (2012). Treatment for severe and enduring anorexia nervosa: a review. *Aust. N. Z. J. Psychiatry* 42, 1136–1144. doi: 10.1177/0004867412450469

Hege, M. A., Stingl, K. T., Kullmann, S., Schag, K., Giel, K. E., Zipfel, S., et al. (2014). Attentional impulsivity in binge eating disorder modulates response inhibition performance and frontal brain networks. *Int. J. Obes. (Lond).* 39, 353–360. doi: 10.1038/ijo.2014.99

Hilbert, A., Bishop, M. E., Stein, R. I., Tanofsky-Kraff, M., Swenson, A. K., Welch, R. R., et al. (2012). Long-term efficacy of psychological treatments for binge eating disorder. *Br. J. Psychiatry* 200, 232–237. doi: 10.1192/bjp.bp.110.089664

Hoffman, E. R., Gagne, D. A., Thornton, L. M., Klump, K. L., Brandt, H., Crawford, S., et al. (2012). Understanding the association of impulsivity, obsessions, and compulsions with binge eating and purging behaviours in anorexia nervosa. *Eur. Eat. Disord. Rev.* 20, e129–e136. doi: 10.1002/erv.2161

Holsen, L., Lawson, E., Blum, J., Ko, E., Makris, N., Fazeli, P., et al. (2012). Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa. *J. Psychiatry Neurosci.* 37, 322–232. doi: 10.1503/jpn.110156

Holtzheimer, P. E., McDonald, W. M., Mufti, M., Kelley, M. E., Quinn, S., Corso, G., et al. (2010). Accelerated repetitive transcranial magnetic stimulation for treatment-resistant depression. *Depress. Anxiety* 27, 960–963. doi: 10.1002/da.20731

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

Hudson, J. I., Hiripi, E., Pope, H. G., and Kessler, R. C. (2007). The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biol. Psychiatry* 61, 348–358. doi: 10.1016/j.biopsych.2006.03.040

Hummel, D., Rudolf, A. K., Brandi, M.-L., Untch, K.-H., Grabhorn, R., Hampel, H., et al. (2013). Neural adaptation to thin and fat bodies in the fusiform body area and middle occipital gyrus: an fMRI adaptation study. *Hum. Brain Mapp.* 34, 3233–3246. doi: 10.1002/hbm.22135

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., et al. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am. J. Psychiatry* 167, 748–751. doi: 10.1176/appi.ajp.2010.09091379

Isserles, M., Shalev, A. Y., Roth, Y., Peri, T., Kutz, I., Zlotnick, E., et al. (2013). Effectiveness of deep transcranial magnetic stimulation combined with a brief exposure procedure in post-traumatic stress disorder–a pilot study. *Brain Stimul.* 6, 377–383. doi: 10.1016/j.brs.2012.07.008

Johnstone, T., van Reekum, C. M., Urry, H. L., Kalin, N. H., and Davidson, R. J. (2007). Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. *J. Neurosci.* 27, 8877–8884. doi: 10.1523/JNEUROSCI.2063-07.2007

Keating, C., Tilbrook, A. J., Rossell, S. L., Enticott, P. G., and Fitzgerald, P. B. (2012). Reward processing in anorexia nervosa. *Neuropsychologia* 50, 567–575. doi: 10.1016/j.neuropsychologia.2012.01.036

Keel, P. K., Fichter, M., Quadflieg, N., Bulik, C. M., Baxter, M. G., Thornton, L., et al. (2004). Application of a latent class analysis to empirically define eating disorder phenotypes. *Arch. Gen. Psychiatry* 61, 192–200. doi: 10.1001/archpsyc.61.2.192

Keel, P. K., and Mitchell, J. E. (1997). Outcome in bulimia nervosa. *Am. J. Psychiatry* 154, 313–321. doi: 10.1176/ajp.154.3.313

Kekic, M., McClelland, J., Campbell, I., Nestler, S., Rubia, K., David, A. S., et al. (2014). The effects of prefrontal cortex transcranial direct current stimulation (tDCS) on food craving and temporal discounting in women with frequent food cravings. *Appetite* 78, 55–62. doi: 10.1016/j.appet.2014.03.010

Kerr, K., Moseman, S., Avery, J., Bodurka, J., Zucker, N., and Kyle Simmons, W. (2015). Altered insula activity during visceral interoception in weight-restored patients with anorexia nervosa. *Neuropsychopharmacology*. 41, 521–528. doi: 10.1038/npp.2015.174

Khedr, E., Elfetoh, N., Ali, A., and Noamany, M. (2014). Anodal transcranial direct current stimulation over the dorsolateral prefrontal cortex improves anorexia nervosa: a pilot study. *Restor. Neurol. Neurosci.* 32, 789–797. doi: 10.3233/RNN-140392

Klein, D. A., Schebendach, J. E., Gershkovich, M., Bodell, L., Foltin, R., and Walsh, B. T. (2010). Behavioral assessment of the reinforcing effect of exercise in women with anorexia nervosa: further paradigm development and data. *Int. J. Eat. Disord.* 43, 611–618. doi: 10.1002/eat.20758

Kozel, F. A., Nahas, Z., deBrux, C., Molloy, M., Lorberbaum, J. P., Bohning, D., et al. (2000). How coil-cortex distance relates to age, motor threshold, and antidepressant response to repetitive transcranial magnetic stimulation. *J. Neuropsychiatry Clin. Neurosci.* 12, 376–384. doi: 10.1176/appi.neuropsych.12.3.376

Kühn, S., Gallinat, J., and Brass, M. (2011). "Keep Calm and Carry On": structural correlates of expressive suppression of emotions. *PLoS ONE* 6:e16569. doi: 10.1371/journal.pone.0016569

Kullmann, S., Giel, K. E., Hu, X., Bischoff, S. C., Teufel, M., Thiel, A., et al. (2014). Impaired inhibitory control in anorexia nervosa elicited by physical activity stimuli. *Soc. Cogn. Affect. Neurosci.* 9, 917–923. doi: 10.1093/scan/nst070

Lao-Kaim, N., Fonville, L., Giampietro, V., Williams, S., Simmons, A., and Tchanturia, K. (2015). Aberrant function of learning and cognitive control networks underlie inefficient cognitive flexibility in anorexia nervosa: a cross-sectional fMRI study. *PLoS ONE* 10:e124027. doi: 10.1371/journal.pone.0124027

Lapenta, O. M., Sierve, K., de Macedo, E. C., Fregni, F., and Boggio, P. S. (2014). Transcranial direct current stimulation modulates ERP-indexed inhibitory control and reduces food consumption. *Appetite* 83, 42–48. doi: 10.1016/j.appet.2014.08.005

Lee, S., Ran Kim, K., Ku, J., Lee, J. H., Namkoong, K., and Jung, Y. C. (2014). Resting-state synchrony between anterior cingulate cortex and precuneus relates to body shape concern in anorexia nervosa and bulimia nervosa. *Psychiatry Res.* 221, 43–48. doi: 10.1016/j.pscychresns.2013.11.004

Leehr, E. J., Krohmer, K., Schag, K., Dresler, T., Zipfel, S., and Giel, K. E. (2015). Emotion regulation model in binge eating disorder and obesity–a systematic review. *Neurosci. Biobehav. Rev.* 49, 125–134. doi: 10.1016/j.neubiorev.2014.12.008

Li, C.-T., Chen, M.-H., Juan, C.-H., Huang, H.-H., Chen, L.-F., Hsieh, J.-C., et al. (2014). Efficacy of prefrontal theta-burst stimulation in refractory depression: a randomized sham-controlled study. *Brain* 137, 2088–2098. doi: 10.1093/brain/awu109

Lilenfeld, L. R., Kaye, W. H., Greeno, C. G., Merikangas, K. R., Plotnicov, K., Pollice, C., et al. (1998). A controlled family study of anorexia nervosa and bulimia nervosa: psychiatric disorders in first-degree relatives and effects of proband comorbidity. *Arch. Gen. Psychiatry* 55, 603–610. doi: 10.1001/archpsyc.55.7.603

Lindquist, K. A., Satpute, A. B., Wager, T. D., Weber, J., and Barrett, L. F. (2015). The brain basis of positive and negative affect: evidence from a meta-analysis of the human neuroimaging literature. *Cereb. Cortex*. doi: 10.1093/cercor/bhv001. [Epub ahead of print].

Lipsman, N., Woodside, D. B., Giacobbe, P., Hamani, C., Carter, J. C., Norwood, S. J., et al. (2013). Subcallosal cingulate deep brain stimulation for treatment-refractory anorexia nervosa: a phase 1 pilot trial. *Lancet* 381, 1361–1370. doi: 10.1016/S0140-6736(12)62188-6

Lock, J., Garrett, A., Beenhakker, J., and Reiss, A. L. (2011). Aberrant brain activation during a response inhibition task in adolescent eating disorder subtypes. *Am. J. Psychiatry* 168, 55–64. doi: 10.1176/appi.ajp.2010.10010056

Luo, Y., Qin, S., Fernández, G., Zhang, Y., Klumpers, F., and Li, H. (2014). Emotion perception and executive control interact in the salience network during emotionally charged working memory processing. *Hum. Brain Mapp.* 35, 5606–5616. doi: 10.1002/hbm.22573

Machii, K., Cohen, D., Ramos-Estebanez, C., and Pascual-Leone, A. (2006). Safety of rTMS to non-motor cortical areas in healthy participants and patients. *Clin. Neurophysiol.* 117, 455–471. doi: 10.1016/j.clinph.2005.10.014

Maeda, F., Keenan, J. P., Tormos, J. M., Topka, H., and Pascual-Leone, A. (2000). Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. *Exp. Brain Res.* 133, 425–430. doi: 10.1007/s002210000432

Mantovani, A., Simpson, H. B., Fallon, B. A., Rossi, S., and Lisanby, S. H. (2010). Randomized sham-controlled trial of repetitive transcranial magnetic stimulation in treatment-resistant obsessive-compulsive disorder. *Int. J. Neuropsychopharmacol.* 13, 217–227. doi: 10.1017/S1461145709990435

Manwaring, J. L., Green, L., Myerson, J., Strube, M. J., and Wilfley, D. E. (2011). Discounting of Various types of rewards by women with and without binge eating disorder: evidence for general rather than specific differences. *Psychol. Rec.* 61, 561–582.

Marsh, R., Horga, G., Wang, Z., Wang, P., Klahr, K. W., Berner, L. A., et al. (2011). An fMRI study of self-regulatory control and conflict resolution in adolescents with bulimia nervosa. *Am. J. Psychiatry* 168, 1210–1220. doi: 10.1176/appi.ajp.2011.11010094

Marsh, R., Steinglass, J. E., Gerber, A. J., Graziano O'Leary, K., Wang, Z., Murphy, D., et al. (2009). Deficient activity in the neural systems that mediate self-regulatory control in bulimia nervosa. *Arch. Gen. Psychiatry* 66, 51–63. doi: 10.1001/archgenpsychiatry.2008.504

McAdams, C. J., and Krawczyk, D. C. (2011). Impaired neural processing of social attribution in anorexia nervosa. *Psychiatry Res.* 194, 54–63. doi: 10.1016/j.pscychresns.2011.06.016

McAdams, C. J., Lohrenz, T., and Montague, P. R. (2015). Neural responses to kindness and malevolence differ in illness and recovery in women with anorexia nervosa. *Hum. Brain Mapp.* 36, 5207–5219. doi: 10.1002/hbm.23005

McClelland, J., Bozhilova, N., Campbell, I., and Schmidt, U. (2013). A systematic review of the effects of neuromodulation on eating and body weight: evidence from human and animal studies. *Eur. Eat. Disord. Rev.* 21, 436–455. doi: 10.1002/erv.2256

McClelland, J., Kekic, M., Campbell, I. C., and Schmidt, U. (2016). Repetitive transcranial magnetic stimulation (rTMS) treatment in enduring anorexia nervosa: a case series. *Eur. Eat. Disord. Rev.* 24, 157–163. doi: 10.1002/erv.2414

Meng, Z., Liu, C., Yu, C., and Ma, Y. (2014). Transcranial direct current stimulation of the frontal-parietal-temporal area attenuates smoking behavior. *J. Psychiatr. Res.* 54, 19–25. doi: 10.1016/j.jpsychires.2014.03.007

Meron, D., Hedger, N., Garner, M., and Baldwin, D. S. (2015). Transcranial direct current stimulation (tDCS) in the treatment of depression: systematic review and meta-analysis of efficacy and tolerability. *Neurosci. Biobehav. Rev.* 57, 46–62. doi: 10.1016/j.neubiorev.2015.07.012

Milos, G., Spindler, A., Schnyder, U., and Fairburn, C. G. (2005). Instability of eating disorder diagnoses: prospective study. *Br. J. Psychiatry* 187, 573–578. doi: 10.1192/bjp.187.6.573

Mischoulon, D., Eddy, K. T., Keshaviah, A., Dinescu, D., Ross, S. L., Kass, A. E., et al. (2011). Depression and eating disorders: treatment and course. *J. Affect. Disord.* 130, 470–477. doi: 10.1016/j.jad.2010.10.043

Mishra, B. R., Nizamie, S. H., Das, B., and Praharaj, S. K. (2010). Efficacy of repetitive transcranial magnetic stimulation in alcohol dependence: a sham-controlled study. *Addiction* 105, 49–55. doi: 10.1111/j.1360-0443.2009.02777.x

Mitchell, J. E., Agras, S., and Wonderlich, S. (2007). Treatment of bulimia nervosa: where are we and where are we going? *Int. J. Eat. Disord.* 40, 95–101. doi: 10.1002/eat.20343

Miyake, Y., Okamoto, Y., Onoda, K., Shirao, N., Mantani, T., and Yamawaki, S. (2009). Neural correlates of alexithymia in response to emotional stimuli: a study of anorexia nervosa patients. *Hiroshima J. Med. Sci.* 58, 1–8.

Miyake, Y., Okamoto, Y., Onoda, K., Shirao, N., Okamoto, Y., Otagaki, Y., et al. (2010). Neural processing of negative word stimuli concerning body image in patients with eating disorders: an fMRI study. *Neuroimage* 50, 1333–1339. doi: 10.1016/j.neuroimage.2009.12.095

Miyake, Y., Okamoto, Y., Onoda, K., Shirao, N., Okamoto, Y., and Yamawaki, S. (2012). Brain activation during the perception of stressful word stimuli concerning interpersonal relationships in anorexia nervosa patients with high degrees of alexithymia in an fMRI paradigm. *Psychiatry Res.* 201, 113–119. doi: 10.1016/j.pscychresns.2011.07.014

Mole, T. B., Irvine, M., A., Worbe, Y., Collins, P., Mitchell, S. P., Bolton, S., et al. (2015). Impulsivity in disorders of food and drug misuse. *Psychol. Med.* 45, 771–782. doi: 10.1017/S0033291714001834

Morris, S. E., and Cuthbert, B. N. (2012). Research Domain Criteria: cognitive systems, neural circuits, and dimensions of behavior. *Dialogues Clin. Neurosci.* 14, 29–37.

Nauczyciel, C., and Drapier, D. (2012). Repetitive transcranial magnetic stimulation in the treatment of obsessive-compulsive disorder. *Rev. Neurol. (Paris).* 168, 655–661. doi: 10.1016/j.neurol.2012.05.006

Nettekoven, C., Volz, L. J., Leimbach, M., Pool, E.-M., Rehme, A. K., Eickhoff, S. B., et al. (2015). Inter-individual variability in cortical excitability and motor network connectivity following multiple blocks of rTMS. *Neuroimage* 118, 209–218. doi: 10.1016/j.neuroimage.2015.06.004

Nitsche, M. A., Koschack, J., Pohlers, H., Hullemann, S., Paulus, W., and Happe, S. (2012). Effects of frontal transcranial direct current stimulation on emotional state and processing in healthy humans. *Front. Psychiatry* 3:58. doi: 10.3389/fpsyt.2012.00058

Oberndorfer, T. A., Frank, G. K. W., Simmons, A. N., Wagner, A., McCurdy, D., Fudge, J. L., et al. (2013a). Altered insula response to sweet taste processing after recovery from anorexia and bulimia nervosa. *Am. J. Psychiatry* 170, 1143–1151. doi: 10.1176/appi.ajp.2013.11111745

Oberndorfer, T. A., Kaye, W. H., Simmons, A. N., Strigo, I. A., and Matthews, S. C. (2011). Demand-specific alteration of medial prefrontal cortex response during an inhibition task in recovered anorexic women. *Int. J. Eat. Disord.* 44, 1–8. doi: 10.1002/eat.20750

Oberndorfer, T., Simmons, A., McCurdy, D., Strigo, I., Matthews, S., Yang, T., et al. (2013b). Greater anterior insula activation during anticipation of food images in women recovered from anorexia nervosa versus controls. *Psychiatry Res.* 214, 132–141. doi: 10.1016/j.pscychresns.2013.06.010

Olmsted, M. P., Kaplan, A. S., and Rockert, W. (2005). Defining remission and relapse in bulimia nervosa. *Int. J. Eat. Disord.* 38, 1–6. doi: 10.1002/eat.20144

Pascual-Leone, A., Tormos, J. M., Keenan, J., Tarazona, F., Cañete, C., and Catalá, M. D. (1998). Study and modulation of human cortical excitability with transcranial magnetic stimulation. *J. Clin. Neurophysiol.* 15, 333–343.

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(Pt 4), 847–858. doi: 10.1093/brain/117.4.847

Racine, S. E., Burt, S. A., Keel, P. K., Sisk, C. L., Neale, M. C., Boker, S., et al. (2015). Examining associations between negative urgency and key components of objective binge episodes. *Int. J. Eat. Disord.* 48, 527–531. doi: 10.1002/eat.22412

Radeloff, D., Willmann, K., Otto, L., Lindner, M., Putnam, K., Leeuwen, S., et al. (2012). High-fat taste challenge reveals altered striatal response in women recovered from bulimia nervosa: a pilot study. *World J. Biol. Psychiatry*, 15, 307–316. doi: 10.3109/15622975.2012.671958

Reineberg, A. E., Andrews-Hanna, J. R., Depue, B. E., Friedman, N. P., and Banich, M. T. (2015). Resting-state networks predict individual differences in common and specific aspects of executive function. *Neuroimage* 104, 69–78. doi: 10.1016/j.neuroimage.2014.09.045

Ritschel, F., King, J. A., Geisler, D., Flohr, L., Neidel, F., Boehm, I., et al. (2015). Temporal delay discounting in acutely ill and weight-recovered patients with anorexia nervosa. *Psychol. Med.* 45, 1229–1239. doi: 10.1017/S0033291714002311

Rossi, S., and Hallett, M. (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

Roth, Y., Amir, A., Levkovitz, Y., and Zangen, A. (2007). Three-dimensional distribution of the electric field induced in the brain by transcranial magnetic stimulation using figure-8 and deep H-coils. *J. Clin. Neurophysiol.* 24, 31–38. doi: 10.1097/WNP.0b013e31802fa393

Roth, Y., Levkovitz, Y., Pell, G. S., Ankry, M., and Zangen, A. (2014). Safety and characterization of a novel multi-channel TMS stimulator. *Brain Stimul.* 7, 194–205. doi: 10.1016/j.brs.2013.09.004

Rothemund, Y., Buchwald, C., Georgiewa, P., Bohner, G., Bauknecht, H. C., Ballmaier, M., et al. (2011). Compulsivity predicts fronto striatal activation in severely anorectic individuals. *Neuroscience* 197, 242–250. doi: 10.1016/j.neuroscience.2011.09.016

Salomons, T. V., Dunlop, K., Kennedy, S. H., Flint, A., Geraci, J., Giacobbe, P., et al. (2014). Resting-state cortico-thalamic-striatal connectivity predicts response to dorsomedial prefrontal rTMS in major depressive disorder. *Neuropsychopharmacology* 39, 488–498. doi: 10.1038/npp.2013.222

Sanders, N., Smeets, P., van Elburg, A., Danner, U., van Meer, F., Hoek, H., et al. (2015). Altered food-cue processing in chronically ill and

recovered women with anorexia nervosa. *Front. Behav. Neurosci.* 9:46. doi: 10.3389/fnbeh.2015.00046

Sato, Y., Saito, N., Utsumi, A., Aizawa, E., Shoji, T., Izumiyama, M., et al. (2013). Neural basis of impaired cognitive flexibility in patients with anorexia nervosa. *PLoS ONE* 8:e61108. doi: 10.1371/journal.pone.0061108

Saxe, R., and Kanwisher, N. (2003). People thinking about thinking people. The role of the temporo-parietal junction in "theory of mind." *Neuroimage* 19, 1835–1842. doi: 10.1016/S1053-8119(03)00230-1

Schebendach, J., Broft, A., Foltin, R., and Walsh, B. T. (2013). Can the reinforcing value of food be measured in bulimia nervosa? *Appetite* 62, 70–75. doi: 10.1016/j.appet.2012.11.009

Schebendach, J. E., Klein, D. A., Foltin, R. W., Devlin, M. J., and Walsh, B. T. (2007). Relative reinforcing value of exercise in inpatients with anorexia nervosa: model development and pilot data. *Int. J. Eat. Disord.* 40, 446–453. doi: 10.1002/eat.20392

Schienle, A., Schäfer, A., Hermann, A., and Vaitl, D. (2009). Binge-Eating disorder: reward sensitivity and brain activation to images of food. *Biol. Psychiatry* 65, 654–661. doi: 10.1016/j.biopsych.2008.09.028

Schutter, D. J. L. G., and van Honk, J. (2006). A standardized motor threshold estimation procedure for transcranial magnetic stimulation research. *J. ECT* 22, 176–178. doi: 10.1097/01.yct.0000235924.60364.27

Seeger, G., Braus, D. F., Ruf, M., Goldberger, U., and Schmidt, M. H. (2002). Body image distortion reveals amygdala activation in patients with anorexia nervosa – a functional magnetic resonance imaging study. *Neurosci. Lett.* 326, 25–28. doi: 10.1016/S0304-3940(02)00312-9

Shapiro, J. R., Berkman, N. D., Brownley, K. A., Sedway, J. A., Lohr, K. N., and Bulik, C. M. (2007). Bulimia nervosa treatment: a systematic review of randomized controlled trials. *Int. J. Eat. Disord.* 40, 321–336. doi: 10.1002/eat

Smink, F. R. E., Van Hoeken, D., Oldehinkel, A. J., and Hoek, H. W. (2014). Prevalence and severity of DSM-5 eating disorders in a community cohort of adolescents. *Int. J. Eat. Disord.* 47, 610–619. doi: 10.1002/eat.22316

Solvason, H. B., Husain, M., Fitzgerald, P. B., Rosenquist, P., McCall, W. V., Kimball, J., et al. (2014). Improvement in quality of life with left prefrontal transcranial magnetic stimulation in patients with pharmacoresistant major depression: acute and six month outcomes. *Brain Stimul.* 7, 219–225. doi: 10.1016/j.brs.2013.10.008

Spangler, D. L., and Allen, M. D. (2012). An fMRI investigation of emotional processing of body shape in bulimia nervosa. *Int. J. Eat. Disord.* 45, 17–25. doi: 10.1002/eat.20899

Steinglass, J. E., Figner, B., Berkowitz, S., Simpson, H. B., Weber, E. U., and Walsh, B. T. (2012). Increased capacity to delay reward in anorexia nervosa. *J. Int. Neuropsychol. Soc.* 18, 773–780. doi: 10.1017/S1355617712000446

Steinhausen, H.-C., and Weber, S. (2009). The outcome of bulimia nervosa: findings from one-quarter century of research. *Am. J. Psychiatry* 166, 1331–1341. doi: 10.1176/appi.ajp.2009.09040582

Striegel-Moore, R. H., Franko, D. L., Thompson, D., Barton, B., Schreiber, G. B., and Daniels, S. R. (2005). An empirical study of the typology of bulimia nervosa and its spectrum variants. *Psychol. Med.* 35, 1563–1572. doi: 10.1017/S0033291705006057

Strigo, I. A., Matthews, S. C., Simmons, A. N., Oberndorfer, T., Klabunde, M., Reinhardt, L. E., et al. (2013). Altered insula activation during pain anticipation in individuals recovered from anorexia nervosa: evidence of interoceptive dysregulation. *Int. J. Eat. Disord.* 46, 23–33. doi: 10.1002/eat.22045

Strober, M., Freeman, R., Lampert, C., Diamond, J., and Kaye, W. (2000). Controlled family study of anorexia nervosa and bulimia nervosa: evidence of shared liability and transmission of partial syndromes. *Am. J. Psychiatry* 157, 393–401. doi: 10.1176/appi.ajp.157.3.393

Suchan, B., Bauser, D. S., Busch, M., Schulte, D., Grönemeyer, D., Herpertz, S., et al. (2013). Reduced connectivity between the left fusiform body area and the extrastriate body area in anorexia nervosa is associated with body image distortion. *Behav. Brain Res.* 241, 80–85. doi: 10.1016/j.bbr.2012.12.002

Suda, M., Brooks, S. J., Giampietro, V., Friederich, H. C., Uher, R., Brammer, M. J., et al. (2013). Functional neuroanatomy of body checking in people with anorexia nervosa. *Int. J. Eat. Disord.* 46, 653–662. doi: 10.1002/eat.22150

Suda, M., Brooks, S. J., Giampietro, V., Uher, R., Mataix-Cols, D., Brammer, M. J., et al. (2014). Provocation of symmetry/ordering symptoms in Anorexia nervosa: a functional neuroimaging study. *PLoS ONE* 9:e97998. doi: 10.1371/journal.pone.0097998

Sullivan, P. F. (1995). Mortality in anorexia nervosa. *Am. J. Psychiatry* 152, 1073–1074.

Sullivan, P. F., Bulik, C. M., and Kendler, K. S. (1998). The epidemiology and classification of bulimia nervosa. *Psychol. Med.* 28, 599–610. doi: 10.1017/S0033291798006576

Sutoh, C., Koga, Y., Kimura, H., Kanahara, N., Numata, N., Hirano, Y., et al. (2016). Repetitive transcranial magnetic stimulation changes cerebral oxygenation on the left dorsolateral prefrontal cortex in bulimia nervosa: a near-infrared spectroscopy pilot study. *Eur. Eat. Disord. Rev.* 24, 83–88. doi: 10.1002/erv.2413

Tapajóz P de Sampaio, F., Soneira, S., Aulicino, A., Harris, P., and Allegri, R. F. (2015). Emotional reactivity to social stimuli in patients with eating disorders. *Psychiatry Res.* 229, 887–894. doi: 10.1016/j.psychres.2015.07.049

Torresan, R. C., Ramos-Cerqueira, A. T. A., Shavitt, R. G., do Rosário, M. C., de Mathis, M. A., Miguel, E. C., et al. (2013). Symptom dimensions, clinical course and comorbidity in men and women with obsessive-compulsive disorder. *Psychiatry Res.* 209, 186–195. doi: 10.1016/j.psychres.2012.12.006

Tsutsumi, R., Hanajima, R., Terao, Y., Shirota, Y., Ohminami, S., Shimizu, T., et al. (2014). Effects of the motor cortical quadripulse transcranial magnetic stimulation (QPS) on the contralateral motor cortex and interhemispheric interactions. *J. Neurophysiol.* 111, 26–35. doi: 10.1152/jn.00515.2013

Uher, R., Murphy, T., Brammer, M. J., Dalgleish, T., Phillips, M. L., Ng, V. W., et al. (2004). Medial prefrontal cortex activity associated with symptom provocation in eating disorders. *Am. J. Psychiatry* 161, 1238–1246. doi: 10.1176/appi.ajp.161.7.1238

Uher, R., Yoganathan, D., Mogg, A., Eranti, S. V., Treasure, J., Campbell, I. C., et al. (2005). Effect of left prefrontal repetitive transcranial magnetic stimulation on food craving. *Biol. Psychiatry* 58, 840–842. doi: 10.1016/j.biopsych.2005.05.043

Ursu, S., Clark, K. A., Stenger, V. A., and Carter, C. S. (2008). Distinguishing expected negative outcomes from preparatory control in the human orbitofrontal cortex. *Brain Res.* 1227, 110–119. doi: 10.1016/j.brainres.2008.06.033

Val-Laillet, D., Aarts, E., Weber, B., Ferrari, M., Quaresima, V., Stoeckel, L. E., et al. (2015). Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. *NeuroImage. Clin.* 8, 1–31. doi: 10.1016/j.nicl.2015.03.016

Van den Eynde, F., Claudino, A. M., Mogg, A., Horrell, L., Stahl, D., Ribeiro, W., et al. (2010). Repetitive transcranial magnetic stimulation reduces cue-induced food craving in bulimic disorders. *Biol. Psychiatry* 67, 793–795. doi: 10.1016/j.biopsych.2009.11.023

Van den Eynde, F., Guillaume, S., Broadbent, H., Campbell, I. C., and Schmidt, U. (2013). Repetitive transcranial magnetic stimulation in anorexia nervosa: a pilot study. *Eur. Psychiatry* 28, 98–101. doi: 10.1016/j.eurpsy.2011.06.002

Via, E., Soriano-Mas, C., Sánchez, I., Forcano, L., Harrison, B. J., Davey, C. G., et al. (2015). Abnormal social reward responses in anorexia nervosa: an fMRI study. *PLoS ONE* 10:e0133539. doi: 10.1371/journal.pone.0133539

Vocks, S., Busch, M., Grönemeyer, D., Schulte, D., Herpertz, S., and Suchan, B. (2010). Neural correlates of viewing photographs of one's own body and another woman's body in anorexia and bulimia nervosa: an fMRI study. *J. Psychiatry Neurosci.* 35, 163–176. doi: 10.1503/jpn.090048

Vocks, S., Herpertz, S., Rosenberger, C., Senf, W., and Gizewski, E. R. (2011). Effects of gustatory stimulation on brain activity during hunger and satiety in females with restricting-type anorexia nervosa: an fMRI study. *J. Psychiatr. Res.* 45, 395–403. doi: 10.1016/j.jpsychires.2010.07.012

Voss, U., Holzmann, R., Hobson, A., Paulus, W., Koppehele-Gossel, J., Klimke, A., et al. (2014). Induction of self awareness in dreams through frontal low current stimulation of gamma activity. *Nat. Neurosci.* 17, 810–812. doi: 10.1038/nn.3719

Wagner, A., Aizenstein, H., Mazurkewicz, L., Fudge, J., Frank, G. K., Putnam, K., et al. (2008). Altered insula response to taste stimuli in individuals recovered from restricting-type anorexia nervosa. *Neuropsychopharmacology* 33, 513–523. doi: 10.1038/sj.npp.1301443

Wagner, A., Aizenstein, H., Venkatraman, V. K., Fudge, J., May, J. C., Mazurkewicz, L., et al. (2007). Altered reward processing in women recovered from anorexia nervosa. *Am. J. Psychiatry* 164, 1842–1849. doi: 10.1176/appi.ajp.2007.07040575

Walpoth, M., Hoertnagl, C., Mangweth-Matzek, B., Kemmler, G., Hinterhölzl, J., Conca, A., et al. (2008). Repetitive transcranial magnetic stimulation in bulimia nervosa: preliminary results of a single-centre, randomised, double-blind, sham-controlled trial in female outpatients. *Psychother. Psychosom.* 77, 57–60. doi: 10.1159/000110061

Walsh, B. T. (2013). The enigmatic persistence of anorexia nervosa. *Am. J. Psychiatry* 170, 477–484. doi: 10.1176/appi.ajp.2012.12081074

Weygandt, M., Schaefer, A., Schienle, A., and Haynes, J. D. (2012). Diagnosing different binge-eating disorders based on reward-related brain activation patterns. *Hum. Brain Mapp.* 33, 2135–2146. doi: 10.1002/hbm.21345

Wierenga, C. E., Ely, A., Bischoff-Grethe, A., Bailer, U. F., Simmons, A. N., and Kaye, W. H. (2014). Are extremes of consumption in eating disorders related to an altered balance between reward and inhibition? *Front. Behav. Neurosci.* 8:410. doi: 10.3389/fnbeh.2014.00410

Wiethoff, S., Hamada, M., and Rothwell, J. C. (2014). Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimul.* 7, 468–475. doi: 10.1016/j.brs.2014.02.003

Wildes, J. E., Forbes, E. E., and Marcus, M. D. (2014). Advancing research on cognitive flexibility in eating disorders: the importance of distinguishing attentional set-shifting and reversal learning. *Int. J. Eat. Disord.* 47, 227–230. doi: 10.1002/eat.22243

Wildes, J. E., Forbush, K. T., and Markon, K. E. (2013). Characteristics and stability of empirically derived anorexia nervosa subtypes: towards the identification of homogeneous low-weight eating disorder phenotypes. *J. Abnorm. Psychol.* 122, 1031–1041. doi: 10.1037/a0034676

Wildes, J., and Marcus, M. (2015). Application of the Research Domain Criteria (RDoC) framework to eating disorders: emerging concepts and research. *Curr. Psychiatry Rep.* 17, 1–10. doi: 10.1007/s11920-015-0572-2

Wonderlich, S. A., Crosby, R. D., Joiner, T., Peterson, C. B., Bardone-Cone, A., Klein, M., et al. (2005). Personality subtyping and bulimia nervosa: psychopathological and genetic correlates. *Psychol. Med.* 35, 649–657. doi: 10.1017/S0033291704004234

Wu, M., Hartmann, M., Skunde, M., Herzog, W., and Friederich, H.-C. (2013). Inhibitory control in bulimic-type eating disorders: a systematic review and meta-analysis. *PLoS ONE* 8:e83412. doi: 10.1371/journal.pone.0083412

Zastrow, A., Kaiser, S., Stippich, C., Walther, S., Herzog, W., Tchanturia, K., et al. (2009). Neural correlates of impaired cognitive-behavioral flexibility in anorexia nervosa. *Am. J. Psychiatry* 166, 608–616. doi: 10.1176/appi.ajp.2008.08050775

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 Neuroscience

ORIGINAL RESEARCH
published: 11 January 2016
doi: 10.3389/fnins.2015.00505

Clinical Value of the Assessment of Changes in MEP Duration with Voluntary Contraction

Marisa Brum[1,2], *Christopher Cabib*[3,4] *and Josep Valls-Solé*[3,4]*

[1] São Bernardo Hospital, Centro Hospitalar de Setúbal, Setúbal, Portugal, [2] Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Portugal, [3] EMG Unit, Neurology Department, Hospital Clinic, University of Barcelona, Barcelona, Spain, [4] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Universitat de Barcelona, Barcelona, Spain

Transcranial magnetic stimulation (TMS) gives rise to muscle responses, known as motor evoked potentials (MEP), through activation of the motor pathways. Voluntary contraction causes facilitation of MEPs, which consists of shortening MEP latency, increasing MEP amplitude and widening MEP duration. While an increase in excitability of alpha motorneurons and the corticospinal tract can easily explain latency shortening and amplitude increase, other mechanisms have to be accounted for to explain the increase in duration. We measured the increase in duration of the MEP during contraction with respect to rest in a group of healthy volunteers and retrospectively assessed this parameter in patients who were examined in a standardized fashion during the past 5 years. We included 25 healthy subjects, 21 patients with multiple sclerosis, 33 patients with acute stroke, 5 patients with hereditary spastic paraparesis, and 5 patients with signs suggesting psychogenic paresis. We found already significant differences among groups in the MEP duration at rest, patients with MS had a significantly longer duration, and patients with stroke had significantly shorter duration, than the other two groups. The increase in MEP duration during voluntary contraction was different in patients and in healthy subjects. It was significantly shorter in MS and significantly longer in stroke patients. It was absent in the five patients with suspected psychogenic weakness. In patients with HSP, an abnormally increase in duration occurred only in leg muscles. Our results suggest that the increase in duration of the MEP during contraction may reveal the contribution of propriospinal interneurons to the activation of alpha motorneurons. This mechanism may be altered in some diseases and, therefore, the assessment proposed in this work may have clinical applicability for the differential diagnosis of weakness.

Keywords: motor evoked potential, contraction-induced facilitation, stroke, multiple sclerosis, spastic paraparesis, psychogenic weakness

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales de Madrid and CEU-San Pablo University, Spain

Reviewed by:
Weien Yuan, Shanghai JiaoTong University, China
Carmen Terranova, University of Messina, Italy
Michele Dileone, Cinac-Hm Hospital Mostoles, Spain

***Correspondence:**
Josep Valls-Solé
jvalls@clinic.ub.es

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

Received: *17 October 2015*
Accepted: *21 December 2015*
Published: *11 January 2016*

Citation:
Brum M, Cabib C and Valls-Solé J (2016) Clinical Value of the Assessment of Changes in MEP Duration with Voluntary Contraction. Front. Neurosci. 9:505. doi: 10.3389/fnins.2015.00505

INTRODUCTION

Transcranial magnetic stimulation (TMS) gives rise to muscle responses, known as motor evoked potentials (MEP), through activation of the motor pathways (Amassian et al., 1987; Day et al., 1987, 1989; Rothwell et al., 1987, 1991). Voluntary contraction causes facilitation of the MEPs, which consists of shortening the MEP latency, increasing the MEP size and widening the MEP duration resulting in an increase in total MEP area (Mills et al., 1987; Valls-Solé et al., 1994a,b). While latency

shortening and amplitude increase can be well explained by assuming that voluntary contraction put more motorneurons near the firing threshold, the rationale behind the increase in MEP duration is less clear. On one side, motorneurons should be firing earlier and more synchronously to give rise to the shortening of latency and increase in peak amplitude. However, MEP duration increases beyond the time that the MEP would end if elicited at rest. Therefore, some motorneurons should receive excitatory inputs with latency longer than at rest. This could be due to activation of slow conducting corticospinal fibers or to the generation of excitatory inputs at spinal level (Brasil-Neto et al., 1992; Di Lazzaro et al., 1999, 2001), but it seems contradictory that slow conducting corticospinal fibers are recruited during voluntary contraction. Many sources of inhibition lead to the silent period after synchronized alpha motorneuron firing. After hyperpolarization prevents motorneurons from immediate reactivation and inhibitory inputs from Renshaw cells should reach the motorneuron within few milliseconds (ms) after the peak. Even if some inhibitory activity may be temporarily switched off during contraction, this does not justify the presence of EMG activity at a time when there are no identifiable excitatory inputs.

The EMG activity that follows the MEP during contraction is not usually taken into account in clinical assessment through TMS. The effects of facilitation are usually evaluated by either the onset latency or the size of the MEP. Onset of silent period is usually measured from more stable marks such as the stimulus artifact or MEP latency onset. We argue that measuring the characteristics of such segment of EMG activity might be of some clinical applicability. We reasoned that, when an MEP is elicited during voluntary contraction, inhibitory inputs generated at spinal level or by the descending volleys should counteract the excitatory commands to effectively end the ongoing EMG activity and give way to the silent period. This may take some time and, meanwhile, the EMG activity will not be suppressed. We considered that such "MEPtail," i.e., the EMG activity that follows the MEP peak and ends at the beginning of the silent period, should indicate the relationship between the strength of the excitatory inputs reaching the alpha motorneurons during voluntary contraction and the inhibitory inputs derived from the synchronized activation of alpha motorneurons at the time of the MEP. Therefore, we determined the extent of contraction-induced facilitation of the MEP in healthy subjects, with special attention to the increase in MEP duration, and performed a retrospective analysis of how such parameter was affected in various neurological disorders involving the motor pathway, i.e., multiple sclerosis (MS), stroke, hereditary spastic paraparesis (HSP), and psychogenic weakness.

METHODS

We performed a retrospective study of the recordings obtained within the last 5 years in healthy subjects and patients in whom we examined facilitation of the MEP in the first dorsal interosseous muscle (FDI), using a standardized method. We included 25 healthy subjects, 21 patients with multiple sclerosis, 33 patients with acute ischemic stroke, 5 patients with hereditary spastic paraparesis, and 5 patients with the clinical diagnosis of probable psychogenic paresis. In 12 healthy subjects and in the 5 patients with hereditary spastic paraparesis, we recorded the MEP also from the tibialis anterior (TA). The overall inclusion criteria were to have consistently elicitable MEPs at rest to cortical stimulation and a complete study carried out by following the protocol summarized below. Patients with multiple sclerosis were all diagnosed according to the McDonald criteria (Polman et al., 2011). They all had signs compatible with mild to moderate involvement of the motor pathway, with a mean Expanded Disability Status Scale of 3.5 (range between 2 and 6) and no one above four for the functional scale on pyramidal signs (Kurtzke, 1983). Patients with stroke were examined within the first 2 weeks after presentation of the lesion and had mild to severe hemiparesis due to a subcortical ischemic middle cerebral artery infarct. Patients with hereditary spastic paraparesis were all genetically mediated, spastin positive (SPG4), each of them belonging to a different family. Patients with psychogenic weakness had all normal diagnostic tests for possible lesions in the motor pathway and clinical evidence of inconsistent weakness, out of proportion of examination findings.

Retrospective data were all collected following a standardized protocol, which contemplated recording at rest and during a voluntary contraction of about 30% of maximum. The stimuli were applied with a Magstim (Magstim Company, Dyfed, UK), equipped with either a figure-of-8 coil for hand muscles or a circular coil for leg muscles. Subjects were sitting, relaxed, and alert. Silver/silver chloride disk electrodes were used for recording all responses. They were attached bilaterally, in a belly-tendon montage, over the FDI for the study of upper limb muscles, which was done in all subjects, and the TA for the study of lower limb muscles in healthy subjects and patients with HSP. EMG signals were filtered and amplified, and traces were recorded using a KeyPointNet electromyograph. Stimulus intensity was fixed at about 120% resting motor threshold, which, based on the recommendations of Rossini et al. (1994), we determined as the minimum stimulus intensity that gave rise to a MEP of at least 50 μV amplitude in at least 50% of trials when TMS was applied to the appropriate scalp location for the target muscle, with the subject at rest. In all instances, we recorded a variable number of MEPs at each stimulation condition (between 2 and 10) and superimposed them at their best fit to facilitate parametric measuring.

In a group of newly recruited 10 healthy subjects, we examined again the effects of facilitation on the MEP to cortical stimulation and added the observation of the effects on the MEP elicited by cervical foraminal stimulation. We asked these subjects to perform two different levels of muscle contraction: mild (10% of maximum voluntary contraction) and strong (30% of their maximum voluntary contraction). At the time of testing, the participants signed an informed consent and the study protocol for retrospective data collection, as well as for the new study in healthy subjects, were approved by the Ethics committee of the Hospital Clinic of Barcelona.

DATA ANALYSIS

In each recording, we measured onset latency at the time that the EMG activity became consistently more than 10% above background amplitude, whether at rest or during contraction. Amplitude was measured from the maximum negative peak to the maximum positive peak. Total MEP duration was measured from MEP onset latency to the time at which the activity returned to baseline. We determined the percentage change for each MEP parameter during contraction with respect to rest. For the statistical comparison among groups, we chose to analyze the recordings from the dominant side in healthy subjects and the most impaired side in patients. In the 10 newly recruited subjects, we examined the effects of level of muscle contraction (mild and strong) on the MEP onset latency, peak-to-peak amplitude, and duration. We compared also the effects of cortical to those of foraminal stimulation. The outcome measure in which we focused our study was the increase in duration that takes place during contraction at the tail of the MEP (tail), as a specific aspect of MEP facilitation. This was measured in ms as the difference between the end of the MEP obtained during contraction and the end of the MEP obtained at rest.

All data were analyzed using SPSS 21.0 (IBM UK, London). We used repeated measures ANOVA for the analysis of the effects of level of muscle contraction in healthy subjects (rest, mild contraction and strong contraction). A One-way analysis of variance (ANOVA) was used to determine whether there were significant differences in retrospective data among the three independent groups with a sizeable number of subjects (healthy controls, multiple sclerosis, and stroke patients). In the other 2 groups of patients (HSP and psychogenic patients) the sample was too small to do the analyses. $P \leq 0.05$ was considered statistically significant.

RESULTS

The newly recruited subjects were 5 men and 5 women, with an age ranging from 32 to 65 (44 ± 7). In the subjects recruited retrospectively, mean age was significantly higher in patients with stroke than in the other groups (**Table 1**). Data were available from all subjects initially recruited, with no data gaps or missing information.

TABLE 1 | Demographic and general clinical characteristics of patients included in the retrospective study.

	Stroke	MS	HSP	Psychogenic
Age	67 (6.4)[a]	52 (3.1)	49 (4.3)	54 (2.4)
Gender (M/F)	21/12	11/10	4/1	5/0
Weakness (MCR)	1–4	2–5	4–5*	0–3**
Most imparied side	14 R/19 L	14 R/7 L	Bilateral*	4R/1L

[a] *significantly higher than in the other groups.*

**In leg muscles.*

***As for the patients first expression, with no encouragement.*

MEPs from the FDI to Cortical and Foraminal Stimulation in Healthy Subjects

Representative examples of recordings in one of the newly recruited healthy subject are shown in **Figure 1** to cortical and foraminal stimulation. A summary of the mean data is reported in **Table 2**. The effects of contraction on the MEP elicited with cortical stimulation were the expected ones: shortening of onset latency, increase in amplitude and increase in duration. The repeated measures ANOVA for values obtained when comparing the three conditions (rest, mild contraction, and strong contraction) showed statistically significant differences [ANOVA; $F_{(2, 27)} = 4.981$; $p = 0.004$]. The *post-hoc* analysis showed that all significant differences were found when comparing contraction to rest ($p < 0.05$ for latency, amplitude and duration), but there were no significant differences between data obtained with mild and strong contraction ($p > 0.05$ for all comparisons). The MEP tail increased a mean of 4.1 ms ($SD = 0.8$ ms) with mild contraction and 4.4 ms ($SD = 0.5$ ms) with strong contraction ($p > 0.05$). With foraminal stimulation there were no significant changes in onset latency, amplitude or duration, although amplitude, and duration increased in a few trials in some subjects (**Table 2**). A burst was consistently seen at a mean latency of 38.7 ms ($SD = 5.1$ ms), interrupting the post-MEP silent period.

Comparison of Data among Groups

Table 3 shows the summary of retrospective data gathered for all groups on the MEP at rest and during contraction as well as the percentage change observed during contraction with respect to rest for onset latency, peak amplitude and MEP duration. Representative recordings of MEPs at rest and during contraction are shown in **Figure 2** for the FDI recordings in each group

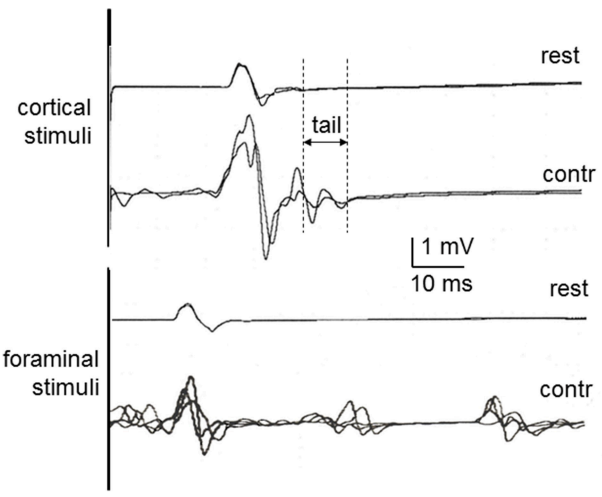


FIGURE 1 | MEPs in healthy control subjects at rest and during contraction, recorded in the first dorsal interosseous muscle to cortical stimulation (above) and cervical foraminal stimulation (below). Rest, Recorded at rest; Contr, Recorded during contraction. The vertical lines illustrate the methods used to measure the MEP tail as one of the aspects of MEP facilitation with contraction.

of patients. There were already significant differences among groups in the characteristics of the MEPs recorded at rest. The repeated measures one-factor ANOVA, run only on data from the three groups with a sizeable number of subjects (healthy subjects, stroke, and MS patients), showed significant differences in onset latency, peak amplitude, and MEP duration [$F_{(2, 76)}$; $p < 0.01$ for all of them]. The *post-hoc* analyses showed that patients with MS and stroke had delayed onset latency and smaller peak amplitude than healthy subjects ($p < 0.05$ for both groups of patients in both comparisons). In regard to MEP duration, patients with MS had a significantly longer duration, while patients with stroke had significantly shorter duration, than the other two groups ($p < 0.005$ for all comparisons).

Muscle contraction induced facilitation to different degrees in healthy subjects and patients (**Table 3**). As expected, the percentage shortening in onset latency, increase in peak amplitude, and lengthening in MEP duration, including the increase in MEP tail, calculated in healthy subjects were all similar to those reported above on the newly recruited subjects. The effects of contraction on the MEP in patients were not uniform, and the percentage change with respect to rest showed significant differences in all comparisons [ANOVA; $F_{(2, 76)}$; $p < 0.05$]. The *post-hoc* analyses indicated a significantly bigger percentage of onset latency shortening, and a significantly larger percentage of peak amplitude increase, in stroke than in MS patients. In MEP duration, differences were found between groups in all comparisons: There was a smaller increase in MS patients than in healthy subjects and stroke patients, and a bigger increase in stroke patients than in healthy subjects and MS patients ($p < 0.05$ for all comparisons). No significant differences with respect to healthy subjects were observed in hand muscles in patients with HSP but they were observed in leg muscles (reported below). In patients with psychogenic weakness, MEP duration was similar at rest and during voluntary contraction, although a moderate shortening of latency and increase in amplitude was observed in some traces.

A more striking difference among groups was observed in the MEP tail (**Table 4**). The contraction-induced increase in MEP duration beyond the end of the MEP at rest was significantly different among most groups, including psychogenic patients, in the FDI.

TABLE 2 | Data gathered from 10 newly recruited healthy volunteers on facilitation of the MEP with mild and strong voluntary muscle contractions.

	Rest	Mild	%	Strong	%
CORTICAL STIMULATION					
Onset latency (ms)	21.5 (1.8)	19.7 (1.3)*	91.6	19.6 (1.1)*	91.1
Amplitude (mV)	1.1 (0.6)	4.1 (0.7)*	372.7	4.4 (0.9)*	401.0
Duration (ms)	12.7 (2.0)	17.7 (3.3)[a]	139.3	17.9 (3.9)*	140.9
FORAMINAL STIMULATION					
Onset latency (ms)	13.4 (1.1)	13.3 (1.1)	99.2	13.3 (1.0)	99.2
Amplitude (mV)	0.9 (0.5)	1.3 (0.6)*	144.4	1.3 (0.7)*	144.6
Duration (ms)	8.4 (1.7)	8.8 (2.1)	104.7	8.8 (2.2)	104.8

Data are represented as mean and 1 standard deviation (within parenthesis). Data in the columns labeled % are the ratio between contraction and rest for mild and strong contractions. Asterisks refer to a significant difference ($p < 0.05$) with respect to rest, in the post-hoc analysis after one factor ANOVA.

MEPs from the Tibialis Anterior

Data were gathered from 12 healthy subjects and the 5 patients with HSP. Although we did not run comparative statistics because of the small number of subjects, differences between the two groups of subjects were already clear at rest (**Table 3**) and more so in the percentage increase of MEP duration with voluntary contraction. The mean MEP tail increase was markedly

TABLE 3 | Mean data on MEPs obtained at rest in the healthy subjects and patients included in the retrospective study.

	Onset latency		Percentage	Peak amplitude		Percentage	MEP duration		Percentage
	Rest	Contr		Rest	Contr		Rest	Contr	
HV (25)	21.3 (1.9)	19.2 (1.4)	90.14	3.1 (0.7)	6.8 (2.7)	219.35	13.1 (2.3)	17.8 (2.3)	135.88
Stroke (33)	23.6 (3.1)[a]	20.5 (2.2)	86.86	0.7 (1.3)[b]	2.2 (3.1)[b]	307.14	9.3 (4.8)[b]	18.2 (3.1)	195.70[a]
MS (21)	25.5 (4.2)[a]	24.5 (3.3)[a]	96.08[c]	1.4 (0.8)[b]	1.9 (2.2)[b]	135.71[d]	19.4 (2.5)[a,d]	21.7 (3.7)	111.86[d]
Psychogenic (5)	21.1 (2.2)	20.3 (3.0)	96.21	1.7 (2.3)	2.3 (3.5)	135.29	14.0 (2.1)	14.3 (2.3)	102.14
HSP (5)	22.0 (3.1)	19.9 (2.8)	90.45	2.3 (1.5)	4.8 (3.0)	208.70	14.5 (2.9)	19.1 (4.1)	131.72
HV TA (12)	30.9 (2.8)	28.5 (2.0)	92.23	1.4 (1.0)	2.9 (2.3)	207.14	19.7 (4.5)	29.4 (5.6)	144.16
HSP TA (5)	35.8 (4.7)	35.4 (4.1)	98.88	0.5 (1.2)	0.6 (1.8)	115.38	35.9 (8.4)	36.2 (9.9)	100.83

Data are the mean and one standard deviation (within parenthesis) measured on the MEP recorded in the first dorsal interosseous (except for HV TA and HSP TA, which were recorded in the tibialis anterior). HV, Healthy volunteers; MS, Multiple sclerosis; HSP, Hereditary spastic paraparesis. The number in parenthesis in the first column refers to the number of patients included in the analysis.

Onset latency and duration are measured in ms; peak amplitude in mV. Percentage refers to the percentage of the MEP characteristics during contraction with respect to rest. Superindices a, b, and c refer to significant differences ($p < 0.05$) found in the comparison among the three groups with a sizeable sample (healthy subjects, patients with stroke, and patients with multiple sclerosis).

[a] *Significantly longer than in healthy subjects.*

[b] *Significantly shorter than in healthy subjects.*

[c] *Significantly longer/larger in MS than in stroke.*

[d] *Significantly smaller/shorter in MS than in stroke.*

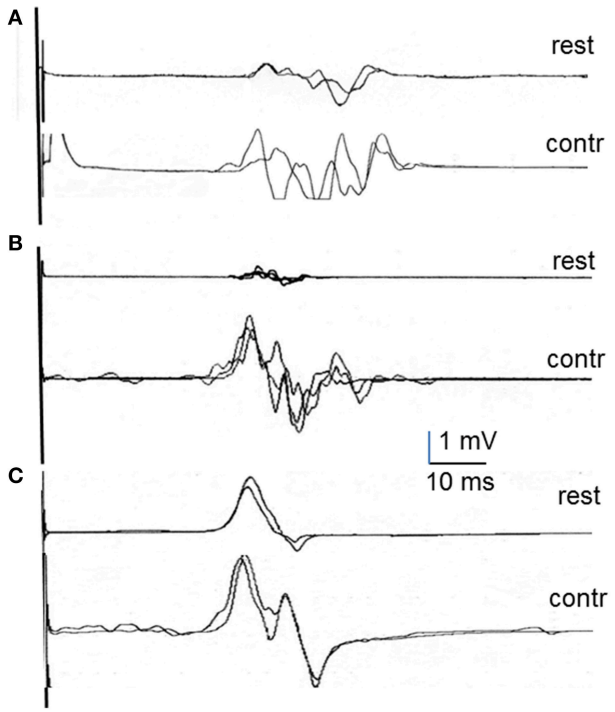


FIGURE 2 | Examples of MEP recordings in first dorsal interosseous at rest and during contraction in multiple sclerosis patients (A), stroke patients (B), and psychogenic weakness patients (C).

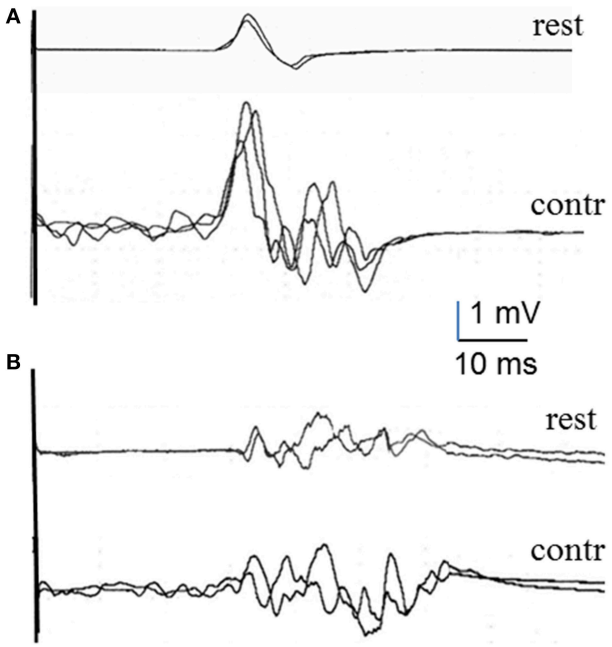


FIGURE 3 | MEPs recorded in the tibialis anterior muscle at rest and during voluntary contraction in a healthy subject (A) and an HSP patient (B).

TABLE 4 | Differences in MEP tail among groups of subjects.

Recording	Group (N)	Increase in MEP tail
FDI	Control (25)	4.7 (0.7)
	MS (21)	1.4 (1.2)[a]
	Stroke (33)	8.5 (1.8)[b]
	Psychogenic (5)	0.8 (2.1)[a]
	HSP (5)	4.5 (0.9)
TA	Control (12)	6.1 (1.1)
	HSP (5)	0.5 (0.9)[a]

Data are the mean calculated among all subjects of the same group. Superindices a and b refer to the statistical significance measured with the post-hoc comparison after one-factor ANOVA including all groups recorded in the same muscle.

[a] *Significantly shorter than in control subjects.*

[b] *Significantly longer than in all other groups.*

larger in healthy subjects than in patients (**Table 4**), with a significant difference in the unpaired t-test ($p < 0.05$). **Figure 3** shows representative TA recordings from healthy controls and patients with HSP.

DISCUSSION

The main physiological mechanism for MEP facilitation is, likely, the increase in spinal motorneuronal excitability. During voluntary contraction, the descending volley will meet with more motoneurons available for firing than at rest (Di Lazzaro et al., 2008). Descending volleys after cortical stimulation in humans have been recorded with epidural electrodes (Di Lazzaro et al., 1999). While a direct (D) wave can be obtained with electrical stimulation, this is usually not so with TMS (Day et al., 1987; Di Lazzaro et al., 1999), where mostly indirect (I) waves are recorded. The I waves have an interpeak latency of about 1.2–2.0 ms, up to about 6 ms after the D wave (Day et al., 1987; Boniface et al., 1991; Nakamura et al., 1997). The ultimate target of such descending volleys is assumedly the alpha motorneuron, but an unknown number of excitatory and inhibitory propriospinal interneurons may lay in between. The number of descending I waves increases little when subjects perform a voluntary contraction (Di Lazzaro et al., 1999). Therefore, the large increase in the MEP duration reveals that facilitation takes place mostly because of summation of inputs from various sources at the alpha motorneuronal level.

Polyphasic MEPs have been reported so far in various diseases. In patients with ALS (Kohara et al., 1999), the authors suggested that polyphasic MEPs were due to activation of additional motor pathways, such as slow monosynaptic pyramidal or even polysynaptic pathways in ALS patients. In patients with DYT11-positive myoclonus-dystonia syndrome (van der Salm et al., 2009), the authors hypothesized that the mutation associated with the disease, SGCE gene, could have caused changes in membrane properties or ion channels, leading to asynchronous discharge timing of spinal motorneurons by the descending corticospinal activity. MEP polyphasia has been also reported in MS, who have already an increase in MEP duration at rest (Kukowski, 1993), compatible with

increased temporal dispersion of the impulses reaching the spinal motorneurons. Recently, in patients with idiopathic generalized epilepsy (IGE) and first-degree relatives, Chowdhury et al. (2015) showed increased polyphasia, attributed to abnormal timing and patterning of the descending volleys in the corticospinal tract, and suggested that this was a novel endophenotype in this pathology. No data are available on changes in MEP duration in patients so far and, specifically in the increase in the MEP tail, beyond the end of the MEP at rest. We found that this parameter was the one discriminating better among our groups of subjects.

Patients with MS and HSP had longer duration of MEP already at rest, with little increase during contraction. In patients with stroke, if their damaged corticospinal tract was still excitable, the MEP increased significantly in amplitude and duration, indicating preservation of spinal mechanisms involved in contraction-induced facilitation. The only group studied in which we found no increase in duration was in the patients with psychogenic weakness. This was probably due to voluntary absence of energization of alpha motorneurons by these patients.

The mechanisms involved in the increase in MEP duration with muscle contraction are not clear but our results are consistent with a role for the excitatory pre-motor interneurons that are activated by descending inputs from contralateral corticospinal tract. Patients with MS and HSP, with lesions in the spinal cord, had increased duration at rest likely because of dispersion of the descending volley reaching the alpha motorneuron. These patients could not increase the synchronization of their motorneuronal firing, and the possible implication of the interneurons is masked by the already long duration of the MEP. In patients with stroke, whose spinal interneurons are not altered, the mechanisms of contraction-induced facilitation are fully activated, provided that the volley reaches the spinal level. In opposition, patients with psychogenic weakness do not perform the maximum voluntary contraction when requested, and may not set their spinal interneurons excitability at the level required for facilitation of the MEP during contraction.

The main limitation of our study is that it is retrospective and, therefore, it was not specifically designed for the study of the effects of duration. Even though the data reported here were generated after standardized recordings with no missing values, replication of the study is needed before firm conclusions. Meanwhile, we can conclude that the increase in duration of the MEP during contraction beyond the end of the resting MEP may reveal the activation of premotor propriospinal interneurons by descending inputs. This mechanism may be altered in some diseases. The patterns of MEP facilitation with voluntary contraction may differ depending on the disease, and the study of this feature can be of some clinical utility in the differential diagnosis of weakness.

AUTHOR CONTRIBUTIONS

MB: Review of patients' reports, Analysis of data, and Writing the first draft. CC: Analysis of data, Statistical analysis, and Revision of the manuscript. JV: Conceptual design of the study and Revision and finalization of the manuscript.

ACKNOWLEDGMENTS

Parts of this work were accomplished thanks to grant 110930 from Fundació Marató TV3.

REFERENCES

Amassian, V. E., Stewart, M., Quirk, G. J., and Rosenthal, J. L. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery* 20, 74–93. doi: 10.1097/00006123-198701000-00022

Boniface, S. J., Mills, K. R., and Schubert, M. (1991). Responses of single spinal motoneurons to magnetic brain stimulation in healthy subjects and patients with multiple sclerosis. *Brain* 114, 643–662. doi: 10.1093/brain/114.1.643

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., and Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *J. Clin. Neurophysiol.* 9, 132–136. doi: 10.1097/00004691-199201000-00014

Chowdhury, F. A., Pawley, A. D., Ceronie, B., Nashef, L., and Elwes, R. D. C. (2015). Motor evoked potential polyphasia A novel endophenotype of idiopathic generalized epilepsy. *Neurology* 84, 1301–1307. doi: 10.1212/WNL.0000000000001413

Day, B. L., Dressler, D., Maertens de Noordhout, A., Marsden, C. D., Nakashima, K., Rothwell, J. C., et al. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *J. Physiol.* 412, 449–473. doi: 10.1113/jphysiol.1989.sp017626

Day, B. L., Rothwell, J. C., Thompson, P. D., Dick, J. P., Cowan, J. M., Berardelli, A., et al. (1987). Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain?* 110, 1191–1209.

Di Lazzaro, V., Oliviero, A., Profice, P., Meglio, M., Cioni, B., Tonali, P., et al. (2001). Descending spinal cord volleys evoked by transcranial magnetic and electrical stimulation of the motor cortex leg area in conscious humans. *J. Physiol.* 537, 1047–1058. doi: 10.1111/j.1469-7793.2001.01047.x

Di Lazzaro, V., Oliviero, A., Profice, P., Insola, A., Mazzone, P., Tonali, P., and Rothwell, J. C. (1999). Effects of voluntary contraction on descending volleys evoked by transcranial electrical stimulation over the motor cortex hand area in conscious humans. *Exp. Brain Res.* 124, 525–528. doi: 10.1007/s002210050649

Di Lazzaro, V., Ziemann, U., and Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation* 1, 345–362. doi: 10.1016/j.brs.2008.07.004

Kohara, N., Kaji, R., Kojima, Y., and Kimura, J. (1999). An electrophysiological study of the corticospinal projections in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 110, 1123–1132.

Kukowski, B. (1993). Duration, configuration and amplitude of the motor response evoked by magnetic brain stimulation in patients with multiple sclerosis. *Electromyogr. Clin. Neurophysiol.* 33, 295–297.

Kurtzke, J. F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 33, 1444–1452.

Mills, K. R., Murray, N. M., and Hess, C. W. (1987). Magnetic and electrical transcranial brain stimulation: physiological mechanisms and clinical applications. *Neurosurgery* 20, 164–168.

Nakamura, H., Kitagawa, H., Kawaguchi, Y., and Tsuji, H. (1997). Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J. Physiol.* 498, 817–823. doi: 10.1113/jphysiol.1997.sp021905

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., et al. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* 69, 292–302. doi: 10.1002/ana.22366

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr. Clin. Neurophysiol.* 91, 79–92. doi: 10.1016/0013-4694(94)90029-9

Rothwell, J. C., Thompson, P. D., Day, B. L., Boyd, S., and Marsden, C. D. (1991). Stimulation of the human motor cortex through the scalp. *Exp. Physiol.* 76, 159–200.

Rothwell, J. C., Thompson, P. D., Day, B. L., Dick, J. P., Kachi, T., Cowan, J. M., et al. (1987). Motor cortex stimulation in intact man. 1. General characteristics of EMG responses in different muscles. *Brain* 110, 1173–1190.

Valls-Solé, J., Alvarez, R., and Tolosa, E. S. (1994a). Responses of the soleus muscle to transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol.* 93, 421–427. doi: 10.1016/0168-5597(94)90148-1

Valls-Solé, J., Pascual-Leone, A., Brasil-Neto, J. P., Cammarota, A., McShane, L., and Hallett, M. (1994b). Abnormal facilitation of the response to transcranial magnetic stimulation in patients with Parkinson's disease. *Neurology* 44, 735–741. doi: 10.1212/WNL.44.4.735

van der Salm, S. M., van Rootselaar, A. F., Foncke, E. M., Koelman, J. H., Bour, L. J., Tijssen, M. A., et al. (2009). Normal cortical excitability in Myoclonus-Dystonia. A TMS study. *Exp. Neurol.* 216, 300–305.

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 Neuroscience

ORIGINAL RESEARCH
published: 08 March 2016
doi: 10.3389/fnins.2016.00088

Combining Robotic Training and Non-Invasive Brain Stimulation in Severe Upper Limb-Impaired Chronic Stroke Patients

Vincenzo Di Lazzaro[1,2], Fioravante Capone[1,2], Giovanni Di Pino[1,2], Giovanni Pellegrino[1], Lucia Florio[1], Loredana Zollo[3], Davide Simonetti[3], Federico Ranieri[1,2], Nicoletta Brunelli[1,2], Marzia Corbetto[1,2], Sandra Miccinilli[4], Marco Bravi[4], Stefano Milighetti[4], Eugenio Guglielmelli[3] and Silvia Sterzi[4]*

[1] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy, [2] Fondazione Alberto Sordi - Research Institute for Ageing, Rome, Italy, [3] Unit of Biomedical Robotics and Biomicrosystems, Department of Engineering, Università Campus Bio-Medico di Roma, Rome, Italy, [4] Unit of Physical and Rehabilitation Medicine, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy

OPEN ACCESS

Edited by:
Marjan Jahanshahi,
UCL Institute of Neurology, UK

Reviewed by:
Giacomo Koch,
Santa Lucia IRCCS, Italy
Michelle Nadine McDonnell,
University of South Australia, Australia

***Correspondence:**
Vincenzo Di Lazzaro
v.dilazzaro@unicampus.it

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

Received: *13 November 2015*
Accepted: *22 February 2016*
Published: *08 March 2016*

Citation:
Di Lazzaro V, Capone F, Di Pino G, Pellegrino G, Florio L, Zollo L, Simonetti D, Ranieri F, Brunelli N, Corbetto M, Miccinilli S, Bravi M, Milighetti S, Guglielmelli E and Sterzi S (2016) Combining Robotic Training and Non-Invasive Brain Stimulation in Severe Upper Limb-Impaired Chronic Stroke Patients. Front. Neurosci. 10:88. doi: 10.3389/fnins.2016.00088

Previous studies suggested that both robot-assisted rehabilitation and non-invasive brain stimulation can produce a slight improvement in severe chronic stroke patients. It is still unknown whether their combination can produce synergistic and more consistent improvements. Safety and efficacy of this combination has been assessed within a proof-of-principle, double-blinded, semi-randomized, sham-controlled trial. Inhibitory continuous Theta Burst Stimulation (cTBS) was delivered on the affected hemisphere, in order to improve the response to the following robot-assisted therapy via a homeostatic increase of learning capacity. Twenty severe upper limb-impaired chronic stroke patients were randomized to robot-assisted therapy associated with real or sham cTBS, delivered for 10 working days. Eight real and nine sham patients completed the study. Change in Fugl-Meyer was chosen as primary outcome, while changes in several quantitative indicators of motor performance extracted by the robot as secondary outcomes. The treatment was well-tolerated by the patients and there were no adverse events. All patients achieved a small, but significant, Fugl-Meyer improvement (about 5%). The difference between the real and the sham cTBS groups was not significant. Among several secondary end points, only the Success Rate (percentage of targets reached by the patient) improved more in the *real* than in the *sham* cTBS group. This study shows that a short intensive robot-assisted rehabilitation produces a slight improvement in severe upper-limb impaired, even years after the stroke. The association with homeostatic metaplasticity-promoting non-invasive brain stimulation does not augment the clinical gain in patients with severe stroke.

Keywords: stroke recovery, robot-assisted rehabilitation, non-invasive brain stimulation, homeostatic plasticity, robotic assessment of motor performance

INTRODUCTION

Severe upper limb impairment in chronic stroke patients does not respond to standard rehabilitation strategies; for this reason there is the need of new treatments that might be effective in patients with drastically limited residual movement capacity. In patients with moderate to severe upper-limb impairment, a slight improvement have been reported using robot-assisted rehabilitative treatment, even years after a stroke (Lo et al., 2010). Another innovative approach for the enhancement of motor recovery is represented by non-invasive human brain stimulation techniques, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). These techniques can induce long-lasting changes in the excitability of central motor circuits via long-term potentiation/depression (LTP/LTD)-like phenomena (Di Pino et al., 2014b). A recent study reported a mild motor improvement after 10 sessions of rTMS in a group of severe chronic stroke patients (Demirtas-Tatlidedea et al., 2015).

Aim of present study was to explore whether the combination of these two approaches might enhance their positive effects on motor recovery. To the end of assessing safety and potential efficacy of the combination of robot-assisted rehabilitation and non-invasive brain stimulation in a group of chronic stroke patients with severe upper limb impairment, we designed a proof-of-principle double blinded semi-randomized sham-controlled trial. We used continuous theta burst stimulation (cTBS), a robust form of inhibitory rTMS inducing LTD-like changes lasting for about 1 h [8]. The choice of employing cTBS on the affected hemisphere was based on the findings of our recent study, which suggested that this inhibitory protocol can improve the response to physical therapy (Di Lazzaro et al., 2013). Moreover, rTMS protocols suppressing cortical excitability have been shown to strongly facilitate motor learning in normal subjects (Jung and Ziemann, 2009). Jung and Ziemann suggested that such enhancement might involve the phenomenon of "homeostatic" plasticity, which can be induced in the human brain using a variety of brain stimulation protocols (Karabanov et al., 2015). Considering the close link between LTP and mammalian learning and memory (Malenka and Bear, 2004), an enhancement of learning after LTD induction might appear a paradox. However, the experimental studies by Rioult-Pedotti et al. demonstrated the existence of a homeostatic balance between learning and the induction of LTP/LTD (Rioult-Pedotti et al., 2000), thus showing that the ease of producing synaptic LTP/LTD depends on the prior history of neural activity. In the context of stroke, this predicts that by delivering a rTMS protocol that induces LTD-like effects on the stroke-affected hemisphere before performing rehabilitation, would luckily result in better relearning (Di Pino et al., 2014a).

MATERIALS AND METHODS

Subjects

The study was performed according to the Oviedo Convention and approved by the Ethics Committee of Università Campus Bio-Medico of Rome. Participants provided written informed consent. Inclusion criteria were: (a) first-ever ischemic stroke at least 1 year earlier; (b) severe hand function impairment, defined as score of 3–28 on the Fugl-Meyer Assessment of sensory motor recovery after stroke, a scale with scores for upper-limb impairment ranging from 0 (no function) to 66 (normal function); (c) ability to give informed consent and comprehend instructions. Exclusion criteria were: (a) concomitant neurological conditions, including any history of epilepsy and significant comorbidities; (b) cognitive impairment or any substantial decrease in alertness, language reception, or attention that might interfere with understanding instructions for motor testing; (c) apraxia; (d) excessive pain in any joint of the paretic extremity; (e) contraindications to TMS such as metal head implants; (f) advanced liver, kidney, cardiac or pulmonary disease; (g) history of significant alcohol or drug abuse; (h) depression or use of neuropsychotropic drugs such as antidepressants or benzodiazepines. The National Institute of Health Stroke Scale (NIHSS) and the Barthel Index (BI) were used to evaluate neurological impairment and disability at the enrolment.

The study was proposed to patients attending the outpatient clinic for cerebrovascular disorders of Campus Bio-Medico University Hospital. From April the first, 2013, to September the 30th, 2014, we screened 143 patients, 13 of whom declined, 110 were excluded, and 20 underwent randomization (**Figure 1**). Common reasons for exclusion of patients from the study were a baseline Fugl-Meyer score outside the required range, history of epilepsy, and haemorrhagic stroke. Other causes of exclusion were previous ischemic strokes, stroke occurring <1 year before, severe cognitive impairment, contraindications to TMS such as metal head implants or pacemaker, use of neuropsychotropic drugs such as antidepressants or benzodiazepines.

Experimental Design

Ten patients were randomized to robot-assisted therapy associated with real cTBS and 10 patients to robot-assisted therapy associated with sham cTBS, through a randomization stratification approach. Patients were stratified by using at baseline measures to ensure that both groups had a similar distribution regarding degree of impairment. Researcher randomizing patients and researchers delivering cTBS were not involved in outcome assessments and data analysis; rehabilitation doctors, patients, and researchers involved in data analysis were blind to the type of cTBS delivered (i.e., sham or real), in order to obtain a double-blinded sham-controlled study design.

Each day, for 10 consecutive working days, each patient received a session of robotic therapy following the real or sham stimulation. Patients were evaluated at four time points: baseline (Baseline), just after the treatment (Post), after 1 (1 Month), and 3 months (3 Months). For all these evaluation points we assessed the Fugl-Meyer score and Robotic measures of motor performance (**Figure 1**). At baseline we also included the following scales: NIHSS, Rankin Scale, Barthel Index, and Modified Ashworth Scale (MAS). Spasticity was assessed by MAS at four different joint of affected arm: shoulder, elbow, wrist, and fingers. For each patient, a cumulative score was obtained by summing the scores obtained in the four joints. The cumulative

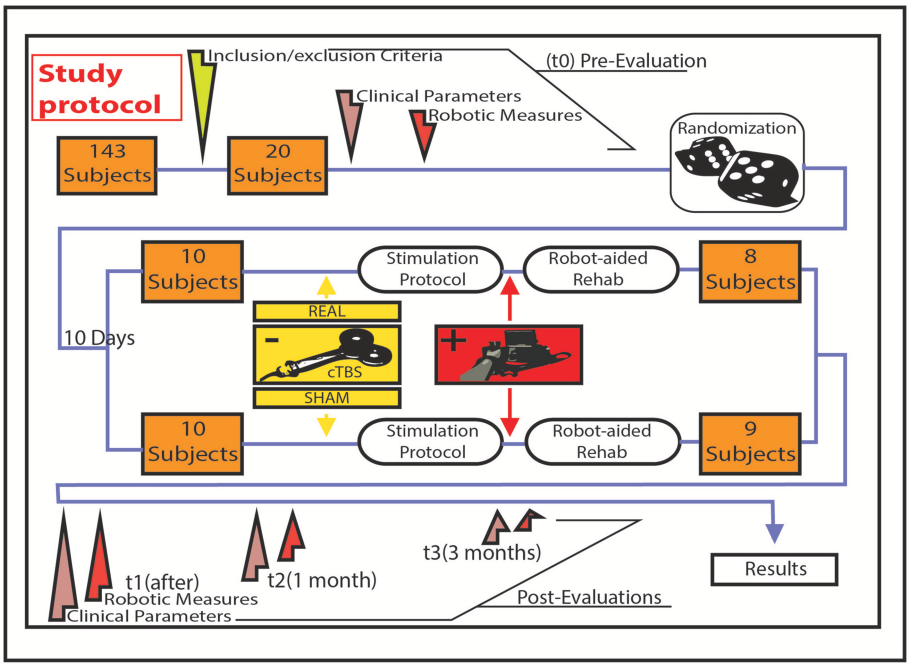


FIGURE 1 | Figurative illustration representing the algorithm of the study design, the evaluations carried out, and the treatments delivered. Treatment (real/sham cTBS + physical therapy) was delivered for 10 consecutive working days. Baseline evaluation was performed in the first day of treatment.

score ranges from 0 (no spasticity) to 16 (maximum spasticity, i.e., score 4 in all the considered joints).

The combined effect of robotic rehabilitation and brain stimulation was evaluated on (a) the Fugl Meyer score after intervention, as compared to baseline (primary outcome measure of clinical improvement) and (b) robot derived measures of motor performance (secondary outcome measures).

After the 2 weeks of intervention, patients did not receive any additional physical therapy until the last follow-up visit (at 3 months). Pharmacological therapy was also unchanged.

Interventions

Transcranial Brain Stimulation

rTMS was applied over the hand motor area of the affected hemisphere using a DUOMAG XT stimulator (DEYMED Diagnostic, Czech Republic) and a figure-of-eight shaped coil, with the handle pointed posteriorly and approximately perpendicular to the central sulcus.

Active rTMS used cTBS, in which 3 pulses are given at 50 Hz, repeated every 200 ms for a total of 600 pulses. Stimulation intensity was 80% active motor threshold (AMT) of the affected hemisphere, defined as the minimum single pulse intensity required to produce a motor evoked potential $>200\,\mu V$ on more than 5 out of 10 trials from the contracted contralateral first dorsal interosseous muscle. Whenever AMT over the affected hemisphere could not be determined because TMS at maximum stimulator output (MSO) failed to evoke any response, cTBS intensity was performed at an intensity corresponding to unaffected hemisphere AMT. Sham rTMS was performed using the same stimulator at an intensity of 3% of MSO and with the coil tilted at 90°; this intensity of stimulation, with this orientation of the coil, produces auditory sensation similar to the active stimulation, but has no stimulating effect on the cortex.

Robotic Therapy

The Robot was exploited for the two-fold purpose of delivering therapy and measuring, objectively and quantitatively, patients' motor performance. Shoulder-elbow robotic therapy was delivered with the InMotion2 robotic machine (Interactive Motion Technologies, Inc.) (Krebs et al., 1998). The InMotion2 (**Figure 2**) is based on a direct-drive five-bar-linkage SCARA mechanism that provides two translational degrees of freedom for elbow and forearm motion. Impedance control enables the robot to move, guide or perturb the patient's movement. Absolute encoders at each motor and a 6-axis force/torque sensor at the end effector allow measuring robot joint position, robot Cartesian position (via forward kinematics) and interaction forces.

In the evaluation phase, the robot was completely passive while position sensors recorded subject kinematic data. Patients were asked to perform five blocks of unassisted 16 point-to-point movements from the center to eight outbound targets along a circle at a distance of 0.14 m. Patients were required to move with a self-paced speed in a maximum time slot of 3 s.

Robot data were offline processed to compute quantitative indicators of temporal and spatial features of motor skill recovery (Zollo et al., 2011a; Papaleo et al., 2013), i.e.:

Motion Accuracy—It is assessed by means of the area index and the normalized mean deviation, defined below:

FIGURE 2 | The InMotion2 robotic machine (Interactive Motion Technologies, Inc.).

- *AREA*. It is the area between the desired and the actual trajectory performed by the patient in the XY plane during the point-to-point motion; it is expected to decrease as movement accuracy increases with recovery.
- *normalized Mean Deviation (nMD)* (Colombo et al., 2008). It is the mean absolute value of the distance between the desired path and the curve actually performed by the patient, normalized on the maximum deviation (or on the length of the theoretical path). As the patient recovers, the deviation from the desired path is expected to decrease;

Motion Direction—It is assessed through the *aiming angle*, i.e., the angular difference between the target direction and the direction of the path performed from the starting point up to peak speed point. It is expected to decrease as movement direction improves during recovery;

Smoothness (Rohrer et al., 2002)—It is a measure of how gradually a movement is changing and it is characterized by peaks and deep valleys in the velocity profile. Smoothness is quantified through the indicators reported below:

- *Speed Metric (SM)*. It is expressed as the ratio between mean speed and peak speed. As patient recovers, the normalized mean velocity increases due to the reduction of peaks and valleys in the velocity profile;
- *Mean Arrest Period Ratio (MAPR)*. It represents the amount of time (i.e., the percentage of samples) that the movement speed exceeds the 10% of the peak speed. The deep valleys (percentage of pauses during the task execution) in the velocity profile of the patient hand are expected to reduce as movement smoothness improves.

Speed—It quantifies the movement velocity by measuring the Deviation from Ratio between Velocities (DRV), defined as the absolute deviation of the ratio between peak velocity and mean velocity from the constant value 1.875 (corresponding to the value obtained in the minimum jerk trajectory) (Flash and Hogan, 1985). It is expected to reduce when patient velocity tends to the bell-shaped velocity profile of the minimum jerk trajectory.

Movement Duration (MD)—It gives a measure of the task execution time, evaluated as the time occurred for performing a point-to-point movement from movement onset to movement termination. Movement onset is defined as the time instant where speed exceeds a predefined threshold of 10% of peak velocity and movement termination is defined as the time instant where velocity goes below a predefined threshold of 10% of peak velocity. As patient recovers, movement duration is expected to decreases as a consequence of the improved efficiency.

Efficiency—It evaluated the measure of patient ability to reach the target during point-to-point movement; it can be assessed by means of the path length index and the percentage of successes:

- *Path Length (PL)*. It is defined as the length ratio between the actual patient curve and the desired straight line, and computed as the line integral of the trajectory over the Movement Duration (MD), normalized with respect to the desired path. It is expected that during recovery the actual patient curve tends to the desired path and, hence, their ratio tends to one;
- *% Successes (SR)* (Panarese et al., 2012). It represents the percentage of times that the patient reaches the target during a therapy session of point-to-point movements. The increase of the SR with recovery is expected.

Each day of robotic treatment consisted of three sessions of 320 assisted point-to-point movements, from the center to eight outbound targets, interspersed by four sessions of 16 unassisted recorded point-to-point movements. Robot assistance at each session was tuned on patients' performance during the 16 point-to-point sessions. Both during evaluations and during training, patients were required to move with a self-paced speed in a maximum time slot of 3 s. Robotic treatment was delivered daily for 10 consecutive working days. A physical and rehabilitation medicine doctor attended and assisted patients both during evaluations and treatment.

Statistics

Statistical analysis was performed using IBM SPSS v22. We verified that at baseline the two groups were matched regarding age, sex, and clinical status. Then we investigated the effect of brain stimulation and robotic rehabilitation on the primary outcome measure, namely Fugl-Meyer scores, using an ANOVA mixed model design, with *Time* (four levels: *Baseline*, *Post*, *1 Month*, *3 Months*) as within subject factor and *Group* (two levels: *real cTBS* and *sham cTBS*) as between subjects factor. For the secondary outcome measures (robot derived measures) we applied a Generalized Estimating Equation approach, as multiple values were available for each cell of the design (Pellegrino et al., 2012; Di Lazzaro et al., 2014). The autoregressive (lag = 1) working correlation within subjects was chosen because measures of motor performance were acquired consecutively. The study of the *Success Rate* was performed by means of the Chi-Square test. The level of significance was set at $p < 0.05$ and the alpha inflation due to multiple comparisons was faced according to Bonferroni's procedure whenever required. Descriptive statistics is reported as Mean ± Standard Error of the Mean (SE).

TABLE 1 | Demographic and clinical characteristics of the patients at baseline.

	Real cTBS ($n = 8$)	Sham cTBS ($n = 9$)	*P*-value
Age (years)	57.88 ± 4.434	56.78 ± 3.202	0.841
Sex (M)	4	4	1.000[a]
Months since stroke	63.25 ± 25.437	61.33 ± 14.716	0.541[b]
NIHSS	5.50 ± 0.779	5.00 ± 0.687	0.636[c]
Rankin	2.88 ± 0.350	3.00 ± 0.333	0.815[b]
Barthel index	76.88 ± 7.130	77.22 ± 4.648	0.743[b]
Modified ashworth scale cumulative score*	5.00 ± 0.597	7.111 ± 1.160	0.140[c]
Fugl-Meyer	14.50 ± 2.428	12.56 ± 2.243	0.565[c]

All data are expressed as mean ± standard error.
[a] *Chi-Square.*
[b] *Mann-Whitney.*
[c] *Two tailed independent sample t-test.*
**Cumulative score was obtained by summing the scores obtained at four different joints of affected arm: shoulder, elbow, wrist, and fingers.*

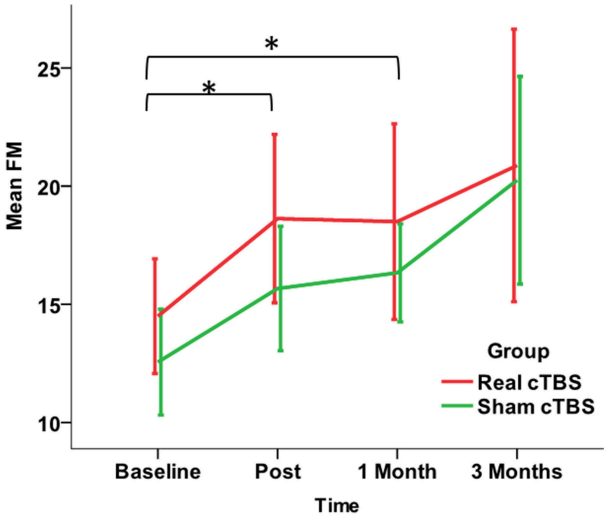


FIGURE 3 | Changes in the primary Outcome Measure (Fugl-Meyer Assessment score) in the *Real* (red line) and the *Sham* (green line) cTBS groups. Compared to Baseline both groups significantly improved at t1 (post-treatment) and t2 (1 month). There is no significant difference between groups. *$p < 0.05$.

RESULTS

Twenty patients underwent randomization (14% of the screened patients): 10 to robot-assisted therapy associated with real cTBS and 10 to robot-assisted therapy associated with sham cTBS. One real patient withdrew consent before the first session of treatment. One real patient and 1 sham patient withdrew because of difficulty in reaching the hospital after the third and after the fifth day of treatment, respectively. Data of these patients was not included in the analysis. A total of 17 patients completed the study including the 3 month follow-up: 8 real cTBS patients (mean age: 57.8 ± 4.4 years) and 9 sham cTBS (mean age: 56.7 ± 3.2 years), therefore, 85% of the screened patients completed the study. For the purposes of the study we applied an on-treatment analysis (**Figure 1**). The real and sham groups were matched regarding age, sex, time elapsed from stroke onset, and clinical status at baseline (**Table 1**).

Physicians inquired about adverse events and pain, each day during the whole stimulation period (10 consecutive working days) and at each outward control. There were no treatment-related adverse events. No patient reported pain in the affected arm subsequent to treatment or required to stop treatment session for pain or any other unpleasant sensation. In particular, patients reported no side effects that could be related either to the robotic treatment (e.g., shoulder, elbow, or wrist pain) or to cTBS (seizure, syncope, transient headache, local pain, neck pain, transient cognitive/neuropsychologial changes; Rossi et al., 2009).

Primary Outcome Measure

The ANOVA Mixed Model with *Time* (four levels: *Baseline*, *Post*, *1 Month*, *3 Months*) as within subject factor and *Group* (two levels: *real cTBS* and *sham cTBS*) as between subjects factor revealed a significant effect of rehabilitation [*FactorTime*: $F_{(1.613,\ 22.586)} = 5.801$, $p = 0.013$], but no effect of the brain stimulation (*Factor Group* and *Group by Time interaction*: $p > 0.200$ consistently). The improvement vs. baseline was statistically significant both soon after the intervention (*Post*) and at *1 Month* follow-up (Bonferroni corrected *post-hoc* $p = 0.30$, $p = 0.19$, respectively). At *3 Months* there was an average additional increase of the Fugl-Meyer score, however the difference toward *Baseline* was not significant (Bonferroni corrected *post-hoc* $p = 0.75$) (**Figure 3**).

Secondary Outcome Measures

Motion Accuracy, Motion Direction, Smoothness, Speed, Movement Duration

The main finding was a rehabilitation-related improvement of the motor performance across multiple domains, including *Motion Accuracy*, *Motion Direction*, *Smoothness, Speed,* and *Movement Duration*. In particular all the robot-derived measures, except normalized Mean Deviation (nMD), consistently showed a significant factor *Time* (*Area*: Wald Chi-Square = 28.019, $df = 3$, $p = 0.000$; *Aiming angle (alpha)*: Wald Chi-Square = 44.608, $df = 3$, $p = 0.000$; *Speed Metric (SM):* Wald Chi-Square = 126.045, $df = 3$, $p = 0.000$; *Mean Arrest Period Ratio (MAPR)*:Wald Chi-Square = 2.796, $df = 3$, $p = 0.000$; *DRV*: Wald Chi-Square = 20.275, $df = 3$, $p = 0.000$; *Movement Duration (MD)*: Wald Chi-Square = 52.088, $df = 3$, $p = 0.000$). The Bonferroni corrected comparisons at all the time points toward *Baseline* showed a consistent and persistent improvement for all these measures (Post intervention, at 1 Month and at 3 Months, $p < 0.05$ consistently). The lack of significant main factor *Group* and *Group by Time interaction* ruled out an effect of cTBS on these parameters (**Figure 4**).

Efficiency

The study of the efficiency showed that cTBS over the affected hemisphere has an impact on the improvement of motor

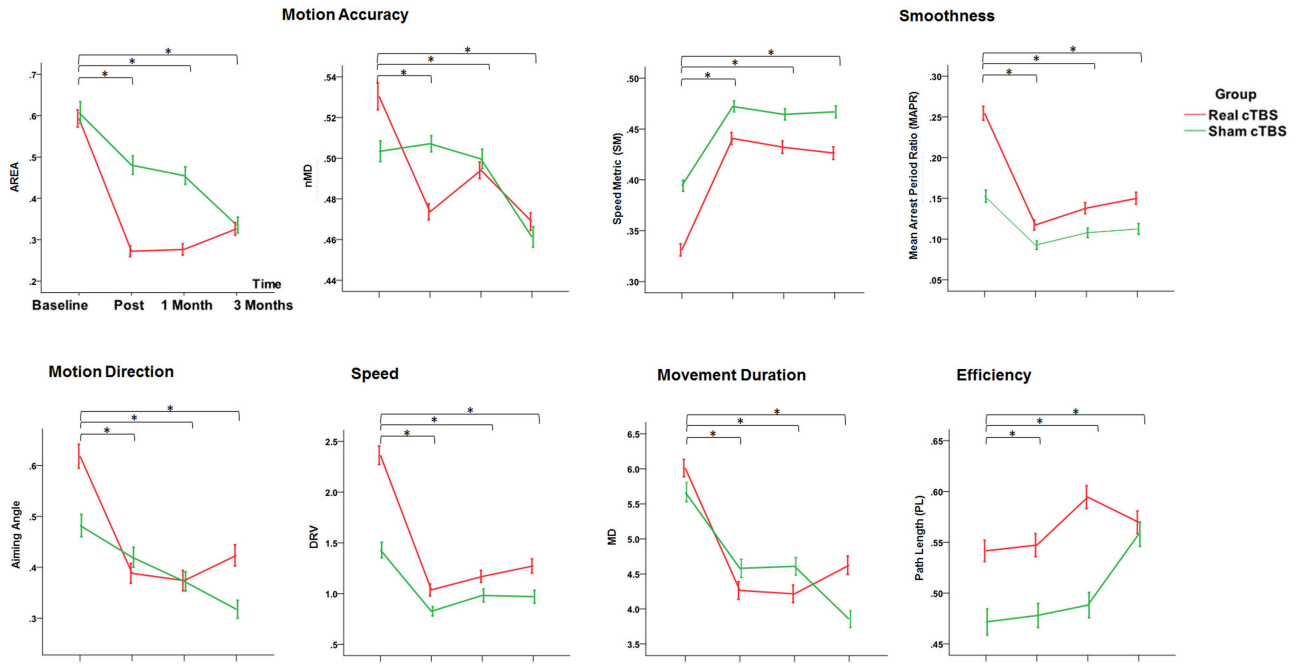


FIGURE 4 | Changes in the Secondary Outcome Measures (motor performance parameters extracted by the robot) in the *Real* (red line) and the *Sham* (green line) cTBS groups. Compared to Baseline both groups significantly improved at t1 (post-treatment), t2 (1 month), and t3 (3 months) *$p < 0.05$. There is no difference between groups.

performance produced by the rehabilitation. Such effect was not unveiled by the measure of the path length (PL), for which both main Factors and interaction were not significant, but became clear at the analysis of the Success Rate. Indeed, the number of errors at *Baseline* (across groups) was 593 and decreased after the intervention, being 342 at *Post*, 364 at *1 Month,* and 313 at *3 Months*. However, the improvement was different in the two *cTBS groups* (Chi-Square = 35.576, $df = 3$, $p = 0.000$). The *Real cTBS group* showed a higher error number (337 vs. 256, Std. Residual −2.4) at *Baseline.* However, in spite of this, the study of the residuals revealed that, compared to the cTBS group, the errors were significantly more in the *sham 1* at *Post* (*Real cTBS* 136, *Sham cTBS* 206, Std. Residuals >1.9) and *1 Month* (Real cTBS 155, Sham cTBS 209, Std residuals = 1.9). Such effect was no more present at *3 Months* (3 Months; Real cTBS 170, Sham cTBS 143, Std residuals = 1.2) (**Figure 5**).

DISCUSSION

The present study shows that a robot-assisted rehabilitation protocol lasting 2 weeks produces a slight, but significant, clinical improvement in chronic stroke patients with severe upper limb motor deficits.

This study also shows that non-invasive brain stimulation delivered as cTBS over the affected hemisphere does not enhance the clinical gains from this treatment. Indeed, considering the primary outcome, there was no significant difference between real and sham-cTBS patients. The improvement in Fugl-Meyer was significant for both groups immediately after the intervention and at 1 month follow-up, while it was not significant at 3 months. At 3 months there was a slight further increase in the average scores, and the lack of significance was probably due to the high variability of the measures.

The mean change in the Fugl-Meyers score was rather limited, about 5% (3–4 points). However, this might be considered meaningful in chronic patients, especially in those with severe impairment (Lo et al., 2010). In a more general sense, a minimum increase of about five points is required to make a clinically significant difference (Page et al., 2012), but this threshold was established in patients with minimum to moderate impairment and does not fit well with our group of patients with severe impairment.

It should also be noted that, assuming that a Fugl-Meyers score difference of at least four points is of clinical interest, in order to find a significant difference between our two groups a much larger sample size might be needed (50 patients per group, Power = 80%, Type I error = 0.05). In any case, the percentage of the patients assigned to real cTBS who achieved a gain of at least five points, was slightly higher than the percentage of patients who achieved this gain in the sham group [3 out of 8 (38%) in the real group vs. 2 out of 9 (22%) in the sham group].

Interestingly, the mean improvement in the Fugl-Meyer score is comparable to what has been achieved previously with longer lasting interventions (12 weeks with 36 1-hour/day sessions of robot-assisted rehabilitative therapy in the study of Lo et al., 2010); 8 weeks with a total 24 sessions in the study by Klamroth-Marganska et al. (2014). In contrast with

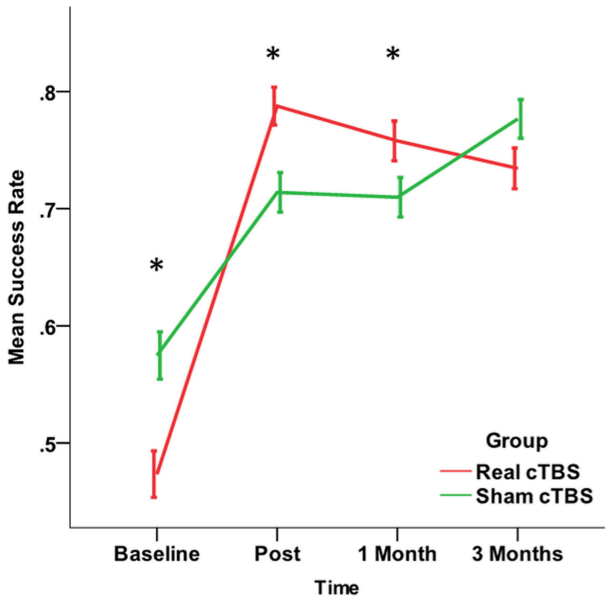


FIGURE 5 | Changes in the Success Rate, a secondary Outcome Measure broader measure of motor performance representing the percentage of times that the patient reaches the target. The improvement in the real cTBS group was higher than in the sham group at t1 (post-treatment) and t2 (1 month). *$p < 0.05$.

present findings, our previous study in chronic stroke patients with moderate upper limb deficits suggested that cTBS might enhance the gain from a late rehabilitation with a standardized protocol of physical rehabilitation (Di Lazzaro et al., 2013). One possible explanation for this discrepancy is that robot assisted rehabilitation attains a maximal benefit in patients with severe deficits and this cannot be enhanced by brain stimulation because of a ceiling effect. Another possibility, is that, as suggested by Daly et al. (2005), a more prolonged robotic therapy is needed to obtain a consistent improvement in patients with severe impairment and thus, it cannot be excluded that prolonging the association of robotic treatment and brain stimulation for a longer period might result more effective. Finally, a further possibility could arise from the fact that the affected and unaffected hemispheres seems to play a different role in mild vs. severe strokes, so that the hemisphere mainly responsible for motor recovery in severe stroke is the unaffected one (Di Pino et al., 2014b). If this is the case, in patients with severe brain damage, it may be not useful to attempt to promote ipsilesional reorganization because the manipulation of the excitability of the affected hemisphere may not produce any advantage in terms of promoting relearning from rehabilitation. Instead, in these patients, our future efforts should target the unaffected hemisphere being its role in recovery more relevant (Di Pino et al., 2014b).

It should be considered that, for safety concerns, stimulus intensity was estimated from the unaffected hemisphere, because this might be hyperexcitable (Di Lazzaro et al., 2010), it might be that this intensity was below the one needed to activate intracortical networks of the affected hemisphere. Although cTBS after effects are produced by stimulus intensities well below motor threshold (Huang et al., 2005), and although this intensity produced significant effects in our previous study in patients with less severe stroke (Di Lazzaro et al., 2013), we cannot exclude that higher intensity cTBS could produce a more pronounced effect also in patients with severe stroke.

The study of the robotic measures of motor performance (secondary outcomes) allowed us a more sensitive and accurate evaluation of the effects of robotic rehabilitation and brain stimulation on motor recovery (Pellegrino et al., 2012). These measures complement the clinical scales and show that our rehabilitation strategy achieves a significant benefit up to 3 months after the end of the treatment, confirming previous studies (Prange et al., 2006; Kwakkel et al., 2008; Lo et al., 2010). A significant improvement was achieved in multiple domains of motor control in both groups (Motion accuracy, Motion Direction, Smoothness, Speed, Movement Duration, Success Rate) with no significant difference between groups. Only the Success Rate, representing the percentage of times that the patient reaches the target, improved significantly more in the *real cTBS* group than in the *sham cTBS* one: this might suggest a mild benefit of cTBS on rehabilitation. Nevertheless, this finding should be taken extremely cautiously, since the difference between real and sham groups was not significant on the other robot-derived measures. Despite the secondary outcome measures have been analyzed in an independent fashion, we cannot rule out that the Success Rate, being a broader measure of motor performance, capitalizes the slight improvements in multiple domains of motor control, resulting statistically significant (Zollo et al., 2011a,b). However, it should also be considered that the success rate was different at baseline between the groups, this imbalance might influence the changes observed in the two groups, and this is a further reason that led to consider with caution the more pronounced improvement in success rate after real cTBS.

CONCLUSIONS

Our study confirms that robot-assisted rehabilitative treatment produces a slight improvement years after a stroke and it shows, for the first time, that an improvement can be obtained even in patients with severe upper-limb impairment treated daily for only 10 working days. Moreover, it shows that non-invasive brain stimulation delivered as cTBS of the affected hemisphere to promote homeostatic metaplasticity, is not effective in patients with severe deficits as those enrolled in present study, while our previous study showed that this approach might be effective in patients with moderate deficits. It might be that in severe patients the unaffected hemisphere is more involved in recovery, thus, the modulation of the excitability of this hemisphere could produce positive effects. In these patients, it could also be that the facilitation of the affected hemisphere is more effective than inhibition. Also, it might be that different strategies for promoting homeostatic plasticity might produce positive effects (e.g., protocols of so-called primed stimulation in which low-frequency rTMS is preceded by a bout of high-frequency rTMS; Cassidy et al., 2015).

The implementation of non-invasive brain stimulation techniques as an additional tool to promote recovery in chronic stroke patients requires further studies in order to identify the subgroups of patients that most likely will respond to a particular intervention.

AUTHOR CONTRIBUTIONS

VD designed the study, made the preliminary recruitment of patients and wrote the manuscript. FC was responsible of patients' recruitment and neuromodulation and revised the manuscript. GD participated to patients' recruitment, neuromodulation and revised the manuscript. GP performed statistic analysis and revised the manuscript. LF, FR, NB, MC performed the experimental sessions and the clinical evaluations. LZ, DS, EG analyzed the data collected by the robot, participated to design the robotic rehabilitative protocol, and wrote the related part of the manuscript. Sandra Miccinilli, MB and SS designed and performed the rehabilitative protocol and revised the manuscript. Stefano Milighetti revised the manuscript. All Authors approved the final version of the manuscript.

REFERENCES

Cassidy, J. M., Chu, H., Anderson, D. C., Krach, L. E., Snow, L., Kimberley, T. J., et al. (2015). A comparison of primed low-frequency repetitive transcranial magnetic stimulation treatments in chronic stroke. *Brain Stimul.* 8, 1074–1084. doi: 10.1016/j.brs.2015.06.007

Colombo, R., Pisano, F., Micera, S., Mazzone, A., Delconte, C., Carrozza, M., et al. (2008). Assessing mechanisms of recovery during robot-aided neurorehabilitation of the upper limb. *Neurorehabil. Neural Repair* 22, 50–63. doi: 10.1177/1545968307303401

Daly, J. J., Hogan, N., Perepezko, E. M., Krebs, H. I., Rogers, J. M., Goyal, K. S., et al. (2005). Response to upper-limb robotics and functional neuromuscular stimulation following stroke. *J. Rehabil. Res. Dev.* 42, 723–736. doi: 10.1682/JRRD.2005.02.0048

Demirtas-Tatlidedea, A., Alonso-Alonsoa, M., Shetty, R. P., Ronenc, I., Pascual-Leonea, A., and Fregnia, F. (2015). Long-term effects of contralesional rTMS in severe stroke: safety, cortical excitability, and relationship with transcallosal motor fibers. *NeuroRehabilitation* 36, 51–59. doi: 10.3233/NRE-141191

Di Lazzaro, V., Rothwell, J. C., Penelope, T., Federico, R., Wallace, A. C., Gabriella, M., et al. (2013). Inhibitory theta burst stimulation of affected hemisphere in chronic stroke: a proof of principle, sham-controlled study. *Neurosci. Lett.* 553, 148–152. doi: 10.1016/j.neulet.2013.08.013

Di Lazzaro, V., Dileone, M., Capone, F., Pellegrino, G., Ranieri, F., Musumeci, G., et al. (2014). Immediate and late modulation of interhemipheric imbalance with bilateral transcranial direct current stimulation in acute stroke. *Brain Stimul.* 7, 841–848. doi: 10.1016/j.brs.2014.10.001

Di Lazzaro, V., Profice, P., Pilato, F., Capone, F., Ranieri, F., Pasqualetti, P., et al. (2010). Motor cortex plasticity predicts recovery in acute stroke. *Cereb. Cortex* 20, 1523–1528. doi: 10.1093/cercor/bhp216

Di Pino, G., Pellegrino, G., Capone, F., and Di Lazzaro, V. (2014a). Human cerebral cortex metaplasticity and stroke recovery. *Austin J. Cerebrovasc. Dis. Stroke* 1:2.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., et al. (2014b). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat. Rev. Neurol.* 10, 597–608. doi: 10.1038/nrneurol.2014.162

Flash, T., and Hogan, N. (1985). The coordination of arm movements: an experimentally confirmed mathematical model. *J. Neurosci.* 5, 1688–1703.

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

Jung, P., and Ziemann, U. (2009). Homeostatic and nonhomeostatic modulation of learning in human motor cortex. *J. Neurosci.* 29, 5597–5604. doi: 10.1523/JNEUROSCI.0222-09.2009

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, 993–1006. doi: 10.1016/j.brs.2015.01.404

Klamroth-Marganska, V., Blanco, J., Campen, K., Curt, A., Dietz, V., Ettlin, T., et al. (2014). Three-dimensional, task-specific robot therapy of the arm after stroke: a multicentre, parallel-group randomised trial. *Lancet Neurol.* 13, 159–166. doi: 10.1016/S1474-4422(13)70305-3

Krebs, H. I., Hogan, N., Aisen, M. L., and Volpe, B. T. (1998). Robot-aided neurorehabilitation. *IEEE Trans. Rehabil. Eng.* 6, 75–87. doi: 10.1109/86.662623

Kwakkel, G., Kollen, B. J., and Krebs, H. I. (2008). Effects of robot-assisted therapy on upper limb recovery after stroke: a systematic review. *Neurorehabil. Neural Repair.* 22, 111–121. doi: 10.1177/1545968307305457

Lo, A. C., Guarino, P. D., Richards, L. G., Haselkorn, J. K., Wittenberg, G. F., Federman, D. G., et al. (2010). Robot-assisted therapy for long-term upper-limb impairment after stroke. *N. Engl. J. Med.* 362, 1772–1783. doi: 10.1056/NEJMoa0911341

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

Page, S. J., Fulk, G. D., and Boyne, P. (2012). Clinically important differences for the upper-extremity Fugl-Meyer Scale in people with minimal to moderate impairment due to chronic stroke. *Phys. Ther.* 92, 791–798. doi: 10.2522/ptj.20110009

Panarese, A., Colombo, R., Sterpi, I., Pisano, F., and Micera, S. (2012). Tracking motor improvement at the subtask level during robot-aided neurorehabilitation of stroke patients. *Neurorehabil. Neural Repair* 26, 822–833. doi: 10.1177/1545968311431966

Papaleo, E., Zollo, L., Spedaliere, L., and Guglielmelli, E. (2013). "Patient-tailored adaptive robotic system for upper-limb rehabilitation," in *2013 IEEE International Conference on Robotics and Automation (ICRA)* (Karlsruhe), 3860–3865.

Pellegrino, G., Tomasevic, L., Tombini, M., Assenza, G., Bravi, M., Sterzi, S., et al. (2012). Inter-hemispheric coupling changes associate with motor improvements after robotic stroke rehabilitation. *Restor. Neurol. Neurosci.* 30, 497–510. doi: 10.3233/RNN-2012-120227

Prange, G. B., Jannink, M. J., Groothuis-Oudshoorn, C. G., Hermens, H. J., and IJzerman, M. J. (2006). Systematic review of the effect of robot-aided therapy on recovery of the hemiparetic arm after stroke. *J. Rehabil. Res. Dev.* 43, 171. doi: 10.1682/JRRD.2005.04.0076

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

Rohrer, B., Fasoli, S., Krebs, H. I., Hughes, R., Volpe, B., Frontera, W. R., et al. (2002). Movement smoothness changes during stroke recovery. *J. Neurosci.* 22, 8297–8304.

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

Zollo, L., Gallotta, E., Guglielmelli, E., and Sterzi, S. (2011a). Robotic technologies and rehabilitation: new tools for upper-limb therapy and assessment in chronic stroke. *Eur. J. Phys. Rehabil. Med.* 47, 223–236.

Zollo, L., Rossini, L., Bravi, M., Magrone, G., Sterzi, S., and Guglielmelli, E. (2011b). Quantitative evaluation of upper-limb motor control in robot-aided rehabilitation. *Med. Biol. Eng. Comput.* 49, 1131–1144. doi: 10.1007/s11517-011-0808-1

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 Neuroscience

ORIGINAL RESEARCH
published: 25 February 2016
doi: 10.3389/fnins.2016.00062

Differential Modulation of Excitatory and Inhibitory Neurons during Periodic Stimulation

Mufti Mahmud[1,2] *and Stefano Vassanelli*[1]*

[1] NeuroChip Laboratory, Department of Biomedical Sciences, University of Padova, Padova, Italy, [2] Institute of Information Technology, Jahangirnagar University, Savar, Dhaka, Bangladesh

Non-invasive transcranial neuronal stimulation, in addition to deep brain stimulation, is seen as a promising therapeutic and diagnostic approach for an increasing number of neurological diseases such as epilepsy, cluster headaches, depression, specific type of blindness, and other central nervous system disfunctions. Improving its effectiveness and widening its range of use may strongly rely on development of proper stimulation protocols that are tailored to specific brain circuits and that are based on a deep knowledge of different neuron types response to stimulation. To this aim, we have performed a simulation study on the behavior of excitatory and inhibitory neurons subject to sinusoidal stimulation. Due to the intrinsic difference in membrane conductance properties of excitatory and inhibitory neurons, we show that their firing is differentially modulated by the wave parameters. We analyzed the behavior of the two neuronal types for a broad range of stimulus frequency and amplitude and demonstrated that, within a small-world network prototype, parameters tuning allow for a selective enhancement or suppression of the excitation/inhibition ratio.

Keywords: brain stimulation, transcranial stimulation, periodic stimulation, extracellular stimulation, excitatory and inhibitory neuron, neuronal network, Hodgkin-Huxley model, neurodegenerative diseases

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales de Madrid and CEU-San Pablo University, Spain

Reviewed by:
Marcus Thomas Wilson, University of Waikato, New Zealand
Mario Rosanova, University of Milan, Italy

***Correspondence:**
Stefano Vassanelli
stefano.vassanelli@unipd.it

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

Received: *24 November 2015*
Accepted: *09 February 2016*
Published: *25 February 2016*

Citation:
Mahmud M and Vassanelli S (2016) Differential Modulation of Excitatory and Inhibitory Neurons during Periodic Stimulation. Front. Neurosci. 10:62. doi: 10.3389/fnins.2016.00062

1. INTRODUCTION

Non-invasive brain stimulation—i.e., transcranial magentic and current stimulation (Antal and Paulus, 2013; Davis and Koningsbruggen, 2013; Dayan et al., 2013; Paulus, 2014; Shin et al., 2015)—as well as invasive deep brain stimulation (DBS) (McConnell et al., 2012; Miocinovic et al., 2013; Green and Aziz, 2014; Coenen et al., 2015) deeply rely on electrically inducing changes of the neuronal transmembrane potential to modify excitability. Electrical stimulation of neurons has been adopted by many clinicians as means for treatment of a range of neurological disorders. For example, recent reports show its increasing use in Parkinson's disease (Krack et al., 2002; Alon et al., 2012; Obeso et al., 2013; Chou et al., 2015; de Hemptinne et al., 2015), epilepsy (Loddenkemper et al., 2001; Boex et al., 2007; Boon et al., 2009; Fisher et al., 2010; Nelson et al., 2011; Alarcón and Valentín, 2012; Berenyi et al., 2012; Orosz et al., 2014; Salanova et al., 2015), certain types of blindness (Rizzo et al., 2003; Freeman, 2010; Freeman et al., 2010; Antal et al., 2011; Gall et al., 2013), cluster headaches (Grover et al., 2009; Sillay et al., 2010; Matharu and Zrinzo, 2011; Piacentino et al., 2014; Hodaj et al., 2015), depression (Miller and Selman, 2009; Jorge and Robinson, 2011; Rizvi et al., 2011; Anderson et al., 2012; Henn, 2012; Cook et al., 2014; Concerto et al., 2015), obsessive-compulsive disorder (Nuttin et al., 2008; Jiménez-Ponce et al., 2009; Kohl et al., 2014; Grassi et al., 2015; Islam et al., 2015), and other movement disorders like essential tremor

(Lozano, 2000; Volkmann and Benecke, 2002; Birdno et al., 2011; Gironell et al., 2014; Lettieri et al., 2015). However, the outcome of the method to many of these applications is reported to be limited due to lack of specificity of the stimulation (Fisher, 2011; Lozano and Lipsman, 2013; Shin et al., 2015). Thus, improvements are to be expected from protocols allowing for selective activation or inhibition of specific target neurons or classes of individual neurons, a strategy that can be broadly referred to as "differential stimulation" and that can be pursued by different approaches.

The "differential stimulation" of neurons can be approached both by means of invasive or non-invasive methods. For example, extracellular microstimulation has been used to selectively activate or inactivate neurons in ganglia using anodic or cathodic currents, respectively (Lu et al., 2008). By an alternative strategy, McIntyre and Grill reported that charge balanced asymmetric biphasic stimuli (McIntyre and Grill, 1999, 2000) can differentially activate neurons or fiber-of-passages by exploiting their difference in voltage thresholds and carefully tuning relevant stimulus parameters, e.g., amplitude, shape, frequency, and localization. They also reported on the effect of stimulus waveform and frequency on central nervous system (CNS) neurons through a detailed computer-based simulation of CNS cells and axons (McIntyre and Grill, 2002), where it was demonstrated that the relative position of the stimulating electrode plays an important role in activating a neuron. Results comparing experimental values and modeling prediction of threshold currents for varied electrode distances have also been reported (Joucla et al., 2012). Intriguingly, sinusoidal stimulation with microelectrodes has emerged as a possible tool to preferentially activate certain retinal cell types (e.g., photoreceptors, bipolar, and ganglion cells) (Freeman et al., 2010) or to induce complex phase-locked firing patterns of cortical pyramidal neurons (Brumberg and Gutkin, 2007).

How neurons are influenced by continuous or alternating electric fields depending on their position with respect to stimulating electrodes, morphology and electrical properties is matter of intense research also in the case of transcranial electrical stimulation approaches, such as the resurgent transcranial current stimulation (TCS) (Ali et al., 2013). For example, studies on transcranial direct current stimulation (tDCS) have shown that exposure to a uniform electric field promotes neuronal bursting and modulates spike timings (Radman et al., 2009). When alternating fields are considered, such as those produced by endogenous oscillations (Fröhlich and McCormick, 2010) and weak external fields (Deans et al., 2007) or by transcranial alterating current stimulation (tACS) (Herrmann et al., 2013; Reato et al., 2013), the general believe is that they can entrain brain oscillations. Furthermore, recent experimental evidence on tACS in humans supports the fascinating idea that excitation/inhibition balance (E/I) can be modulated by tuning the intensity of the stimulation current (Moliadze et al., 2012). The observation could be explained assuming that inhibitory neurons are more sensitive to alternating electrical stimulation and are already activated at low intensities, whereas excitatory neurons would require stronger stimulation. Recently, a similar capability on E/I modulation has been postulated also for tDCS (Krause et al., 2013).

As a matter of fact, understanding how excitatory and inhibitory neurons respond to extracellular electrical stimulation is still an open challenge. A particularly intriguing and clinically relevant aspect is their response to sinusoidal stimuli, such as those employed in tACS, and how it varies by tuning stimulus intensity and over the frequency range (Antal and Paulus, 2013).

This study reports simulation results of excitatory and inhibitory neurons' responses upon sinusoidal stimulation using varied frequencies and amplitudes. We focus on the effect of the extracellular field generated by the stimulus on action potentials firing. We found that it is possible, by careful selection of specific frequencies and amplitudes of the stimulus, to selectively enhance and inhibit either excitatory or inhibitory neurons. We show that the approach can be exploited to differentially modulate neuronal excitability within a network, suggesting its potential usefulness for non-invasive (e.g., tACS Kanai et al., 2008; Zaehle et al., 2010; Liew et al., 2014) as well as invasive brain stimulation (Coenen et al., 2015). Outside the tACS context, the concept can apply to neuroprosthetic devices (e.g., retinal stimulation using multicapacitor / multielectrode array, Eickenscheidt et al., 2012; Ghezzi, 2015; Lewis et al., 2015 and to brain-chip interfacing applications, Vassanelli et al., 2012; Vassanelli, 2014).

2. METHODS

Single compartment Hodgkin-Huxley (HH) neuron models (Hodgkin and Huxley, 1952) representing two main cortical neuron classes were implemented, the "regular spiking" (RS) excitatory neurons and the "fast spiking" (FS) interneurons (Connors and Gutnick, 1990). The HH model was chosen as it more faithfully describes membrane conductances dynamics with respect to, e.g., an Izhikevich model. This was of primary importance in our context were the neuronal response was investigated across a wide range of frequencies. In the implemented HH model all neurons had two main voltage-dependent ion channels, the Na^+ and the K^+, whose conductances, in conjunction with an adjustable leak conductance, were sufficient to generate action potentials. Synaptic interactions were described by conductance-based synaptic currents that implement ionotropic glutamate receptors (AMPA and NMDA) and GABA receptors ($GABA_A$) (Destexhe et al., 1994; Börgers et al., 2005). A small-world network of neurons was created by randomly connecting a predefined number of neighboring neurons assigned with a decided connection probability. The following subsections detail the models of the two classes of neurons, their synapses, and the network formation.

2.1. Model Neuron

Each neuron of both classes (RS and FS) was modeled using single compartment HH type model taken from the literature. There are many variants of kinetic models in the literature to govern the generation of action potentials (Herz et al., 2006) and we selected a model that describes the dynamics of membrane conductances and was previously adopted to match experimental

findings (Fröhlich and McCormick, 2010). The constants and parameters used in the model to generate action potentials (see **Table 1**) were taken from the literature (for RS neuron: Traub et al., 1991 and Mainen et al., 1995; for FS neuron: Wang and Buzsáki, 1996). The membrane potential, V, was generated using Equation (1).

$$C_m \frac{dV}{dt} = I_{Na} + I_K + I_L + I_{syn} + I_{app} \quad (1)$$

Here I_{Na}, I_K, I_L, I_{syn}, and I_{app} are the sodium, potassium, leakage, synaptic, and applied currents, respectively and were calculated using Equation (2). All differential equations were solved using second-order Runge-Kutta method (Press et al., 2007) with a step size (dt) of 0.05 ms.

$$\begin{aligned} I_{Na} &= \overline{g_{Na}} m^3 h (V_{Na} - V) \\ I_K &= \overline{g_K} n^4 (V_K - V) \\ I_L &= \overline{g_L} (V_L - V) \\ I_{syn} &= I_{AMPA} + I_{NMDA} + I_{GABA_A} \\ I_{app} &= C_m \frac{dV_s}{dt} \end{aligned} \quad (2)$$

TABLE 1 | Constants and parameters for individual neuron classes with their units.

Parameters	Excitatory neuron	Inhibitory neuron	Unit
Membrane capacitance, C_m	1	1	μF/cm^2
Sodium reversal potential, V_{Na}	60	55	mV
Potassium reversal potential, V_K	−90	−90	mV
Leakage reversal potential, V_L	−65	−65	mV
Max. sodium conductance, $\overline{g_{Na}}$	30	35	mS/cm^2
Max. potassium conductance, $\overline{g_K}$	100	9	mS/cm^2
Max. leakage conductance, $\overline{g_L}$	0.1	0.1	mS/cm^2

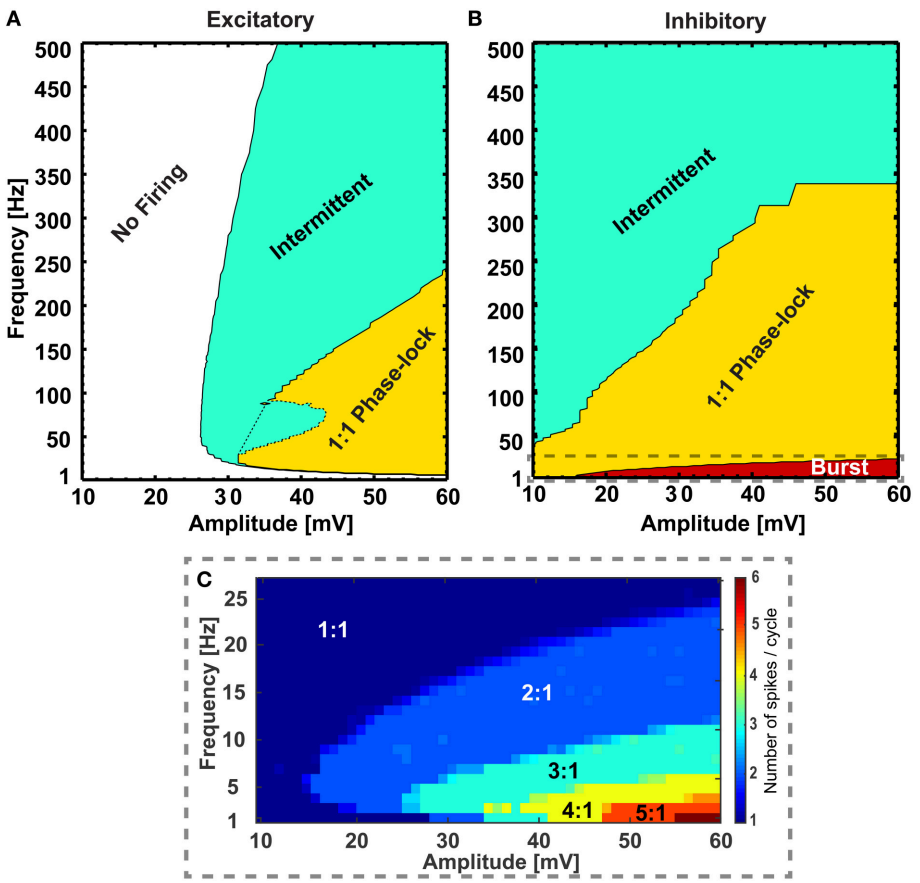


FIGURE 1 | Different firing regimes during sinusoidal stimulation. Excitatory **(A)** and inhibitory **(B)** neurons show different firing behaviors upon sinusoidal stimulation with different frequency and amplitude. The excitatory neurons do not respond to a range of amplitudes of sinusoidal stimulations (10– ∼ 26 mV, "No Firing" region) and at higher amplitudes (> 26 mV) with frequencies greater than ∼ 10 Hz they show phase lock ("1:1 Phase-lock" region) and intermittent ("Intermittent" region) firing behavior. Also, at a range of frequencies and amplitudes the excitatory neuron switches between "1:1 Phase-lock" and "Intermittent" firing [the dotted region in **(A)**, referred as "Knee"]. On the other hand, the inhibitory neurons show bursting behavior in low frequencies ["Burst" region, zoomed in **(C)**], with gradual transition to phase-lock ("1:1 Phase-lock" region) and intermittent ("Intermittent" region) firing behavior with increasing amplitude and frequencies of the sinusoidal stimulations. In the burst region, the inhibitory neuron emits a varied number of action-potentials per cycle of stimulation **(C)** starting from 2 : 1 to 6 : 1 for the explored range of frequency and amplitude.

The symbols $\overline{g_{Na}}$, $\overline{g_K}$, and $\overline{g_L}$ denote the maximum sodium, potassium, and leakage conductances respectively; V_{Na}, V_K, and V_L denote the reversal potentials of those channels; m, h, and n denote the channel gating variables; I_{AMPA}, I_{NMDA}, and I_{GABA_A} denote the synaptic receptor mediated currents; and C_m, V, and V_s represent membrane capacitance density, membrane voltage, and applied voltage (i.e., as generated by transcranial or intracranial stimulation), respectively. The parameters and units of the entities are listed in **Table 1** and V was calculated in mV. For the sake of brevity units will be omitted in the rest of the text.

The channel gating variables, i.e., the activation and inactivation variables for the sodium current (m and h) and activation variable for the potassium current (n) were calculated using Equation (3), where $x \in \{m, h, n\}$, and α_x and β_x are the voltage dependent transition rates that govern the values taken by activation and inactivation variables.

$$x_\infty(V) = \frac{\alpha_x(V)}{\alpha_x(V) + \beta_x(V)} \text{ and } \frac{dx}{dt} = \alpha_x(1-x) - \beta_x x \tag{3}$$

2.1.1. Excitatory Neuron

For the RS neuron, the gating variables were calculated using Equation (3) with m_∞, h_∞, and n_∞ being the initial states of the sodium activation, sodium inactivation, and potassium activation variables, respectively. The voltage dependent transition rates for each of the m, h, and n gating variables were updated using Equations (4, 5, 6), respectively.

$$\alpha_m(V) = \frac{0.182(V\ +\ 35)}{1 - \exp\left[\frac{-(V+35)}{9}\right]} \text{ and } \beta_m(V) = \frac{-0.124(V\ -\ 35)}{1 - \exp\left[\frac{(V-35)}{9}\right]} \tag{4}$$

$$\alpha_h(V) = \frac{0.024(V\ +\ 50)}{1 - \exp\left[\frac{-(V+50)}{5}\right]} \text{ and } \beta_h(V) = \frac{-0.0091(V\ -\ 75)}{1 - \exp\left[\frac{(V-75)}{5}\right]} \tag{5}$$

$$\alpha_n(V) = \frac{0.2(V\ -\ 20)}{1 - \exp\left[\frac{-(V-20)}{9}\right]} \text{ and } \beta_n(V) = \frac{-0.002(V\ -\ 20)}{1 - \exp\left[\frac{(V-20)}{9}\right]} \tag{6}$$

2.1.2. Inhibitory Neuron

On the other hand, the voltage dependent transition rates for each of the m, h, and n gating variables were calculated using Equations (7, 8, 9), respectively. The initial states of these gating variables (m_∞, h_∞, and n_∞) were obtained similarly using Equation (3) with their own transition rates.

$$\alpha_m(V) = \frac{-0.1(V\ +\ 35)}{-1 + \exp\left[\frac{-(V+35)}{10}\right]} \text{ and}$$
$$\beta_m(V) = 4\exp\left[\frac{-(V+60)}{18}\right] \tag{7}$$

$$\alpha_h(V) = 0.07\exp\left[\frac{-(V+58)}{20}\right] \text{ and}$$
$$\beta_h(V) = \frac{1}{1 + \exp\left[\frac{-(V+28)}{10}\right]} \tag{8}$$

$$\alpha_n(V) = \frac{-0.01(V\ +\ 34)}{-1 + \exp\left[\frac{-(V+34)}{10}\right]} \text{ and}$$
$$\beta_n(V) = 0.125\exp\left[\frac{-(V+44)}{80}\right] \tag{9}$$

2.2. Model Synapses

Both GABAergic and glutamatergic synapses were designed to provide inhibition and excitation in the network. The inhibitory synapses were mediated by $\gamma-$aminobutyric acid ($GABA_A$) receptors, whereas the excitatory synapses were mediated by a combination of $\alpha-$amino$-3-$hydroxy$-5-$methyl$-4-$isoxazolepropionic acid (AMPA) and N–methyl–D–aspartate (NMDA) receptors (Börgers et al., 2005).

The $GABA_A$ mediated synaptic currents were modeled by Equation (10) and summed up for the postsynaptically connecting $GABA_A$ mediated synapses.

$$I_{GABA_A} = \frac{g}{N_I \sum s_i(t)(V_I - V)}$$
with
$$g = \begin{cases} g_{IE}, & \text{if current neuron is excitatory, and} \\ g_{II}, & \text{if current neuron is inhibitory.} \end{cases} \tag{10}$$

Here g is the strength of the synaptic coupling, N_I is the number of presynaptic inhibitory neurons, V_I is the resting potential of the inhibitory neuron (constant value of -70 was used), V is the current neuron's membrane potential, and s_i is the gating

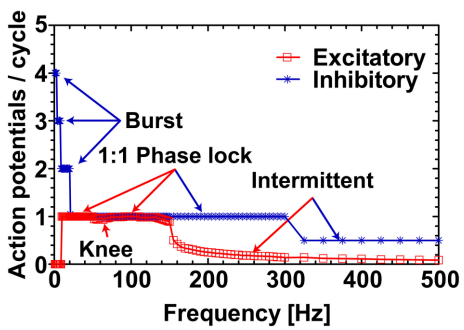


FIGURE 2 | Excitatory and inhibitory neurons response profile at 38 mV. The response profile of excitatory and inhibitory neurons as a function of number of spikes generated per cycle of sinusoidal stimulation at 38 mV. The applied frequency was from 1 to 500 Hz with a step of 1 Hz for 1–50 Hz, 2 Hz for 51–150 Hz, 5 Hz for 151–300 Hz, and 25 Hz for 301–500 Hz. The firing behavior outlined ("Burst," "1:1 Phase lock," and "Intermittent") are in complete agreement with the reference map shown in **Figure 1**. The "Knee" corresponds to the switching behavior (i.e., from 1:1 phase-lock to intermittent and back to 1:1 phase-lock) of the excitatory neuron in the frequency range ~50– ~90 Hz as noticed in **Figure 1**.

variable calculated using Equation (11). The g_{IE} and g_{II} denote the synaptic coupling strength of inhibitory to excitatory and inhibitory to inhibitory synapses, respectively.

$$\frac{ds}{dt} = \frac{1 + tanh(V_{pre}/10)}{2}\frac{1-s}{\tau_R} - \frac{s}{\tau_D} \tag{11}$$

With τ_R being the rise time constant (= 0.5 ms), τ_D being the decay time constant (= 10 ms) and V_{pre} being the membrane potential of the presynaptic neuron.

The AMPA and NMDA mediated synaptic currents were modeled by Equation (12) and summed up for the postsynaptically connecting AMPA and NMDA mediated synapses based on the synapse type under consideration.

$$I_{AMPA/NMDA} = \frac{g}{N_E \sum s_i(t)(V_E - V)}$$

with

$$g = \begin{cases} g_{EE}, & \text{if current neuron is excitatory, and} \\ g_{EI}, & \text{if current neuron is inhibitory.} \end{cases} \tag{12}$$

Here N_E is the number of presynaptic excitatory neurons with either AMPA or NMDA type synapses, V_E is the resting potential of the excitatory neuron (constant value of −70 was used), V is the current neuron's membrane potential, and s_i is the gating variable calculated using either Equation (11) (in case of AMPA mediated synapses) or Equation (13) (in case of NMDA mediated synapses). The g_{EE} and g_{EI} denote the synaptic coupling strength of excitatory to excitatory and excitatory to inhibitory synapses, respectively.

$$\frac{ds}{dt} = \frac{1}{1 + 3.57\exp(-0.062V_{post})}\frac{1 + tanh(V_{pre}/10)}{2}\frac{1-s}{\tau_R} - \frac{s}{\tau_D} \tag{13}$$

While calculating gating variables for the AMPA receptors, Equation (11) with $\tau_R = 0.2$ ms and $\tau_D = 2$ ms was used. On the other hand, the NMDA receptors' gating variables were calculated using Equation (13) with rise time constant $\tau_R = 1$ ms, decay time constant $\tau_D = 100$ ms, and V_{post} as the postsynaptic neuron's membrane potential.

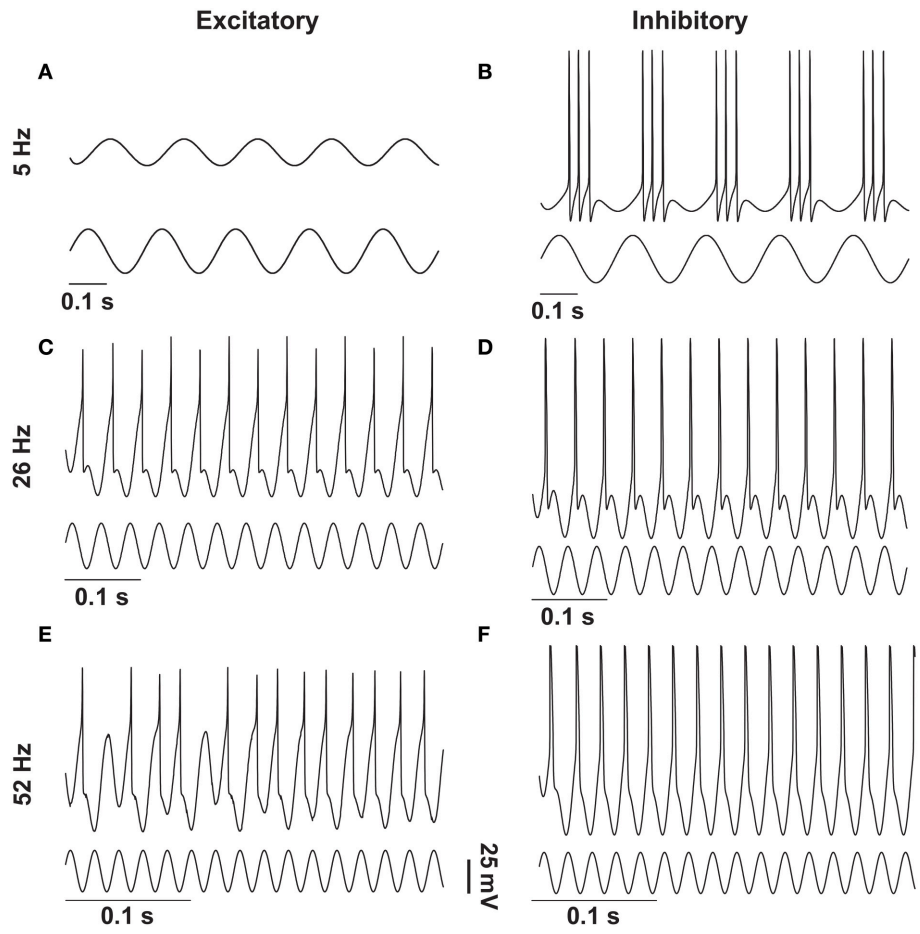


FIGURE 3 | Response of excitatory and inhibitory neurons to different frequencies of sinusoidal stimulations. Stimulating resting state excitatory and inhibitory neurons with different frequencies (5 Hz, 26 Hz, and 52 Hz) of sinusoids of 38 mV elicited different firing patterns as outlined in **Figure 1**. In case of the excitatory neuron 5 Hz stimulation was not enough to elicit action potentials **(A)**, whereas the inhibitory neuron produced bursting activity **(B)**. For 26 Hz action potentials were elicited in both types of neurons in a phase-locked fashion **(C,D)**, and for 52 Hz the excitatory neuron moved to the intermittent region **(E)** and the inhibitory neuron still showed the phase-locked firing **(F)**.

2.3. Model Small-World Network

A small-world (SW) network topology was considered as a prototype model of brain neuronal network (Watts and Strogatz, 1998). As per the definition of SW topology, the network is generated from a ring lattice where each neuron is connecting to K neighbors at random with a probability p ($0 < p < 1$). Zero probability ($p = 0$) makes the network regular and maximum probability ($p = 1$) makes it a random network (Sun et al., 2011) (see **Figure 4A**).

The neuronal network consisted of RS excitatory neurons (**E**) and FS inhibitory neurons (**I**) at a ratio of 4:1. The **E** and **I** were synaptically connected using SW topology with $K = 33$ neurons and $p = 0.165$. Noteworthy, all the neurons of the SW network were subject to the same applied potential, as it can be reasonably assumed within a small volume of brain tissue that is exposed to an electric field. The choice of single compartment neurons is justified by the fact that the region of the axon hillock (considered to be isopotential with the soma) is by far the most sensitive to external electric stimulation with respect to action potential triggering (Nowak and Bullier, 1998).

The design of synapses are described in the Model Synapses subsection (See Section 2.2). We considered all possible synaptic connections in the network (i.e., **E**→**E**, **E**→**I**, **I**→**E**, and **I**→**I**) with predefined input strengths of arbitrary units (**E**→**E**: 0.1, **E**→**I**: 0.1, **I**→**E**: 0.05, and **I**→**I**: 0.06) to create the connectivity weight matrices without recurrent connectivity. We further used a scaling factor for the excitatory and inhibitory synapses with values 0.03 and 0.06, respectively.

2.4. Model Background Network

The SW network with RS and FS neurons (see Section 2.3) remained at rest without external input. To simulate a background noise input and drive the SW network to spontaneous activity, we used an external neuronal population not exposed to electric stimulation (called "background population," see **Figure 4B**) consisting of 30 Izhikevich neurons (see Equations 14, 15) (Izhikevich, 2003) randomly connecting to 50% neurons in the target population through AMPA mediated synapses (see Equations 12, 11). In this case, Izhikevich neurons were preferred to HH neurons because computationally favorable and considering that they solely represented a source of spikes.

$$\begin{aligned} \frac{dv}{dt} &= 0.04v^2 + 5v + 140 - u + I \\ \frac{du}{dt} &= a(bv - u) \end{aligned} \tag{14}$$

with an after-spike resetting function defined by Equation (15).

$$\text{if } v \geq 30 \text{ mV, then } \begin{cases} v \leftarrow c \\ u \leftarrow u + d \end{cases} \tag{15}$$

Here, v, u, t, and I are membrane potential, membrane recovery variable representing the Na^+ and K^+ channel kinetics, time, and injected current respectively. a is the time scale of u, b is the sensitivity of u, c is the after-spike resetting value of v, and d is the after-spike resetting value of u. The values used for a, b, c, and d are 0.1, 0.2, −65, and 2 respectively. The values of v and c are expressed in mV, and t in ms.

Each of the neurons in the background population constantly received a zero-mean Gaussian noise (I in Equations 14) with a variance of 4.6 that generated enough AMPA mediated excitation

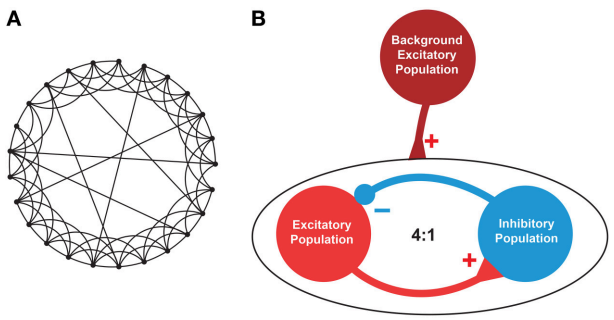


FIGURE 4 | Architecture of the neuronal network. (A). An instance of a representative small-world network with 25 nodes out of which 6 (K) nodes were linked via random rewiring with probability $p = 0.24$. **(B)**. A network consisting of excitatory (**E**) and inhibitory (**I**) neurons at 4 : 1 ratio was created with **E**→**E**, **E**→**I**, **I**→**E**, and **I**→**I** synaptic connectivity. To mimic the background activity or driving force, another population of excitatory neurons was used (maroon circle). This background population provided excitatory inputs to 50% of the excitatory (red circle) and inhibitory (blue circle) neurons to maintain a stable firing pattern. The excitatory-inhibitory population consisted of total N (= 200, E = 160, I = 40) neurons, each randomly connected to other K (= 33) neurons with p (= 0.165) connection probability using undirected edges creating a small-world network as described in Watts and Strogatz (1998).

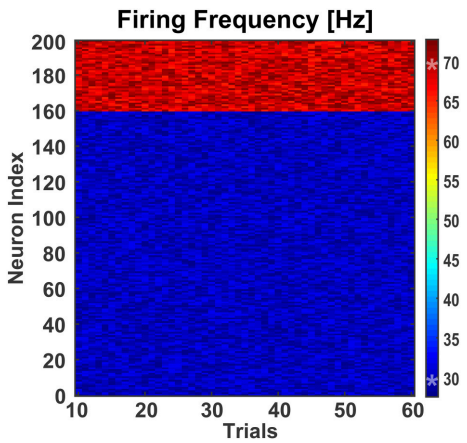


FIGURE 5 | Effect of background activity on network. To test the effect of differential modulation of excitatory and inhibitory neurons, the small-world network was fed with excitatory synaptic inputs from a background population consisting of 30 Izhikevich neurons. These neurons were stimulated with a randomly generated zero-mean Gaussian noise of variance 4.6. This generated enough excitatory synaptic conductance to activate the target population of excitatory and inhibitory neurons. Over 60 trials, the mean firing frequencies of the excitatory and inhibitory neurons were 30.00 Hz (±0.11 Hz) and 68.98 Hz (±0.27 Hz), respectively. The two asterisks ('*') on the colorbar represent the mean firing frequencies.

to activate the target population which then maintained a steady firing pattern.

3. RESULTS AND DISCUSSION

We first examined how the individual RS excitatory and FS inhibitory neurons respond to sinusoidal modulation of their transmembrane potential, e.g., as a result of extracellular stimulation with alternating current. We assessed different frequencies and strengths and created maps of firing patterns for the two types of neurons (see **Figure 1**). Maps show how the amplitude-frequency relationship affects the neuronal firing. Four possible modes were found: (i) non-firing, (ii) phase-lock firing with one action potential per peak (i.e., following a 1:1 relation), (iii) an intermediate condition where peaks of the sinusoidal modulation were not all associated to an action potential (i.e., intermittent firing in **Figure 1**) and (iv) bursting (i.e., with multiple action potentials per peak). Interestingly, excitatory neurons displayed the first three modes of response (**Figure 1A**): the no firing mode in the very low frequency range and for low stimulation amplitudes, the phase-locked behavior in the intermediate frequency range and for high amplitudes, and the intermittent firing in between. Conversely, inhibitory neurons were characterized by either bursting (**Figure 1C**), phase-locked or intermittent behavior when moving from the low to the high frequency range (**Figure 1B**).

3.1. Differential Modulation of Single Neurons

Due to intrinsic differences in the membrane properties between the two classes of neurons, they exhibit variation in their firing

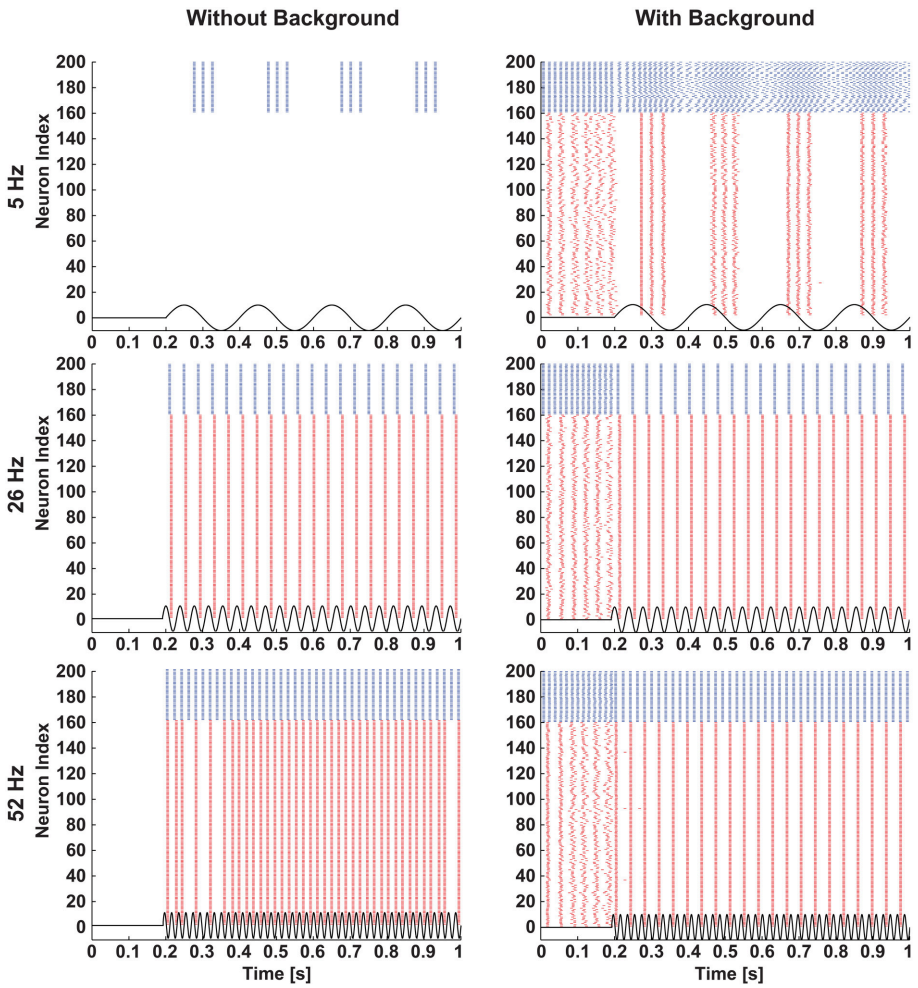


FIGURE 6 | Selective modulation of excitatory and inhibitory neurons in a network. A small-world network of excitatory and inhibitory neurons was stimulated with three different frequencies of sinusoids (5 Hz: top row, 26 Hz: middle row, and 52 Hz: bottom row) of 38 mV amplitude when the network was silent (left column) and activated by background excitatory synaptic inputs (right column). Raster plots of the network illustrate that when stimulated with sinusoids, the silent network showed similar firing patterns as in single neurons (see **Figure 2**), and the spontaneously active network showed selective modulation of excitatory and inhibitory neurons. The blue and red colors represent activities of inhibitory and excitatory neurons, respectively.

patterns while subject to the same periodic modulation as seen in **Figure 1**. After simulating the firing behavior of neurons from both classes for a large range of stimulating sinusoidal waveforms (frequency range: 1 to 500 Hz, and amplitude range: 10 to 60 mV), invariant to background perturbations (i.e., with or without sub-threshold background inputs to the single neurons), the RS neurons remained silent for a range of amplitudes and frequencies of the input signals ($10 \leq$ amplitude ≤ 26 mV, $1 \leq$ frequency ≤ 10 Hz), but in the same range the FS neurons exhibited firing patterns that varied from bursting to 1:1 phase lock to intermittent, depending on input signal's amplitude and frequency. Furthermore, though the RS neurons responded to input signals with amplitude > 26 mV and frequency > 10 Hz by firing action potentials, the firing pattern was irregular for the range ($26 \leq$ amplitude ≤ 44 mV, $10 \leq$ frequency ≤ 80 Hz), that is the pattern was switching between intermittent and 1:1 phase lock depending on specific amplitude-frequency combinations (dotted area in **Figure 1A**). On the contrary, in this range of stimulation, the FS neurons show steady progression in firing patterns (i.e., either "1:1 phase lock to intermittent" or "burst to 1:1 phase lock to intermittent") with increasing stimulus strength and frequency.

To exemplify the firing behavior mapped in **Figure 1**, individual neuronal classes were stimulated at 38 mV for the whole frequency range ($1 - 500$ Hz). As expected, when the number of elicited action potentials per sinusoidal cycle was plotted against the applied frequencies, the individual neuronal classes showed different firing patterns within given frequency ranges **Figure 2**. The inhibitory neuron (blue circled line) showed bursting behavior (with 4:1, 3:1, and 2:1 action potentials per cycle ratio) for input signals up to 20 Hz and the excitatory neuron (red squared line) remained silent for the first 10 Hz. However, during the subsequent range of intermediate frequencies ($20- \sim 300$ Hz), while the inhibitory neuron fired 1:1 phase-locked action potentials, the excitatory neuron first fired in a 1:1 phase-locked mode ($10- \sim 110$ Hz) with a short knee-shaped interval to an intermittent state ($\sim 50- \sim 90$ Hz), and then stably reverted to an intermittent regime. In fact, for the frequencies above 300 Hz both the neuronal classes showed intermittent firing in agreement with the reference maps.

To clearly visualize the differential responses of the two neuronal types to sinusoidal stimulation, we selected three representative frequencies of sinusoidal input signals (5, 26, and 52 Hz) from **Figure 2**. As seen in **Figure 3**, at 5 Hz, the inhibitory neuron fires bursts of action potentials (**Figure 3B**) but the excitatory neuron remains silent (**Figure 3A**); at 26 Hz, both types of neurons fire action potentials 1:1 phase locked to the stimulation signals (**Figure 3C,D**); and at 52 Hz, the excitatory neuron exhibits intermittent firing (**Figure 3E**) while the inhibitory neuron still fires in phase to the input signal (**Figure 3F**).

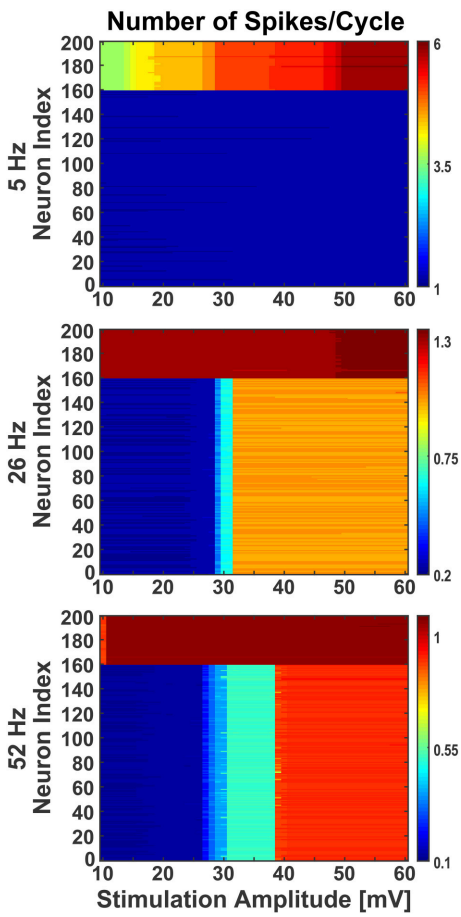


FIGURE 7 | Effect of stimulation amplitude on active network. The network was stimulated with varied amplitude of sinusoids (10 mV to 60 mV with step of 1) at three different frequencies (5 Hz, 26 Hz, and 52 Hz), as in **Figure 6**, when the network was activated by synaptic inputs from the background population. Even during the presence of background activity, stimulating the network with different amplitudes of sinusoids, the individual neurons' firing behavior was comparable to the ones noticed at 38 mV. The Y-axis shows the neurons index where 1 to 160 are excitatory and 161 to 200 are inhibitory. The colorbar shows the number of spikes per cycle.

3.2. Differential Modulation of Neuronal Network

We further investigated the effect of differential sinusoidal stimulation on neurons forming a neuronal network with SW topology (**Figure 4A**), a condition more closely resembling real brain circuits with respect to isolated neurons (Bullmore and Sporns, 2012). We assessed two distinct network conditions: (i) when the SW network was silent, i.e., without any external supply except the test inputs; and (ii) when the SW network was driven to be spontaneously active.

The latter condition was achieved by adding an external "background" neuronal population driving the SW network to a basal activity regime (**Figure 4B**). The background neuronal population was activated by perturbing it with zero-mean Gaussian noise, which caused spikes uniformly distributed in the population. 50% neurons from both classes in the SW network received AMPA mediated excitatory synaptic inputs from the background population which were enough to drive the SW

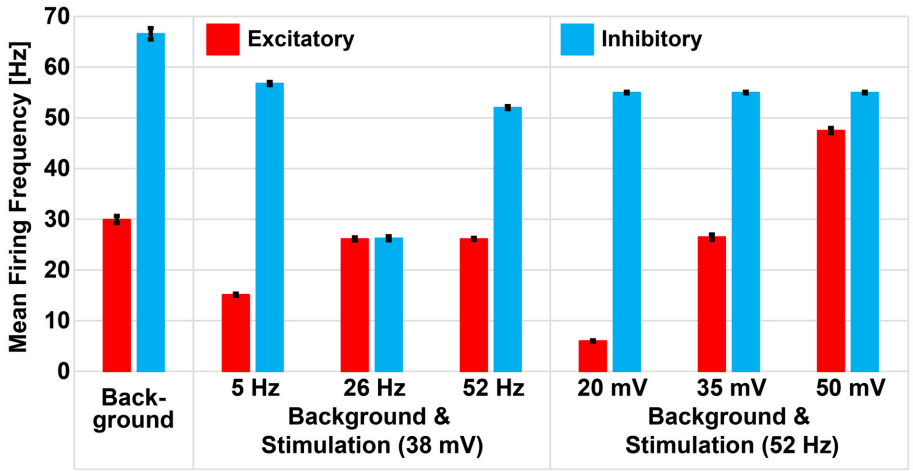


FIGURE 8 | Repetitive stimulation changes mean firing rate of excitatory and inhibitory neurons in presence of background activity . Excitatory and inhibitory neurons of the small-world network had mean firing frequencies of 29.94 Hz (± 0.7 Hz) and 66.56 Hz (± 1.12 Hz), respectively, as a result of excitation received from the excitatory background population. Sinusoidal stimulation at 38 mV selectively modulated the mean firing rate of excitatory and inhibitory neurons to 15.12 Hz (± 0.2 Hz) and 56.75 Hz (± 0.32 Hz) during 5 Hz, 26.12 Hz (± 0.1 Hz) and 26.24 Hz (± 0.1 Hz) during 26 Hz, and 26.12 Hz (± 0.14 Hz) and 51.99 Hz (± 0.1 Hz) during 52 Hz stimulation. However, fixing the stimulation frequency at 52 Hz and varying the amplitudes, we noticed gradual enhancement of excitatory neuron only (i.e., from 6.02 ± 0.12 Hz to 26.47 ± 0.51 Hz to 47.47 ± 0.52 Hz) while the inhibitory neuron remained unchanged (54.97 ± 0.16 Hz).

network (see **Figure 5**) and generate excitatory and inhibitory synaptic conductances comparable to those of a real biological network (Guillamon et al., 2006).

In both network conditions (i.e., silent and spontaneously active) the individual neurons belonging to different classes were differentially modulated (see **Figures 6**, **7**). In the spontaneously active network, differential modulation was forcing the spontaneously active SW network to a highly synchronized state (**Figure 6**, right column). This phenomenon can be attributed to the modulation of synaptic coupling in the SW network (Breakspear et al., 2003), and selective amplification of cortical cells' responses at preferred frequencies by intra-network inputs from similarly tuned neurons (Liu et al., 2007; Rotstein and Nadim, 2014). Conversely, in the case of the silent network condition, the modulation (**Figure 6**, left column) closely matched the single-neuron reference map (see **Figure 1**).

Moreover, in response to sinusoidal stimulation with given amplitudes and frequencies, the neurons exhibit either an increase or a decrease in their spiking rates with respect to background activity. The inhibitory neurons in the network were found to be more susceptible to sinusoids at lower intensities, matching qualitatively previous experimental observations (Moliadze et al., 2012), and frequencies (Reato et al., 2013). As seen in **Figure 7**, at low frequency (i.e., 5 Hz) with increasing intensity, the inhibitory neurons show frequent change in action potential firing rate (indicated by color stripes in figure) compared to excitatory neurons which fire steadily (indicated by uniform color in figure). The reverse happens at higher frequencies (i.e., 26 and 52 Hz), where the excitatory neurons show an increasing enhancement of firing rates (indicated by color stripes in figure), while the inhibitory ones fire invariably (indicated by uniform color in figure). This differential activation of excitatory and inhibitory neurons gives rise to a change of the excitation/inhibition ratio (E/I) in the network which is dependent form the amplitude and frequency of the stimulus, and that may represent a mechanism behind experimental and clinical observations during tACS (Antal and Paulus, 2013; Herrmann et al., 2013).

Dysregulation of E/I has been associated to many CNS disorders (Eichler and Meier, 2008), characterized by inefficient information exchange in brain regions. This inefficacy could be caused by loss of homeostatic control of excitation and inhibition (Krause et al., 2013), making it crucial to find therapeutic approaches to restore physiological E/I. To this aim, also on the basis of our results, the E/I may be modulated by finely tuning the amplitude and frequency of sinusoidal stimulation. The concept is evidenced in **Figure 8** were we show how, by changing stimulation parameters, the average activity of excitatory and inhibitory neurons in a spontaneoulsy active network can be tuned modulating, in turn, the E/I.

4. CONCLUSION

We provide evidence that, leveraging the different properties of voltage-dependent membrane conductances in excitatory and inhibitory neurons, sinusoidal stimuli can be used to differentially modulate their firing. In particular, basing on simulations of a network of excitatory and inhibitory neurons exposed to a sinusoidal modulation of the extracellular potential, we showed that sinusoidal stimulation could modulate the E/I. In practice, all electrical stimulation methods adopted in the experimental and clinical context and causing sinusoidal voltage changes in the extracellular fluid of the brain tissue could be

suitable for the purpose. These include implanted electrodes, such as in DBS, and transcranial non-invasive stimulation approaches such as tACS. However, further elaboration will be necessary to assess the real potential of the approach in clinics. First of all, an unknown contribution will exist from fibers stimulation by the electric field (Roth and Basser, 1990; Herrmann et al., 2013). Second, synaptic plasticity phenomena may also influence network dynamics upon sinusoidal stimulation, as proposed by Antal and Paulus (2013); Zaehle et al. (2010). Finally, it will be crucial to precisely estimate the transmembrane potential in neurons during tACS, taking into account the impedence of the neuronal membrane and its shunting influence at higher frequencies. In fact, despite technical advances to strengthen stimulation (Herrmann et al., 2013), the transmembrane potential modulation caused by tACS may turn out to be too weak to control E/I for clinical usage. Despite these unknowns, and in future perspective, differential sinusoidal stimulation may prove to be a versatile approach in clinics to restore physiological balance between excitation and inhibition in a number of neurological disorders.

AUTHOR CONTRIBUTIONS

SV conceived the basic idea of differential sinusoidal stimulation; MM developed the model and wrote the code to assess the differential sinusoidal stimulation concept. MM and SV wrote the manuscript. Both authors have contributed to, seen and approved the final manuscript.

FUNDING

Financial support from the 7th Framework Programme of the European Commission through "CyberRat" (http://www.vassanellilab.eu/projects/cyberrat/, GA no. 216528) and "RAMP" projects (www.rampproject.eu, GA no. 612058) are acknowledged.

ACKNOWLEDGMENTS

The authors are particularly thankful to Dr. M. Mostafizur Rahman and Mr. Giacomo Bassetto for their help and fruitful discussion.

REFERENCES

Alarcón, G., and Valentín, A. (2012). Cortical stimulation with single electrical pulses in human epilepsy. *Clin. Neurophysiol.* 123, 223–224. doi: 10.1016/j.clinph.2011.07.001

Ali, M. M., Sellers, K. K., and Fröhlich, 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., Yungher, D. A., Shulman, L. M., and Rogers, M. W. (2012). Safety and immediate effect of noninvasive transcranial pulsed current stimulation on gait and balance in parkinson disease. *Neurorehabil. Neural Repair.* 26, 1089–1095. doi: 10.1177/1545968312448233

Anderson, R. J., Frye, M. A., Abulseoud, O. A., Lee, K. H., McGillivray, J. A., Berk, M., et al. (2012). Deep brain stimulation for treatment-resistant depression: efficacy, safety and mechanisms of action. *Neurosci. Biobehav. Rev.* 36, 1920–1933. doi: 10.1016/j.neubiorev.2012.06.001

Antal, A., and Paulus, W. (2013). Transcranial alternating current stimulation (tacs). *Front. Hum. Neurosci.* 7:317. doi: 10.3389/fnhum.2013.00317

Antal, A., Paulus, W., and Nitsche, M. A. (2011). Electrical stimulation and visual network plasticity. *Restor. Neurol. Neurosci.* 29, 365–374. doi: 10.3233/rnn-2011-0609

Berényi, A., Belluscio, M., Mao, D., and Buzsaki, G. (2012). Closed-loop control of epilepsy by transcranial electrical stimulation. *Science* 337, 735–737. doi: 10.1126/science.1223154

Birdno, M. J., Kuncel, A. M., Dorval, A. D., Turner, D. A., Gross, R. E., and Grill, W. M. (2011). Stimulus features underlying reduced tremor suppression with temporally patterned deep brain stimulation. *J. Neurophysiol.* 107, 364–383. doi: 10.1152/jn.00906.2010

Boëx, C., Vulliémoz, S., Spinelli, L., Pollo, C., and Seeck, M. (2007). High and low frequency electrical stimulation in non-lesional temporal lobe epilepsy. *Seizure* 16, 664–669. doi: 10.1016/j.seizure.2007.05.009

Boon, P., Raedt, R., Herdt, V., Wyckhuys, T., and Vonck, K. (2009). Electrical stimulation for the treatment of epilepsy. *Neurotherapeutics* 6, 218–227. doi: 10.1016/j.nurt.2008.12.003

Börgers, C., Epstein, S., and Kopell, N. J. (2005). Background gamma rhythmicity and attention in cortical local circuits: a computational study. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7002–7007. doi: 10.1073/pnas.0502366102

Breakspear, M., Terry, J. R., and Friston, K. J. (2003). Modulation of excitatory synaptic coupling facilitates synchronization and complex dynamics in a biophysical model of neuronal dynamics. *Network* 14, 703–732. doi: 10.1088/0954-898X_14_4_305

Brumberg, J. C., and Gutkin, B. S. (2007). Cortical pyramidal cells as non-linear oscillators: experiment and spike-generation theory. *Brain Res.* 1171, 122–137. doi: 10.1016/j.brainres.2007.07.028

Bullmore, E., and Sporns, O. (2012). The economy of brain network organization. *Nat. Rev. Neurosci.* 13, 336–349. doi: 10.1038/nrn3214

Chou, Y., Hickey, P. T., Sundman, M., Song, A. W., and Chen, N. (2015). Effects of repetitive transcranial magnetic stimulation on motor symptoms in parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* 72, 432–440. doi: 10.1001/jamaneurol.2014.4380

Coenen, V. A., Amtage, F., Volkmann, J., and Schläpfer, T. E. (2015). Deep brain stimulation in neurological and psychiatric disorders. *Dtsch. Arztebl. Int.* 112, 519–526. doi: 10.3238/arztebl.2015.0519

Concerto, C., Lanza, G., Cantone, M., Ferri, R., Pennisi, G., Bella, R., et al. (2015). Repetitive transcranial magnetic stimulation in patients with drug-resistant major depression: a six-month clinical follow-up study. *Int. J. Psychiatry Clin. Pract.* 19, 252–258. doi: 10.3109/13651501.2015.1084329

Connors, B. W., and Gutnick, M. J. (1990). Intrinsic firing patterns of diverse neocortical neurons. *Trends Neurosci.* 13, 99–104. doi: 10.1016/0166-2236(90)90185-D

Cook, I. A., Espinoza, R., and Leuchter, A. F. (2014). Neuromodulation for depression: invasive and noninvasive (deep brain stimulation, transcranial magnetic stimulation, trigeminal nerve stimulation). *Neurosurg. Clin. N. Am.* 25, 103–116. doi: 10.1016/j.nec.2013.10.002

Davis, N. J., and Koningsbruggen, M. V. (2013). 'Non-invasive' brain stimulation is not non-invasive. *Front. Syst. Neurosci.* 7:76. doi: 10.3389/fnsys.2013.00076

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 Hemptinne, C., Swann, N. C., Ostrem, J. L., Ryapolova-Webb, E. S., San Luciano, M., Galifianakis, N. B., et al. (2015). Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. *Nat. Neurosci.* 18, 779–786. doi: 10.1038/nn.3997

Deans, J. K., Powell, A. D., and Jefferys, J. G. R. (2007). Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *J. Physiol.* 583, 555–565. doi: 10.1113/jphysiol.2007.137711

Destexhe, A., Mainen, Z. F., and Sejnowski, T. J. (1994). An efficient method for computing synaptic conductances based on a kinetic model of receptor binding. *Neural Comput.* 6, 14–18. doi: 10.1162/neco.1994.6.1.14

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

Eickenscheidt, M., Jenkner, M., Thewes, R., Fromherz, P., and Zeck, G. (2012). Electrical stimulation of retinal neurons in epiretinal and subretinal configuration using a multicapacitor array. *J. Neurophysiol.* 107, 2742–2755. doi: 10.1152/jn.00909.2011

Fisher, R., Salanova, V., Witt, T., Worth, R., Henry, T., Gross, R., et al. (2010). Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. *Epilepsia* 51, 899–908. doi: 10.1111/j.1528-1167.2010.02536.x

Fisher, R. S. (2011). Neurostimulation for epilepsy: do we know the best stimulation parameters? *Epilepsy. Curr.* 11, 203–204. doi: 10.5698/1535-7511-11.6.203

Freeman, D. (2010). Electric stimulation with sinusoids and white noise for neural prostheses. *Front. Neurosci.* 4:28. doi: 10.3389/neuro.20.001.2010

Freeman, D. K., Eddington, D. K., Rizzo, J. F., and Fried, S. I. (2010). Selective activation of neuronal targets with sinusoidal electric stimulation. *J. Neurophysiol.* 104, 2778–2791. doi: 10.1152/jn.00551.2010

Fröhlich, 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

Gall, C., Antal, A., and Sabel, B. A. (2013). Non-invasive electrical brain stimulation induces vision restoration in patients with visual pathway damage. *Graefes Arch. Clin. Exp. Ophthalmol.* 251, 1041–1043. doi: 10.1007/s00417-012-2084-7

Ghezzi, D. (2015). Retinal prostheses: progress toward the next generation implants. *Front. Neurosci.* 9:290. doi: 10.3389/fnins.2015.00290

Gironell, A., Martinez-Horta, S., Aguilar, S., Torres, V., Pagonabarraga, J., Pascual-Sedano, B., et al. (2014). Transcranial direct current stimulation of the cerebellum in essential tremor: a controlled study. *Brain Stimul.* 7, 491–492. doi: 10.1016/j.brs.2014.02.001

Grassi, G., Godini, L., Grippo, A., Piccagliani, D., and Pallanti, S. (2015). Enhancing cognitive-behavioral therapy with repetitive transcranial magnetic stimulation in refractory obsessive-compulsive-disorder: a case report. *Brain Stimul.* 8, 160–161. doi: 10.1016/j.brs.2014.10.007

Green, A. L., and Aziz, T. Z. (2014). Steering technology for deep brain stimulation. *Brain* 137(Pt 7), 1854–1856. doi: 10.1093/brain/awu126

Grover, P. J., Pereira, E. A., Green, A. L., Brittain, J.-S., Owen, S. L., Schweder, P., et al. (2009). Deep brain stimulation for cluster headache. *J. Clin. Neurosci.* 16, 861–866. doi: 10.1016/j.jocn.2008.10.012

Guillamon, A., McLaughlin, D. W., and Rinzel, J. (2006). Estimation of synaptic conductances. *J. Physiol.* 100, 31–42. doi: 10.1016/j.jphysparis.2006.09.010

Henn, F. A. (2012). Circuits, cells, and synapses: toward a new target for deep brain stimulation in depression. *Neuropsychopharmacology* 37, 307–308. doi: 10.1038/npp.2011.193

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

Herz, A. V., Gollisch, T., Machens, C. K., and Jaeger, D. (2006). Modeling single-neuron dynamics and computations: a balance of detail and abstraction. *Science* 314, 80–85. doi: 10.1126/science.1127240

Hodaj, H., Alibeu, J. P., Payen, J. F., and Lefaucheur, J. P. (2015). Treatment of chronic facial pain including cluster headache by repetitive transcranial magnetic stimulation of the motor cortex with maintenance sessions: a naturalistic study. *Brain Stimul.* 8, 801–807. doi: 10.1016/j.brs.2015.01.416

Hodgkin, A. L., and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117, 500–544. doi: 10.1113/jphysiol.1952.sp004764

Islam, L., Franzini, A., Messina, G., Scarone, S., and Gambini, O. (2015). Deep brain stimulation of the nucleus accumbens and bed nucleus of stria terminalis for obsessive-compulsive disorder: a case series. *World Neurosurg.* 83, 657–663. doi: 10.1016/j.wneu.2014.12.024

Izhikevich, E. (2003). Simple model of spiking neurons. *IEEE Trans. Neural Netw.* 14, 1569–1572. doi: 10.1109/TNN.2003.820440

Jiménez-Ponce, F., Velasco-Campos, F., Castro-Farfán, G., Nicolini, H., Velasco, A. L., Salín-Pascual, R., et al. (2009). Preliminary study in patients with obsessive-compulsive disorder treated with electrical stimulation in the inferior thalamic peduncle. *Neurosurgery* 65, ons203–ons209. doi: 10.1227/01.neu.0000345938.39199.90

Jorge, R. E., and Robinson, R. G. (2011). Treatment of late-life depression: a role of non-invasive brain stimulation techniques. *Int. Rev. Psychiatry* 23, 437–444. doi: 10.3109/09540261.2011.633501

Joucla, S., Branchereau, P., Cattaert, D., and Yvert, B. (2012). Extracellular neural microstimulation may activate much larger regions than expected by simulations: a combined experimental and modeling study. *PLoS ONE* 7:e41324. doi: 10.1371/journal.pone.0041324

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

Kohl, S., Schonherr, D. M., Luigjes, J., Denys, D., Mueller, U. J., Lenartz, D., et al. (2014). Deep brain stimulation for treatment-refractory obsessive compulsive disorder: a systematic review. *BMC Psychiatry* 14:214. doi: 10.1186/s12888-014-0214-y

Krack, P., Fraix, V., Mendes, A., Benabid, A.-L., and Pollak, P. (2002). Postoperative management of subthalamic nucleus stimulation for parkinson's disease. *Move. Disord.* 17, S188–S197. doi: 10.1002/mds.10163

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

Lettieri, C., Rinaldo, S., Devigili, G., Pisa, F., Mucchiut, M., Belgrado, E., et al. (2015). Clinical outcome of deep brain stimulation for dystonia: constant-current or constant-voltage stimulation? a non-randomized study. *Eur. J. Neurol.* 22, 919–926. doi: 10.1111/ene.12515

Lewis, P. M., Ackland, H. M., Lowery, A. J., and Rosenfeld, J. V. (2015). Restoration of vision in blind individuals using bionic devices: a review with a focus on cortical visual prostheses. *Brain Res.* 1595, 51–73. doi: 10.1016/j.brainres.2014.11.020

Liew, S. L., Santarnecchi, E., Buch, E. R., and Cohen, L. G. (2014). Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery. *Front. Hum. Neurosci.* 8:378. doi: 10.3389/fnhum.2014.00378

Liu, B. H., Wu, G. K., Arbuckle, R., Tao, H. W., and Zhang, L. I. (2007). Defining cortical frequency tuning with recurrent excitatory circuitry. *Nat. Neurosci.* 10, 1594–1600. doi: 10.1038/nn2012

Loddenkemper, T., Pan, A., Neme, S., Baker, K. B., Rezai, A. R., Dinner, D. S., et al. (2001). Deep brain stimulation in epilepsy. *J. Clin. Neurophysiol.* 18, 514–532. doi: 10.1097/00004691-200111000-00002

Lozano, A. M. (2000). Vim thalamic stimulation for tremor. *Arch. Med. Res.* 31, 266–269. doi: 10.1016/S0188-4409(00)00081-3

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

Lu, H., Chestek, C. A., Shaw, K. M., and Chiel, H. J. (2008). Selective extracellular stimulation of individual neurons in ganglia. *J. Neural Eng.* 5, 287–309. doi: 10.1088/1741-2560/5/3/003

Mainen, Z. F., Joerges, J., Huguenard, J. R., and Sejnowski, T. J. (1995). A model of spike initiation in neocortical pyramidal neurons. *Neuron* 15, 1427–1439. doi: 10.1016/0896-6273(95)90020-9

Matharu, M. S., and Zrinzo, L. (2011). Deep brain stimulation in cluster headache. *Exp. Rev. Neurother.* 11, 473–475. doi: 10.1586/ern.11.35

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

McIntyre, C. C., and Grill, W. M. (1999). Excitation of central nervous system neurons by nonuniform electric fields. *Biophys. J.* 76, 878–888. doi: 10.1016/S0006-3495(99)77251-6

McIntyre, C. C., and Grill, W. M. (2000). Selective microstimulation of central nervous system neurons. *Ann. Biomed. Eng.* 28, 219–233. doi: 10.1114/1.262

McIntyre, C. C., and Grill, W. M. (2002). Extracellular stimulation of central neurons: influence of stimulus waveform and frequency on neuronal output. *J. Neurophysiol.* 88, 1592–1604. Available online at: http://jn.physiology.org/content/88/4/1592.long

Miller, J. P., and Selman, W. R. (2009). Deep brain stimulation for depression. *J. Neurosurg.* 111, 1207–1208. doi: 10.3171/2008.1.JNS081353

Miocinovic, S., Somayajula, S., Chitnis, S., and Vitek, J. L. (2013). History, applications, and mechanisms of deep brain stimulation. *JAMA Neurol.* 70, 163–171. doi: 10.1001/2013.jamaneurol.45

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

Nelson, T. S., l. Suhr, C., Freestone, D. R., Lai, A., Halliday, A. J., Mclean, K. J., et al. (2011). Closed-loop seizure control with very high frequency electrical stimulation at seizure onset in the gaers model of absence epilepsy. *Int. J. Neural Syst.* 21, 163–173. doi: 10.1142/S0129065711002717

Nowak, L. G., and Bullier, J. (1998). Axons, but not cell bodies, are activated by electrical stimulation in cortical gray matter. ii. evidence from selective inactivation of cell bodies and axon initial segments. *Exp. Brain Res.* 118, 489–500. doi: 10.1007/s002210050305

Nuttin, B. J., GabriAls, L. A., Cosyns, P. R., Meyerson, B. A., Andréewitch, S., Sunaert, S. G., et al. (2008). Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. *Neurosurgery* 62(Suppl. 3), SHC966–SHC977. doi: 10.1227/01.neu.0000333764.20575.d6

Obeso, I., Wilkinson, L., Rodrguez-Oroz, M.-C., Obeso, J., and Jahanshahi, M. (2013). Bilateral stimulation of the subthalamic nucleus has differential effects on reactive and proactive inhibition and conflict-induced slowing in parkinsonas disease. *Exp. Brain Res.* 226, 451–462. doi: 10.1007/s00221-013-3457-9

Orosz, I., McCormick, D., Zamponi, N., Varadkar, S., Feucht, M., Parain, D., et al. (2014). Vagus nerve stimulation for drug-resistant epilepsy: a european long-term study up to 24 months in 347 children. *Epilepsia* 55, 1576–1584. doi: 10.1111/epi.12762

Paulus, W. (2014). Transcranial brain stimulation: potential and limitations. *e-Neuroforum* 5, 29–36. doi: 10.1007/s13295-014-0056-6

Piacentino, M., D'Andrea, G., Perini, F., and Volpin, L. (2014). Drug-resistant cluster headache: long-term evaluation of pain control by posterior hypothalamic deep-brain stimulation. *World Neurosurg.* 81:442e11-5. doi: 10.1016/j.wneu.2013.01.130

Press, W. H., Teukolsky, S. A., Vetterling, W. T., and Flannery, B. P. (2007). *Numerical Recipes The Art of Scientific Computing*. New York, NY: Cambridge University Press.

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, 228e1–3. doi: 10.1016/j.brs.2009.03.007

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

Rizvi, S. J., Donovan, M., Giacobbe, P., Placenza, F., Rotzinger, S., and Kennedy, S. H. (2011). Neurostimulation therapies for treatment resistant depression: a focus on vagus nerve stimulation and deep brain stimulation. *Int. Rev. Psychiatry* 23, 424–436. doi: 10.3109/09540261.2011.630993

Rizzo, J. F., Wyatt, J., Loewenstein, J., Kelly, S., and Shire, D. (2003). Perceptual efficacy of electrical stimulation of human retina with a microelectrode array during short-term surgical trials. *Invest. Ophthalmol. Vis. Sci.* 44, 5362. doi: 10.1167/iovs.02-0817

Roth, B. J., and Basser, P. J. (1990). A model of the stimulation of a nerve fiber by electromagnetic induction. *IEEE Trans. Biomed. Eng.* 37, 588–597. doi: 10.1109/10.55662

Rotstein, H. G., and Nadim, F. (2014). Frequency preference in two-dimensional neural models: a linear analysis of the interaction between resonant and amplifying currents. *J. Comput. Neurosci.* 37, 9–28. doi: 10.1007/s10827-013-0483-3

Salanova, V., Witt, T., Worth, R., Henry, T. R., Gross, R. E., Nazzaro, J. M., et al. (2015). Long-term efficacy and safety of thalamic stimulation for drug-resistant partial epilepsy. *Neurol* 84, 1017–1025. doi: 10.1212/WNL.0000000000001334

Shin, Y.-I., Foerster, A., and Nitsche, M. A. (2015). Transcranial direct current stimulation (tdcs) application in neuropsychology. *Neuropsychologia* 69, 154–175. doi: 10.1016/j.neuropsychologia.2015.02.002

Sillay, K. A., Sani, S., and Starr, P. A. (2010). Deep brain stimulation for medically intractable cluster headache. *Neurobiol. Dis.* 38, 361–368. doi: 10.1016/j.nbd.2009.05.020

Sun, X. J., Lei, J. Z., Perc, M., Lu, Q. S., and Lv, S. J. (2011). Effects of channel noise on firing coherence of small-world hodgkin-huxley neuronal networks. *Eur. Phys. J. B* 79, 61–66. doi: 10.1140/epjb/e2010-10031-3

Traub, R. D., Wong, R. K., Miles, R., and Michelson, H. (1991). A model of a ca3 hippocampal pyramidal neuron incorporating voltage-clamp data on intrinsic conductances. *J. Neurophysiol.* 66, 635–650.

Vassanelli, S. (2014). *Multielectrode and Multitransistor Arrays for In Vivo Recording* New York, NY: Springer.

Vassanelli, S., Mahmud, M., Girardi, S., and Maschietto, M. (2012). On the way to large-scale and high-resolution brain-chip interfacing. *Cogn. Comput.* 4, 71–81. doi: 10.1007/s12559-011-9121-4

Volkmann, J., and Benecke, R. (2002). Deep brain stimulation for dystonia: patient selection and evaluation. *Move. Disord.* 17, S112–S115. doi: 10.1002/mds.10151

Wang, X. J., and Buzsáki, G. (1996). Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model. *J. Neurosci.* 16, 6402–6413.

Watts, D. J., and Strogatz, S. H. (1998). Collective dynamics of 'small-world' networks. *Nature* 393, 440–442. doi: 10.1038/30918

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 Neuroscience

ORIGINAL RESEARCH
published: 29 January 2016
doi: 10.3389/fnins.2016.00010

Human Motor Cortex Functional Changes in Acute Stroke: Gender Effects

Vincenzo Di Lazzaro[1,2], Giovanni Pellegrino[1,3], Giovanni Di Pino[1,2], Federico Ranieri[1,2], Fiorenza Lotti[4], Lucia Florio[1,2] and Fioravante Capone[1,2]*

[1] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy, [2] Fondazione Alberto Sordi – Research Institute for Ageing, Rome, Italy, [3] Multimodal Functional Imaging Laboratory, Montreal Neurological Institute – McGill University, Montreal, QC, Canada, [4] Orthopaedic and Trauma Surgery Unit, Università Campus Bio-Medico di Roma, Rome, Italy

The acute phase of stroke is accompanied by functional changes in the activity and interplay of both hemispheres. In healthy subjects, gender is known to impact the functional brain organization. We investigated whether gender influences also acute stroke functional changes. In thirty-five ischemic stroke patients, we evaluated the excitability of the affected (AH) and unaffected hemisphere (UH) by measuring resting and active motor threshold (AMT) and motor-evoked potential amplitude under baseline conditions and after intermittent theta burst stimulation (iTBS) of AH. We also computed an index of the excitability balance between the hemispheres, laterality indexes (LI), to evidence hemispheric asymmetry. AMT differed significantly between AH and UH only in the male group ($p = 0.004$), not in females ($p > 0.200$), and both LI_{AMT} and LI_{RMT} were significantly higher in males than in females (respectively $p = 0.033$ and $p = 0.042$). LTP-like activity induced by iTBS in AH was more frequent in females. Gender influences the functional excitability changes that take place after human stroke and the level of LTP that can be induced by repetitive stimulation. This knowledge is of high value in the attempt of individualizing to different genders any non-invasive stimulation strategy designed to foster stroke recovery.

Keywords: acute cerebral infarction, gender, neurophysiology, stroke, transcranial magnetic stimulation

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales de Madrid and CEU-San Pablo University, Spain

Reviewed by:
Diane Ruge, University College London, UK
Antonio Suppa, Sapienza University of Rome, Italy

****Correspondence:***
Vincenzo Di Lazzaro
v.dilazzaro@unicampus.it

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

Received: *17 November 2015*
Accepted: *11 January 2016*
Published: *29 January 2016*

Citation:
Di Lazzaro V, Pellegrino G, Di Pino G, Ranieri F, Lotti F, Florio L and Capone F (2016) Human Motor Cortex Functional Changes in Acute Stroke: Gender Effects. Front. Neurosci. 10:10. doi: 10.3389/fnins.2016.00010

INTRODUCTION

Gender related functional asymmetries between the two cerebral hemispheres have been documented in healthy human brain (Tomasi and Volkow, 2012). It has been suggested that they might be responsible for gender differences in cognitive styles (Proust-Lima et al., 2008), in the incidence of neuropsychiatric disorders (Narr et al., 2001; Baron-Cohen et al., 2005), and for a gender-specific influence on the functional outcome after unilateral cerebral lesion (Draca, 2010). Sex-related differences have been also reported after stroke with a worse functional outcome in women (Lisabeth et al., 2015), however the causes of this sex disparity in stroke outcome are still largely unknown because demographics, prestroke and clinical factors cannot explain it. One possibility is that gender has a significant influence on the functional changes underlying recovery that take place in the brain after a stroke. Non-invasive brain stimulation techniques provide the opportunity for the functional evaluation of the human brain. Thanks to these techniques it has been shown that pronounced asymmetrical functional changes take place in cortex in the acute phase of stroke (Di Pino et al., 2014a,b).

These changes involve both the affected (AH) and unaffected (UH) hemispheres and might be correlated with long term recovery (for review see Di Pino et al., 2014a). Along this line, it is still unknown whether gender has an effect in stroke-related acute functional changes in the excitability of AH and UH.

The present study aims at investigating whether gender influences the cortical functional changes observed in the acute phase of stroke. To this end, in patients with acute stroke we evaluated motor cortex excitability by using single pulse transcranial magnetic stimulation (TMS) and the propensity of the cortex to undergo LTP- and LTD-like plasticity by means of a repetitive TMS (rTMS) paradigm, known as intermittent theta burst stimulation (iTBS). iTBS produces LTP-like changes in the stimulated hemisphere and LTD-like changes in the contralateral hemisphere (Di Lazzaro et al., 2008; Suppa et al., 2008). Similar interhemispheric effects have been observed also using a different TBS protocol known as continuous TBS, a rTMS paradigm that produces opposite effects on cortical excitability with LTD-like changes in the stimulated hemisphere and LTP-like changes in the contralateral hemisphere (Stefan et al., 2008).

Electrophysiological findings after single pulse TMS and after iTBS were compared between genders.

MATERIALS AND METHODS

Patients

Thirty-five patients with first-ever stroke were recruited (mean age = 71.4 years, SER = 1.96, 15F). Inclusion criteria were: (1) single ischemic stroke (both cortical and subcortical) involving the middle cerebral artery territory; (2) less than 10 days post-stroke; (3) hand weakness; (4) recordable muscle evoked potential (MEP) after TMS of the AH. Exclusion criteria were: (1) history of seizure; (2) hemorrhagic stroke; (3) concomitant neurological or other severe medical problems; (4) complete paralysis of the hand; (5) inability to give informed consent; (6) concomitant treatment with drugs acting on the central nervous system; (7) contraindications for TMS studies. In order to identify at risk patients for post-stroke epilepsy, all patients underwent an EEG before entering the study and none of them showed any epileptic abnormality (Rossini and Johnston, 2005). The evaluation of neurological impairment was based on the National Institutes of Health Stroke Scale (NIHSS).

All patients underwent brain MRI with a 1.5-T scanner (GE Signa; General Electric, Milwaukee, WI), and lesion size was estimated by using the Alberta Stroke Program Early CT Score (ASPECTS) (Barber et al., 2000).

All the patients signed a written informed consent form. This study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the Ethics Committee of Campus Bio-Medico University of Rome.

Magnetic Stimulation

Motor Cortex Excitability to Single Pulse TMS

Magnetic stimulation was performed with a high-power Magstim 200 (MagstimCo., Whitland, Dyfed). A figure-of-eight coil with external loop diameters of 9 cm was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous muscle (FDI). The induced current flowed in a postero-anterior direction. We evaluated the threshold and amplitude of MEPs. The resting motor threshold (RMT) was defined as the minimum stimulus intensity, expressed as the percentage of the maximal output intensity deliverable by the stimulator, which produced a liminal MEP (about 50 μV in 50% of 10 trials) at rest (Rossini, 2014). The active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 200 μV in 50% of 10 trials) during isometric contraction of the tested muscle (Rossini, 2014). We evaluated the RMT, AMT, and MEP amplitude elicited stimulating both the AH and UH. The MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Audio-visual feedback of the electromyographic (EMG) signal at high gain was given to subjects in order to assist them in maintaining complete relaxation; trials contaminated by EMG activity were discarded. Ten data sweeps were collected, and the mean peak-to-peak amplitude of the MEPs was calculated.

Intermittent Theta Burst Stimulation

iTBS was delivered over the affected motor cortex "hot spot" for MEPs in the contralateral FDI muscle using a MagPro stimulator (Medtronic A/S Denmark) connected to a figure-of-eight coil (MCF B65). The magnetic stimulus had a biphasic waveform with a pulse width of about 280 μs and a maximum magnetic field strength of 1.5 T. The initial direction of the current induced in the brain was anterior to posterior. The stimulation intensity was defined in relation to the AMT evaluated using the MagPro stimulator. An intensity of 80% AMT was used. We applied the iTBS protocol in which 10 bursts of high frequency stimulation (3 pulses at 50 Hz) are delivered at 5 Hz every 10 s, for a total of 600 pulses (Huang et al., 2005). iTBS effects on both hemispheres were assessed by evaluating the changes of the RMT, AMT, and MEP amplitude stimulating the AH and UH, before and immediately after iTBS. MEP amplitude was evaluated as detailed above.

Statistical Analysis

Main aim of the statistical analysis is to assess the effect of gender on excitability and plasticity measures. Baseline and iTBS-dependent excitability changes were tested on RMT, AMT, MEP amplitude and on the Laterality Index (LI) (Cramer et al., 1997; Di Lazzaro et al., 2015). The latter is a derived compound estimate of inter-hemispheric excitability imbalance. In the case of MEP amplitude, LI is expressed by the following equation: LI_{MEP}= (MEP_{UH}- MEP_{AH})/(MEP_{UH}+ MEP_{AH}). On the contrary, in the case of AMT and RMT the correlation with excitability is opposite (the lower are the thresholds the higher is the excitability). Thus, LI is calculated as follow: LI_{RMT}= (MEP_{AH} - MEP_{UH})/(MEP_{UH}+ MEP_{AH}) and LI_{AMT}= (MEP_{AH} - MEP_{UH})/(MEP_{UH} + MEP_{AH}). LI ranges between −1 and +1; positive values always indicate higher excitability of the UH. The bigger the difference from 0, the higher is the inter-hemispheric imbalance. Gender effect on baseline RMT, AMT, and MEP is evaluated applying a mixed model ANOVA with *Hemisphere*

(two levels: *Affected -AH and Unaffected -UH*) as within-subjects factor and *Gender* (two levels: *Female and Male*) as between-subjects factor. A two-tailed independent sample t-test is used to assess the LI between groups difference. iTBS effect is tested on RMT, AMT, and MEP amplitude by using a mixed model ANOVA with *Hemisphere* (*Affected -AH and Unaffected -UH*) and iTBS (*Pre and Post*) as within-subjects factor and *Gender* (*Female and Male*) as between-subjects factor. The same model without the factor hemisphere is applied to study iTBS-related LI changes. Differences between females and males for non-normal distributed data were checked applying Mann-Whitney tests. In order to better address the variability of iTBS effects on MEP amplitude, the proportion of iTBS-induced AH excitability increase and UH excitability decrease are compared between groups, by means of Chi-Square test. The correlation between the clinical status and the neurophysiological data was performed using Pearson's correlation coefficients and partial correlations. The statistical distribution of all the variables is tested by means of Kolmogorov and Smirnov test. The significance level is set to 0.05. Descriptive statistic is reported as Mean ± Standard Error of the Mean (SEM).

TABLE 1 | Summary of the gender-related difference in basal and iTBS-induced changes.

DIFFERENT POST-STROKE FUNCTIONAL CHANGES	
On MTs	Males have higher AMT in the AH than in the UH ($p = 0.004$) Females have higher AMT in the UH than in the AH ($p = 0.056$)
On inter hemispheric balance	Males have higher inter-hemispheric asymmetry than females (LI_{AMT} $p = 0.033$ and LI_{RMT} $p = 0.042$) Males and Females have opposite inter-hemispheric balance (positive LI in males and negative in females)
DIFFERENT PROPENSITY TO UNDERGO PLASTIC CHANGES	
Pooling both hemispheres together	Females undergo a cumulative (pooling AH and UH together) increase of brain excitability, while males a decrease of it
Rate of iTBS effect	In the female group there is a higher rate of increase of AH excitability than in the male group ($p = 0.022$)

RESULTS

The average NIHSS at onset was 5.21 ± 0.413. Gender groups were matched regarding age (F age = 69.87 ± 3.16; M age = 72.55 ± 2.50, $p = 0.505$) and clinical status (NIHSS) at stroke onset (F NIHSS = 4.73 ± 0.66; M NIHSS = 5.58 ± 0.53, $p = 0.317$). Groups were also matched regarding the percentage of patients with different lesion site (subcortical or cortical–subcortical), a pure cortical lesion was present in 3 out of 15 female patients (20%) and in 4 out of 20 male patients (20%). This is relevant because functional changes in cortical excitability may be influenced by stroke location and distribution (Ameli et al., 2009). Lesion size, as evaluated with the ASPECT score, was comparable in the two groups ($p > 0.200$) and resulted 7.47 ± 0.47 for females and 7.40 ± 0.36 for males. In a subgroup of 7 females and 8 males we measured the stroke volume using the procedure described in Di Lazzaro et al. (2010). The median stroke volume was 1463 mmc (range 653–26,514) for females and 2614 mmc (range 576–30,102) for males and it was not significantly different between males and females (Mann-Whitney *U*-test=27.000, $p = 0.955$).

Baseline Brain Excitability Measures

Table 1 summarizes the gender-related difference in basal and iTBS-induced changes. Considering all patients together, UH excitability is higher than AH excitability, as probed by RMT, AMT and MEP amplitude [Factor *Hemisphere*: $p = 0.001$, $p = 0.001$, $p < 0.001$, respectively. **Figure 1** Upper Panel]. The effect of *Gender* on hemispheric excitability asymmetry, revealed by the *Hemisphere by Gender* interaction, is significant for AMT [$F_{(1, 32)} = 4.449$, $p = 0.043$], with a trend toward significance for RMT [$F_{(1, 32)} = 3.412$, $p = 0.074$], not significant for MEP amplitude [$F_{(1, 32)} = 0.511$, $p = 0.480$]. The *post-hoc* analysis reveals that AMT is significantly lower over the UH only in the *Male* group ($p = 0.004$), not in the *Female* group ($p > 0.200$) [**Figure 1** Lower Panel and **Tables 1, 2**].

Laterality Index

Both LI_{AMT} and LI_{RMT} show significant higher hemispheric asymmetry for the *Male* group ($p = 0.042$), while no significant difference has been found for LI_{MEP} (**Figure 2** and **Table 2**).

iTBS Effects

Our analysis shows an *iTBS* by *Hemisphere* interaction [RMT $p = 0.025$; AMT $p = 0.001$; MEP $p < 0.001$], suggesting that there is an effect of iTBS characterized by an excitability increase over the AH and an excitability decrease over the UH (**Figure 3**, **Tables 2, 3**). RMT shows that *Gender* influences *iTBS* effects on brain excitability [*iTBS* by *Gender* interaction $F_{(1, 32)} = 7.860$, $p = 0.009$]. This effect does not differ depending on the *Hemisphere* [*iTBS* by *Gender* by *Hemisphere* interaction $F_{(1, 32)} = 0.081$, $p = 0.777$]. The significant *iTBS* by *Gender* interaction is motivated by a cumulative (both hemispheres together) mild increase of brain excitability for *Females* and decrease of brain excitability for *Males* (**Figure 3**, **Tables 2, 3**). *Gender* does not significantly impact on the effect of *iTBS* on AMT and MEP.

Laterality Index

iTBS reduces the LI (LI_{RMT}, LI_{AMT}, LI_{MEP}) regardless of the *Gender* [*iTBS* by *Gender* interaction: LI_{RMT} $F_{(1, 33)} = 0.030$, $p = 0.864$; LI_{AMT} $F_{(1, 33)} = 0.223$, $p = 0.640$; LI_{MEP} $F_{(1, 33)} = 0.001$, $p = 0.979$]. More in details:

- LI_{RMT}: *Pre-iTBS* 0.04 ± 0.04; *Post-iTBS* 0.03 ± 0.04, $p = 0.022$;
- LI_{AMT}:*Pre-iTBS* 0.05 ± 0.04; *Post-iTBS* 0.03 ± 0.04, $p = 0.001$; after *iTBS* LI_{AMT} remains negative for *Female* and positive for

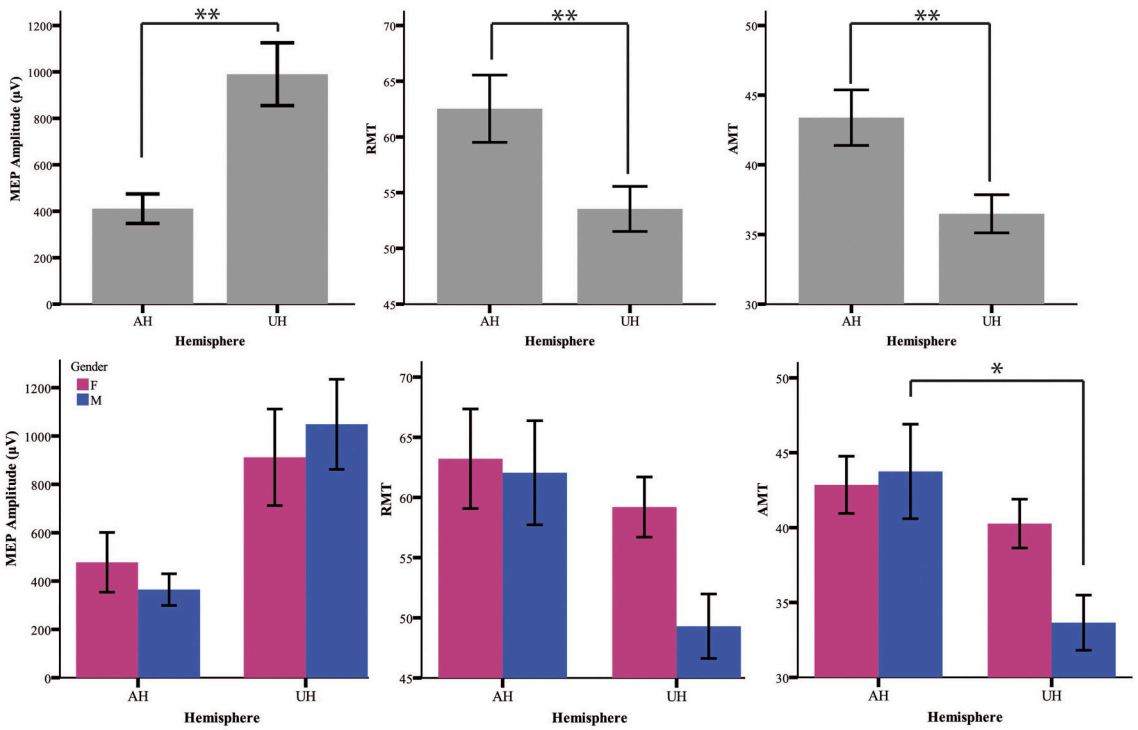


FIGURE 1 | Upper Panel: Baseline excitability measures (MEP, RMT, AMT) in the different hemispheres pooling genders together. The statistical significance refers to the factor Hemisphere of the ANOVA model. **Lower Panel:** baseline value of excitability measures divided for gender (female = pink and male = blue). *$p < 0.05$; **$p < 0.001$ Error Bars = SER.

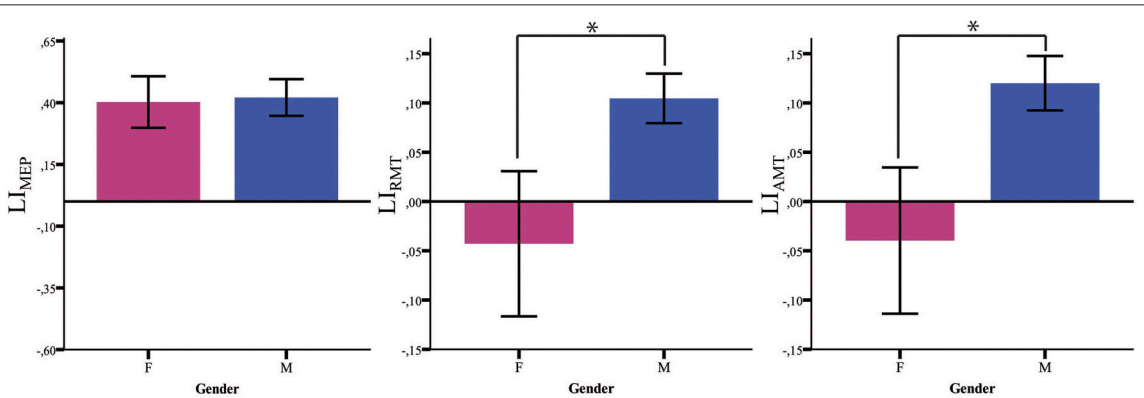


FIGURE 2 | Baseline LI measures. LI baseline measures (MEP, RMT, AMT) in males (blue) and females (pink). *$p < 0.05$; Error Bars = SER.

Male [factor *Gender*: $F_{(1, 33)} = 4.842$, $p = 0.035$; *Post-iTBS* LI_{AMT} *Male* = 0.092 ± 0.028; *Female* = -0.060 ± 00.4];

- LI_{MEP}: *Pre-iTBS* 0.41 ± 0.06; *Post-iTBS* 0.30 ± 0.08; $p = 0.014$.

To better characterize iTBS effects, we also looked at the rate of subjects reporting iTBS-related effects in the two groups. The rate of iTBS-induced AH excitability increase is higher in *Females* (14 out of 15, 93%) than in *Males* (11 out of 20, 55%; Chi-square $p = 0.022$). Even if the comparison does not reach a significant level (Chi-square $p = 0.266$), the rate of iTBS-induced UH excitability decrease is higher in *Males* (16 out of 20, 80%) than in *Females* (9 out of 15 female, 60%).

The individual level of brain excitability and iTBS effects are reported in **Supplementary Figure 1**.

Relationship between Clinical Condition and Neurophysiological Measures

Pooling together all patients, NIHSS showed a significant correlation with the AMT LI both before iTBS (AMT LI pre-iTBS Pearson's $R = 0.328$, $p = 0.029$) and after iTBS (AMT LI post-iTBS Person's $R = 0.327$, $p = 0.030$). This correlation pattern did not survive the correction by sex, suggesting that sex might in fact play also a role in the relationship between measure of

TABLE 2 | Excitability measures for both AH and UH under baseline conditions and after iTBS.

		RMT						AMT						MEP					
		PRE			POST			PRE			POST			PRE			POST		
		AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI	AH	UH	LI
F	Mean	63.21	59.20	−0.04	61.71	59.33	−0.06	42.86	40.27	−0.04	41.57	40.60	−0.06	477.42	911.95	0.40	634.75	792.27	0.29
	SER	4.14	2.50	0.07	4.35	2.49	0.07	1.91	1.63	0.07	1.94	1.63	0.07	123.64	199.45	0.10	170.69	160.87	0.12
M	Mean	62.05	49.30	0.10	62.05	50.70	0.09	43.75	33.65	0.12	42.90	34.70	0.09	364.51	1048.34	0.42	368.69	807.60	0.31
	SER	4.32	2.68	0.03	4.17	2.63	0.03	3.16	1.84	0.03	3.14	1.68	0.03	65.43	186.41	0.07	66.99	130.89	0.11
M+F	Mean	62.53	53.54	0.04	61.91	54.40	0.03	43.38	36.49	0.05	42.35	37.23	0.03	411.00	989.89	0.41	478.25	801.03	0.30
	SER	3.02	2.02	0.04	2.99	1.96	0.04	1.99	1.37	0.04	1.99	1.27	0.04	63.43	135.09	0.06	82.25	100.20	0.08

RMT, resting motor threshold, expressed as percentage intensity of maximal stimulator output; AMT, active motor threshold expressed as percentage intensity of stimulator output; MEP, amplitude of motor evoked potentials at 120% of RMT (μ V).

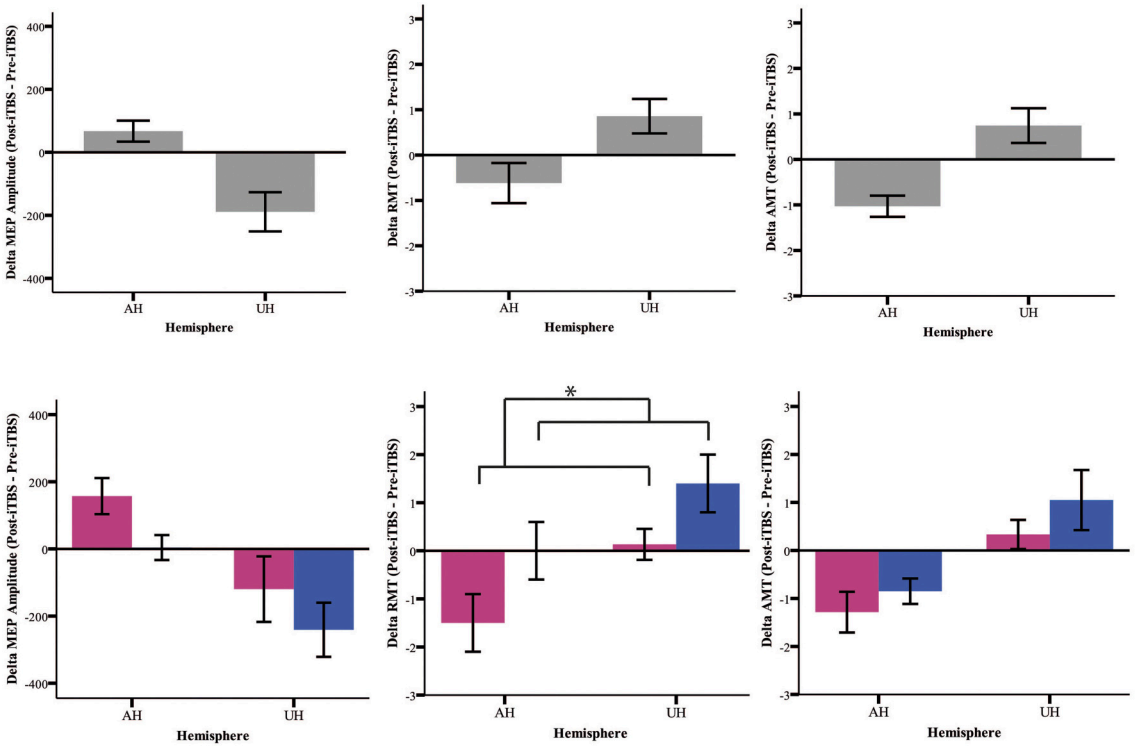


FIGURE 3 | Upper Panel: iTBS effects on excitability measures (MEP, RMT, AMT) on both groups together expressed as Post-iTBS—Pre-iTBS excitability change. Lower Panel: iTBS effects on both hemispheres (AH and UH) and groups (female = pink and male = blue). iTBS effect on RMT is gender dependent [iTBS by Gender interaction $F_{(1, 32)} = 7.860$, $^*p = 0.009$].

cortical excitability and clinical status. The subsequent analysis performed independently on the two groups showed: (i) absence of correlation in the male group (ii) strong correlation in the female group. More in details, in this subgroup we confirmed the relationship between LI and NIHSS (AMT LI Pre-iTBS Pearson's $R = 0.500$, $p = 0.029$, AMT LI Post-iTBS Pearson's $R = 0.530$, $p = 0.021$). In other words, a worst clinical condition is associated to stronger interhemispheric unbalance toward higher excitability of the UH. Additionally, both before and after iTBS higher NIHSS scores were associated to higher AH AMT (Person's $R = 0.601$, $p = 0.011$ before iTBS; Person's $R = 0.649$, $p = 0.006$, after iTBS).

DISCUSSION

Several studies have reported an asymmetry in the excitability of the AH and UH to non-invasive brain stimulation after stroke (Liepert et al., 2000; Manganotti et al., 2002; Shimizu et al., 2002; Cicinelli et al., 2003; Di Lazzaro et al., 2010, 2014). This is the first study evaluating the effects of gender on the changes in human

TABLE 3 | Summary of the ANOVA Mixed Model on iTBS effects on brain excitability measures.

	RMT		AMT		MEP	
Source	$F_{(1, 32)}$	p	$F_{(1, 32)}$	p	$F_{(1, 32)}$	p
Hemisphere	9.462	0.004	8.779	0.006	12.778	0.001
iTBS	0.040	0.843	0.493	0.488	2.284	0.140
Gender	1.192	0.283	0.854	0.362	0.238	0.629
Hemisphere * iTBS	5.558	0.025	13.761	0.001	17.568	0.000
Hemisphere * Gender	3.465	0.072	4.316	0.046	0.834	0.368
iTBS * Gender	7.860	0.009	1.395	0.246	2.575	0.118
Hemisphere * iTBS * Gender	0.081	0.777	0.013	0.909	0.226	0.638

brain excitability observed in the acute phase of stroke. We found sex differences in the functional changes that take place in AH and UH. The AH showed a lower excitability than the UH in both men and women, but males have higher excitability in the UH (lower AMT) and higher inter-hemispheric asymmetry than females. At a group level, the excitability of the AH is always lower than of the UH. However, the study of the LI, which takes into consideration and normalizes subject by subject for the level of excitability of both hemispheres, reveals that males and females have opposite inter-hemispheric balance, with higher excitability of UH in males, *vice versa* in females.

The meaning of these findings is still uncertain; we can speculate that they might be correlated with the existence of gender-related differences in the organizational patterns of functional cortical connectivity between different brain areas. Several studies have demonstrated sex differences in the connectivity of the brain (Gong et al., 2011). The results of the analysis of the structural connectome of the human brain suggest that male brains are structured to facilitate intra-hemispheric cortical connectivity, while female brain displays higher strength of inter-hemispheric connectivity (Ingalhalikar et al., 2014). Thus, we can speculate that to facilitate within-hemisphere connectivity in males there is a higher level of inter-hemispheric inhibition and, in case of a mono-hemispheric brain lesion, a lower level of inter-hemispheric inhibition from AH to UH makes the UH hyper-excitable to transcranial stimulation.

When testing the effects of iTBS of the AH, a rTMS protocol capable of inducing LTP-like changes in the stimulated hemisphere, females undergo a cumulative (pooling AH and UH together) increase of brain excitability, while males a decrease of it. In the female group, there is a higher rate of increase of AH excitability than in the male group and a tendency for a lower rate of decrease of UH excitability. Again men showed a pronounced effect in UH with a partial correction of the hyper-excitability associated with a comparable increase in the excitability of AH. In women, instead, we observed a more pronounced increase in the excitability of the AH that was associated with a slight increase in excitability, and not a suppression, of UH.

This is a further demonstration that the establishment of inter-hemispheric imbalance after stroke should not be given for grant, rather it is strictly dependent on patient's individuality. We recently showed that also the haplotype of Brain-Derived Neurotrophic Factor (BDNF) gene has a profound influence on the inter-hemispheric imbalance in cortical excitability (Di Lazzaro et al., 2015). Indeed, the presence of the Val66Met BDNF polymorphism is associated with a nine-fold weaker inter-hemispheric imbalance in cortical excitability as evaluated by comparing the RMT of the AH and the UH.

Is the imbalance in cortical excitability deleterious for recovery? As we suggested for BDNF polymorphism (Di Pino et al., 2014a, 2016), the hyper-excitability of UH might contribute to, or might interfere with, recovery depending on the level of impairment of AH. UH over-activity, observed more commonly in males, might interfere with paretic limb function in patients with less severe damage, while it might have a compensatory role in severely affected patients (Bradnam et al., 2013; Di Pino et al., 2014a). On the other hand, the limited imbalance between the hemispheres in females might represent an advantage in case of limited damage, facilitating the recovery of AH in the absence of a potentially deleterious interference from the UH. However, in more severe lesions the compensatory role of UH seems to be prevalent, and this might be limited in females by their lower UH hyper-excitability. Overall, this would reduce the impact of mild stroke in females and of more severe stroke in males, in line with a lower incidence, but a poorer prognosis of stroke in females and *vice versa* in males (Gibson, 2013).

Moreover, the differential functional changes that take place in the AH and UH in males and females might be adaptive or maladaptive depending on the degree of corticospinal tract damage. Gender influences many aspects of stroke including risk/incidence, diagnosis, symptoms, treatment and outcomes (Reeves et al., 2008; Appelros et al., 2009; Haast et al., 2012; Gibson, 2013); our study strongly contributes to highlight that it also influences the brain response to the damage.

Those considerations warrant further studies aimed at characterizing the interactions that gender and inter-hemispheric imbalance have on recovery.

In conclusion, our study suggests the existence of gender-dependent differences in the functional brain changes that take place after human stroke, in that it seems that male brain has greater asymmetry than the female's. This perfectly fits the recently advanced hypothesis of a higher strength of inter-hemispheric connection owned by the female's healthy brain (Ingalhalikar et al., 2014).

Male and female individuality could conceivably arise from a complex interaction of some sort of gender-specific base with a mosaic of environmental factors. Stroke and its strong plasticity-inducing potential are, in our opinion, optimal examples of events that might unveil and amplify those gender-specific differences, that otherwise might remain unrevealed. Our findings should suggest to be cautious in designing stroke studies, especially since sex differences in stroke that might affect recovery and brain plasticity probably result from a combination of factors, including elements intrinsic to the sex chromosomes, as well as the effects of sex hormone exposure, and not less important cultural and social factors (Cox et al.,

2006; Vagnerova et al., 2008; Cesaroni et al., 2009; Liu et al., 2009; Gibson, 2013). For instance, animal model are often used to provide a better understanding of stroke and of specific brain recovery patterns (Alkayed et al., 1998; Bacigaluppi et al., 2010). However, the majority of experimental stroke studies keeps focusing on using only male animals (Fisher et al., 2009; Gibson, 2013), despite the Stroke Therapy Academy Industry Roundtable (STAIR) recommends that neuro-protective studies should be performed in both male and female rodents (Fisher et al., 2009). Moreover, the impacts of gender on the weight of age and hormone-related risk factors needs to be clarified, since epidemiological studies document an association between the female gender during the premenopausal years and a reduced risk of stroke addressing hormonal factors as potential protective treatments (Gibson et al., 2006, 2009; Suzuki et al., 2009; Liu and Yang, 2013). We envisage that a greater experimental plan and the understanding of the mechanisms underlying gender-related differences in stroke and responsiveness to neuroprotection and brain plasticity will lead to more appropriate treatment strategies for patients of both sexes.

AUTHOR CONTRIBUTIONS

VD designed the study and wrote the manuscript. GP was in charge of statistic and revised the manuscript, GDP, FR, FL, LF, and FC participated to patients' recruitment, neuromodulation and revised the manuscript. All Authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnins.2016.00010

Supplementary Figure 1 | Scatterplot graph of individual brain excitability levels (Post-iTBS over Pre-iTBS) for RTM, AMT and MEP Amplitude for the Affected (AH) and Unaffected (UH) hemispheres.

REFERENCES

Alkayed, N. J., Harukuni, I., Kimes, A. S., London, E. D., Traystman, R. J., and Hurn, P. D. (1998). Gender-linked brain injury in experimental stroke. *Stroke* 29, 159–165. Discussion: 166. doi: 10.1161/01.STR.29.1.159

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

Appelros, P., Stegmayr, B., and Terént, A. (2009). Sex differences in stroke epidemiology: a systematic review. *Stroke* 40, 1082–1090. doi: 10.1161/STROKEAHA.108.540781

Bacigaluppi, M., Comi, G., and Hermann, D. M. (2010). Animal models of ischemic stroke. Part one: modeling risk factors. *Open Neurol. J.* 4, 26–33. doi: 10.2174/1874205x01004010026

Barber, P. A., Demchuk, A. M., Zhang, J., and Buchan, A. M. (2000). Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. *Lancet* 355, 1670–1674. doi: 10.1016/S0140-6736(00)02237-6

Baron-Cohen, S., Knickmeyer, R. C., and Belmonte, M. K. (2005). Sex differences in the brain: implications for explaining autism. *Science* 310, 819–823. doi: 10.1126/science.1115455

Bradnam, L. V., Stinear, C. M., and Byblow, W. D. (2013). Ipsilateral motor pathways after stroke: implications for non-invasive brain stimulation. *Front. Hum. Neurosci.* 7:184. doi: 10.3389/fnhum.2013.00184

Cesaroni, G., Agabiti, N., Forastiere, F., and Perucci, C. A. (2009). Socioeconomic differences in stroke incidence and prognosis under a universal healthcare system. *Stroke* 40, 2812–2819. doi: 10.1161/STROKEAHA.108.542944

Cicinelli, P., Pasqualetti, P., Zaccagnini, M., Traversa, R., Oliveri, M., and Rossini, P. M. (2003). interhemispheric asymmetries of motor cortex excitability in the postacute stroke stage a paired-pulse transcranial magnetic stimulation study. *Stroke* 34, 2653–2658. doi: 10.1161/01.STR.0000092122.96722.72

Cox, A. M., Mckevitt, C., Rudd, A. G., and Wolfe, C. D. (2006). Socioeconomic status and stroke. *Lancet Neurol.* 5, 181–188. doi: 10.1016/S1474-4422(06)70351-9

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

Di Lazzaro, V., Dileone, M., Capone, F., Pellegrino, G., Ranieri, F., Musumeci, G., et al. (2014). Immediate and late modulation of interhemipheric imbalance with bilateral transcranial direct current stimulation in acute stroke. *Brain Stimul.* 7, 841–848. doi: 10.1016/j.brs.2014.10.001

Di Lazzaro, V., Pellegrino, G., Di Pino, G., Corbetto, M., Ranieri, F., Brunelli, N., et al. (2015). Val66Met BDNF gene polymorphism influences human motor cortex plasticity in acute stroke. *Brain Stimul.* 8, 92–96. doi: 10.1016/j.brs.2014.08.006

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Oliviero, A., Mazzone, P., et al. (2008). The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J. Physiol.* 586, 3871–3879. doi: 10.1113/jphysiol.2008.152736

Di Lazzaro, V., Profice, P., Pilato, F., Capone, F., Ranieri, F., Pasqualetti, P., et al. (2010). Motor cortex plasticity predicts recovery in acute stroke. *Cereb. Cortex* 20, 1523–1528. doi: 10.1093/cercor/bhp216

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., et al. (2014a). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat. Rev. Neurol.* 10, 597–608. doi: 10.1038/nrneurol.2014.162

Di Pino, G., Pellegrino, G., Capone, F., Assenza, G., Florio, L., Falato, E., et al. (2016). Val66Met BDNF polymorphism implies a different way to recover from stroke rather than a worse overall recoverability. *Neurorehabil. Neural Repair* 30, 3–8. doi: 10.1177/1545968315583721

Di Pino, G., Pellegrino, G., Capone, F., and Di Lazzaro, V. (2014b). Human cerebral cortex metaplasticity and stroke recovery. *Austin J. Cerebrovasc. Dis. Stroke* 1:1001.

Draca, S. (2010). Gender-specific functional cerebral asymmetries and unilateral cerebral lesion sequelae. *Rev. Neurosci.* 21, 421–425. doi: 10.1515/REVNEURO.2010.21.6.421

Fisher, M., Feuerstein, G., Howells, D. W., Hurn, P. D., Kent, T. A., Savitz, S. I., et al. (2009). Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke* 40, 2244–2250. doi: 10.1161/STROKEAHA.108.541128

Gibson, C. L. (2013). Cerebral ischemic stroke: is gender important? *J. Cereb. Blood Flow Metab.* 33, 1355–1361. doi: 10.1038/jcbfm.2013.102

Gibson, C. L., Coomber, B., and Rathbone, J. (2009). Is progesterone a candidate neuroprotective factor for treatment following ischemic stroke? *Neuroscientist* 15, 324–332. doi: 10.1177/1073858409333069

Gibson, C. L., Gray, L. J., Murphy, S. P., and Bath, P. M. (2006). Estrogens and experimental ischemic stroke: a systematic review. *J. Cereb. Blood Flow Metabolism* 26, 1103–1113. doi: 10.1038/sj.jcbfm.9600270

Gong, G., He, Y., and Evans, A. C. (2011). Brain connectivity: gender makes a difference. *Neuroscientist* 17, 575–591. doi: 10.1177/1073858410386492

Haast, R. A., Gustafson, D. R., and Kiliaan, A. J. (2012). Sex differences in stroke. *J. Cereb. Blood Flow Metabolism* 32, 2100–2107. doi: 10.1038/jcbfm.2012.141

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

Ingalhalikar, M., Smith, A., Parker, D., Satterthwaite, T. D., Elliott, M. A., Ruparel, K., et al. (2014). Sex differences in the structural connectome of the human brain. *Proc. Natl. Acad. Sci. U.S.A.* 111, 823–828. doi: 10.1073/pnas.1316909110

Liepert, J., Hamzei, F., and Weiller, C. (2000). Motor cortex disinhibition of the unaffected hemisphere after acute stroke. *Muscle Nerve* 23, 1761–1763. doi: 10.1002/1097-4598(200011)23:11<1761::AID-MUS14>3.0.CO;2-M

Lisabeth, L. D., Reeves, M. J., Baek, J., Skolarus, L. E., Brown, D. L., Zahuranec, D. B., et al. (2015). Factors influencing sex differences in poststroke functional outcome. *Stroke* 46, 860–863. doi: 10.1161/STROKEAHA.114.007985

Liu, M., Dziennis, S., Hurn, P. D., and Alkayed, N. J. (2009). Mechanisms of gender-linked ischemic brain injury. *Restor. Neurol. Neurosci.* 27, 163–179. doi: 10.3233/RNN-2009-0467

Liu, R., and Yang, S. H. (2013). Window of opportunity: estrogen as a treatment for ischemic stroke. *Brain Res.* 1514, 83–90. doi: 10.1016/j.brainres.2013.01.023

Manganotti, P., Patuzzo, S., Cortese, F., Palermo, A., Smania, N., and Fiaschi, A. (2002). Motor disinhibition in affected and unaffected hemisphere in the early period of recovery after stroke. *Clin. Neurophysiol.* 113, 936–943. doi: 10.1016/S1388-2457(02)00062-7

Narr, K., Thompson, P., Sharma, T., Moussai, J., Zoumalan, C., Rayman, J., et al. (2001). Three-dimensional mapping of gyral shape and cortical surface asymmetries in schizophrenia: gender effects. *Am. J. Psychiatry* 158, 244–255. doi: 10.1176/appi.ajp.158.2.244

Proust-Lima, C., Amieva, H., Letenneur, L., Orgogozo, J. M., Jacqmin-Gadda, H., and Dartigues, J. F. (2008). Gender and education impact on brain aging: a general cognitive factor approach. *Psychol. Aging* 23, 608–620. doi: 10.1037/a0012838

Reeves, M. J., Bushnell, C. D., Howard, G., Gargano, J. W., Duncan, P. W., Lynch, G., et al. (2008). Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurol.* 7, 915–926. doi: 10.1016/S1474-4422(08)70193-5

Rossini, P. M. (2014). 1994-2014 Twenty years from the first guidelines for electrical and magnetic stimulation of brain, spinal cord and spinal roots. *Clin. Neurophysiol.* 125, 865–866. doi: 10.1016/j.clinph.2014.01.004

Rossini, P. M., and Johnston, C. S. (2005). Facilitating acute stroke recovery with magnetic fields? *Neurology* 65, 353–354. doi: 10.1212/01.wnl.0000173428.48059.c7

Shimizu, T., Hosaki, A., Hino, T., Sato, M., Komori, T., Hirai, S., et al. (2002). Motor cortical disinhibition in the unaffected hemisphere after unilateral cortical stroke. *Brain* 125, 1896–1907. doi: 10.1093/brain/awf183

Stefan, K., Gentner, R., Zeller, D., Dang, S., and Classen, J. (2008). Theta-burst stimulation: remote physiological and local behavioral after-effects. *Neuroimage* 40, 265–274. doi: 10.1016/j.neuroimage.2007.11.037

Suppa, A., Ortu, E., Zafar, N., Deriu, F., Paulus, W., Berardelli, A., et al. (2008). Theta burst stimulation induces after−effects on contralateral primary motor cortex excitability in humans. *J. Physiol.* 586, 4489–4500. doi: 10.1113/jphysiol.2008.156596

Suzuki, S., Brown, C. M., and Wise, P. M. (2009). Neuroprotective effects of estrogens following ischemic stroke. *Front. Neuroendocrinol.* 30, 201–211. doi: 10.1016/j.yfrne.2009.04.007

Tomasi, D., and Volkow, N. D. (2012). Laterality patterns of brain functional connectivity: gender effects. *Cereb. Cortex* 22, 1455–1462. doi: 10.1093/cercor/bhr230

Vagnerova, K., Koerner, I. P., and Hurn, P. D. (2008). Gender and the injured brain. *Anesth. Analg.* 107, 201–214. doi: 10.1213/ane.0b013e31817326a5

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 Neuroscience

ORIGINAL RESEARCH
published: 08 March 2016
doi: 10.3389/fnins.2016.00079

Influence of Corticospinal Tracts from Higher Order Motor Cortices on Recruitment Curve Properties in Stroke

*Kelsey A. Potter-Baker[1], Nicole M. Varnerin[1], David A. Cunningham[1,2], Sarah M. Roelle[1], Vishwanath Sankarasubramanian[1], Corin E. Bonnett[1], Andre G. Machado[3], Adriana B. Conforto[4,5], Ken Sakaie[6] and Ela B. Plow[1,3,7]**

[1] Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA, [2] School of Biomedical Sciences, Department of Neuroscience, Kent State University, Kent, OH, USA, [3] Center for Neurological Restoration, Neurosurgery, Neurological Institute, Cleveland Clinic Foundation, Cleveland ,OH, USA, [4] Neurology Clinical Division, Neurology Department, Clinics Hospital, São Paulo University, São Paulo, Brazil, [5] Hospital Israelita Albert Einstein, São Paulo, Brazil, [6] Department of Diagnostic Radiology, Imaging Institute, Cleveland Clinic Foundation, Cleveland, OH, USA, [7] Department of Physical Medicine and Rehabilitation, Neurological Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

OPEN ACCESS

Edited by:
Ignacio Obeso, Centro Integral en Neurociencias AC, HM Hospitales, Spain

Reviewed by:
Michele Dileone, Centro Integral en Neurociencias AC, HM Hospitales, Spain
Manuel Gomez-Choco, Imperial College Healthcare Trust, UK

***Correspondence:**
Ela B. Plow
plowe2@ccf.org

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

Received: *09 December 2015*
Accepted: *18 February 2016*
Published: *08 March 2016*

Citation:
Potter-Baker KA, Varnerin NM, Cunningham DA, Roelle SM, Sankarasubramanian V, Bonnett CE, Machado AG, Conforto AB, Sakaie K and Plow EB (2016) Influence of Corticospinal Tracts from Higher Order Motor Cortices on Recruitment Curve Properties in Stroke. Front. Neurosci. 10:79. doi: 10.3389/fnins.2016.00079

Background: Recruitment curves (RCs) acquired using transcranial magnetic stimulation are commonly used in stroke to study physiologic functioning of corticospinal tracts (CST) from M1. However, it is unclear whether CSTs from higher motor cortices contribute as well.

Objective: To explore whether integrity of CST from higher motor areas, besides M1, relates to CST functioning captured using RCs.

Methods: RCs were acquired for a paretic hand muscle in patients with chronic stroke. Metrics describing gain and overall output of CST were collected. CST integrity was defined by diffusion tensor imaging. For CST emerging from M1 and higher motor areas, integrity (fractional anisotropy) was evaluated in the region of the posterior limb of the internal capsule, the length of CST and in the region of the stroke lesion.

Results: We found that output and gain of RC was related to integrity along the length of CST emerging from higher motor cortices but not the M1.

Conclusions: Our results suggest that RC parameters in chronic stroke infer function primarily of CST descending from the higher motor areas but not M1. RCs may thus serve as a simple, in-expensive means to assess re-mapping of alternate areas that is generally studied with resource-intensive neuroimaging in stroke.

Keywords: stimulus-response curve, recruitment curve, TMS, DTI, stroke

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a popular non-invasive technique to assess physiology of corticospinal tracts (CST; Di Lazzaro, 2004). TMS is able to gauge such physiology based on the principle of electromagnetic induction. Specifically, rapidly alternating currents form the basis for TMS. These are created by discharging a large capacitor into an insulated coiled wire. The

produced currents then generate magnetic fields over the scalp and skull. Electrical currents are induced, which pass unimpeded to excite superficial areas like the primary motor cortices (M1). In M1, induced currents trigger volleys along descending CST pathways that produce motor evoked potentials (MEP) in contralateral muscles (Di Lazzaro, 2004). The resultant MEP amplitude is believed to reflect output of the CST pathways. With increasing TMS intensities, MEP amplitudes typically increase. By applying a range of increasing intensities, one can study incremental gains in MEPs that are plotted commonly as a stimulus-response or a recruitment curve. The slope of the curve and sum of MEP amplitudes signify gain and output of the descending CST (Devanne et al., 1997; Ridding and Rothwell, 1997; Boroojerdi et al., 2001; Monti et al., 2001; Ward et al., 2006).

TMS techniques are particularly relevant in stroke. This is because TMS can index function and recovery of the paretic upper limb by evaluating CST damage that is typical of stroke affecting the territory of the middle cerebral artery (Bogousslavsky and Regli, 1990; Johansen-Berg et al., 2002; Buffon et al., 2005). For example, several groups have established that the mere presence or absence of MEPs in paretic muscles can inform about clinical function (Ward et al., 2006; Stinear et al., 2007, 2012; Ward, 2011; Levy et al., 2016). Beyond the binary outcome, recruitment curves offer several additional advantages. By definition, recruitment curves assay MEPs at a range of increasing TMS intensities, and as such, illustrate a graded profile of CST function (Thickbroom et al., 2002). As a result, increases or decreases in slope or gain of the recruitment curve can signify recovery more closely than binary outcomes signaling the presence or absence of MEPs. For example, numerous studies have shown that decreases in recruitment curve parameters are indicative of more substantial CST damage, functional impairment, or poor recovery potential in patients with stroke (Devanne et al., 1997; Carroll et al., 2001; Liepert et al., 2005; Talelli et al., 2006; Ward et al., 2006; Lindberg et al., 2007; Lotze et al., 2012; Cunningham et al., 2014). In fact, graded increases in the slope of the recruitment curve have been associated with graded functional gains in recovery (Hummel et al., 2005) suggesting that metrics that are not binary, but rather based on an interval scale may serve as an effective monitor for rehabilitation-related recovery.

Recruitment curves are especially popular in stroke because they are believed to reflect CST gain and output from the region most linked to motor function, despite inherent damage, the primary motor cortex (M1; Devanne et al., 1997). However, given that other secondary motor cortices contribute to paretic hand function and recovery in stroke, it is possible that recruitment curves may also represent functioning of CST from higher motor areas beyond M1. For example, higher motor areas like the supplementary motor area (SMA) and premotor cortex (PMC) can support paretic hand function and recovery via re-mapping and plasticity changes proportional to the level of damage to CST from M1 (Weiller et al., 1992; Fries et al., 1993; Seitz et al., 1998; Liu and Rouiller, 1999; Fridman et al., 2004; Dancause et al., 2005; Ward et al., 2006, 2007; Bhatt et al., 2007; Takeuchi et al., 2007; Calautti et al., 2010; Zeiler et al., 2013; Plow et al., 2014). Indeed, SMA and PMC can offer alternate CST to the paretic upper limb, contributing in the range of 20–40% of entire CST (Dum and Strick, 1991; Schulz et al., 2012).

Understanding if there is a role of CST from secondary motor areas on recruitment curve properties is critical. TMS is already relevant for neurorehabilitation since it is simple and in-expensive. Therefore, by gaining this understanding, we could realize if using TMS generated recruitment curves could accurately and in-expensively interpret which areas re-map and contribute to overall CST function during recovery. For this reason, here we explored whether integrity of CST from PMC and SMA, besides M1, related to CST function as captured by recruitment curves in patients with chronic stroke. CST integrity was measured using diffusion tensor imaging (DTI) [fractional anisotropy (FA)] due to its long-standing use in neurology and generally accepted accuracy (Chenevert et al., 1990; Alexander et al., 2007; Soares et al., 2013). We argued that if recruitment curve properties were to reflect integrity of CST from higher motor cortices, then any increase in gain/output of the recruitment curve would signify their remapping in recovery. As such, our finding would create an opportunity to target PMC/SMA with techniques like cortical stimulation that are believed to boost recovery by boosting functioning of CST recovery (Fregni and Pascual-Leone, 2007). In addition, our results could help show that recruitment curves may serve as a simple, in-expensive means to assess function from areas generally studied with more resource-intensive structural and functional imaging in patients with stroke.

To our knowledge, only a pilot study by Lindberg et al. has directly investigated the relationship between CST integrity captured using DTI and recruitment curves in stroke. Within their study, Lindberg and colleagues found that a greater loss of integrity at the level of the cerebral peduncle was correlated with a reduced recruitment curve slope (Lindberg et al., 2007). However, because recent research has suggested that DTI indices describing CST integrity vary with extent and location of the lesion, it is critical to capture integrity not just in a single region but across several regions, and along the length of CST (Liepert et al., 2005; Zhu et al., 2010; Lindenberg et al., 2012; Schulz et al., 2012). Therefore, here, we chose to assess CST integrity at different regions along the path of CST. We captured FA at the most commonly used regions for analysis—the posterior limb of internal capsule (PLIC) and mean along the length of CST (Stinear et al., 2007; Allendorfer et al., 2012; Lindenberg et al., 2012). We also captured CST integrity in the region of the stroke lesion. We aimed to learn whether CST integrity at a specific region- PLIC, lesion or the length of CST pathways-closely related to neurophysiologic measurement of CST function described using the recruitment curve. We postulated that by identifying, which regions of CST most contribute to CST function, it would become possible to use recruitment curves as means to understand lesion characteristics, lesion load, and accordingly derive prognosis.

Abbreviations: TMS, Transcranial Magnetic Stimulation; DWI, Diffusion Weighted Imaging; DTI, Diffusion Tensor Imaging; CST, Corticospinal Tract; M1, Primary Motor Cortex; PMC, Premotor Cortex; SMA, Supplementary Motor Area; MEP, Motor evoked potential.

METHODS

Participants

Twelve patients who suffered a first-ever stroke, and eight healthy control subjects (68.3 ± 12.4 years) were enrolled (**Table 1**). Lesion locations for each patient are demonstrated in **Figure 1**. Patients were ≥21 years of age, in the chronic phase (>6 months) after a unilateral hemorrhagic or ischemic stroke and possessed at least a trace movement at the wrist, any of the fingers or the thumb of the paretic upper limb. Patients with contraindications to TMS, such as intracranial metallic implants, history of alcohol/substance abuse, seizures, neuro- and psycho-active medications lowering threshold for seizures, or cardiac pacemakers were excluded from the study, following published recommendations (Nitsche et al., 2008; Rossi et al., 2009; Shellock, 2014). All study procedures were approved by the Institutional Review Board (IRB) of the Cleveland Clinic Foundation. All participants provided written informed consent.

Study Design

A schematic of the study design is shown in **Figure 2** and study outcomes are diagrammed in **Table 2**. Clinical impairment was evaluated using the Upper Extremity Fugl Meyer score (UEFM), a commonly used scale that rates distal and proximal movements and upper limb coordination and reflexes on an ordinal scale (0–2) for a maximum score of 66 (Fugl-Meyer et al., 1975; Gladstone et al., 2002). Patients subsequently underwent magnetic resonance imaging (MRI) and TMS. T1-weighted MRI was used to quantify lesion locations and lesion volume. Diffusion-weighted/tensor imaging (DWI/DTI) was acquired to study CST integrity. In addition, patients underwent functional MRI (fMRI) during self-paced flexion-extension of the fingers of each hand. fMRI was acquired to provide for neuro-navigated TMS (Neggers et al., 2004) to help shorten testing and simplify thresholding needed to identify optimal site for TMS (details are provided in Cunningham et al., 2014). Specifically, given that substantial cortical remapping can occur, by employing fMRI-guidance, we aimed to add an additional layer of accuracy in our methodology (Lotze et al., 2006). Finally, fMRI-guided TMS was used to generate a stimulus-response or recruitment curve from the first dorsal interosseous (FDI) muscle.

Transcranial Magnetic Stimulation (TMS)

fMRI-guided, single pulse TMS (Magstim 200^2, Wales, U.K.) was delivered using a figure-of-eight coil (diameter 70 mm; Cunningham et al., 2014). Individuals were seated comfortably in a chair that allowed them to rest their forearms and hands on a flat surface. MEPs were recorded in the FDI muscle using surface electromyography. EMG was acquired using bipolar Ag/AgCl electrodes (8 mm diameter) positioned over the muscle belly, with a reference electrode placed on the lateral epicondyle. All EMG signals were recorded using PowerLab 4/25 set at ± 10 mV, subsequently band-pass filtered (10–2000 Hz) and then recorded at a sampling rate of 4000 Hz.

Cranial landmarks (nasion, left ear, right ear) of each individual were registered with respective sites in the MRI via Brainsight. The voxel with peak fMRI activation in the region of M1 (or an adjacent site when M1 was damaged) was chosen as the initial site to study with TMS. Although the fMRI peak was used as an initial guide, we identified the optimal site for TMS using careful thresholding. The optimal site (motor hotspot) was determined by applying TMS across a 10 mm-resolution grid. The motor hot spot was defined as the site that evoked MEPs of at least 50 μV peak-to-peak amplitude in the FDI muscle in three out of five trials at the lowest TMS intensity. The intensity used to elicit criterion MEPs in the resting state of the muscle was called the resting motor threshold (RMT) commonly expressed as % maximum stimulator output, or %MSO (Rossini and Rossi, 2007). We confirmed that resting-state EMG activity was ≤10 μV in all patients and controls. Recruitment curves were acquired in resting state at the hotspot. Ten serial MEPs were collected at gradient increases in TMS intensity ranging from 90 to 150% of the RMT. Intensities were presented in a randomized order.

MEPs could not be generated from the ipsilesional hemisphere in the resting state of paretic FDI in patients 1, 9, and 11; the absence of resting-state MEPs in not uncommon in patients with severe loss of corticospinal output (Harris-Love et al., 2011). These patients were thus excluded from analysis of ipsilesional recruitment curves though they were included in the analysis of contralesional recruitment curves (Talelli et al., 2006).

Compound muscle action potentials or M-waves were acquired to normalize MEP data. Maximum M-waves (M-MWaves) were elicited by applying a supramaximal electrical stimulus to the ulnar nerve at the wrist. Electrodes (Ultratrace 1690) were placed at the distal end of the ulnar nerve ∼2 inches away from the wrist. Increasing stimulus intensities were applied ranging from 1 to 15 mV for 0.5 to 1.5 ms until a maximum EMG response was noted.

Diffusion Tensor Imaging (DTI)

DTI was used to quantify CST integrity. Here, a High Angular Resolution Diffusion Weighted Imaging (HARDI-DWI) dataset was acquired on a Siemens 3T TIM Trio with 71 diffusion-weighting gradients ($b = 1000$ s/mm^2) and 8 image volumes ($b = 0$ s/mm^2) for a total scan time of 12 min. Each DWI scan allotted for whole brain coverage and 2-mm isotropic voxels (field of view: 256 × 256 mm, image matrix: 128 × 128, and 52 2-mm thick slices).

Data Analysis

Lesion Volume and Location

The anatomical location of each patient's stroke lesion was determined on T1-weighted MRI images by a trained neurologist (AM). Lesion volume was defined using MRIcro, a free, readily-downloadable software (http://www.mccauslandcenter.sc.edu/mricro/). Lesion volumes were found using manually drawn regions of interest (ROI) along a single plane of the stroke lesion (Zhu et al., 2010).

TMS Analysis

Recruitment curves were plotted as intensity (90–150% RMT) vs. MEP size noted as peak-to-peak amplitude (expressed as raw values in mV and %M-MWave; **Figure 2**) (Rossini and Rossi, 2007). For each intensity, MEP size was averaged across all 10

TABLE 1 | Patient Characteristics.

Patient	Age/Gender/ Handedness	Edinburgh Handedness Inventory	Time Since Stroke (Months)	Side of Paresis	Stroke Location	Stroke Volume (cm^3)	Stroke Subtype	Diabetic?	History of Smoking?	Medication Controlled Hypertension?	Gait Impaired?	Aphasia?	Stroke-Related PMC Damage	Stroke-Related M1 Damage	UEFM (Max 66)	% MSO at Ipsi MT (Max 100%)
1	68/F/R	60	32	L	Basal Ganglia	2.2	Ischemic	N	N	N	Y	N	N	N	35	95
2	66/M/R	100	19	L	Cortical	9.4	Ischemic	N	N	Y	N	N	Y	Y	50	73
3	58/F/L	−40	24	L	Caudate Nucleus, Basal Ganglia	2.8	Ischemic	N	Y	Y	Y	N	N	N	54	76
4	69/M/R	100	23	R	Thalamus, Internal Capsule	1.5	Hemorrhagic	Y	Y	Y	Y	Y	Y	N	46	40
5	54/M/R	70	29	R	Caudate Nucleus, Basal Ganglia	2.4	Ischemic	N	N	Y	Y	Y	N	N	44	44
6	72/M/R	100	84	L	Pontine, Mesencephalus	0.01	Ischemic	Y	N	Y	Y	Y	N	N	59	40
7	55/F/R	80	48	R	Thalamus, Internal Capsule	1.8	Hemorrhagic	N	N	Y	Y	N	N	N	47	55
8	59/F/R	100	23	L	Temporal lobe, Basal Ganglia, Caudate Nucleus	80.8	Ischemic	N	N	N	N	Y	N	N	51	46
9	76/M/R	45.45	24	R	Basal Ganglia, Internal Capsule, Corona Radiata	0.9	Ischemic	N	Y	Y	Y	N	Y	N	15	100
10	50/M/R	100	54	L	Thalamus, Internal Capsule	1.0	Ischemic	N	N	Y	Y	Y	N	N	44	42
11	45/M/R	100	12	R	Temporal lobe, Internal Capsule	60.7	Hemorrhagic	N	Y	N	Y	Y	Y	Y	26	95
12	63/M/R	100	228	R	Striatum	48.6	Ischemic	N	Y	Y	Y	Y	Y	Y	53	48
Mean	61.3 8 (M) / 4 (F) 11 (R) / 1 (L)	76.3	50	6 (L) / 6 (R)		17.8	Ischemic (9) Hemorrhagic (3)	2 (Y)/ 10 (N)	7 (N) / 5 (Y)	3 (N) / 9 (Y)	10 (Y) / 2 (N)	7 (Y) / 5 (N)	5 (Y) / 7 (N)	3 (Y) / 9 (N)	43.3	67.3
St Dev	9.4	41.3	59.4			28.5									12.2	24.3

%MSO at Ipsi MT denotes the stimulation intensity used to arrive at the RMT. Intensity is expressed as a % maximum stimulator output (MSO).

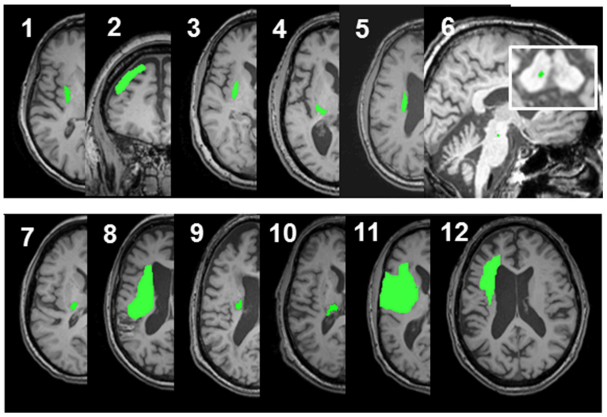


FIGURE 1 | Lesion locations for the 12 enrolled stroke patients. Images were adjusted so the lesion would appear in the left hemisphere. Lesion for patient #6 is enlarged to demonstrate location and size. Lesion volumes are noted in **Table 1**.

TMS trials. Two main parameters were computed: area under the recruitment curve (RC_{AUC}) and slope (RC_{Slope}). For analysis of the ipsilesional hemisphere, nine patients were studied whereas, 12 patients were evaluated for the contralesional hemisphere.

RC_{AUC}: Overall CST output.

RC_{AUC} values for each hemisphere were quantified using the trapezoidal area method (**Figure 2**). RC_{AUC} for the ipsilesional hemisphere was expressed relative to RC_{AUC} of the contralesional hemisphere following Equation 1, with values <1 indicating a RC_{AUC} for the ipsilesional hemisphere. We chose to normalize values to the contralesional hemisphere to control for inter-subject differences (Lindberg et al., 2007; Lotze et al., 2012).

$$RC\ AUC_{\ Ipsi/Contra} = \frac{Ipsilesional\ RC\ AUC}{Contralesional\ RC\ AUC} \quad (1)$$

RC_{Slope}: Descending CST gain.

To quantify the gain of the stimulus-response curve, all recruitment curves were fitted, as described previously (Devanne et al., 1997; Kaelin-Lang and Cohen, 2000; Carroll et al., 2001; Carson et al., 2013), using a nonlinear sigmoidal model (Boltzmann Equation) shown in Equation 2.

$$y = \frac{A_t - A_b}{1 + e^{(-\frac{x-x_0}{w})}} \quad (2)$$

The function parameters A_t and A_b denote the asymptotic y-values of the sigmoidal function, where the x-range of the sigmoidal slope was defined as w and the midpoint of the slope as x_o. The x-values were taken to be the %RMT, ranging from 90 to 150%. Therefore, the four function parameters were adjusted to best fit the modeled y-value to the experimental y-value. The slope of the sigmoidal curve, the midpoint (inflection point) and R^2 of the fit were recorded (**Figure 2**; **Table 2**). A fit above 0.7 was considered to be indicative of an accurate model (Carson et al., 2013). The inflection point was defined as the point of 50% of the maximal MEP for each patient (**Table 2**). Similar to the AUC, RC_{Slope} of the ipsilesional hemisphere was expressed relative to RC_{Slope} of the contralesional hemisphere following Equation 3.

$$RC\ Slope_{Ipsi/Contra} = \frac{Ipsilesional\ Slope}{Contralesional\ Slope} \quad (3)$$

DTI Analysis

DTI images were corrected for eddy currents and head motion using FSL (Jenkinson et al., 2012). Whole brain diffusion tensor maps of FA were calculated by first least-squares fitting of the 71 acquired diffusion profiles to each of the six independent tensor elements and then determining the final tensor-based value. Fiber orientation distribution functions (FOD) were used to account for crossing fibers on the 71 acquired diffusion profiles, as previously described by our group (Sakaie and Lowe, 2007; Lowe et al., 2008). Prior to tractography, we applied a threshold value of 0.2 on all FA maps. A threshold value of 0.2 has been used extensively. It is believed to be an optimal level to ensure that all ROI in the brain, including the centrum semiovale (known to have inherently low FA values) remain in the FA skeleton in patients with stroke (Kunimatsu et al., 2004; Zhu et al., 2010).

CSTs were virtually reconstructed using a three-dimensional random walk probabilistic tracking method (Sakaie and Lowe, 2007; Lowe et al., 2008; Zhang et al., 2013). Briefly, the track density value of each voxel was used as the probability distribution to generate stepping directions. Structural masks of the right and left hemisphere were applied during tracking and tracts branching outside the brain tissue hemisphere mask were terminated and not included in analysis. Our probabilistic tractography was constrained to voxels with more than 95% of the individual tract-specific connectivity probability, wherein voxels outside of the 95th percentile were assumed to have a track density value of zero. A "slice-by-slice" analysis was established by calculating a mean FA across all non-zero voxels at each z-slice (Lowe et al., 2008).

Thresholded probabilistic tracking was performed from the PLIC to the M1, PMC or SMA separately. Seeds were defined at the level of the PLIC, since the internal capsule is mainly comprised of CST which control voluntary movement as opposed to other tracts such as the corticobulbar (Holodny et al., 2005). ROI for PLIC were defined directly on the axial plane of the FA map at the appropriate level of the foramen of Monroe. ROIs for M1, PMC, and SMA (**Figure 2**) were drawn on ACPC aligned T1-weighted images based on guidelines (Bhatt et al., 2007) and then transformed into DTI ($b0$) space. Errors due to between-space transformations were corrected manually**.**

Structural integrity of CST originating from the M1, SMA, or PMC was compared between the ipsilesional and contralesional hemispheres using the asymmetry index of FA (described in Equation 4). The asymmetry index is given by Equation (4), where values range from −1 to +1; values >0 denoted increased CST damage on the ipsilesional side. For control subjects, the right hemisphere (non-dominant) was considered the ipsilesional side and the left hemisphere (dominant) was the contralesional.

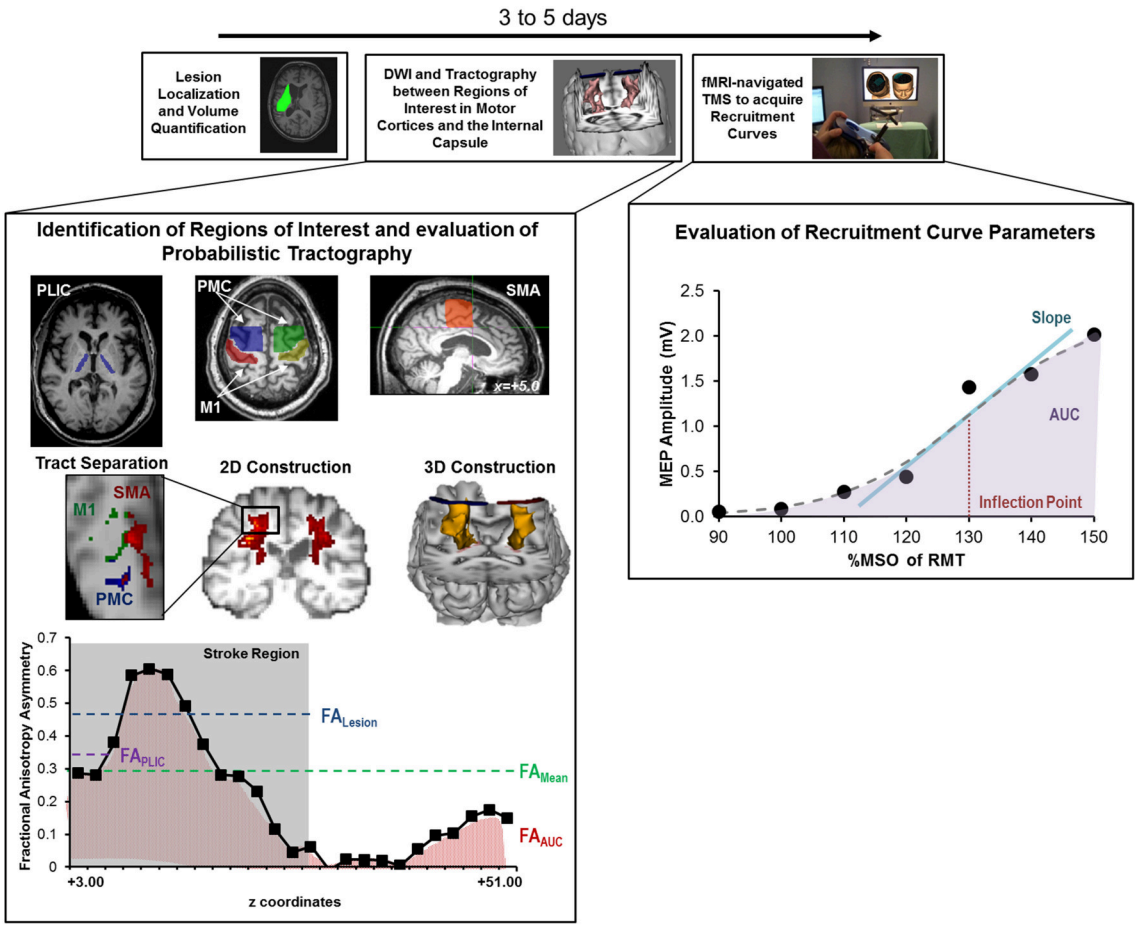


FIGURE 2 | Study design and quantitative analysis for assessing relationship between CST integrity assessed using DTI and recruitment curves captured using TMS. (Top) General flow of experiments for the present study. **(Bottom Left)** Regions of interest were defined for the posterior limb of the internal capsule (PLIC), motor cortex (M1), premotor cortex (PMC), and supplementary motor area (SMA). Probabilistic tractography was performed between the PLIC and each respective motor cortex. Fractional anisotropy (FA) was determined along the length of the reconstructed CST and four indices of asymmetry between ipsilesional vs. contralesional CST were computed- FA_{Lesion}, FA_{PLIC}, FA_{AUC}, and FA_{Mean}. Indices are denoted in the figure at the bottom left, where FA_{Lesion} refers to the asymmetry index within the stroke region, FA_{PLIC} refers to the asymmetry index at the region of the PLIC, and FA_{AUC} and FA_{Mean} are a net or average asymmetry index for the determined CST. Coordinates denote the location of the brain/region of interest in respect to AC-PC alignment, where-in z = +0.00, denotes the level of alignment between the anterior commissure and posterior commissure. **(Bottom Right)** Recruitment curves acquired using TMS were modeled using the Boltzmann Equation and parameters of the curve (Slope, Inflection Point) were extracted for further comparisons. In addition, the area under the recruitment curve (AUC) was calculated using the trapezoidal area method. Recruitment curve slope and AUC were quantified as an asymmetry measure between the ipsilesional and contralesional hemisphere.

$$FA_{asymmetry} = \frac{FA_{contralesional} - FA_{ipsilesional}}{FA_{contralesional} + FA_{ipsilesional}} \quad (4)$$

Since CST integrity can be influenced by stroke location, overlap with the lesion, and degeneration, we determined FA asymmetry values for CST integrity at several different regions (**Figure 2**). First, we computed the average FA asymmetry along the entire length of the tract (termed FA_{Mean}). Second, a FA index was determined at the region of the PLIC (termed FA_{PLIC}), which represents one of the most common forms of analysis (Stinear et al., 2007). Here, PLIC was defined at the level of three consecutive axial slices along the CST (Sidaros et al., 2008; Puig et al., 2011; Park et al., 2013). Third, the entire area under the FA asymmetry curve was calculated using the trapezoidal rule (FA_{AUC}) in Matlab (Mathworks, Inc.). Finally, we evaluated FA asymmetry within the stroke region (termed FA_{Lesion}; Grandin et al., 2001; Granziera et al., 2007, 2010) because substantial reductions in CST integrity occur near the lesion site (Lindenberg et al., 2012). To help determine FA_{Lesion} an expert neurologist (AM) localized the region of stroke on z-slice levels for each patient. A second blinded rater (EP) repeated the analysis with excellent inter-rater reliability [ICC(2) = 0.925]. The mean FA asymmetry across the stroke region and mirror region in the contralesional hemisphere was recorded. For all DTI comparisons, patient 6 was excluded since their stroke (pontine/mesencephalic) was outside of the investigated CST region and could potentially affect the accuracy of FA measures within the PLIC due to retrograde

TABLE 2 | Significance of Outcome Measures.

Analyzed metric	Measureable outcomes	Physiological representation	Significance of change in measurement
Recruitment curve	Slope	Gain of descending CST	↓ Slope suggests a reduction in the overall strength of output of structurally available axonal tracts (Devanne et al., 1997)
	Area Under the Curve (AUC)	Overall CST output	↓ AUC suggests an overall reduction in CST recruitment and/or poor synchronization of descending volleys (Talelli et al., 2008)
	Fit to model (R^2)	Accuracy of orderly CST recruitment	Fit values above 0.7 indicate orderly recruitment ($R = 0.84$; Carson et al., 2013)
	Inflection point	%RMT at which maximum gain is demonstrated	↓ Inflection point suggests loss of mid- and/or high threshold motoneuron populations and/or increased inhibitory components that alter recruitment (Talelli et al., 2006)
Fractional anisotropy (DTI)	FA_{PLIC}	Average structural integrity of CST within the PLIC	↑ Fractional anisotropy suggests increased demyelination and axonal loss within the CST (at the respective measured location; Alexander et al., 2007)
	FA_{Mean}	Average structural integrity of CST between the seed and target region of interest	
	FA_{Lesion}	Average structural integrity of CST within the stroke region	
	FA_{AUC}	Overall structural integrity of CST	

FA, Fractional Anisotropy; CST, Corticospinal tract; AUC, Area Under the Curve; DTI, Diffusion Tensor Imaging.

degeneration (Kobayashi et al., 2005; Liang et al., 2008; Schulz et al., 2012).

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS Inc.). All data was tested for normality using the Shapiro–Wilk test. For normally distributed data, a student's *t*-test was utilized to analyze differences between patients and controls. For non-normal data, the Mann–Whitney *U*-test was used to determine differences between patients and controls. Corrections for multiple comparisons were incorporated when applicable. For all comparisons between healthy and stroke patients, comparisons were only examined between the ipsilesional and right control hemisphere (non-dominant), and the contralesional and left control hemisphere (dominant; Cunningham et al., 2015).

We utilized a repeated-measures analysis of variance (ANOVA) with polynomial contrast to determine differences between FA asymmetry collected at different regions (FA_{PLIC}, FA_{Mean}, $FA_{AUC,}$ and FA_{Lesion}) in patients with stroke. If Mauchly's test of sphericity was violated, we applied a Greenhouse-Geisser correction for final *F*-Values. To identify if FA asymmetry at which particular regions was different, we used pair-wise comparisons with a Bonferroni confidence interval adjustment.

We examined the bivariate correlation between FA asymmetry values and parameters of the recruitment curves (RC $AUC_{Ipsi/Contra}$, RC $Slope_{Ipsi/Contra}$) using the Pearson's correlation coefficient. Based on the Pearson's correlation coefficient criteria, a small (0.1–0.3), medium (0.3–0.5), or large (0.5–1) association was determined. Inter-rater reliability was determined using intraclass correlation coefficients (ICCs). ICCs were calculated using a two-way random-model with consistency agreement. ICC values >0.8 were defined as excellent agreement between raters (Danielian et al., 2010). All utilized tests were two-sided, where $p \leq 0.05$ was considered statistically significant.

RESULTS

Clinical Assessment

Patient characteristics are presented in **Table 1**. Age differences between controls (68.3 ± 12.4 years) and patients (61.3 ± 9.4 years) were not significant (**Table 1**; $t = 1.44$, $p = 0.166$). All controls and 11 of the 12 patients were right handed as determined by the Edinburgh Handedness Inventory (EHI; EHI Patients = 76.3 ± 41.3; Oldfield, 1971). The average lesion volume was 17.67 ± 8.22 cm^3 (s.e.m.), where strokes affecting the basal ganglia ($n = 5$) or PLIC ($n = 5$) were most common. The average UEFM score was 43.3 ± 12.2 (range 15–59).

Recruitment Curve Properties in Patients with Chronic Stroke

Average RMT for the ipsilesional hemisphere (67.3 ± 24.3%) was significantly higher than RMT for the contralesional hemisphere (46.6 ± 11.4%; $t = 2.667$; df = 18; $p = 0.014$) and RMT for controls (42.8 ± 6.1%; $t = -2.773$; df = 18; $p = 0.013$). M-Mwaves were also not different between patients and controls [ipsilesional ($t = 1.254$; df = 18; $p = 0.226$) and contralesional ($F = 3.405$; $t = -1.152$; df = 18; $p = 0.265$)].

With regards to recruitment curves, controls had an average fit (R^2) of 0.77 ± 0.072 and 0.95 ± 0.007 in the right and left hemispheres and patients had a fit of 0.80 ± 0.06 and 0.82 ± 0.05 in the ipsilesional ($n = 9$) and contralesional ($n = 12$) hemispheres. Interestingly, fit accuracy was significantly different between hemispheres in healthy controls ($U = 4.5$; $Z = -2.89$; $p = 0.004$) due partially to hand dominance (Wittenberg and

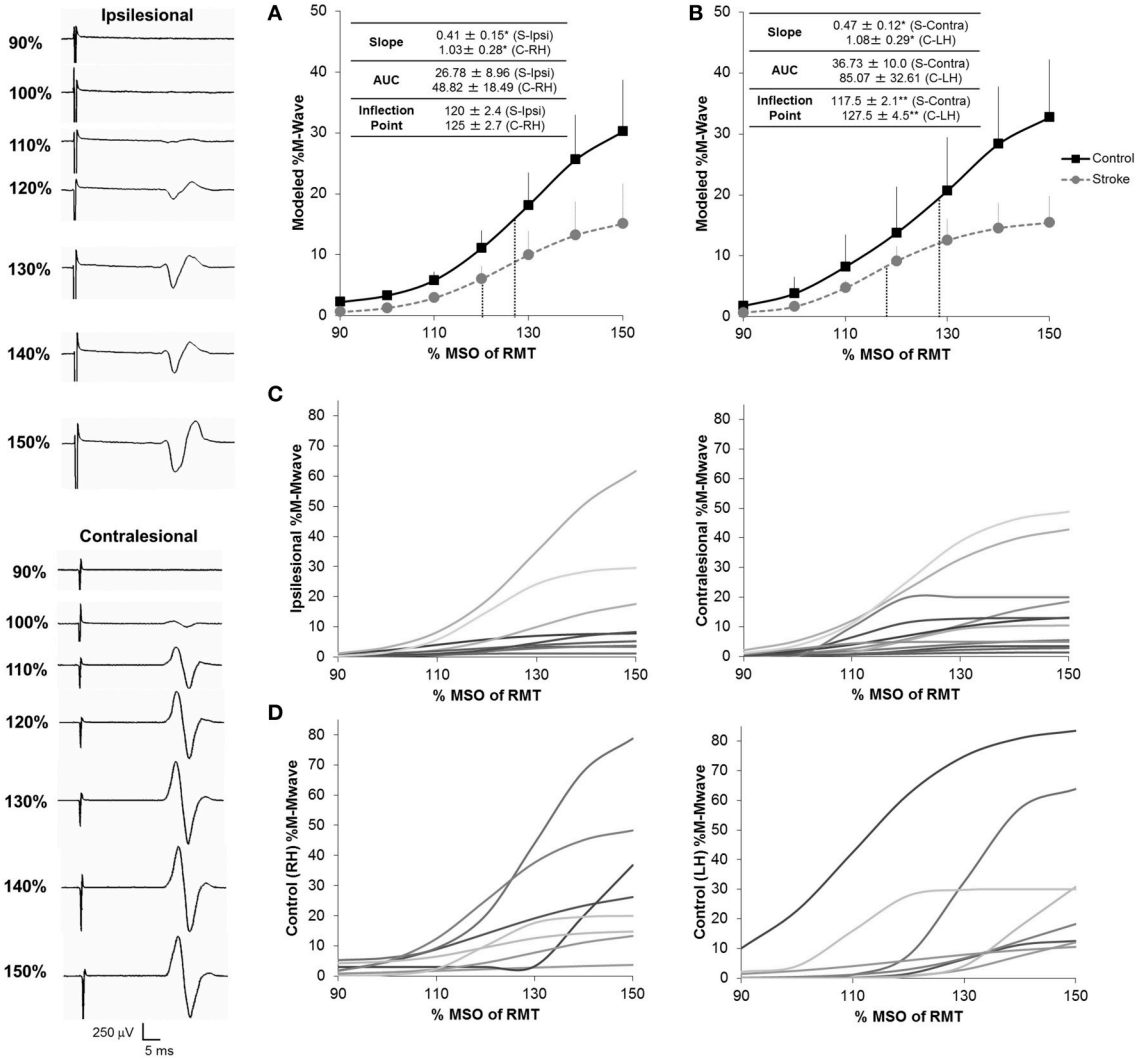


FIGURE 3 | Recruitment curves for stroke subjects and healthy controls. We noted a significant reduction in the gain of descending CST, as shown by the slope of the recruitment curve, between stroke and healthy controls in both the ipsilesional ($n = 9$) **(A)** and contralesional ($n = 12$) **(B)** hemispheres. In addition, the contralesional hemisphere displayed a reduced inflection point in comparison to controls suggesting either loss of higher threshold motoneuron populations and/or increased inhibitory components in this hemisphere. No significant differences for overall CST output, as shown by the area under the curve (AUC) were noted between stroke patients and controls in either hemisphere. Inflection points are shown in blue dashes for all conditions. Data in the ipsilesional hemisphere is only shown for patients eliciting a resting state recruitment curve ($n = 9$), since muscle activation can influence recruitment curve gradients. In contrast, since all patients elicited a resting state recruitment curve in the contralesional hemisphere, all patients are presented in panels **(B,C)**. Data was averaged across each subject population following mathematical modeling, normalized to the max %Maximum MWave (M-Mwave) and plotted ± s.e.m. for each assessed intensity. Recruitment curves are presented for all patients with stroke **(C)** and controls **(D)**. Representational motor evoked potentials from patient 10 are shown to the left of the plotted data. *$p = 0.05$; **$p = 0.07$ for control vs. stroke. S, Stroke; Ipsi, Ipsilesional; Contra, Contralesional; RH, Right Hemisphere; LH, Left Hemisphere.

Schaechter, 2009). Recruitment curve parameters in patients significantly differed from those in healthy controls (**Figure 3**). We noted a significantly reduced RC_{Slope} in the ipsilesional ($U = 16$; $Z = -1.925$; $p = 0.05$) and contralesional ($U = 21$; $Z = -2.083$; $p = 0.03$) hemispheres (**Figures 3A,B**; **Table 2**). No significant differences in the inflection point were noted between controls and patients. In addition, while we noted a slight reduction in RC AUC between patients and controls, significance was not reached between groups. Taken collectively, we noted that patients demonstrated reduced CST gain (RC_{Slope}) and output (RC_{AUC}) in comparison to controls. Recruitment curves for all stroke subjects and controls are shown in **Figures 3C,D**, respectively.

CST Integrity

Next, we assessed CST integrity for tracts descending from the M1, PMC, and SMA. For these sets of tracts, we studied FA asymmetry in different regions (FA_{PLIC}, FA_{Mean}, FA_{AUC}, FA_{Lesion}), and raw FA diffusivity values (**Figure 4**).

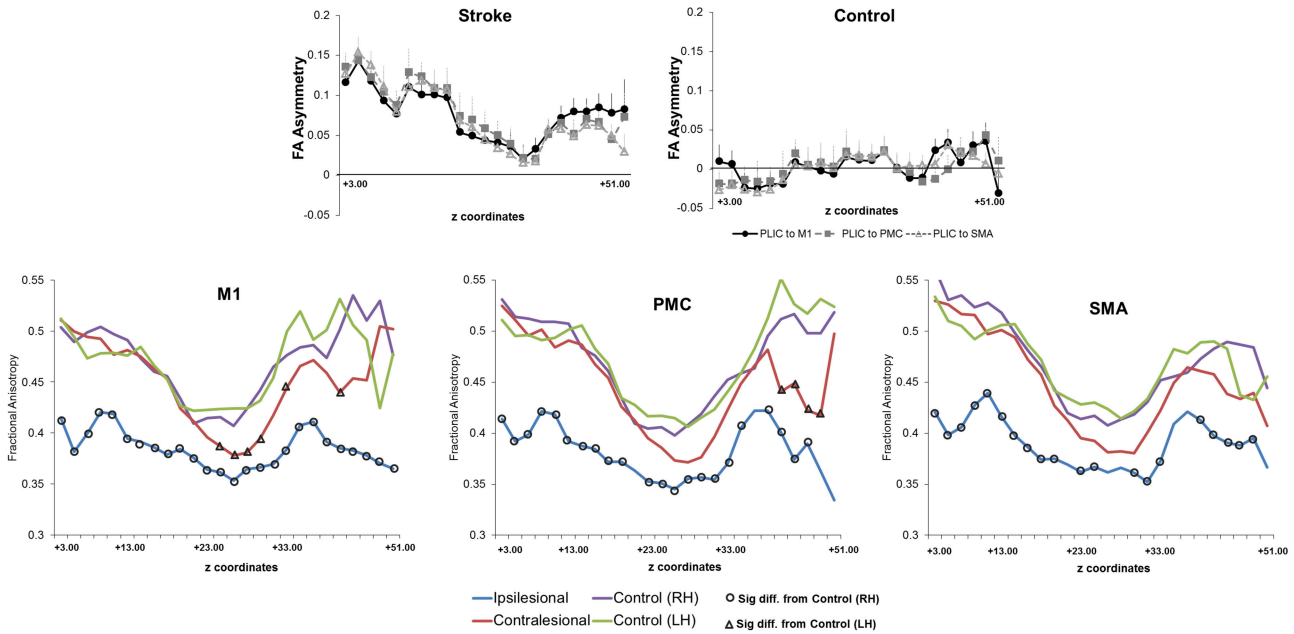


FIGURE 4 | Fractional anisotropy and fractional anisotropy asymmetry across three sets of corticospinal tracts (CSTs) in patients with stroke and healthy controls. (Top) Average CST tractography FA asymmetry values between seed and target(s) ($n = 11$). Higher values of FA asymmetry, indicative of greater CST damage, were noted along the entire tract in comparison to healthy controls. The CST integrity between tracts descending from the M1, PMC, or SMA was similar in either patients or controls ($n = 8$). **(Bottom)** Raw fractional anisotropy values were plotted along the length from the M1 **(left)**, PMC **(middle)**, and SMA **(right)**. Patients displayed significantly higher levels of CST damage, as indicated by a decreased area of fractional anisotropy, in both the ipsilesional (differences are shown with black circles) and contralesional (differences are shown with black triangles) hemispheres in comparison to controls. Significance was defined as $p = 0.05$. Coordinates denote the location of the brain/region of interest in respect to AC-PC alignment, where-in z = +0.00, denotes plane of the anterior commissure and the posterior commissure.

Visual reconstructions of tractography are demonstrated in **Figures 2**, **5**.

We first compared CST integrity (FA asymmetry) across tracts descending from the M1, PMC, and SMA. Though differences were not significant in controls, in patients, integrity in the region of the lesion differed [FA_{Lesion} ($F_{(1.833,20.159)} = 6.942$; $p < 0.006$)]. CST from M1 demonstrated significantly more damage at the region of the lesion (FA_{Lesion}) in comparison to CST from PMC ($p = 0.04$) and SMA ($p = 0.02$); albeit noted differences were minor, with an average difference in means of 0.015. Next, we compared CST integrity (FA asymmetry) between patients and controls. We found increased FA asymmetry in patients across all regions of analysis- FA_{PLIC}, FA_{Mean} and FA_{AUC}, and FA_{Lesion}. FA asymmetry differed based on which region was studied in patients. We found significant differences between FA_{Lesion} and FA_{Mean}, and FA_{PLIC} and FA_{Mean} for CST descending from the M1 [$F_{(2, 22)} = 9.826$; $p < 0.001$], PMC [$F_{(2, 22)} = 19.660$; $p < 0.0001$], and SMA [$F_{(2, 22)} = 14.838$; $p < 0.0001$; all $p < 0.05$; **Table 3**]. Therefore, CST integrity denoted as FA_{Lesion} and FA_{PLIC} was indicative of most damage. Besides FA asymmetry, we compared values of raw FA between patients and controls (see **Table 4** for values). We found that raw FA was reduced along the majority of CST from ipsilesional M1, PMC and SMA in patients vs. controls (all $p < 0.05$; **Figure 4**). In addition, we found that the contralesional hemisphere showed regions of reduced FA in CST descending from M1 and the PMC in comparison to controls (all $p < 0.05$; **Figure 4**; Buffon et al., 2005; Schaechter et al., 2009; Crofts et al., 2011; Dacosta-Aguayo et al., 2014).

Relationship between Recruitment Curves and CST Integrity

We next examined the relationship between recruitment curves and integrity of CST from M1, PMC and SMA in patients. Notably, we found that RC $AUC_{Ipsi/Contra}$ was related with FA asymmetry ($p = 0.05$) of tracts from PMC and SMA but not of M1. Specifically, as depicted in **Figure 6**, RC $AUC_{Ipsi/Contra}$ ($n = 8$) was negatively correlated with FA_{Mean} and FA_{AUC} for CST from the PMC and the SMA. RC $Slope_{Ipsi/Contra}$ too was only related to FA asymmetry for tracts from SMA but not from M1. In particular, we found only one significant correlation between RC Slope $_{Ipsi/Contra}$ and FA_{Mean} for CST descending from the SMA. Taken collectively, we observed that patients who had both a reduced gain (RC $Slope_{Ipsi/Contra}$) and reduced output from the recruitment curve (RC $AUC_{Ipsi/Contra}$) presented with high levels of CST damage within the PMC and SMA, as illustrated in **Figure 6**.

Finally, similar to other reports, we noted that higher UEFM (less impairment) was significantly correlated with less CST damage of ipsilesional tracts and a trending increase in ipsilesional recruitment curve slope (**Figure 7**; Ward et al., 2006; Lindberg et al., 2007; Stinear et al., 2007, 2012).

TABLE 3 | Fractional Anisotropy (FA) asymmetry indices in patients with stroke ($n = 11$) and healthy controls ($n = 8$).

Patient group				Control group			
Parameter	**M1**	**PMC**	**SMA**	**Parameter**	**M1**	**PMC**	**SMA**
FA_{PLIC}	0.126 ± 0.015^a	0.134 ± 0.014^a	0.140 ± 0.015^a	FA_{PLIC}	0.009 ± 0.019	-0.018 ± 0.018	-0.027 ± 0.018
FA_{Mean}	0.078 ± 0.009^b	0.076 ± 0.012^b	0.065 ± 0.01^b	FA_{Mean}	0.005 ± 0.003	0.004 ± 0.004	-0.002 ± 0.003
FA_{Lesion}	0.096 ± 0.01	0.112 ± 0.012	0.109 ± 0.009	FA_{Lesion}	–	–	–
FA_{AUC}	1.773 ± 0.255	1.791 ± 0.323	1.653 ± 0.234	FA_{AUC}	0.074 ± 0.055	0.112 ± 0.092	-0.018 ± 0.079

[a] *Significantly different from FA_{Mean} ($p \leq 0.01$).*
[b] *Significantly different from FA_{Lesion} ($p \leq 0.04$).*

TABLE 4 | Non-normalized (raw) fractional anisotropy (FA) diffusivity values for patients with stroke and healthy controls.

Ipsilesional (Patient)				Contralesional (Patient)			
Parameter	**M1**	**PMC**	**SMA**	**Parameter**	**M1**	**PMC**	**SMA**
FA_{PLIC}	0.394 ± 0.014	0.394 ± 0.013	0.397 ± 0.014	FA_{PLIC}	0.505 ± 0.009	0.514 ± 0.013	0.526 ± 0.015
FA_{Mean}	0.382 ± 0.008	0.379 ± 0.008	0.386 ± 0.007	FA_{Mean}	0.448 ± 0.008	0.445 ± 0.009	0.443 ± 0.010
FA_{Lesion}	0.387 ± 0.009	0.379 ± 0.011	0.387 ± 0.011	FA_{Lesion}	–	–	–
Right Hemisphere (Control)				**Left Hemisphere (Control)**			
FA_{PLIC}	0.487 ± 0.018	0.508 ± 0.019	0.537 ± 0.018	FA_{PLIC}	0.491 ± 0.014	0.498 ± 0.016	0.517 ± 0.018
FA_{Mean}	0.459 ± 0.012	0.464 ± 0.013	0.468 ± 0.014	FA_{Mean}	0.465 ± 0.009	0.468 ± 0.012	0.468 ± 0.013

DISCUSSION

The goal of the present study was to assess if recruitment curves reflect integrity of CST from motor regions beyond M1 in patients with chronic stroke. Specifically, we aimed to determine whether CST descending from the PMC and SMA, in addition to M1, made unique contributions to recruitment curve properties. The main findings from our study show that output of the recruitment curve (RC $AUC_{Ipsi/Contra}$) is most intimately associated with CST integrity from the premotor areas but not the primary motor cortex (M1). Association varied based on which regions of CST integrity were investigated (**Table 2**). For example, recruitment curve output was most related to CST integrity measured along the entire length of CST (FA_{AUC} and FA_{Mean}), but not integrity studied at specific regions, like internal capsule or lesion territory (FA_{Lesion} and FA_{PLIC}). Based on our observations, recruitment curves could sensitively capture re-mapping of function to higher motor cortices, and help comprehensively infer damage and degeneration that occurs typically along the length of CST in chronic recovery. As such, future studies should explore whether TMS-based recruitment curves can serve as a less expensive, and easy-to-administer proxy for functional and structural imaging in stroke.

Contribution of Higher Motor Areas to Recruitment Curve Properties

We have identified that in patients with chronic stroke, CST function captured by recruitment curves (RC $AUC_{Ipsi/Contra}$) is most representative of integrity of CST from higher motor areas (PMC and SMA). This finding is conceivable given that higher motor areas re-map to contribute to recovery in chronic stroke (Seitz et al., 1998; Fridman et al., 2004; Dancause et al., 2005; Ward et al., 2006; Plow et al., 2014). Indeed, re-mapping has been shown to increase with damage to territories in M1 and loss of its CST (Weiller et al., 1992; Seitz et al., 1998; Bhatt et al., 2007; Ward et al., 2007). Thus, in our sample, since raw FA diffusivity of CST indicated greater damage to CST from M1 than that to CST from PMC and SMA (**Figure 5**), it is explainable that recruitment curve properties were related to residual CST from PMC and SMA but not damaged CST from M1. Our results suggest that chronic stroke patients may rely on intact CST from re-mapped territories in higher motor areas to elicit output in paretic muscles.

Our results are also possible given that TMS can excite higher motor areas via cortico-cortical projections from M1 (Lemon, 1999; Klöppel et al., 2008). Indeed, both animal experiments and human studies have suggested that cortico-cortical projections between the premotor-motor cortices remain an important mechanism for trans-synaptic excitation of fast-conducting pyramidal cells in M1 (Ghosh and Porter, 1988; Klöppel et al., 2008). However, recruitment of higher motor areas, whether through remapping or through cortico-cortical connections, would not result in complete motor recovery given that they project CST that contain polysynaptic, less-myelinated, slow-conducting axons than M1 (Boudrias et al., 2006; Ward, 2011). For example, our patients had a UEFM of 43.3 ± 12.2 (max = 66) indicating that they were moderately affected, but were still in chronic recovery (Duncan et al., 1983). Thus, role of higher motor areas could be a reasonable proxy given that CST from M1 are damaged most commonly (Bogousslavsky

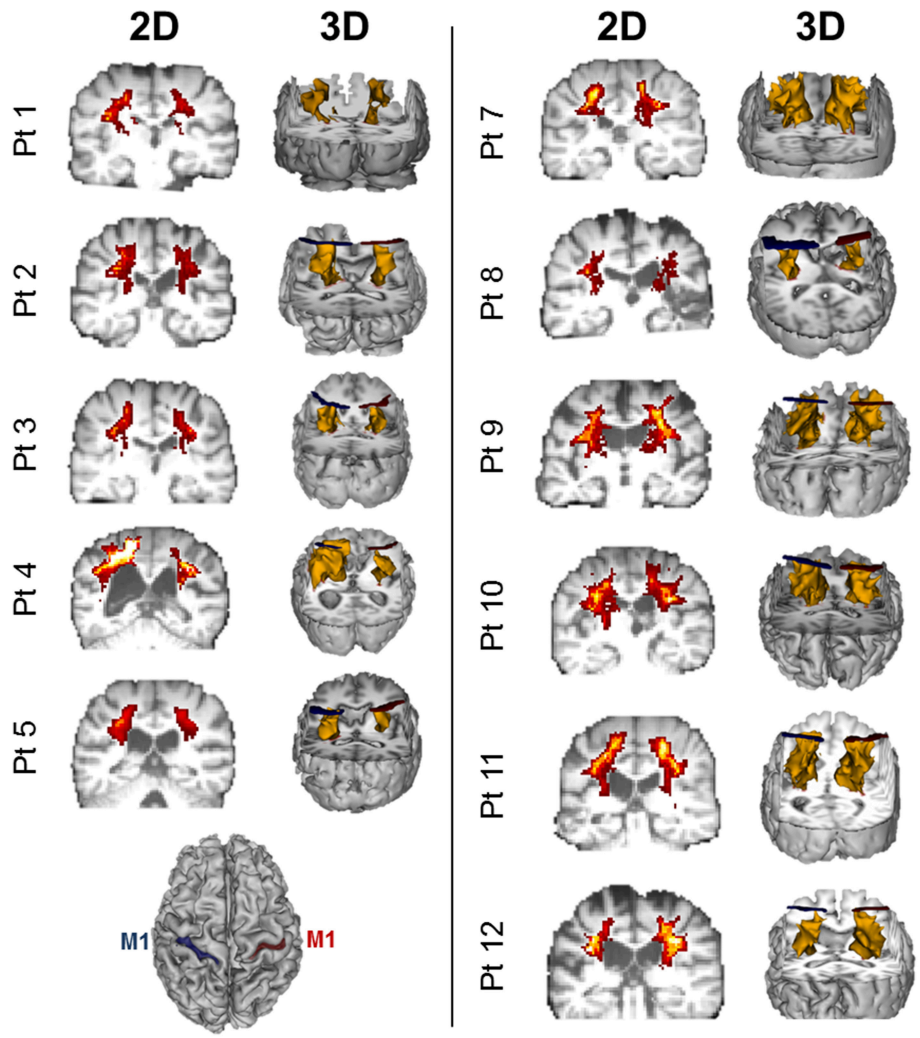


FIGURE 5 | Diffusion weighted imaging (DWI)-based probablistic tractography across patients from primary motor cortex to posterior limb of the interal capsule. Probablistic tractography was performed across all patients from the posterior limb of the internal capsule (PLIC) to the primary motor cortex (M1) for each hemisphere. Regions of interest for tractography were defined as outlined in **Figure 2** and Section Statistical Analysis. Two dimensional (2D) corticospinal tract (CST) density maps are shown for each patient, with yellow/orange denoting more dense CSTs. 3D reconstruction of tractography is also displayed for each patient in yellow. Blue ROIs denote the left hemisphere M1 (see **bottom left**), while red ROIs denote the right hemisphere M1.

and Regli, 1990; Johansen-Berg et al., 2002; Buffon et al., 2005).

An interesting finding was that the gain of the recruitment curve (RC $Slope_{Ipsi/Contra}$) was only associated with integrity of CST descending from the SMA. One possibility for this result is the hierarchy of CST recruitment. Traditionally, motor unit recruitment following TMS begins with low-threshold, large diameter CST emerging from M1 (Devanne et al., 1997; Ward et al., 2006). Then, as the stimulus intensity increases, higher-threshold, small diameter fibers, such as those from the PMC and then SMA are recruited (Devanne et al., 1997; Henderson et al., 2006). Given this order, damage of select high-threshold fibers in the SMA, which may occur with more extreme damage (Lukács et al., 2008), could eventually influence the slope of the recruitment curve and consequently, motor function.

Other Factors Explaining Contribution of Higher Motor Areas to Recruitment Curve Properties

Some could argue that our result that recruitment curves represent CST function from anteriorly located higher motor territories is surprising given that the location of the motor hotspot, most likely in M1, did not significantly differ between patients and controls (data not shown). However, we note that beyond neurophysiologic influence, several other factors may have affected our observed results. First, TMS using a figure-eight coil is inherently non-focal. Indeed, it has previously been shown that while the electrical field strength is maximal under the middle of the coil, the spatial derivative of the electric field is also highest below the center of each lobe (Civardi et al., 2001). Thus, fibers aligned between the middle of the coil and the center of the anteriorly directed lobe may have become preferentially

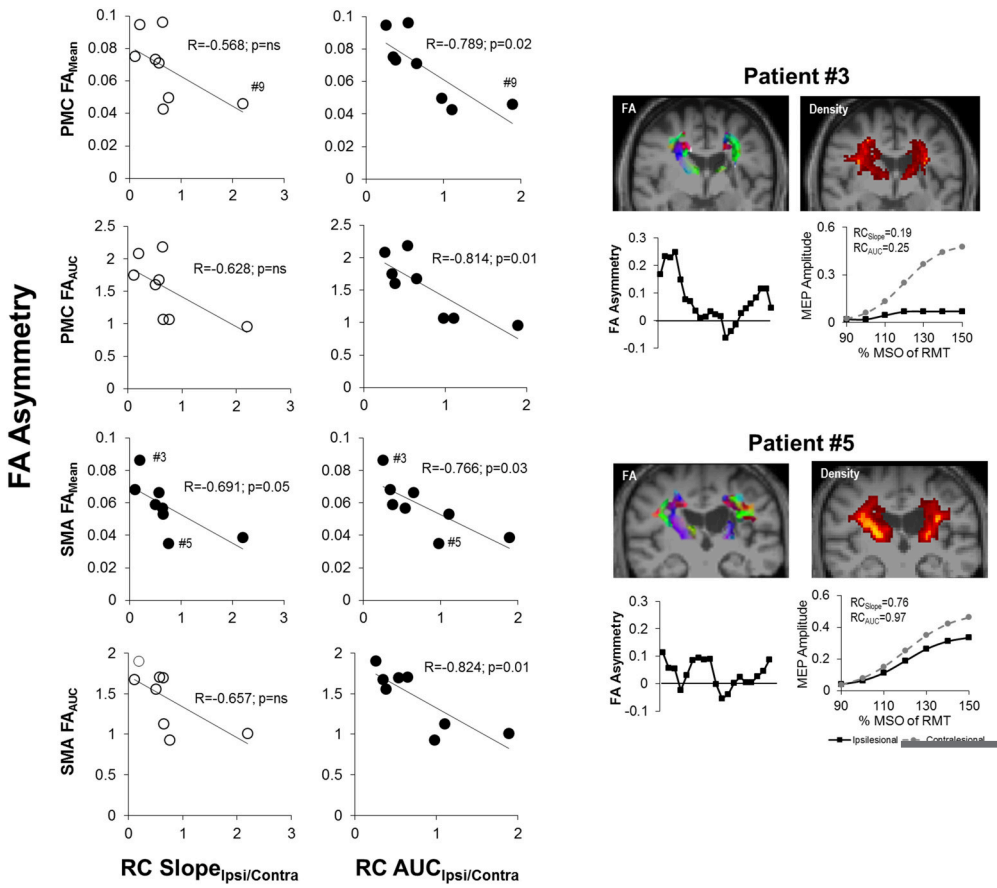


FIGURE 6 | Recruitment curve slope and area under the curve predicts level of corticospinal tract (CST) damage in patients with stroke. (Left) The gain of descending CST, as indicated by the slope (RC $Slope_{Ipsi/Contra}$), was found to be significantly negatively correlated with the average structural integrity of CST (FA_{Mean}) for CST descending from the SMA ($p = 0.05$; $n = 8$). **(Middle)** The area under the recruitment curve, a representation of the overall output of stimulated CST (RC $AUC_{Ipsi/Contra}$), however, had an even stronger relationship with CST damage. Specifically, RC $AUC_{Ipsi/Contra}$ was significantly negatively correlated with overall CST integrity (FA_{Mean} and FA_{AUC}) for CST originating from either the PMC or SMA ($p = 0.03$). Removal of Patient #9 from analysis did not change observed results. Filled circles denote significant relationships. **(Right)** Sample data of stroke patient with severe CST damage (upper; Patient #3) in comparison to patient with moderate damage (lower; Patient #5), as marked by #3 and #5 in SMA FA_{Mean} plots, and their respective FA asymmetry and recruitment curves. Fractional anisotropy maps (FA) are shown for each patient. Red denotes fibers in the x-axis (left to right), green denotes fibers in the y-axis (anterior to posterior) and blue denotes fibers in the z-axis (superior to inferior). CST density maps are shown in the top right for each patient, with yellow/orange denoting more dense CSTs.

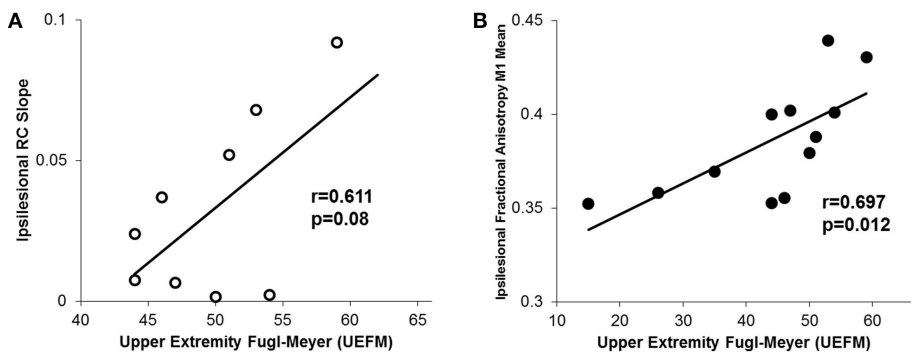


FIGURE 7 | Relationship between motor impairment (UEFM) and CST integrity and recruitment curves. (A) We observed a trending positive relationship between the ipsilesional recruitment curve slope and upper extremity fugl-meyer (UEFM). **(B)** Similarly, a significant positive correlation was noted between UEFM and the ipsilesional mean raw fractional anisotropy in CST from M1. Higher fractional anisotropy values here denote a more intact tract.

activated (MacCabee et al., 1993). Therefore, while M1 was likely activated at the hotspot by TMS, inherent diffuse electric field spreads that would occur within ∼15 mm from the middle of the coil may have activated other structures like the PMC and SMA (MacCabee et al., 1993). Second, several of our patients ($n = 5$) had characteristic damage to the posterior part of their PLIC (Kobayashi et al., 2005; Liang et al., 2008; Schulz et al., 2012). As a result, M1 CST traveling via the posterior edge of the PLIC may have been preferentially lost. Thus, variable function of severely damaged CST from M1 may have rendered its contribution toward recruitment curve properties moot. In such cases, CST from less–damaged territories located anteriorly in higher motor areas could have become recruited due to functional remapping and/or current spreads (Holodny et al., 2005; Ino et al., 2007; Park et al., 2008).

The finding that recruitment curve properties were associated with CST integrity from SMA and PMC can also be understood when considering differences in TMS intensities. Since the ipsilesional hemisphere required a significantly higher intensity to acquire recruitment curves, it is possible that diffuse electric field spreads recruited greater degree of CST from ipsilesional than contralesional higher motor territories (Gerschlager et al., 2001; Teitti et al., 2008). For example, Gerschlager et al. have suggested that stimulation intensities used to induce common neurophysiological metrics from the motor cortex, e.g., MEPs, can activate low threshold premotor pathways as a result of current spreading. Specifically, since the dorsal premotor cortex is located more superficially on the surface of the precentral gyrus in comparison to M1, neurons within these regions are much more likely to have a lower threshold than neuronal components deeper within the central sulcus (M1; Geyer et al., 1996; Klöppel et al., 2008). Thus, even at relatively low RMT, neuronal ensembles from premotor areas may have been preferentially activated in our model.

Regardless of other influences, however, we submit that our observed findings were likely the main result of neurophysiological phenomenon in patients with chronic stroke. Indeed, because we found that the hotspot used for TMS was similar between both the ipsilesional and contralesional hemisphere in patients and between patients and controls ($p < 0.05$ for all comparisons), current spreads may not have contributed as fully. Specifically, given that the distance of current spreads is <15 mm but that the distance from M1 to the PMC is ∼25 mm (Rizzo et al., 2004; Boros et al., 2008), the major contributions were likely due to re-mapping of higher motor areas in response to the commonly damaged M1.

Alterations in Recruitment Curve Properties in the Contralesional Hemisphere

While we expected that the gain and output of descending tracts would be weaker for the ipsilesional hemisphere, our finding of decreased output in the contralesional hemisphere is surprising (**Figure 3**). A recent study by Bowden et al has suggested that such a reduced contralesional recruitment curve may reflect limitations in CST function (Bowden et al., 2014). Bowden concluded that recruitment order hierarchy, rather than structural properties, were more affected in the contralesional hemisphere since patients elicited less output from lower threshold fibers than controls, despite comparable MEPs. In contrast, however, we cannot discount that here the contralesional hemisphere presented with reduced CST integrity in certain segments (**Figure 4**) from both the M1 and PMC. Indeed, reductions in CST integrity in the contralesional hemisphere have been shown to occur as early as 6 months after stroke (Schaechter et al., 2009). Recent work has also suggested that "mirroring damage," wherein the region homologous to the stroke region on the contralesional side becomes altered, can occur as early as 3 months following a stroke (Granziera et al., 2007, 2010; Crofts et al., 2011). In addition, co-morbidities associated with stroke (e.g., hypertension, diabetes, history of smoking) have been suggested to contribute to small-vessel disease; a condition that could cause subclinical lesions in the contralesional hemisphere (Prins et al., 2005). Thus, since the majority of our patients ($n = 10$) and some of the control subjects ($n = 3$) had small-vessel disease risk factors, this may have also facilitated reduced contralesional integrity. Therefore, taken collectively, along with functional losses, it is conceivable that inherent damage in the contralesional hemisphere either due from the stroke or possible small-vessel disease could have directly impacted measured output.

Strengths of Chosen DTI and TMS Metrics

A notable observation was that recruitment curve properties were most related to CST integrity captured along the length of the CST (FA_{Mean} and FA_{AUC}). One likely explanation for this finding is based on research inferring that recruitment curve output is influenced by the amount of residual or intact CST function (**Table 2**; Devanne et al., 1997; Talelli et al., 2006). Thus, only those levels of analysis that accounted for all of the surviving, damaged and degenerated regions within CST, i.e., FA_{Mean} and FA_{AUC}, showed significant relationships with recruitment curve metrics. We also observed that analysis of varying regions of CST integrity resulted in significantly different output values. Of note, FA_{PLIC} and FA_{Lesion} were most indicative of damage and only metrics assessing the entire CST (FA_{Mean} and FA_{AUC}) were reflective of CST function measured by recruitment curves. Such a result, while explainable given the distinctive definitions of each of the metrics, has implications on future use of DTI metrics in longitudinal studies. Specifically, future researchers should exercise caution when determining how to relate DTI metrics of CST integrity to neurophysiological or functional outcome measures. For example, studies that aim to understand the role of CST damage may benefit from using metrics indicative of regions of most damage (e.g., FA_{PLIC} and FA_{Lesion}), while DTI metrics that assess the net integrity of the CST (e.g., FA_{Mean} and FA_{AUC}) may be more suitable for studies that evaluate relationships with CST function.

Taken collectively, while our findings here have potential to inform future DTI studies in stroke, they also create enthusiasm for the field of TMS. This is because recruitment curve properties can more closely reflect the graded range of CST damage following stroke in comparison to binary TMS metrics

(e.g., RMT, MEP absence/presence; data not shown). Thus, recruitment curves collected using simple, easy-to-administer TMS techniques can closely reflect CST function from areas generally studied with more resource-intensive structural and functional imaging in patients with stroke. For example, physical and occupational therapists could employ the use of recruitment curves to better understand the dynamic and graded changes in CST integrity that occur immediately after stroke in order to maximize a patient's rehabilitation program.

Limitations

Although we attempted to account for potential problems in our experimental design, our study still suffers from some inherent limitations. First, as a preliminary study, our results only included analysis from 12 patients with chronic stroke, wherein we were unable to record recruitment curves in 3 patients since the severity of their deficit limited our ability to use existing TMS methodology to acquire the curve. Thus, even though our sample size was comparable to sample sizes of other DTI studies in stroke (Ward et al., 2006; Lindberg et al., 2007; Qiu et al., 2011; Allendorfer et al., 2012; Lindenberg et al., 2012; Groisser et al., 2014), future studies with larger sample sizes would be needed to validate the results found here. Similarly, based on methodology from previously conducted work in other groups (Civardi et al., 2001; Butefisch et al., 2003; Rossini and Rossi, 2007), our entire analysis was done in a resting state of the target muscle (FDI). Thus, it is unclear if the relationships noted here can be translated to data collected in an active state (Talelli et al., 2006). Further, we acknowledge that our CST integrity measures encompassed all motor pathways. Thus, any lower extremity deficits or damage to lower extremity motor CST in our patients may have influenced our FA measures, particularly in the SMA. However, while we acknowledge this limitation, we remain optimistic about our findings. This is because our results still emphasize the strength of a more specific modality (TMS) as an ultimate tool to replace structural imaging. We also cannot discount that inclusion of patients with hemorrhagic stroke may have increased data variance; although heterogeneity of lesion size, etiology, and location was similar to reports by other groups (Ward et al., 2006; Lindenberg et al., 2012; Demirtas-Tatlidede et al., 2015). In addition, since comparisons between controls and patients with stroke did not take into account hand dominance, our results may have added confound if patients with stroke displayed a lesion contralateral to their dominant hand. Another limitation in our study is the use of PLIC as the tractography seed. While we chose our seed as to focus on CST controlling voluntary movement (Holodny et al., 2005), future work would need to evaluate the relationship between recruitment curves and tracts descending below the PLIC. Finally, by employing a tractography based analysis, we were unable to relate recruitment curve properties to a specific region of interest that incorporated all descending CST (e.g., non-segmented PLIC). Future work would need to expound upon the results here in order to determine if overall CST FA displayed a similar role in recruitment curve output.

CONCLUSIONS

Our study shows that recruitment curves in patients with chronic stroke may reflect information about CST function mainly from premotor areas but not those from the primary motor cortex. Specifically, we noted that CST integrity from premotor regions was correlated to the output of the recruitment curve (RC $AUC_{Ipsi/Contra}$). This finding is conceivable since higher motor cortices undergo remapping in chronic recovery while M1 CST are substantially damaged (Weiller et al., 1992; Seitz et al., 1998; Bhatt et al., 2007; Ward et al., 2007). Therefore, we suggest caution when interpreting areas that contribute to recovery based on changes in CST function. For example, it may be that even if recruitment curves acquired in the territory of M1 show gains in properties, PMC/SMA located anteriorly could have remapped and instead contributed to recovery.

Another notable finding was that recruitment curve properties were sensitive to integrity along the entire length of CST, taking into account not just the lesion, but also degenerated regions. Based on these results, we suggest that recruitment curves may serve as a viable alternative to time- and cost-intensive imaging modalities when trying to understand CST integrity in a chronic stroke population. We conclude that since different regions of CST damage can uniquely define properties of the recruitment curve, unlike simple TMS metrics that convey binary decisions like recovery or no recovery based on mere presence or absence of MEPs, recruitment curves may serve as a simple, in-expensive means to infer an understanding about damage and degeneration occurring throughout the CST, particularly from re-mapped higher motor regions.

AUTHOR CONTRIBUTIONS

The initial conception and design of the work was done by KP, DC, AM, AC, and EP. Data was collected and analyzed by KP, NV, SR, VS, CB, and KS. All scripts and analysis of DTI was done by KP and KS. Authors that were in charge of collecting the data were also involved in the interpretation of the data. The primary author of the manuscript was KP. Revisions and critical evaluations, including the addition of substantial intellectual content, were then provided by all authors over the course of several drafts. All authors gave their final approval of the version to be published and are in an agreement to be accountable for any questions related to the accuracy of the work.

ACKNOWLEDGMENTS

We acknowledge support by the National Institutes of Health (K01HD069504) and American Heart Association (13BGIA17120055 and 16GRNT27720019) grants to EP and NIH's Clinical and Translational Science Collaborative grant (RPC2014-1067) to DC.

REFERENCES

Alexander, A. L., Lee, J. E., Lazar, M., and Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics* 4, 316–329. doi: 10.1016/j.nurt.2007.05.011

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

Bhatt, E., Nagpal, A., Greer, K. H., Grunewald, T. K., Steele, J. L., Wiemiller, J. W., et al. (2007). Effect of finger tracking combined with electrical stimulation on brain reorganization and hand function in subjects with stroke. *Exp. Brain Res.* 182, 435–447. doi: 10.1007/s00221-007-1001-5

Bogousslavsky, J., and Regli, F. (1990). Anterior cerebral artery territory infarction in the lausanne stroke registry: clinical and etiologic patterns. *Arch. Neurol.* 47, 144–150. doi: 10.1001/archneur.1990.00530020040012

Boroojerdi, B., Battaglia, F., Muellbacher, W., and Cohen, L. G. (2001). Mechanisms influencing stimulus-response properties of the humancorticospinal system. *Clin. Neurophysiol.* 112, 931–937. doi: 10.1016/S1388-2457(01)00523-5

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

Boudrias, M. H., Belhaj-Saïf, A., Park, M. C., and Cheney, P. D. (2006). Contrasting properties of motor output from the supplementary motor area and primary motor cortex in rhesus macaques. *Cereb. Cortex.* 16, 632–638. doi: 10.1093/cercor/bhj009

Bowden, J. L., Taylor, J. L., and McNulty, P. A. (2014). Voluntary activation is reduced in both the more- and less-affected upper limbs after unilateral stroke. *Front. Neurol.* 5:239. doi: 10.3389/fneur.2014.00239

Buffon, F., Molko, N., Hervé, D., Porcher, R., Denghien, I., Pappata, S., et al. (2005). Longitudinal diffusion changes in cerebral hemispheres after MCA infarcts. *J. Cereb. Blood Flow Metab.* 25, 641–650. doi: 10.1038/sj.jcbfm.9600054

Bütefisch, C. M., Netz, J., Webling, M., Seitz, R. J., and Hömberg, V. (2003). Remote changes in cortical excitability after stroke. *Brain* 126, 470–481. doi: 10.1093/brain/awg044

Calautti, C., Jones, P. S., Guincestre, J. Y., Naccarato, M., Sharma, N., Day, D. J., et al. (2010). The neural substrates of impaired finger tapping regularity after stroke. *Neuroimage* 50, 1–6. doi: 10.1016/j.neuroimage.2009.12.012

Carroll, T. J., Riek, S., and Carson, R. G. (2001). Reliability of the input–output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *J. Neurosci. Methods* 112, 193–202. doi: 10.1016/S0165-0270(01)00468-X

Carson, R. G., Nelson, B. D., Buick, A. R., Carroll, T. J., Kennedy, N. C., and Cann, R. M. (2013). Characterizing changes in the excitability of corticospinal projections to proximal muscles of the upper limb. *Brain Stimul.* 6, 760–768. doi: 10.1016/j.brs.2013.01.016

Chenevert, T. L., Brunberg, J. A., and Pipe, J. G. (1990). Anisotropic diffusion in human white matter: demonstration with MR techniques *in vivo*. *Radiology* 177, 401–405. doi: 10.1148/radiology.177.2.2217776

Civardi, C., Cantello, R., Asselman, P., and Rothwell, J. C. (2001). Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. *Neuroimage* 14, 1444–1453. doi: 10.1006/nimg.2001.0918

Crofts, J. J., Higham, D. J., Bosnell, R., Jbabdi, S., Matthews, P. M., Behrens, T. E., et al. (2011). Network analysis detects changes in the contralesional hemisphere following stroke. *Neuroimage* 54, 161–169. doi: 10.1016/j.neuroimage.2010.08.032

Cunningham, D. A., Machado, A., Janini, D., Varnerin, N., Bonnett, C., Yue, G., et al. (2014). The assessment of inter-hemispheric imbalance using imaging and non-invasive brain stimulation in patients with chronic stroke. *Arch. Phys. Med. Rehabil.* 96(4 Suppl), S94–S103. doi: 10.1016/j.apmr.2014.07.419

Cunningham, D. A., Varnerin, N., Machado, A., Bonnett, C., Janini, D., Roelle, S., et al. (2015). Stimulation targeting higher motor areas in stroke rehabilitation: A proof-of-concept, randomized, double-blinded placebo-controlled study of effectiveness and underlying mechanisms. *Restor. Neurol. Neurosci.* 33, 911–926. doi: 10.3233/RNN-150574

Dacosta-Aguayo, R., Graña, M., Fernández-Andújar, M., López-Cáncio, E., Caceres, C., Bargalló, N., et al. (2014). Structural integrity of the contralesional hemisphere predicts cognitive impairment in ischemic stroke at three months. *PLoS ONE* 9:e86119. doi: 10.1371/journal.pone.0086119

Dancause, N., Barbay, S., Frost, S. B., Plautz, E. J., Chen, D., Zoubina, E. V., et al. (2005). Extensive cortical rewiring after brain injury. *J. Neurosci.* 25, 10167–10179. doi: 10.1523/JNEUROSCI.3256-05.2005

Danielian, L. E., Iwata, N. K., Thomasson, D. M., and Floeter, M. K. (2010). Reliability of fiber tracking measurements in diffusion tensor imaging for longitudinal study. *Neuroimage* 49, 1572–1580. doi: 10.1016/j.neuroimage.2009.08.062

Demirtas-Tatlidede, A., Alonso-Alonso, M., Shetty, R. P., Ronen, I., Pascual-Leone, A., and Fregni, F. (2015). Long-term effects of contralesional rTMS in severe stroke: safety, cortical excitability, and relationship with transcallosal motor fibers. *Neurorehabilitation* 36, 51–59. doi: 10.3233/NRE-141191

Devanne, H., Lavoie, B. A., and Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Exp. Brain Res.* 114, 329–338. doi: 10.1007/PL00005641

Di Lazzaro, V. (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

Dum, R. P., and Strick, P. L. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *J. Neurosci.* 11, 667–689.

Duncan, P. W., Propst, M., and Nelson, S. G. (1983). Reliability of the fugl-meyer assessment of sensorimotor recovery following cerebrovascular accident. *Phys. Ther.* 63, 1606–1610.

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

Fridman, E. A., Hanakawa, T., Chung, M., Hummel, F., Leiguarda, R. C., and Cohen, L. G. (2004). Reorganization of the human ipsilesional premotor cortex after stroke. *Brain* 127, 747–758. doi: 10.1093/brain/awh082

Fries, W., Danek, A., Scheidtmann, K., and Hamburger, C. (1993). Motor recovery following capsular stroke: Role of descending pathways from multiple motor areas. *Brain* 116, 369–382. doi: 10.1093/brain/116.2.369

Fugl-Meyer, A. R., Jääskö, L., Leyman, I., Olsson, S., and Steglind, S. (1975). The post-stroke hemiplegic patient: I. A method for evaluation of physical performance. *Scand. J. Rehab. Med.* 7, 13–31.

Gerschlager, W., Siebner, H. R., and Rothwell, J. (2001). Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. *Neurology* 57, 449–455. doi: 10.1212/WNL.57.3.449

Geyer, S., Ledberg, A., Schleicher, A., Kinomura, S., Schormann, T., Bürgel, U., et al. (1996). Two different areas within the primary motor cortex of man. *Nature* 382, 805–807. doi: 10.1038/382805a0

Ghosh, S., and Porter, R. (1988). Corticocortical synaptic influences on morphologically identified pyramidal neurones in the motor cortex of the monkey. *J. Physiol.* 400, 617–629. doi: 10.1113/jphysiol.1988.sp017139

Gladstone, D. J., Danells, C. J., and Black, S. E. (2002). The fugl-meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil. Neural Repair* 16, 232–240. doi: 10.1177/154596802401105171

Grandin, C. B., Duprez, T. P., Smith, A. M., Mataigne, F., Peeters, A., Oppenheim, C., et al. (2001). Usefulness of magnetic resonance–derived quantitative measurements of cerebral blood flow and volume in prediction of infarct growth in hyperacute stroke. *Stroke* 32, 1147–1153. doi: 10.1161/01.STR.32.5.1147

Granziera, C., Ay, H., Koniak, S. P., Krueger, G., and Sorensen, A. G. (2010). Diffusion tensor imaging shows structural remodeling of stroke mirror region: results from a pilot study. *Eur. Neurol.* 67, 370–376. doi: 10.1159/000336062

Granziera, C., D'Arceuil, H., Zai, L., Magistretti, P. J., Sorensen, A. G., and de Crespigny, A. J. (2007). Long-term monitoring of post-stroke plasticity after transient cerebral ischemia in mice using *in vivo* and *ex vivo* diffusion tensor MRI. *Open Neuroimag. J.* 1, 10–17. doi: 10.2174/1874440000701010010

Groisser, B. N., Copen, W. A., Singhal, A. B., Hirai, K. K., and Schaechter, J. D. (2014). Corticospinal tract diffusion abnormalities early after stroke predict motor outcome. *Neurorehabil. Neural Repair.* 28, 751–760. doi: 10.1177/1545968314521896

Harris-Love, M. L., Morton, S. M., Perez, M. A., and Cohen, L. G. (2011). Mechanisms of short-term training-induced reaching improvement in severely

hemiparetic stroke patients: a TMS study. *Neurorehabil. Neural Repair* 25, 398–411. doi: 10.1177/1545968310395600

Henderson, R. D., Ridall, G. R., Pettitt, A. N., McCombe, P. A., and Daube, J. R. (2006). The stimulus-response curve and motor unit variability in normal subjects and subjects with amyotrophic lateral sclerosis. *Muscle Nerve* 34, 34–43. doi: 10.1002/mus.20561

Holodny, A. I., Gor, D. M., Watts, R., Gutin, P. H., and Ulug, A. M. (2005). Diffusion-tensor mr tractography of somatotopic organization of corticospinal tracts in the internal capsule: initial anatomic results in contradistinction to prior reports. *Radiology* 234, 649–653. doi: 10.1148/radiol.2343032087

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C., et al. (2005). Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain* 128, 490–499. doi: 10.1093/brain/awh369

Ino, T., Nakai, R., Azuma, T., Yamamoto, T., Tsutsumi, S., and Fukuyama, H. (2007). Somatotopy of corticospinal tract in the internal capsule shown by functional MRI and di¡usion tensor images. *NeuroReport* 18, 665–668. doi: 10.1097/WNR.0b013e3280d943e1

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., and Smith, S. M. (2012). Fsl. *Neuroimage* 62, 782–790. doi: 10.1016/j.neuroimage.2011.09.015

Johansen-Berg, H., Rushworth, M. F., Bogdanovic, M. D., Kischka, U., Wimalaratna, S., and Matthews, P. M. (2002). 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

Kaelin-Lang, A., and Cohen, L. G. (2000). Enhancing the quality of studies using transcranial magnetic and electrical stimulation with a new computer-controlled system. *J. Neurosci. Methods* 102, 81–89. doi: 10.1016/S0165-0270(00)00284-3

Klöppel, S., Baumer, T., Kroeger, J., Koch, M. A., Buchel, C., Münchau, A., et al. (2008). The cortical motor threshold reflects microstructural properties of cerebral white matter. *Neuroimage* 40, 1782–1791. doi: 10.1016/j.neuroimage.2008.01.019

Kobayashi, S., Hasegawa, S., Maki, T., and Murayama, S. (2005). Retrograde degeneration of the corticospinal tract associated with pontine infarction. *J. Neurol. Sci.* 236, 91–93. doi: 10.1016/j.jns.2005.04.018

Kunimatsu, A., Aoki, S., Masutani, Y., Abe, O., Hayashi, N., Mori, H., et al. (2004). The optimal trackability threshold of fractional anisotropy for diffusion tensor tractography of the corticospinal tract. *Magn. Reson. Med. Sci.* 3, 11–17. doi: 10.2463/mrms.3.11

Lemon, R. N. (1999). Neural control of dexterity: what has been achieved? *Exp. Brain Res.* 128, 6–12. doi: 10.1007/s002210050811

Levy, R. M., Harvey, R. L., Kissela, B. M., Winstein, C. J., Lutsep, H. L., Parrish, T. B., et al. (2016). Epidural electrical stimulation for stroke rehabilitation: results of the prospective, multicenter, randomized, single-blinded everest trial. *Neurorehabil. Neural Repair*. 30, 107–117. doi: 10.1177/1545968315575613

Liang, Z., Zeng, J., Zhang, C., Liu, S., Ling, X., Xu, A., et al. (2008). Longitudinal investigations on the anterograde and retrograde degeneration in the pyramidal tract following pontine infarction with diffusion tensor imaging. *Cerebrovasc. Dis.* 25, 209–216. doi: 10.1159/000113858

Liepert, J., Restemeyer, C., Kucinski, T., Zittel, S., and Weiller, C. (2005). Motor strokes: the lesion location determines motor excitability changes. *Stroke* 36, 2648–2653. doi: 10.1161/01.STR.0000189629.10603.02

Lindberg, P. G., Skejø, P. H., Rounis, E., Nagy, Z., Schmitz, C., Wernegren, H., et al. (2007). Wallerian degeneration of the corticofugal tracts in chronic stroke: a pilot study relating diffusion tensor imaging, transcranial magnetic stimulation, and hand function. *Neurorehabil. Neural Repair* 21, 551–560. doi: 10.1177/1545968307301886

Lindenberg, R., Zhu, L. L., Ruber, T., and Schlaug, G. (2012). Predicting functional motor potential in chronic stroke patients using diffusion tensor imaging. *Hum. Brain Mapp.* 33, 1040–1051. doi: 10.1002/hbm.21266

Liu, Y., and Rouiller, E. M. (1999). Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. *Exp. Brain Res.* 128, 149–159. doi: 10.1007/s002210050830

Lotze, M., Beutling, W., Loibl, M., Domin, M., Platz, T., Schminke, U., et al. (2012). Contralesional motor cortex activation depends on ipsilesional corticospinal tract integrity in well-recovered subcortical stroke patients. *Neurorehabil. Neural Repair* 26, 594–603. doi: 10.1177/1545968311427706

Lotze, M., Laubis-Herrmann, U., and Topka, H. (2006). Combination of TMS and fMRI reveals a specific pattern of reorganization in M1 in patients after complete spinal cord injury. *Restor. Neurol. Neurosci.* 24, 97–107.

Lowe, M. J., Beall, E. B., Sakaie, K. E., Koenig, K. A., Stone, L., Marrie, R. A., et al. (2008). Resting state sensorimotor functional connectivity in multiple sclerosis inversely correlates with transcallosal motor pathway transverse diffusivity. *Hum. Brain Mapp.* 29, 818–827. doi: 10.1002/hbm.20576

Lukács, M., Vécsei, L., and Beniczky, S. (2008). Large motor units are selectively affected following a stroke. *Clin. Neurophysiol.* 119, 2555–2558. doi: 10.1016/j.clinph.2008.08.005

MacCabee, P. J., Amassian, V. E., Eberle, L. P., and Cracco, R. Q. (1993). Magnetic coil stimulation of straight and bent amphibian and mammalian peripheral nerve *in vitro*: locus of excitation. *J. Physiol.* 460, 201–219. doi: 10.1113/jphysiol.1993.sp019467

Monti, R. J., Roy, R. R., and Edgerton, V. R. (2001). Role of motor unit structure in defining function. *Muscle Nerve* 24, 848–866. doi: 10.1002/mus.1083

Neggers, S. F., Langerak, T. R., Schutter, D. J., Mandl, R. C., Ramsey, N. F., Lemmens, P. J., et al. (2004). A stereotactic method for image-guided transcranial magnetic stimulation validated with fMRI and motor-evoked potentials. *Neuroimage* 21, 1805–1817. doi: 10.1016/j.neuroimage.2003.12.006

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

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

Park, C. H., Kou, N., Boudrias, M. H., Playford, E. D., and Ward, N. S. (2013). Assessing a standardised approach to measuring corticospinal integrity after stroke with DTI. *Neuroimage Clin.* 2, 521–533. doi: 10.1016/j.nicl.2013.04.002

Park, J. K., Kim, B. S., Choi, G., Kim, S. H., Choi, J. C., and Khang, H. (2008). Evaluation of the somatotopic organization of corticospinal tracts in the internal capsule and cerebral peduncle: results of diffusion-tensor MR tractography. *Korean J. Radiol.* 9, 191–195. doi: 10.3348/kjr.2008.9.3.191

Plow, E. B., Cunningham, D. A., Varnerin, N., and Machado, A. (2014). *Rethinking stimulation of the brain in stroke* rehabilitation: why higher motor areas might be better alternatives for patients with greater impairments. *Neuroscientist*. doi: 10.1177/1073858414537381

Prins, N. D., van Dijk, E. J., den Heijer, T., Vermeer, S. E., Jolles, J., Koudstaal, P. J., et al. (2005). Cerebral small-vessel disease and decline in information processing speed, executive function and memory. *Brain* 128, 2034–2041. doi: 10.1093/brain/awh553

Puig, J., Pedraza, S., Blasco, G., Daunis, I. E. J., Prados, F., Remollo, S., et al. (2011). Acute damage to the posterior limb of the internal capsule on diffusion tensor tractography as an early imaging predictor of motor outcome after stroke. *AJNR Am. J. Neuroradiol.* 32, 857–863. doi: 10.3174/ajnr.A2400

Qiu, M., Darling, W. G., Morecraft, R. J., Ni, C. C., Rajendra, J., and Butler, A. J. (2011). White matter integrity is a stronger predictor of motor function than BOLD response in patients with stroke. *Neurorehabil. Neural Repair* 25, 275–284. doi: 10.1177/1545968310389183

Ridding, M. C., and Rothwell, J. C. (1997). Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electrocephalogr. Clin. Neurophysiol.* 105, 340–344. doi: 10.1016/S0924-980X(97)00041-6

Rizzo, V., Siebner, H. R., Modügno, N., Pesenti, A., Munchau, A., Gerschlager, W., et al. (2004). Shaping the excitability of human motor cortex with premotor rTMS. *J. Physiol.* 554, 483–495. doi: 10.1113/jphysiol.2003.048777

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., and Safety of TMS Consensus Group. (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

Rossini, P. M., and Rossi, S. (2007). Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. *Neurology* 68, 484–488. doi: 10.1212/01.wnl.0000250268.13789.b2

Sakaie, K. E., and Lowe, M. J. (2007). An objective method for regularization of fiber orientation distributions derived from diffusion-weighted MRI. *Neuroimage* 34, 169–176. doi: 10.1016/j.neuroimage.2006.08.034

Schaechter, J. D., Fricker, Z. P., Perdue, K. L., Helmer, K. G., Vangel, M. G., Greve, D. N., et al. (2009). Microstructural status of ipsilesional and contralesional

corticospinal tract correlates with motor skill in chronic stroke patients. *Hum. Brain Mapp.* 30, 3461–3474. doi: 10.1002/hbm.20770

Schulz, R., Park, C. H., Boudrias, M. H., Gerloff, C., Hummel, F. C., and Ward, N. S. (2012). Assessing the integrity of corticospinal pathways from primary and secondary cortical motor areas after stroke. *Stroke* 43, 2248–2251. doi: 10.1161/STROKEAHA.112.662619

Seitz, R. J., Höflich, P., Binkofski, F., Tellmann, L., Herzog, H., and Freund, H.-J. (1998). Role of the premotor cortex in recovery from middle cerebral artery infarction. *Arch. Neurol.* 55, 1081–1088. doi: 10.1001/archneur.55.8.1081

Shellock, F. G. (2014). Available online at: www.MRIsafety.com

Sidaros, A., Engberg, A. W., Sidaros, K., Liptrot, M. G., Herning, M., Petersen, P., et al. (2008). Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. *Brain* 131, 559–572. doi: 10.1093/brain/awm294

Soares, J. M., Marques, P., Alves, V., and Sousa, N. (2013). A hitchhiker's guide to diffusion tensor imaging. *Front. Neurosci.* 7:31. doi: 10.3389/fnins.2013.00031

Stinear, C. M., Barber, P. A., Petoe, M., Anwar, S., and Byblow, W. D. (2012). The PREP algorithm predicts potential for upper limb recovery after stroke. *Brain* 135, 2527–2535. doi: 10.1093/brain/aws146

Stinear, C. M., Barber, P. A., Smale, P. R., Coxon, J. P., Fleming, M. K., and Byblow, W. D. (2007). Functional potential in chronic stroke patients depends on corticospinal tract integrity. *Brain* 130, 170–180. doi: 10.1093/brain/awl333

Takeuchi, N., Tada, T., Chuma, T., Matsuo, Y., and Ikoma, K. (2007). Disinhibition of the premotor cortex contributes to a maladaptive change in the affected hand after stroke. *Stroke* 38, 1551–1556. doi: 10.1161/STROKEAHA.106.470187

Talelli, P., Greenwood, R. J., and Rothwell, J. C. (2006). Arm function after stroke: neurophysiological correlates and recovery mechanisms assessed by transcranial magnetic stimulation. *Clin. Neurophysiol.* 117, 1641–1659. doi: 10.1016/j.clinph.2006.01.016

Talelli, P., Ewas, A., Waddingham, W., Rothwell, J. C., and Ward, N. S. (2008). Neural correlates of age-related changes in cortical neurophysiology. *NeuroImage* 40, 1772–1781. doi: 10.1016/j.neuroimage.2008.01.039

Teitti, S., Maatta, S., Säisänen, L., Könönen, M., Vanninen, R., Hannula, H., et al. (2008). Non-primary motor areas in the human frontal lobe are connected directly to hand muscles. *Neuroimage* 40, 1243–1250. doi: 10.1016/j.neuroimage.2007.12.065

Thickbroom, G. W., Byrnes, M. L., Archer, S. A., and Mastaglia, F. L. (2002). Motor outcome after subcortical stroke: MEPs correlate with hand strength but not dexterity. *Clin. Neurophysiol.* 113, 2025–2029. doi: 10.1016/S1388-2457(02)00318-8

Ward, N. (2011). Assessment of cortical reorganisation for hand function after stroke. *J. Physiol.* 589, 5625–5632. doi: 10.1113/jphysiol.2011.220939

Ward, N. S., Newton, J. M., Swayne, O. B., Lee, L., Frackowiak, R. S., Thompson, A. J., et al. (2007). The relationship between brain activity and peak grip force is modulated by corticospinal system integrity after subcortical stroke. *Eur. J. Neurosci.* 25, 1865–1873. doi: 10.1111/j.1460-9568.2007.05434.x

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

Weiller, C., Chollet, F., Friston, K. J., Wise, R. J. S., and Frackowiak, R. S. J. (1992). Functional Reorganization of the brain in recovery from striatocapsular idarction in man. *Ann. Neurol.* 31, 463–472. doi: 10.1002/ana.410310502

Wittenberg, G. F., and Schaechter, J. D. (2009). The neural basis of constraint-induced movement therapy. *Curr. Opin. Neurol.* 22, 582–588. doi: 10.1097/WCO.0b013e3283320229

Zeiler, S. R., Gibson, E. M., Hoesch, R. E., Li, M. Y., Worley, P. F., O'Brien, R. J., et al. (2013). Medial premotor cortex shows a reduction in inhibitory markers and mediates recovery in a mouse model of focal stroke. *Stroke* 44, 483–489. doi: 10.1161/STROKEAHA.112.676940

Zhang, M., Sakaie, K. E., and Jones, S. E. (2013). Logical foundations and fast implementation of probabilistic tractography. *IEEE Trans. Med. Imag.* 32, 1397–1410. doi: 10.1109/TMI.2013.2257179

Zhu, L. L., Lindenberg, R., Alexander, M. P., and Schlaug, G. (2010). Lesion load of the corticospinal tract predicts motor impairment in chronic stroke. *Stroke* 41, 910–915. doi: 10.1161/STROKEAHA.109.577023

Conflict of Interest Statement: AM has the following conflicts of interest to disclose: Intelect medical (advisory board, consultant, shareholder), ATI, Enspire and Cardionomics (distribution rights from intellectual property), Functional Neurostimulation (consultant), Deep Brain Innovations (consultant), Medtronic (Fellowship support). 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.

The reviewer MD and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Neuroscience

REVIEW
published: 18 November 2015
doi: 10.3389/fnins.2015.00440

Non-invasive Central and Peripheral Stimulation: New Hope for Essential Tremor?

Moussa A. Chalah[1,2], *Jean-Pascal Lefaucheur*[1,2] *and Samar S. Ayache*[1,2,3]*

[1] EA 4391, Excitabilité Nerveuse et Thérapeutique, Université Paris-Est-Créteil, Créteil, France, [2] Service de Physiologie – Explorations Fonctionnelles, Hôpital Henri Mondor, Assistance Publique – Hôpitaux de Paris, Créteil, France, [3] Neurology Division, University Medical Center Rizk Hospital, Beirut, Lebanon

Essential tremor (ET) is among the most frequent movement disorders. It usually manifests as a postural and kinematic tremor of the arms, but may also involve the head, voice, lower limbs, and trunk. An oscillatory network has been proposed as a neural correlate of ET, and is mainly composed of the olivocerebellar system, thalamus, and motor cortex. Since pharmacological agents have limited benefits, surgical interventions like deep brain stimulation are the last-line treatment options for the most severe cases. Non-invasive brain stimulation techniques, particularly transcranial magnetic or direct current stimulation, are used to ameliorate ET. Their non-invasiveness, along with their side effects profile, makes them an appealing treatment option. In addition, peripheral stimulation has been applied in the same perspective. Hence, the aim of the present review is to shed light on the emergent use of non-invasive central and peripheral stimulation techniques in this interesting context.

Keywords: essential tremor, tremor, tDCS, rTMS, TBS, TENS, non-invasive brain stimulation

OPEN ACCESS

Edited by:
Ignacio Obeso,
CEU San Pablo University, Spain

Reviewed by:
Guglielmo Foffani,
Drexel University, USA
Lydia Vela,
Hospital Universitario Fundación Alcorcón, Spain

***Correspondence:**
Samar S. Ayache
samarayache@gmail.com

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

Received: *17 July 2015*
Accepted: *01 November 2015*
Published: *18 November 2015*

Citation:
Chalah MA, Lefaucheur J-P and Ayache SS (2015) Non-invasive Central and Peripheral Stimulation: New Hope for Essential Tremor? Front. Neurosci. 9:440. doi: 10.3389/fnins.2015.00440

INTRODUCTION

Essential tremor (ET) is among the most frequent movement disorders in individuals above 40 years of age (Louis et al., 1995; Dogu et al., 2003). Clinically, it manifests as postural and action tremor of the arms, but may also involve the head, voice, lower limbs, and trunk (Deuschl et al., 1998; Bain et al., 2000; Elble, 2000; Raethjen and Deuschl, 2012). From an etiological perspective, it is classified as sporadic or hereditary (Kuhlenbäumer et al., 2014). Despite its high prevalence, its underlying pathophysiological mechanisms are still not well elucidated. Data from neuroimaging and neurophysiological studies have put into evidence the existence of a cerebello-thalamo-cortical (CTC) network for ET (Pinto et al., 2003; Popa et al., 2013; Hallett, 2014). The latter includes the sensorimotor cortex, olivocerebellar system, red nucleus, and thalamus (Colebatch et al., 1990; Jenkins et al., 1993; Hallett and Dubinsky, 1993; Wills et al., 1995; Bucher et al., 1997; Boecker and Brooks, 1998; Deuschl et al., 2000; Pinto et al., 2003; Raethjen et al., 2007; Quattrone et al., 2008; Shin et al., 2008; Schnitzler et al., 2009; Cerasa et al., 2010; Park et al., 2010; Bagepally et al., 2012; Paris-Robidas et al., 2012; Raethjen and Deuschl, 2012; Fang et al., 2013; Buijink et al., 2015; Choi et al., 2015; Shin et al., 2015). The presence of such a circuit was further confirmed by reports documenting ET disappearance following strokes that involved some of its components (Dupuis et al., 2010; Lim et al., 2010; Chalah et al., 2015).

Although ET is commonly thought to be a benign condition, affected patients represent a heterogeneous population (Louis, 2009) and severe cases could be very disabling (Louis, 2005).

In this context, pharmacological agents have yielded modest benefits (Findley, 1987; Louis, 2000; Deuschl et al., 2011), and non-adherence to ET medications has been reported (Louis, 2015). Surgical interventions, like the deep brain stimulation, are the last-line treatment options for the most severe cases (Chopra et al., 2013). However, they have some limitations related to their cost and potential side effects (Grimaldi and Manto, 2008). Nowadays, there is a growing interest in using non-invasive central and peripheral stimulation techniques as alternatives to pharmacological and surgical interventions. Hence, in the present review, we shed light on the emergent use of these techniques in treating ET. Conversely, we excluded all data regarding invasive interventions, namely cortical, or deep brain stimulation.

PRINCIPLES OF NON-INVASIVE BRAIN STIMULATION TECHNIQUES

In the recent years, two non-invasive brain stimulation (NIBS) techniques, i.e., repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), gained interest for their potential implication in treating various neuropsychiatric symptoms (Kuo et al., 2014; Lefaucheur et al., 2014). These techniques are based on different principles.

To start, rTMS consists of a transcranial delivery of an electromagnetic field by a stimulation coil positioned on the patient's scalp. The induced intracortical current is strong enough to trigger action potentials according to Faraday's law of electromagnetic induction (Lefaucheur, 2012). Thus, it acts by modulating the cortical excitability in a frequency-dependent manner, as low (LF) and high (HF) stimulation frequencies (<1 vs. >5 Hz) have been shown to induce inhibitory and excitatory effects, respectively (Lefaucheur et al., 2014). Other than the frequency, various stimulation parameters, such as the selected cortical target, can influence the clinical effects of rTMS (Lefaucheur, 2008, 2009, 2012).

In addition to rTMS, new stimulation paradigms are being developed, of which theta burst stimulation (TBS) is the most popular (Lefaucheur, 2009). Practically, TBS consists of short bursts delivered at 5 Hz (within the theta range), each burst consisting of three magnetic pulses delivered at HF (50 Hz). TBS is either applied continuously (cTBS) for 40 s or intermittently (iTBS) during 2 s every 10 s for a total stimulation time of 200 s. Similar to rTMS, the action of TBS primarily depends on the stimulation pattern: cTBS and iTBS respectively induce long-term synaptic depression-like and potentiation-like effects, when applied over the primary motor cortex (M1) of healthy individuals (Huang et al., 2005, 2007; Teo et al., 2007; Huang, 2010; Wischnewski and Schutter, 2015).

Beside magnetic stimulation, tDCS has emerged as a promising neuromodulatory technique. It consists of delivering an electric current of low intensity (1–2 mA) over few minutes via two electrodes (anode and cathode) positioned over the scalp. By doing so, it could induce prolonged yet reversible shifts in cortical excitability and might modulate the connectivity of various neural circuits (Priori et al., 1998; Nitsche and Paulus, 2000, 2001; Priori, 2003; Nitsche et al., 2003a). The polarity of tDCS protocols determines the neurophysiological outcomes at the level of the exposed tissues: a depolarization or a hyperpolarization of the resting membrane potentials would occur following anodal or cathodal tDCS, respectively (Creutzfeldt et al., 1962; Purpura and McMurtry, 1965; Nitsche and Paulus, 2011; Paulus et al., 2013; Filmer et al., 2014). Several parameters mainly related to the electrodes properties (size, polarity, position), the used current (strength and shape), and the stimulation duration, can account for the tDCS effects (Creutzfeldt et al., 1962; Nitsche and Fregni, 2007; Nitsche et al., 2008). tDCS is safe, easily performed, well-tolerated by the patients with little or no side effects (Poreisz et al., 2007; Nitsche et al., 2008; Brunoni et al., 2012), and presents an easier and indistinguishable implementation of sham sessions compared to rTMS protocols (Gandiga et al., 2006).

FUNCTIONAL UNDERPINNINGS OF ESSENTIAL TREMOR

Quite before their therapeutic implications, NIBS techniques have been used to explore the cortical excitability in various pathologies. For example, using transcranial electrical stimulation, one study has shown a normal central motor conduction time in four ET patients, from a series of patients with various movement disorders (Thompson et al., 1986). Using transcranial magnetic stimulation, another study has revealed normal patterns of cortical excitability in ET patients, as expressed by motor thresholds and motor evoked potentials amplitude (Romeo et al., 1998). In other works, ET patients exhibited normal patterns of intracortical inhibition (Hanajima et al., 1998; Romeo et al., 1998; Shukla et al., 2003; Chuang et al., 2014) and cerebello-thalamo-cortical inhibition (Pinto et al., 2003); the latter is a neurophysiological parameter that reflects the degree of reduction of the motor cortical output via the activation of cerebellar inhibitory projections (Pinto and Chen, 2001).

It was not until recently that cortical excitability studies have unveiled abnormal CTC functioning in patients with ET (Chuang et al., 2014; Bologna et al., 2015). Such results are of particular interest since they are in line with functional neuroimaging studies which revealed altered patterns of cortical activation and inter-regional connectivity within the CTC pathways and non-motor cortices (Wills et al., 1995; Bucher et al., 1997; Cerasa et al., 2010; Passamonti et al., 2011; Fang et al., 2013; Popa et al., 2013; Buijink et al., 2015).

In light of this evidence, the neurotransmitters imbalance has been speculated to contribute to the pathophysiology of ET. In this perspective, the glutamatergic metabolism has lately received some attention, but genetic studies have revealed controversial outcomes (Thier et al., 2012; García-Martín et al., 2013; Tan et al., 2013; Yu et al., 2013; Ross et al., 2014). The role of dopamine was also assessed in a number of studies that tried anti-psychotics (Pakkenberg and Pakkenberg, 1986; Ceravolo et al., 1999; Micheli et al., 2002; Yetimalar et al., 2003, 2005) and dopaminergic drugs (Koller, 1981; Manyam, 1984; Gironell et al., 2006) in ET management; tremor improvement was only

observed in the two studies involving olanzapine (Yetimalar et al., 2003, 2005). Additionally, multidisciplinary studies are supporting the role of an aberrant GABAergic transmission in ET production (Louis, 1999; Zesiewicz et al., 2007, 2013; Boecker et al., 2010; Gironell et al., 2012; Paris-Robidas et al., 2012; Shill et al., 2012; Boecker, 2013; Helmich et al., 2013; Chuang et al., 2014; Gironell, 2014; Schneider and Deuschl, 2014). Therefore, acting on such a neurochemical imbalance might be helpful in improving ET. Interestingly, some studies have reported that NIBS after-effects take place through the modulation of the glutamatergic, GABAergic and dopaminergic transmissions (Liebetanz et al., 2002; Nitsche et al., 2003b, 2006; Stagg et al., 2009; Monte-Silva et al., 2011; Foerster et al., 2015). In addition, studies coupling NIBS techniques with functional neuroimaging have shown that rTMS, TBS, and tDCS are able to improve the functional connectivity of various cortico-subcortical networks (Bestmann et al., 2004; Grefkes et al., 2010; Eldaief et al., 2011; Keeser et al., 2011; Polanía et al., 2011, 2012a,b; Halko et al., 2014; Valchev et al., 2015).

Taken together, these data suggest that NIBS techniques would ameliorate ET by (i) acting on the neurochemical imbalance at the site of stimulation, (ii) subsequently modulating the local cortical excitability and by doing so, (iii) restoring the functional integrity of the CTC network of ET.

NIBS STUDIES AND ESSENTIAL TREMOR

Early neurophysiological studies have assessed the role of NIBS techniques in modulating the physiological parameters in ET. Single-pulse TMS over M1 was successively able to reset ongoing tremor activity (Britton et al., 1993a; Pascual-Leone et al., 1994; Yu et al., 2001). In a recent study, ET resetting resulted from applying single-pulse or paired-pulse TMS over M1 or the supplementary motor area (SMA), but not over the cerebellum (Lu et al., 2015).

Electrical stimulation was also used in the same setting. Although early reports have documented a failure of transcranial motor electrical stimulation in resetting ET (Pascual-Leone et al., 1994), a new study have provided evidence regarding the ability of transcranial alternating current stimulation to induce tremor entrainment when applied over the cerebellum of ET patients (Brittain et al., 2015).

These data altogether have pushed the research toward studying the possible therapeutic implementations of NIBS in terms of ET. A PubMed search using the keywords rTMS/TBS/tDCS and essential tremor has identified seven English papers. Their designs and outcomes are reported in **Table 1**.

rTMS and Essential Tremor

The first published study involved 10 patients with ET of the upper limbs, in a double-blind, crossover, and sham-controlled design (Gironell et al., 2002). The patients received two sessions of either active or sham 1 Hz rTMS over the cerebellum separated by 1-week free interval. Compared to sham, significant short-term effects were observed following real rTMS session, as expressed by the improvement of the tremor clinical rating scale and tremor frequency on accelerometric studies. However, such an improvement did not last more than 5 min following the session.

In a second study, 11 ET patients underwent a single session of 1 Hz rTMS over the cerebellum to evaluate the potential modulation of motor behavior during repetitive finger tapping movements of the right hand using a sensor-engineered glove (Avanzino et al., 2009). Seven patients also received sham session at least 2 weeks apart from the real one. Compared to healthy controls, ET patients presented lower inter-tapping interval (ITI), increased coefficient of variation of ITI (ITICV), and longer touch duration (TD). The latter represents the time when the thumb and another finger are in contact, before their separation which results in generation of the rhythmic movement. It is probably the sum of the time required for the thumb to get an adequate perception of another touched finger (sensory time), and the time needed to plan for the next movement (preparatory time), and by doing so, to maintain the rhythmic tapping (Georgiou et al., 1995). The cerebellum participates in the timing of movement and sensation (Rao et al., 2001), and increased ITICV was previously reported in the context of ET (Farkas et al., 2006). In the absence of sensory deficits in ET patients (Nahab et al., 2007), the abnormal TD values hint toward pathological phenomena at the level of sensorimotor integration, where the sensory information is used for the initiation of motor planning (Avanzino et al., 2009). In this study, rTMS reduced the TD values and normalized the ITI/ITICV values in ET patients in a transient manner. However, in contrast to the first study by Gironell et al. (2002), 1 Hz rTMS was unable to modify the frequency or the intensity of ET, which might be explained in part by the lower stimulation intensity adopted in this study.

A third study included eleven ET patients and eleven healthy controls (Popa et al., 2013). Here, the resting-state functional connectivity (rs-FC) of the CTC circuits and default brain network (DBN) was assessed before and after the application of five consecutive daily sessions of 1 Hz rTMS over the posterior cerebellar cortex. Stimulation was performed using a neuronavigation system to target the lobule VIII of both cerebellar hemispheres. Tremor was assessed using clinical scales and accelerometric recordings. A significant improvement was observed on clinical scales, and was associated with a reduction in tremor amplitude, but not frequency. This improvement persisted for 3 weeks after the last rTMS session and was associated with a near-normal restoration of the connectivity within the CTC network, but not within the DBN. These findings could reflect pronounced neuroplasticity effects that might have resulted from the repetition of the stimulation sessions. In addition, unlike the two previous studies, this one adopted a neuronavigation-guided paradigm, which might have an important role in optimizing rTMS protocols (Lefaucheur, 2010).

TBS and Essential Tremor

Two studies have applied cTBS over the motor and premotor cortices which are the key elements for movement preparation, selection, and execution. The first one assessed the effects of cTBS on tremor and cortical excitability in 10 patients with ET

TABLE 1 | Non-invasive brain stimulation studies in essential tremor.

References	Population	Intervention	Measured outcome	Results
Gironell et al., 2002	10 ET patients	Single session of active or sham 1 Hz rTMS over the cerebellum (on the midline, 2 cm below inion) separated by 1 week interval Each session: 20 min, 300 pulses, 100% of the maximum output intensity	Clinical (TCRS) and accelerometric evaluation before (−5 min), immediately after (+5 min), and 1 h after (+60 min) each session	Short-term clinical and accelerometric improvement, disappearing within 5 min after the end of the active sessio
Avanzino et al., 2009	15 ET patients vs. 11 HCs	Comparing the motor behavior of both groups during repetitive finger tapping movements of the right hand by the means of a sensor-engineered glove	Timing properties and motor behavior	Longer TD, lower ITI, and increased ITICV in ET patients compared to HCs
	11 ET	One session of active or sham† 1 Hz rTMS over the right ipsilateral cerebellum (3 cm lateral and 1 cm beneath the inion) separated by at least 2 week interval Each session: 10 min, 600 pulses, 90% of RMT		Transient reduction of TD values and normalization of ITI and ITICV values No effects of rTMS on the frequency and intensity of tremor
Popa et al., 2013	11 ET patients vs. 11 HCs	5 consecutive daily active sessions of neuronavigated bilateral 1 Hz rTMS over the posterior cerebellar cortex (targeting lobule VIII of each cerebellar hemisphere) Each session: 15 min, 900 pulses, 90% of the RMT	Rs-FC of the CTC network and DBN (as control) before and after rTMS (day 1 and 5) Clinical (FTM) and neurophysiological (electromyographic and accelerometric) tremor assessment at baseline, day 5, day 12, and day 29 after the last session	Improvement in rs-FC within CTC network, but not within DBN Long-term effects lasting for 3 weeks after the last session, consisting in clinical scores improvement and a reduction in tremor amplitude (but not frequency)
Hellriegel et al., 2012	10 ET patients vs. 10 HCs	2 cTBS sessions: one real (80% of AMT), one control (30 % of AMT) over the left hand motor area separated by at least 1 week interval Each session: Two 20-s trains with inter-train interval of 1 min, bursts being repeated every 200 ms	Corticospinal excitability parameter Clinical (FTM) and quantitative (accelerometric) rating of tremor before and at 10, 25, and 40 min after cTBS	Reduction of corticospinal excitability in the stimulated M1 following real cTBS in HCs, but not in ET patients Reduction in tremor amplitude, but not frequency following real cTBS, lasting for at least 45 min No significant clinical reduction of ET after real cTBS
Chuang et al., 2014	13 ET patients vs. 18 HCs	3 cTBS sessions: 2 real (80% of AMT) over the left M1 or PM and 1 sham£ (60% of AMT, flipped coil) over M1) separated by at least 1 week Each session: One 40 s-train, bursts being repeated every 200 ms	Excitability parameters (SICI, CSP, ICF) Accelerometric tremor recording before and 22–25 min after cTBS	Reduced cTBS suppressive effect on motor cortical excitability in ET patients compared with HCs Reduction in tremor amplitude, but not frequency following motor, premotor, but not sham session
Bologna et al., 2015	16 ET patients vs. 11 HCs	2 cTBS sessions: one real (80% of AMT) over the right cerebellar hemisphere (3 cm laterally to and 1 cm below the inion) and one sham over the neck muscles separated by at least 1 week interval Each session: One 40 s-train, bursts being repeated every 200 ms	Excitability parameters (input/output curve) Assessment of tremor and reaching movements at baseline, and at 5 and 45 min after cTBS	Reduced cTBS suppressive effect on motor cortical excitability in ET patients compared with HCs No significant change in tremor severity and reaching movements after any session

(Continued)

TABLE 1 | Continued

References	Population	Intervention	Measured outcome	Results
Gironell et al., 2014	10 ET patients	10 consecutive daily sessions of either active or sham-cathodal cerebellar tDCS separated by a 3-month wash-out period. (two cathodal electrodes placed symmetrically over both cerebellar hemispheres, 3 cm lateral to the inion; and two anodal electrodes positioned over Fp1 and Fp2 EEG leads position) Each session: 20 min; 2 mA	Clinical (TCRS) and accelerometric tremor evaluation before (at day 1), during (10 min after onset) and 60 min after the first session Clinical (TCRS) and accelerometric tremor assessment; and disability scale evaluation before the first session, and at day 10 and 40 after the last session	No significant acute or long-lasting tDCS effects in any outcome measure

AMT, active motor threshold; CSP, cortical silent period; cTBS, continuous theta burst stimulation; CTC, cerebello-thalamo-cortical; DBN, default brain network; ET, essential tremor; FTM, Fahn Tolosa Marin tremor rating scale; HCs, healthy controls; ICF, intracortical facilitation; ITI, inter tapping interval; ITICV, coefficient of variation of the inter tapping interval; MEPs, Motor evoked potentials; Min, minutes; M1, primary motor cortex; PM, premotor cortex; RMT, resting motor threshold; rs-FC, resting state functional connectivity; rTMS, Repetitive transcranial magnetic stimulation; SICI, short interval intracortical inhibition; TCRS, Tremor clinical rating scale; TD, touch duration; tDCS, Transcranial direct current stimulation.

†Sham design was undescribed and performed in seven patients only.

£Sham session was performed in 10 patients only.

and 10 healthy controls (Hellriegel et al., 2012). Each participant randomly received two sessions of real or control cTBS over the left hand motor area separated by at least 1 week. A subclinical reduction in tremor amplitude, but not frequency, was observed following real cTBS session and lasted for at least 45 min. Hereby, the absence of significant clinical improvement could be justified by the logarithmic relationship between accelerometric and clinical tremor assessment (Elble et al., 2006). In line with the first study, a second cTBS study has found an exclusive reduction in tremor amplitude following cTBS delivered to the motor and premotor cortices in 13 patients with ET (Chuang et al., 2014).

Interestingly, in both studies, motor cortical, or corticospinal excitability was assessed using different variables (**Table 1**); and it was shown that the suppressive cTBS effects on cortical excitability was either reduced or absent in ET patients compared to healthy controls. This suggests that the observed improvement in tremor amplitude appears to be independent of the modulation of the corticospinal motor output. Such observation is in accordance with recent evidence hinting toward the occurrence of cTBS-induced behavioral or rs-FC changes, unrelated to those of cortical excitability (Silvanto et al., 2007; Gentner et al., 2008; Nettekoven et al., 2014).

The third cTBS study was a randomized, sham-controlled, double-blind study that assessed the effects of right cerebellar cTBS in ET patients and healthy controls (Bologna et al., 2015). The authors did not find any effect of cTBS on clinical or kinematic measures of tremor. However, as in the two previously published trials, the suppressive effects of cTBS on cortical excitability were lost in ET patients compared to their healthy counterparts.

tDCS and Essential Tremor

Similar to rTMS, the ability of tDCS to modulate the cerebellar excitability has been previously reported (Galea et al., 2009). Gironell and colleagues have studied the effects of cathodal cerebellar tDCS in ten patients with ET (Gironell et al., 2014). Each patient randomly received two blocks, each consisting of 10 consecutive sessions of either active or sham bilateral cerebellar tDCS separated by at least 3 months of washout interval. Clinical and accelerometric studies did not reveal any short-term or long-term benefits following the real tDCS sessions. However, this study suffers from some limitations related to the small sample size and the high intra-subject variability of accelerometric measures.

Peripheral Stimulation and Essential Tremor

Besides trying to act at the level of the central oscillators, an alternative would be to focus on the ET substrates in charge of transmitting and displaying the symptom, namely the peripheral nerves and muscles. The efficacy of symptomatic interventions was tested in tremulous patients regardless of the tremor origin.

Transcutaneous Electrical Nerve Stimulation

Transcutaneous electrical nerve stimulation (TENS) is a non-invasive, cheap, and safe technique that consists of delivering

an electrical current at various frequencies, intensities and pulse duration on a limited skin surface (Sluka and Walsh, 2003; Lim et al., 2010). TENS can modulate motor cortex excitability by acting on the sensory afferent input and the sensorimotor integration at the cortical level (Tinazzi et al., 2005a). In clinical practice, TENS is mainly applied to treat pain syndromes of various origins. In the field of movement disorders, TENS was also found to have some efficacy in dystonic tremor (Bending and Cleeves, 1990), writer's cramp dystonia (Tinazzi et al., 2005b, 2006), and psychogenic movement disorders (Ferrara et al., 2011).

As for ET, the first study to assess the effects of peripheral nerve stimulation was reported by Britton et al. (1993b). Here, the application of supramaximal median nerve shocks at the elbow (0.5 ms square-wave electrical pulse applied as five stimuli, randomly delivered at 5–8 s of interval; with sufficient intensity able to produce maximal EMG responses at the flexor carpi radialis) was able to cause acute inhibition, then synchronization of the EMG activity in 10 patients with ET, nine patients with Parkinson's disease (PD) tremor and nine healthy controls mimicking wrist tremor.

In another study, Munhoz and colleagues assessed TENS effects in five patients with ET and two patients suffering from tremor attributed to peripheral neuropathies (Munhoz et al., 2003). For this purpose, the cathode was placed over the brachial plexus with the reference electrode over the C7 spinous process. A 15-min stimulation was performed, using different settings (frequencies: 5, 10, 50, and 100 Hz; one side vs. the other side vs. both sides simultaneously). No significant improvement was observed at any endpoint (accelerometric variables, tremor rating, and self-reported impression scales) (Munhoz et al., 2003).

Functional Electrical Stimulation

Another alternative to alleviate tremor would be through performing muscular contraction either voluntarily (Dietz et al., 1974; Héroux et al., 2010), or through neurostimulation using the so-called "closed-loop functional electrical stimulation" (FES) (Elek and Prochazka, 1989; Javidan et al., 1990, 1992; Prochazka et al., 1992; Gillard et al., 1999). The earliest study was performed by Javidan and colleagues and involved three patients with ET, four patients with PD and six patients with multiple sclerosis (MS) suffering from cerebellar tremor (Javidan et al., 1992). The authors documented attenuation in tremor amplitude by 73% in ET, 62% in PD, and 38% in MS. Interestingly, a minor shift in tremor frequency was observed in MS group, without any changes in ET or PD patients.

In an attempt to counteract tremor, this method consists of monitoring joint displacement using a miniature displacement transducer. The next step is to use the signals acquired from the joint angle excursions to perform an out-of-phase stimulation, in order to activate the antagonist muscle during involuntary activation of the agonist one. Counteracting tremor is possible via a feedback filter with bandpass characteristics designed to selectively attenuate tremor (2–5 Hz) while barely affecting the slow voluntary movements. Unfortunately, such a technique has some limitations. For instance, the FES ability to act on a given antagonistic pair restricts its role in patients with ET where the symptom is often multidirectional and involves multiple joints. In addition, there is still uncertainty regarding the optimal way of electrodes positioning aiming to stimulate specific muscle groups. Moreover, despite the positive outcome of the preliminary study by Javidan et al. (1992), there is an intra-individual variation in tremor amplitude and frequency, which might limit the efficacy of FES to a specific frequency range and hence requires repeated calibrations of the feedback filter (Javidan et al., 1992). Furthermore, a certain degree of discomfort and fatigue might result from applying the phasic electrical stimulation, which makes the technique less appropriate for daily life usage. In this view, many FES studies have suggested some solutions to circumvent the faced difficulties.

One of the studies tried to explore if the type of feedback filter might affect the clinical outcome (Gillard et al., 1999). For example, a digital filter was found to be superior to its analog counterpart in terms of tremor attenuation in PD patients (84 vs. 65%, respectively). Other studies proposed that a way to improve the FES system would be by implementing a control algorithm that chiefly relies on feedback from inertial sensors and EMG (Zhang and Ang, 2007; Bó et al., 2008; Widjaja et al., 2009; Rocon et al., 2010). This issue was further addressed by a group of authors who applied a new FES system consisting of hardware and software, in three ET patients, four PD patients and five healthy controls (Popović Maneski et al., 2011). In this system, two gyroscopes served the purpose of inertial sensors that provided real-time estimation of tremor, the data of which being digitized and delivered to a computer system that implements an algorithm mainly relying on a Butterworth second-order adaptive bandpass filter (Popović et al., 2010). Via a high-speed USB, the computer controls a battery-driven programmable multichannel stimulator that supports asynchronous activation of several electrodes. The latter are located over the dorsal and volar sides of the forearm, and perform a specific out-of-phase stimulation. The experimental protocol on healthy controls has proven its efficiency in activating the antagonistic muscles in a strong and asynchronous manner, which could not be voluntarily suppressed by the individuals. The intervention was beneficial in only two of the three ET patients. The current design aimed to control several upper extremity joints (fingers, wrist, elbow, and shoulder) and thus was able to overcome the limitations of the mono-joint design discussed in the first work (Javidan et al., 1992). Furthermore, it permitted the stimulation of one muscle group using multi-pad electrodes rather than the previously used single cathode, which could ensure the selectivity (Popović-Bijelić et al., 2005; Popović and Popović, 2009; Popovic et al., 2009; Malešević et al., 2010b) and decrease the stimulation-induced fatigue (Popovic and Malesevic, 2009; Malešević et al., 2010a,b). Following the same principles, other studies combined FES with a brain-computer interface (Grimaldi and Manto, 2010; Rocon et al., 2010). This allows for a multimodal detection of the movement intentionality by fusing signals from EEG, EMG and kinematic sensors (in particular gyroscopes and accelerometers). Another group of authors has relied on EMG detection combined with an iterative Hilbert transform to apply FES in six patients with ET or PD (Dosen et al., 2015). In this study, the tremor was reduced by 46–81 and 35–48% when using the motor and sensory

stimulation, respectively, in five of the six studied patients. Thus, using electrical stimulation below motor threshold seems to be more effective than the sensory one, and prevents muscle fatigue and discomfort.

Finally, fixed-intensity FES was suggested as an alternative to the classical closed-loop FES (Bó et al., 2014). The rationale was that fixing the intensity might make the intervention more comfortable and accepted by patients. Keeping in mind that in ET, the tremor propagates from proximal to peripheral joints, this technique is intrinsically stabilizing compared to the antiphase FES stimulation, where an unstable proximal performance might increase the distal tremor. A single session of fixed-intensity FES was applied to the wrist or fingers of 10 ET patients (pulse width: 150 μs; frequency: 40 Hz; with manual regulation of stimulation intensity respecting patient's feeling of discomfort). The system was similar to the previous ones in a way that it relies on inertial sensors (gyroscopes and accelerometer) and high-pass filter. Tremor was suppressed in eight patients, did not significantly change in one patient, and was exacerbated in one of them.

CONCLUSION

Although only few data are available, some of the preliminary results would pave the way for future studies on a larger scale.

Concerning NIBS, the discrepancy encountered in the results could arise from many factors. On the one hand, all of these studies had assessed the effects of different NIBS techniques in small samples (ranging from 10 to 16 ET patients), and adopted different number of sessions (ranging from 1 to 10 consecutive daily sessions). On the other hand, the fact that ET patients represent a heterogeneous population with regard to the functional brain topography, tremor site, and severity, age of onset, disease duration, pharmacological interventions at the time of protocol, and the patients' sensibility to these treatments, can partly explain the subsequent variation in response to the performed NIBS interventions. In fact, the variation in pre-interventional brain connectivity or genetic polymorphisms in neurotrophic factors can influence NIBS effects (Antal et al., 2010; Cárdenas-Morales et al., 2014; Nettekoven et al., 2014).

Therefore, improving the outcome of NIBS techniques in ET patients can be obtained by acting on different parameters, such as rTMS frequency, TBS pattern, or tDCS polarity. Particularly, increasing the duration or the number of stimulation sessions might enhance the therapeutic effect to a meaningful clinical level, based on the likely dose-dependent effects of these interventions (Nettekoven et al., 2014). Moreover, considering the different functional topography seen in ET patients, a smart attempt to optimize NIBS protocols would be by individualizing them. This could be achieved by performing a baseline functional neuroimaging and neurophysiological interventions in each patient. This approach would improve the definition of the optimal NIBS targets for image-guided procedures. Furthermore, future studies should not be limited to targeting M1 or the cerebellum, but rather should assess the potential value of other targets in terms of motor or cognitive improvement.

Besides acting on the disturbed central networks, peripheral stimulation constitutes a symptomatic approach that proved some benefits in ET. FES can significantly improve tremor of various etiologies, but its use is limited by its practical and esthetic profile. Finally, concerning TENS techniques, only preliminary data are available and further studies are required before drawing any conclusion.

REFERENCES

Antal, A., Chaieb, L., Moliadze, V., Monte-Silva, K., Poreisz, C., Thirugnanasambandam, N., et al. (2010). Brain-derived neurotrophic factor (BDNF) gene polymorphisms shape cortical plasticity in humans. *Brain. Stimul.* 3, 230–237. doi: 10.1016/j.brs.2009.12.003

Avanzino, L., Bove, M., Tacchino, A., Ruggeri, P., Giannini, A., Trompetto, C., et al. (2009). Cerebellar involvement in timing accuracy of rhythmic finger movements in essential tremor. *Eur. J. Neurosci.* 30, 1971–1979. doi: 10.1111/j.1460-9568.2009.06984.x

Bagepally, B. S., Bhatt, M. D., Chandran, V., Saini, J., Bharath, R. D., Vasudev, M. K., et al. (2012). Decrease in cerebral and cerebellar gray matter in essential tremor: a voxel-based morphometric analysis under 3T MRI. *J. Neuroimaging* 22, 275–278. doi: 10.1111/j.1552-6569.2011.00598.x

Bain, P., Brin, M., Deuschl, G., Elble, R., Jankovic, J., Findley, L., et al. (2000). Criteria for the diagnosis of essential tremor. *Neurology* 4, S7.

Bending, J., and Cleeves, L. (1990). Effect of electrical nerve stimulation on dystonic tremor. *Lancet* 336, 1385–1386. doi: 10.1016/0140-6736(90)92946-F

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., and Frahm, J. (2004). Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur. J. Neurosci.* 19, 1950–1962. doi: 10.1111/j.1460-9568.2004.03277.x

Bó, A. P., Azevedo-Coste, C., Geny, C., Poignet, P., and Fattal, C. (2014). On the use of fixed-intensity functional electrical stimulation for attenuating essential tremor. *Artif. Organs.* 38, 984–991. doi: 10.1111/aor.12261

Bó, A. P., Poignet, P., Widjaja, F., and Ang, W. T. (2008). Online pathological tremor characterization using extended Kalman filtering. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2008, 1753–1756. doi: 10.1109/IEMBS.2008.4649516

Boecker, H. (2013). Imaging the role of GABA in movement disorders. *Curr. Neurol. Neurosci. Rep*. 13:385. doi: 10.1007/s11910-013-0385-9

Boecker, H., and Brooks, D. J. (1998). Functional imaging of tremor. *Mov. Disord.* 13, 64e72.

Boecker, H., Weindl, A., Brooks, D. J., Ceballos-Baumann, A. O., Liedtke, C., Miederer, M., et al. (2010). GABAergic dysfunction in essential tremor: an 11C-flumazenil PET study. *J. Nucl. Med*. 51, 1030–1035. doi: 10.2967/jnumed.109.074120

Bologna, M., Rocchi, L., Leodori, G., Paparella, G., Conte, A., Kahn, N., et al. (2015). Cerebellar continuous theta burst stimulation in essential tremor. *Cerebellum* 14, 133–141. doi: 10.1007/s12311-014-0621-0

Brittain, J. S., Cagnan, H., Mehta, A. R., Saifee, T. A., Edwards, M. J., and Brown, P. (2015). Distinguishing the central drive to tremor in Parkinson's disease and essential tremor. *J. Neurosci.* 35, 795–806. doi: 10.1523/JNEUROSCI.3768-14.2015

Britton, T. C., Thompson, P. D., Day, B. L., Rothwell, J. C., Findley, L. J., and Marsden, C. D. (1993a). Modulation of postural wrist tremors by magnetic stimulation of the motor cortex in patients with Parkinson's disease or essential tremor and in normal subjects mimicking tremor. *Ann. Neurol.* 33, 473–479. doi: 10.1002/ana.410330510

Britton, T. C., Thompson, P. D., Day, B. L., Rothwell, J. C., Findley, L. J., and Marsden, C. D. (1993b). Modulation of postural tremors at the wrist by supramaximal electrical median nerve shocks in essential tremor, Parkinson's

disease and normal subjects mimicking tremor. *J. Neurol. Neurosurg. Psychiatry* 56, 1085–1089. doi: 10.1136/jnnp.56.10.1085

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., et al. (2012). Clinical research with transcranial direct current stimulation(tDCS): challenges and future direc-tions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Bucher, S. F., Seelos, K. C., Dodel, R. C., Reiser, M., and Oertel, W. H. (1997). Activation mapping in essential tremor with functional magnetic resonance imaging. *Ann. Neurol.* 41, 32–40. doi: 10.1002/ana.410410108

Buijink, A. W., Broersma, M., van der Stouwe, A. M., van Wingen, G. A., Groot, P. F., Speelman, J. D., et al. (2015). Rhythmic finger tapping reveals cerebellar dysfunction in essential tremor. *Parkinsonism. Relat. Disord.* 21, 383–388. doi: 10.1016/j.parkreldis.2015.02.003

Cárdenas-Morales, L., Volz, L. J., Michely, J., Rehme, A. K., Pool, E. M., Nettekoven, C., et al. (2014). Network connectivity and individual responses to brain stimulation in the human motor system. *Cereb. Cortex* 24, 1697–1707. doi: 10.1093/cercor/bht023

Cerasa, A., Passamonti, L., Novellino, F., Salsone, M., Gioia, M. C., Morelli, M., et al. (2010). Fronto-parietal overactivation in patients with essential tremor during Stroop task. *Neuroreport* 21, 148–151. doi: 10.1097/WNR.0b013e328335b42c

Ceravolo, R., Salvetti, S., Piccini, P., Lucetti, C., Gambaccini, G., and Bonuccelli, U. (1999). Acute and chronic effects of clozapine in essential tremor. *Mov. Disord.* 14, 468–472.

Chalah, M. A., Ayache, S. S., Riachi, N., and Ahdab, R. (2015). Permanent reversal of essential tremor following a frontal lobe stroke. *J. Neurol. Sci.* 354, 133–134. doi: 10.1016/j.jns.2015.04.055

Choi, S. M., Kim, B. C., Chang, J., Choi, K. H., Nam, T. S., Kim, J. T., et al. (2015). Comparison of the brain volume in essential tremor and Parkinson's disease tremor using an automated segmentation method. *Eur. Neurol.* 73, 303–309. doi: 10.1159/000381708

Chopra, A., Klassen, B. T., and Stead, M. (2013). Current clinical application of deep-brain stimulation for essential tremor. *Neuropsychiatr. Dis. Treat.* 9, 1859–1865. doi: 10.2147/NDT.S32342

Chuang, W. L., Huang, Y. Z., Lu, C. S., and Chen, R. S. (2014). Reduced cortical plasticity and GABAergic modulation in essential tremor. *Mov. Disord.* 29, 501–507. doi: 10.1002/mds.25809

Colebatch, J. G., Findley, L. J., Frackowiak, R. S., Marsden, C. D., and Brooks, D. J. (1990). Preliminary report: activation of the cerebellum in essential tremor. *Lancet* 336, 1028–1030. doi: 10.1016/0140-6736(90)92489-5

Creutzfeldt, O. D., Fromm, G. H., and Kapp, H. (1962). Influence of transcortical D-C currents on cortical neuronal activity. *Exp. Neurol.* 5, 436–452. doi: 10.1016/0014-4886(62)90056-0

Deuschl, G., Bain, P., and Brin, M. (1998). Consensus statement of the movement disorder society on tremor. *Ad hoc* scientific committee. *Mov. Disord.* 3, S2–S23. doi: 10.1002/mds.870131303

Deuschl, G., Raethjen, J., Hellriegel, H., and Elble, R. (2011). Treatment of patients with essential tremor. *Lancet Neurol.* 10, 148–161. doi: 10.1016/S1474-4422(10)70322-7

Deuschl, G., Wenzelburger, R., Löffler, K., Raethjen, J., and Stolze, H. (2000). Essential tremor and cerebellar dysfunction clinical and kinematic analysis of intention tremor. *Brain* 123, 1568–1580. doi: 10.1093/brain/123.8.1568

Dietz, V., Hillesheimer, W., and Freund, H. J. (1974). Correlation between tremor, voluntary contraction, and firing pattern of motor units in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 37, 927–937. doi: 10.1136/jnnp.37.8.927

Dogu, O., Sevim, S., Camdeviren, H., Sasmaz, T., Bugdayci, R., Aral, M., et al. (2003). Prevalence of essential tremor. Door-to-door neurologic exams in Mersin Province, Turkey. *Neurology* 61, 1804–1806. doi: 10.1212/01.WNL.0000099075.19951.8C

Dosen, S., Muceli, S., Dideriksen, J. L., Romero, J. P., Rocon, E., Pons, J., et al. (2015). Online tremor suppression using electromyography and low-level electrical stimulation. *IEEE Trans. Neural Syst. Rehabil. Eng.* 23, 385–395. doi: 10.1109/TNSRE.2014.2328296

Dupuis, M. J., Evrard, F. L., Jacquerye, P. G., Picard, G. R., and Lermen, O. G. (2010). Disappearance of essential tremor after stroke. *Mov. Disord.* 25, 2884–2887. doi: 10.1002/mds.23328

Elble, R. J. (2000). Diagnostic criteria for essential tremor and differential diagnosis. *Neurology* 4, S2–S6.

Elble, R. J., Pullman, S. L., Matsumoto, J. Y., Raethjen, J., Deuschl, G., and Tintner, R. (2006). Tremor amplitude is logarithmically related to 4- and 5-point tremor rating scales. *Brain* 129, 2660–2666. doi: 10.1093/brain/awl190

Eldaief, M. C., Halko, M. A., Buckner, R. L., and Pascual-Leone, A. (2011). Transcranial magnetic stimulation modulates the brain's intrinsic activity in a frequency-dependent manner. *Proc. Natl. Acad. Sci. U.S.A.* 108, 21229–21234. doi: 10.1073/pnas.1113103109

Elek, J., and Prochazka, A. (1989). Attenuation of wrist tremor with closed-loop electrical stimulation of muscles. *J. Physiol.* 414, 17P.

Fang, W., Lv, F., Luo, T., Cheng, O., Liao, W., Sheng, K., et al. (2013). Abnormal regional homogeneity in patients with essential tremor revealed by resting-state functional MRI. *PLoS ONE* 8:e69199. doi: 10.1371/journal.pone.0069199

Farkas, Z., Szirmai, I., and Kamondi, A. (2006). Impaired rhythm generation in essential tremor. *Mov. Disord.* 21, 1196–1199. doi: 10.1002/mds.20934

Ferrara, J., Stamey, W., Strutt, A. M., Adam, O. R., and Jankovic, J. (2011). Transcutaneous electrical stimulation (TENS) for psychogenic movement disorders. *J. Neuropsychiatry. Clin. Neurosci.* 23, 141–148. doi: 10.1176/jnp.23.2.jnp141

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

Findley, L. J. (1987). "The pharmacology of essential tremor," in *Movements Disorders*, Vol. 2, eds C. D. Marsden and S. Fahn (London: Butterworths), 438–458.

Foerster, B. R., Nascimento, T. D., DeBoer, M., Bender, M. A., Rice, I. C., Truong, D. Q., et al. (2015). Excitatory and inhibitory brain metabolites as targets of motor cortex transcranial direct current stimulation therapy and predictors of its efficacy in fibromyalgia. *Arthritis. Rheumatol.* 67, 576–581. doi: 10.1002/art.38945

Galea, J. M., Jayaram, G., Ajagbe, L., and Celnik, P. (2009). Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. *J. Neurosci.* 29, 9115–9122. doi: 10.1523/JNEUROSCI.2184-09.2009

Gandiga, P. C., Hummel, F. C., and Cohen, L. G. (2006). TranscranialDC stimulation (tDCS): a tool for dou-ble-blindsham-controlled clinical studies in brain stimulation. *Clin. Neurophysiol.* 117, 845–850. doi: 10.1016/j.clinph.2005.12.003

García-Martín, E., Martínez, C., Alonso-Navarro, H., Benito-León, J., Lorenzo-Betancor, O., Pastor, P., et al. (2013). No association of the SLC1A2 rs3794087 allele with risk for essential tremor in the Spanish population. *Pharmacogenet. Genomics* 23, 587–590. doi: 10.1097/FPC.0b013e328364db9d

Gentner, R., Wankerl, K., Reinsberger, C., Zeller, D., and Classen, J. (2008). Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. *Cereb. Cortex* 18, 2046–2053. doi: 10.1093/cercor/bhm239

Georgiou, N., Bradshaw, J. L., Phillips, J. G., Bradshaw, J. A., and Chiu, E. (1995). Advance information and movement sequencing in Gilles de la Tourette's syndrome. *J. Neurol. Neurosurg. Psychiatry* 58, 184–191. doi: 10.1136/jnnp.58.2.184

Gillard, D. M., Cameron, T., Prochazka, A., and Gauthier, M. J. (1999). Tremor suppression using functional electrical stimulation: a comparison between digital and analog controllers. *IEEE Trans. Rehabil. Eng.* 7, 385–388. doi: 10.1109/86.788474

Gironell, A. (2014). The GABA hypothesis in essential tremor: lights and shadows. *Tremor. Other Hyperkinet. Mov. (N.Y.)* 4:254. doi: 10.7916/D8SF2T9C

Gironell, A., Figueiras, F. P., Pagonabarraga, J., Herance, J. R., Pascual-Sedano, B., Trampal, C., et al. (2012). Gaba and serotonin molecular neuroimaging in essential tremor: a clinical correlation study. *Parkinsonism. Relat. Disord.* 18, 876–880. doi: 10.1016/j.parkreldis.2012.04.024

Gironell, A., Kulisevsky, J., Lorenzo, J., Barbanoj, M., Pascual-Sedano, B., and Otermin, P. (2002). Transcranial magnetic stimulation of the cerebellum in essential tremor: a controlled study. *Arch. Neurol.* 59, 413–417. doi: 10.1001/archneur.59.3.413

Gironell, A., Kulisevsky, J., Pascual-Sedano, B., and Flamarich, D. (2006). Effect of amantadine in essential tremor: a randomized, placebo-controlled trial. *Mov. Disord.* 21, 441–445. doi: 10.1002/mds.20676

Gironell, A., Martínez-Horta, S., Aguilar, S., Torres, V., Pagonabarraga, J., Pascual-Sedano, B., et al. (2014). Transcranial direct current stimulation of the

cerebellum in essential tremor: a controlled study. *Brain Stimul.* 7, 491–492. doi: 10.1016/j.brs.2014.02.001

Grefkes, C., Nowak, D. A., Wang, L. E., Dafotakis, M., Eickhoff, S. B., and Fink, G. R. (2010). Modulating cortical connectivity in stroke patients by rTMS assessed with fMRI and dynamic causal modeling. *Neuroimage* 50, 233–242. doi: 10.1016/j.neuroimage.2009.12.029

Grimaldi, G., and Manto, M. (2008). *Tremor: From Pathogenesis to Treatment.* San Rafael, CA: Mongan & Claypool.

Grimaldi, G., and Manto, M. (2010). "Old" and emerging therapies of human tremor. *Clin. Med. Insights. Ther.* 2, 169–178. doi: 10.4137/CMT.S2999

Halko, M. A., Farzan, F., Eldaief, M. C., Schmahmann, J. D., and Pascual-Leone, A. (2014). Intermittent theta-burst stimulation of the lateral cerebellum increases functional connectivity of the default network. *J. Neurosci.* 34, 12049–12056. doi: 10.1523/JNEUROSCI.1776-14.2014

Hallett, M. (2014). Tremor: pathophysiology. *Parkinsonism Relat. Disord.* 20, S118–S122. doi: 10.1016/S1353-8020(13)70029-4

Hallett, M., and Dubinsky, R. M. (1993). Glucose metabolism in the brain of patients with essential tremor. *J. Neurol. Sci.* 114, 45–48. doi: 10.1016/0022-510X(93)90047-3

Hanajima, R., Ugawa, Y., Terao, Y., Sakai, K., Furubayashi, T., Machii, K., et al. (1998). Cortico-cortical inhibition of the motor cortical area projecting to sternocleidomastoid muscle in normals and patients with spasmodic torticollis or essential tremor. *Electroencephalogr. Clin. Neurophysiol.* 109, 391–396. doi: 10.1016/S0924-980X(98)00036-8

Hellriegel, H., Schulz, E. M., Siebner, H. R., Deuschl, G., and Raethjen, J. H. (2012). Continuous theta-burst stimulation of the primary motor cortex in essential tremor. *Clin. Neurophysiol.* 123, 1010–1015. doi: 10.1016/j.clinph.2011.08.033

Helmich, R. C., Toni, I., Deuschl, G., and Bloem, B. R. (2013). The pathophysiology of essential tremor and Parkinson's tremor. *Curr. Neurol. Neurosci. Rep.* 13:378. doi: 10.1007/s11910-013-0378-8

Héroux, M. E., Pari, G., and Norman, K. E. (2010). The effect of contraction intensity on force fluctuations and motor unit entrainment in individuals with essential tremor. *Clin. Neurophysiol.* 121, 233–239. doi: 10.1016/j.clinph.2009.10.015

Huang, Y. Z. (2010). The modulation of cortical motor circuits and spinal reflexes using theta burst stimulation in healthy and dystonic subjects. *Restor. Neurol. Neurosci.* 28, 449–457. doi: 10.3233/RNN-2010-0554

Huang, Y. Z., Chen, R. S., Rothwell, J. C., and Wen, H. Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin. Neurophysiol.* 118, 1028–1032. doi: 10.1016/j.clinph.2007.01.021

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

Javidan, M., Elek, J., and Prochazka, A. (1990). Tremor reduction by functional electrical stimulation. *Neurol* 40, 369.

Javidan, M., Elek, J., and Prochazka, A. (1992). Attenuation of pathological tremors by functional electrical stimulation. II. Clinical evaluation. *Ann. Biomed. Eng.* 20, 225–236. doi: 10.1007/BF02368522

Jenkins, I. H., Bain, P. G., Colebatch, J. G., Thompson, P. D., Findley, L. J., Frackoviak, R. S., et al. (1993). A positron emission tomography study of essential tremor: evidence for overactivity of cerebellar connections. *Ann. Neurol.* 34, 82–90. doi: 10.1002/ana.410340115

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

Koller, W. C. (1981). Amantadine in essential tremor. *Ann. Neurol.* 16, 621–622. doi: 10.1002/ana.410160517

Kuhlenbäumer, G., Hopfner, F., and Deuschl, G. (2014). Genetics of essential tremor: meta-analysis and review. *Neurology* 82, 1000–1007. doi: 10.1212/WNL.0000000000000211

Kuo, M. F., Paulus, W., and Nitsche, M. A. (2014). Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 3, 948–960. doi: 10.1016/j.neuroimage.2013.05.117

Lefaucheur, J. P. (2008). Principles of therapeutic use of transcranial and epidural cortical stimulation. *Clin. Neurophysiol.* 119, 2179–2184. doi: 10.1016/j.clinph.2008.07.007

Lefaucheur, J. P. (2009). Methods of therapeutic cortical stimulation. *Neurophysiol. Clin.* 39, 1–14. doi: 10.1016/j.neucli.2008.11.001

Lefaucheur, J. P. (2010). Why image-guided navigation becomes essential in the practice of transcranial magnetic stimulation. *Neurophysiol. Clin.* 40, 1–5. doi: 10.1016/j.neucli.2009.10.004

Lefaucheur, J. P. (2012). Neurophysiology of cortical stimulation. *Int. Rev. Neurobiol.* 107, 57–85. doi: 10.1016/B978-0-12-404706-8.00005-X

Lefaucheur, J. P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., et al. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin. Neurophysiol.* 125, 2150–2206. doi: 10.1016/j.clinph.2014.05.021

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, S. Y., Poon, Y. Y., Chen, R., Mikulis, D., Lozano, A. M., Moro, E., et al. (2010). Marked reduction of tremor in essential tremor after putaminal infarct. *J. Neurol. Neurosurg. Psychiatry* 81, 1172–1173. doi: 10.1136/jnnp.2008.171223

Louis, E. D. (1999). A new twist for stopping the shakes? Revisiting GABAergic therapy for essential tremor. *Arch. Neurol.* 56, 807–808. doi: 10.1001/archneur.56.7.807

Louis, E. D. (2000). Essential tremor. *Arch. Neurol.* 57, 1522–1524. doi: 10.1001/archneur.57.10.1522

Louis, E. D. (2005). Essential tremor. *Lancet Neurol.* 4, 100–110. doi: 10.1016/S1474-4422(05)00991-9

Louis, E. D. (2009). Essential tremors: a family of neurodegenerative disorders? *Arch. Neurol.* 66, 1202–1208. doi: 10.1001/archneurol.2009.217

Louis, E. D. (2015). Medication non-adherence in essential tremor. *Parkinsonism Relat. Disord.* 21, 138–141. doi: 10.1016/j.parkreldis.2014.12.001

Louis, E. D., Marder, K., Cote, L., Pullman, S., Ford, B., Wilder, D., et al. (1995). Differences in the prevalence of essential tremor among elderly African Americans, whites, and Hispanics in northern Manhattan. *N.Y. Arch. Neurol.* 52, 1201–1205. doi: 10.1001/archneur.1995.00540360079019

Lu, M. K., Chiou, S. M., Ziemann, U., Huang, H. C., Yang, Y. W., and Tsai, C. H. (2015). Resetting tremor by single and paired transcranial magnetic stimulation in Parkinson's disease and essential tremor. *Clin. Neurophysiol.* doi: 10.1016/j.clinph.2015.02.010. [Epub ahead of print].

Malešević, N., Popović, L., Schwirtlich, L., and Popović, D. B. (2010a). Distributed low frequency functional electrical stimulation delays muscle fatigue compared to conventional stimulation. *Muscle Nerve* 42, 556–562. doi: 10.1002/mus.21736

Malešević, N., Popović, L., Bijeliæ, G., and Kvaščev, G. (2010b). Muscle twitch responses for shaping the multi-pad electrode for functional electrical stimulation. *J. Automatic Control* 20, 53–57. doi: 10.2298/JAC1001053M

Manyam, B. V. (1984). Amantadine in essential tremor. *Ann. Neurol.* 9, 198–199. doi: 10.1002/ana.410090219

Micheli, F., Cersósimo, M. G., Raina, G., and Gatto, E. (2002). Quetiapine and essential tremor. *Clin. Neuropharmacol.* 25, 303–306. doi: 10.1097/00002826-200211000-00004

Monte-Silva, K., Ruge, D., Teo, J. T., Paulus, W., Rothwell, J. C., and Nitsche, M. A. (2011). D2 receptor block abolishes θ burst stimulation-induced neuroplasticity in the human motor cortex. *Neuropsychopharmacology* 36, 2097–2102. doi: 10.1038/npp.2011.100

Munhoz, R. P., Hanajima, R., Ashby, P., and Lang, A. E. (2003). Acute effect of transcutaneous electrical nerve stimulation on tremor. *Mov. Disord.* 18, 191–194. doi: 10.1002/mds.10311

Nahab, F. B., Peckham, E., and Hallett, M. (2007). Essential tremor, deceptively simple. *Pract. Neurol.* 7, 222–233. doi: 10.1136/jnnp.2007.124693

Nettekoven, C., Volz, L. J., Kutscha, M., Pool, E. M., Rehme, A. K., Eickhoff, S. B., et al. (2014). Dose-dependent effects of theta burst rTMS on cortical excitability and resting-state connectivity of the human motor system. *J. Neurosci.* 34, 6849–6859. doi: 10.1523/JNEUROSCI.4993-13.2014

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. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., and Fregni, F. (2007). Transcranial direct current stimulation- an adjuvant tool for the treatment of neuropsychiatric disease. *Curr. Psychiatry Rev.* 3, 222–232. doi: 10.2174/157340007781369649

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., et al. (2003b). 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., 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., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., and Paulus, W. (2003a). 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. (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

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

Pakkenberg, H., and Pakkenberg, B. (1986). Clozapine in the treatment of tremor. *Acta Neurol. Scand.* 73, 295–297. doi: 10.1111/j.1600-0404.1986.tb03279.x

Paris-Robidas, S., Brochu, E., Sintes, M., Emond, V., Bousquet, M., Vandal, M., et al. (2012). Defective dentate nucleus GABA receptors in essential tremor. *Brain* 135, 105–116. doi: 10.1093/brain/awr301

Park, Y. G., Park, H. Y., Lee, C. J., Choi, S., Jo, S., Choi, H., et al. (2010). Ca(V)3.1 is a tremor rhythm pacemaker in the inferior olive. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10731–10736. doi: 10.1073/pnas.1002995107

Pascual-Leone, A., Valls-Solé, J., Toro, C., Wassermann, E. M., and Hallett, M. (1994). Resetting of essential tremor and postural tremor in Parkinson's disease with transcranial magnetic stimulation. *Muscle Nerve* 17, 800–807. doi: 10.1002/mus.880170716

Passamonti, L., Novellino, F., Cerasa, A., Chiriaco, C., Rocca, F., Matina, M. S., et al. (2011). Altered cortical-cerebellar circuits during verbal working memory in essential tremor. *Brain* 134, 2274–2286. doi: 10.1093/brain/awr164

Paulus, W., Peterchev, A. V., and Ridding, M. (2013). Transcranial electric and magnetic stimulation: technique and paradigms. *Handb. Clin. Neurol.* 116, 329–342. doi: 10.1016/B978-0-444-53497-2.00027-9

Pinto, A. D., and Chen, R. (2001). Suppression of the motor cortex by magnetic stimulation of the cerebellum. *Exp. Brain Res.* 140, 505–510. doi: 10.1007/s002210100862

Pinto, A. D., Lang, A. E., and Chen, R. (2003). The cerebellothalamocortical pathway in essential tremor. *Neurology* 60, 1985–1987. doi: 10.1212/01.WNL.0000065890.75790.29

Polanía, R., Paulus, W., Antal, A., and Nitsche, M. A. (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

Polanía, R., Paulus, W., and Nitsche, M. A. (2012a). 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

Polanía, R., Paulus, W., and Nitsche, M. A. (2012b). Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Hum. Brain. Mapp.* 33, 2499–2508. doi: 10.1002/hbm.21380

Popa, T., Russo, M., Vidailhet, M., Roze, E., Lehéricy, S., Bonnet, C., et al. (2013). Cerebellar rTMS stimulation may induce prolonged clinical benefits in essential tremor, and subjacent changes in functional connectivity: an open label trial. *Brain Stimul.* 6, 175–179. doi: 10.1016/j.brs.2012.04.009

Popović, D. B., and Popović, M. B. (2009). Automatic determination of the optimal shape of a surface electrode: selective stimulation. *J. Neurosci. Methods* 178, 174–181. doi: 10.1016/j.jneumeth.2008.12.003

Popović-Bijelić, A., Bijelić, G., Jorgovanović, N., Bojanić, D., Popović, M. B., and Popović, D. B. (2005). Multi-field surface electrode for selective electrical stimulation. *Artif. Organs.* 29, 448–452. doi: 10.1111/j.1525-1594.2005.29075.x

Popovic, L. Z., and Malesevic, N. M. (2009). Muscle fatigue of quadriceps in paraplegics: comparison between single vs. multi-pad electrode surface stimulation. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009, 6785–6788. doi: 10.1109/iembs.2009.5333983

Popovic, L. Z., Malesevic, N. M., and Popovic, M. B. (2009). "Optimization of multi-pad surface electrode: selective stimulation of wrist," *The IEEE Region 8 EUROCON Conference, IEEE Eurocon 2009, Saint-Petersburg, Russia.* (Saint-Petersburg), 142–145.

Popović, L. Z., Sekara, T. B., and Popović, M. B. (2010). Adaptive band-pass filter (ABPF) for tremor extraction from inertial sensor data. *Comput. Methods Prog. Biomed.* 99, 298–305. doi: 10.1016/j.cmpb.2010.03.018

Popović Maneski, L., Jorgovanović, N., Ilić, V., Došen, S., Keller, T., Popović, M. B., et al. (2011). Electrical stimulation for the suppression of pathological tremor. *Med. Biol. Eng. Comput.* 49, 1187–1193. doi: 10.1007/s11517-011-0803-6

Poreisz, C., Boros, K., Antal, A., and Paulus, W. (2007). Safety aspects of transcranial direct current stimula-tion concerning healthy subjects and patients. *Brain Res. Bull.* 72, 208–214. doi: 10.1016/j.brainresbull.2007.01.004

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

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. doi: 10.1097/00001756-199807130-00020

Prochazka, A., Elek, J., and Javidan, M. (1992). Attenuation of pathological tremors by functional electrical stimulation. I. Method. *Ann. Biomed. Eng.* 20, 205–224. doi: 10.1007/BF02368521

Purpura, D. P., and McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during po-larization of motor cortex. *J. Neurophysiol.* 28, 166–185.

Quattrone, A., Cerasa, A., Messina, D., Nicoletti, G., Hagberg, G. E., Lemieux, L., et al. (2008). Essential head tremor is associated with cerebellar vermis atrophy: a volumetric and voxel-based morphometry MR imaging study. *Am. J. Neuroradiol.* 29, 1692–1697. doi: 10.3174/ajnr.a1190

Raethjen, J., and Deuschl, G. (2012). The oscillating central network of Essential tremor. *Clin. Neurophysiol.* 123, 61–64. doi: 10.1016/j.clinph.2011.09.024

Raethjen, J., Govindan, R., Kopper, F., Muthuraman, M., and Deuschl, G. (2007). Cortical involvement in the generation of essential tremor. *J. Neurophysiol.* 97, 3219–3228. doi: 10.1152/jn.00477.2006

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

Rocon, E., Gallego, J. A., Barrios, L., Victoria, A. R., Ibanez, J., Farina, D., et al. (2010). Multimodal BCI-mediated FES suppression of pathological tremor. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2010, 3337–3340. doi: 10.1109/iembs.2010.5627914

Romeo, S., Berardelli, A., Pedace, F., Inghilleri, M., Giovannelli, M., and Manfredi, M. (1998). Cortical excitability in patients with essential tremor. *Muscle Nerve* 21, 1304–1308.

Ross, J. P., Rayaprolu, S., Bernales, C. Q., Soto-Ortolaza, A. I., van Gerpen, J., Uitti, R. J., et al. (2014). SLC1A2 rs3794087 does not associate with essential tremor. *Neurobiol. Aging* 35, e9–e10. doi: 10.1016/j.neurobiolaging.2013.09.022

Schneider, S. A., and Deuschl, G. (2014). The treatment of tremor. *Neurotherapeutics* 11, 128–138. doi: 10.1007/s13311-013-0230-5

Schnitzler, A., Münks, C., Butz, M., Timmermann, L., and Gross, J. (2009). Synchronized brain network associated with essential tremor as revealed by magnetoencephalography. *Mov. Disord.* 24, 1629–1635. doi: 10.1002/mds.22633

Shill, H. A., Adler, C. H., Beach, T. G., Lue, L. F., Caviness, J. N., Sabbagh, M. N., et al. (2012). Brain biochemistry in autopsied patients with essential tremor. *Mov. Disord.* 27, 113–117. doi: 10.1002/mds.24004

Shin, D. H., Han, B. S., Kim, H. S., and Lee, P. H. (2008). Diffusion tensor imaging in patients with essential tremor. *Am. J. Neuroradiol.* 29, 151–153. doi: 10.3174/ajnr.A0744

Shin, H., Lee, D. K., Lee, J. M., Huh, Y. E., Youn, J., Louis, E. D., et al. (2015). Atrophy of the cerebellar vermis in essential tremor: segmental volumetric MRI analysis. *Cerebellum.* doi: 10.1007/s12311-015-0682-8. [Epub ahead of print].

Shukla, G., Bhatia, M., Pandey, R. M., and Behari, M. (2003). Cortical silent period in essential tremor. *Electromyogr. Clin. Neurophysiol.* 43, 329–333.

Silvanto, J., Muggleton, N. G., Cowey, A., and Walsh, V. (2007). Neural activation state determines behavioral susceptibility to modified theta burst transcranial

magnetic stimulation. *Eur. J. Neurosci.* 26, 523–528. doi: 10.1111/j.1460-9568.2007.05682.x

Sluka, K. A., and Walsh, D. (2003). Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. *J. Pain* 4, 109–121. doi: 10.1054/jpai.2003.434

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

Tan, E. K., Foo, J. N., Tan, L., Au, W. L., Prakash, K. M., Ng, E., et al. (2013). SLC1A2 variant associated with essential tremor but not Parkinson disease in Chinese subjects. *Neurology* 80, 1618–1619. doi: 10.1212/WNL.0b013e31828f1903

Teo, J. T., Swayne, O. B., and Rothwell, J. C. (2007). Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. *Clin. Neurophysiol.* 118, 1649–1651. doi: 10.1016/j.clinph.2007.04.010

Thier, S., Lorenz, D., Nothnagel, M., Poremba, C., Papengut, F., Appenzeller, S., et al. (2012). Polymorphisms in the glial glutamate transporter SLC1A2 are associated with essential tremor. *Neurology.* 79, 243–248. doi: 10.1212/WNL.0b013e31825fdeed

Thompson, P. D., Dick, J. P., Day, B. L., Rothwell, J. C., Berardelli, A., Kachi, T., et al. (1986). Electrophysiology of the corticomotoneurone pathways in patients with movement disorders. *Mov. Disord.* 1, 113–117. doi: 10.1002/mds.870010205

Tinazzi, M., Farina, S., Bhatia, K., Fiaschi, A., Moretto, G., Bertolasi, L., et al. (2005b). TENS for the treatment of writer's cramp dystonia: a randomized, placebo-controlled study. *Neurology* 64, 1946–1948. doi: 10.1212/01.WNL.0000163851.70927.7E

Tinazzi, M., Zarattini, S., Valeriani, M., Romito, S., Farina, S., Moretto, G., et al. (2005a). Long-lasting modulation of human motor cortex following prolonged transcutaneous electrical nerve stimulation (TENS) of forearm muscles: evidence of reciprocal inhibition and facilitation. *Exp. Brain Res.* 161, 457–464. doi: 10.1007/s00221-004-2091-y

Tinazzi, M., Zarattini, S., Valeriani, M., Stanzani, C., Moretto, G., Smania, N., et al. (2006). Effects of transcutaneous electrical nerve stimulation on motor cortex excitability in writer's cramp: neurophysiological and clinical correlations. *Mov. Disord.* 21, 1908–1913. doi: 10.1002/mds.21081

Valchev, N., Ćurčić-Blake, B., Renken, R. J., Avenanti, A., Keysers, C., Gazzola, V., et al. (2015). cTBS delivered to the left somatosensory cortex changes its functional connectivity during rest. *Neuroimage* 114, 386–397. doi: 10.1016/j.neuroimage.2015.04.017

Widjaja, F., Shee, C. Y., Poignet, P., and Ang, W. T. (2009). Filtering of intended motion for real-time tremor compensation in human upper limb using surface electromyography. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009, 2996–2999. doi: 10.1109/iembs.2009.5332523

Wills, A. J., Jenkins, I. H., Thompson, P. D., Findley, L. J., and Brooks, D. J. (1995). A positron emission tomography study of cerebral activation associated with essential and writing tremor. *Arch. Neurol.* 52, 299–305. doi: 10.1001/archneur.1995.00540270095025

Wischnewski, M., and Schutter, D. J. (2015). Efficacy and time course of theta burst stimulation in healthy humans. *Brain Stimul.* 8, 685–692. doi: 10.1016/j.brs.2015.03.004

Yetimalar, Y., Irtman, G., Gürgör, N., and Basoglu, M. (2003). Olanzapine efficacy in the treatment of essential tremor. *Eur. J. Neurol.* 10, 79–82. doi: 10.1046/j.1468-1331.2003.00534.x

Yetimalar, Y., Irtman, G., Kurt, T., and Basoglu, M. (2005). Olanzapine versus propranolol in essential tremor. *Clin. Neurol. Neurosurg.* 108, 32–35. doi: 10.1016/j.clineuro.2005.01.002

Yu, H. Y., Chen, J. T., Lee, Y. C., Guo, Y. C., Kao, C. D., Shan, D. E., et al. (2001). Single-pulse transcranial magnetic stimulation reset the rhythm of essential tremor but not heart beat. *Zhonghua Yi Xue Za Zhi* 64, 271–276.

Yu, S. W., Chen, C. M., Chen, Y. C., Chang, C. W., Chang, H. S., Lyu, R. K., et al. (2013). SLC1A2 variant is associated with essential tremor in Taiwanese population. *PLoS ONE* 8:e71919. doi: 10.1371/journal.pone.0071919

Zesiewicz, T. A., Sullivan, K. L., Hinson, V., Stover, N. P., Fang, J., Jahan, I., et al. (2013). Multisite, double-blind, randomized, controlled study of pregabalin for essential tremor. *Mov. Disord.* 28, 249–250. doi: 10.1002/mds.25264

Zesiewicz, T. A., Ward, C. L., Hauser, R. A., Salemi, J. L., Siraj, S., Wilson, M. C., et al. (2007). A pilot, double-blind, placebo-controlled trial of pregabalin (Lyrica) in the treatment of essential tremor. *Mov. Disord.* 22, 1660–1663. doi: 10.1002/mds.21629

Zhang, D., and Ang, W. T. (2007). Reciprocal EMG controlled FES for pathological tremor suppression of forearm. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2007, 4810–4813. doi: 10.1109/IEMBS.2007.4353416

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 Neuroscience

REVIEW
published: 11 February 2016
doi: 10.3389/fnins.2016.00018

Potential Mechanisms Supporting the Value of Motor Cortex Stimulation to Treat Chronic Pain Syndromes

Marcos F. DosSantos[1], Natália Ferreira[2], Rebecca L. Toback[3], Antônio C. Carvalho[2] and Alexandre F. DaSilva[3]**

[1] Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2] Departamento de Radiologia, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3] Headache and Orofacial Pain Effort, Department of Biologic and Materials Sciences and Michigan Center for Oral Health Research, School of Dentistry, University of Michigan, Ann Arbor, MI, USA

Throughout the first years of the twenty-first century, neurotechnologies such as motor cortex stimulation (MCS), transcranial magnetic stimulation (TMS), and transcranial direct current stimulation (tDCS) have attracted scientific attention and been considered as potential tools to centrally modulate chronic pain, especially for those conditions more difficult to manage and refractory to all types of available pharmacological therapies. Interestingly, although the role of the motor cortex in pain has not been fully clarified, it is one of the cortical areas most commonly targeted by invasive and non-invasive neuromodulation technologies. Recent studies have provided significant advances concerning the establishment of the clinical effectiveness of primary MCS to treat different chronic pain syndromes. Concurrently, the neuromechanisms related to each method of primary motor cortex (M1) modulation have been unveiled. In this respect, the most consistent scientific evidence originates from MCS studies, which indicate the activation of top-down controls driven by M1 stimulation. This concept has also been applied to explain M1-TMS mechanisms. Nevertheless, activation of remote areas in the brain, including cortical and subcortical structures, has been reported with both invasive and non-invasive methods and the participation of major neurotransmitters (e.g., glutamate, GABA, and serotonin) as well as the release of endogenous opioids has been demonstrated. In this critical review, the putative mechanisms underlying the use of MCS to provide relief from chronic migraine and other types of chronic pain are discussed. Emphasis is placed on the most recent scientific evidence obtained from chronic pain research studies involving MCS and non-invasive neuromodulation methods (e.g., tDCS and TMS), which are analyzed comparatively.

Keywords: chronic pain, headache, migraine, motor cortex stimulation, neuromodulation, transcranial direct current stimulation, transcranial magnetic stimulation

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales de Madrid and CEU San Pablo University, Spain

Reviewed by:
Jean-Pascal Lefaucheur, Université Paris-Est-Créteil & Hôpital Henri Mondor, Assistance Publique – Hôpitaux de Paris, France
John Rothwell, University College London, UK

****Correspondence:***
Marcos F. DosSantos
santosmfh@gmail.com;
Alexandre F. DaSilva
adasilva@umich.edu

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

Received: *27 October 2015*
Accepted: *14 January 2016*
Published: *11 February 2016*

Citation:
DosSantos MF, Ferreira N, Toback RL, Carvalho AC and DaSilva AF (2016) Potential Mechanisms Supporting the Value of Motor Cortex Stimulation to Treat Chronic Pain Syndromes. Front. Neurosci. 10:18. doi: 10.3389/fnins.2016.00018

INTRODUCTION

Pain is clinically identified as an early and disabling symptom, extremely frequent and common to various diseases. However, rather than simply a sensory phenomenon, pain is better characterized as a complex experience extending beyond the sensory-discriminative component of pain, or the individual capacity to identify the nature (e.g., intensity,

location, and duration) of a particular noxious stimuli. The affective-emotional aspect of pain (e.g., unpleasantness), as well the involvement of attention, memory of previous experiences, and anticipation, termed the cognitive-evaluative pain dimension, are fundamental pieces of this still challenging and complex puzzle (Melzack and Casey, 1968; Merskey et al., 1994; McMahon, 2013).

According to a widely applied definition, pain can be differentiated into either acute or chronic. Acute pain is produced by tissue injury and concurrent activation of local nociceptive transducers. Usually related to trauma, invasive procedures, or as a symptom occurring during the course of some pathological process, acute pain characteristically lasts for only a limited amount of time and resolves as soon as its primary source ceases. While chronic pain may also be initiated by local injury or disease, it usually persists for a longer period of time and tends to be maintained by factors not directly linked to the original event (Fishman et al., 2010). In fact, the International Association for the Study of Pain (IASP) defines chronic pain as pain experienced every day for 3 months over a period of 6 months (Merskey et al., 1994). Chronic pain is not only a clinical struggle but also a social burden, with enormous economic costs to healthcare systems across the globe (Patel et al., 2012). Due to its high prevalence (Verhaak et al., 1998; Elliott et al., 1999, 2002; Breivik et al., 2006) and deleterious impact on patients' quality of life (Patel et al., 2012), chronic pain receives considerable attention from both clinicians and researchers worldwide. Most of this attention is focused on better comprehending the multifaceted biological aspects of chronic pain and developing novel therapies that will permit more adequate relief from such an incapacitating condition. In this respect, recent years have seen an increased research interest in the study of different methods to modulate the activity of neurocircuits with the purpose of treating chronic pains. These methods include both surgical and non-invasive approaches, and their treatment effects have been studied alone and when combined with pharmacological therapies. While the clinical application of brain stimulation techniques dates back to the last century, the related technologies have evolved considerably as scientific evidence accumulated within the field (Kumar and Rizvi, 2014). Furthermore, the efficacy and reliability of different neuromodulatory methods, with stimulation delivered to distinct cortical/subcortical and even peripheral zones, have been tested in the treatment of several chronic pain disorders. Intriguingly, when retrospectively analyzing the scientific evidence accumulated throughout the last 25 years, the stimulation of motor cortical areas, mainly the primary motor cortex (M1), either non-invasively or by implanted electrodes has been consistently reported as an effective analgesic strategy to provide chronic pain relief, especially those of predominantly neuropathic origins (Tsubokawa et al., 1991a; Hosomi et al., 2013; Hagenacker et al., 2014; Ngernyam et al., 2015; Radic et al., 2015).

The advent of neuroimaging has allowed for the identification of an intricate network of brain structures that contributes to the pain experience and their specific roles in each dimension of the whole phenomenon. Most of those brain areas are multimodal, responding to both noxious and salient non-noxious stimuli (Mouraux et al., 2011). It has been recognized that this network includes the primary and secondary somatosensory cortices (SI and SII), the cingulate cortex, the posterior parietal cortex, and the pre-frontal cortex. Also taking part in this network are the thalamus, insula, and several brainstem structures, in addition to other interconnected brain areas. Not surprisingly, there is relatively scarce information regarding the contribution of the motor cortex to this process. Although the effects of pain on motor function have been well-documented, the participation of motor brain areas in the mechanisms that lead to chronic pain is still not completely understood (Farina et al., 2003). Therefore, one question remains unsolved: Why and how is motor cortex stimulation, in particular M1 stimulation, effective in treating chronic pain patients?

Based on scientific evidence currently available, this paper provides a critical review on the topic by exploring the putative mechanisms that explain the effectiveness of two methods of non-invasive neuromodulation, transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), when applied to the motor cortex for the treatment of chronic pains. To this purpose, the scientific evidence obtained with the invasive procedure, termed motor cortex stimulation (MCS), is always used as a reference.

ARE THEY EFFECTIVE? STATE-OF-THE-ART NON-INVASIVE NEUROMODULATORY TECHNOLOGIES AVAILABLE TO AMELIORATE CHRONIC PAIN

Given the clinical challenges that chronic pain management presents, scientific pain researchers have directed their focus toward the development of novel technologies and enhancement of known strategies that permit the modulation of cortical excitability in humans through non-invasive or minimally invasive procedures. Over the past years, several studies have investigated the analgesic effects of epidural/subdural MCS, especially in refractory or intractable neuropathic pain (Meyerson et al., 1993; Tsubokawa et al., 1993; Nguyen et al., 1999, 2008, 2009; Saitoh et al., 2003; Nuti et al., 2005; Rasche et al., 2006; Velasco et al., 2008; Fontaine et al., 2009; Lefaucheur et al., 2009). Regarding non-invasive procedures, the first study demonstrating the analgesic effects of high-frequency rTMS of the motor cortex was performed in neuropathic pain patients (Lefaucheur et al., 2001). Later, the analgesic effects of anodal tDCS applied to the motor cortex was again reported in patients with neuropathic pain due to spinal cord injury (Fregni et al., 2006a) and also in fibromyalgia patients (Fregni et al., 2006b). In the following years, substantial data has emerged suggesting that distinct chronic migraine and pain syndromes can be successfully treated by tDCS (Antal et al., 2010, 2011; Mendonca et al., 2011; DaSilva et al., 2012; Jensen et al., 2013; Kim et al., 2013; Viganò et al., 2013; Villamar et al., 2013; Wrigley et al., 2013; Hagenacker et al., 2014; Schabrun et al., 2014; Bolognini et al., 2015; Donnell et al., 2015) and/or TMS (Lefaucheur et al., 2010b; Picarelli et al., 2010; Mhalla et al., 2011; Lee et al., 2012; Hosomi

et al., 2013; Tzabazis et al., 2013; Hasan et al., 2014). Moreover, the value of rTMS to predict the long-term effects of MCS has been reported (Lefaucheur et al., 2004, 2011; André-Obadia et al., 2006, 2014; Hosomi et al., 2008).

Nevertheless, findings from systematic reviews of the methodology and results of studies investigating the role of non-invasive neuromodulation for pain control suggest that more clinical trials with rigorously designed protocols and larger samples sizes are still necessary to draw more accurate conclusions (Klein et al., 2015; **Table 1**). As reported in a recent meta-analysis, low or very low-quality evidence indicate that prefrontal low-frequency repetitive TMS (rTMS) is not effective for pain control, while a single dose of high-frequency motor cortex TMS provides short-term pain improvement. Conversely, according to an international group of experts, in cases of neuropathic pain the production of analgesic effects by high-frequency (≥5 Hz) rTMS of the motor cortex contralateral to the pain side has a level A of evidence (Lefaucheur et al., 2014). However, this statement cannot be extended to other stimulation settings, targets, or pain conditions. In addition, it is important to highlight the importance of long-term effects of rTMS protocols in pain therapy. Because of the short-lasting duration of the analgesic effects produced, it is still necessary to define and optimize maintenance protocols before considering rTMS as a valuable technique for the treatment of neuropathic pain in routine practice. So far, only a few studies have shown clinical improvement lasting several months from rTMS in patients with chronic pain syndromes (Mhalla et al., 2011; Hodaj et al., 2015).

Regarding tDCS, low-quality evidence does not yet suggest that it is effective for chronic pain control (O'connell et al., 2014). On the other hand, it is imperative to consider the high heterogeneity of the research protocols evaluated, including important differences with respect to the cortical targets chosen for stimulation [e.g., motor cortex and dorsolateral prefrontal cortex (DLPFC)]; differences in the number of stimulations per subject, with the presence of single and multiple-dose studies; application of low (≤1 Hz) or high frequency (≥5 Hz) stimulation, in the case of TMS; differences in the current intensity (usually 1 or 2 mA), in relation to tDCS; and of particular relevance, the type of pain disorder evaluated (e.g., nociceptive or neuropathic).

Indeed, chronic pain does not represent a single entity but a spectrum of disorders, triggered, and maintained by complex mechanisms (Basbaum et al., 2009). Therefore, it is possible to infer that TMS or tDCS could produce differential effects on each type of chronic pain disorder. For example, one systematic review focused on clinical research protocols that investigated the effects of low and high frequency (LF and HF, respectively) TMS and anodal tDCS (at intensities of 1 or 2 mA) in patients diagnosed with fibromyalgia. The review concluded that HF rTMS as well as anodal tDCS stimulation of M1 (M1-tDCS) offer similar pain improvements when compared to the FDA-approved fibromyalgia pharmaceuticals. The authors advocate that rTMS and tDCS should be considered when treating fibromyalgia patients, especially those individuals who are refractory to other (pharmacological) therapies or who do not

TABLE 1 | Summary of the systematic reviews that investigated the methodology and results of studies exploring the effects of tDCS and TMS on pain management.

	Transcranial Magnetic Stimulation (TMS)	Transcranial Direct Current Stimulation (tDCS)
Types of study	Systematic review and meta-analysis.	Systematic review and meta-analysis.
Quality of evidence	Low or very low-quality.	Low-quality.
Main findings	A single dose of high-frequency motor cortex TMS provides short-term pain improvement.	Not effective for chronic pain control (O'connell et al., 2014).
		Presence of scientific evidence indicating that anodal M1-tDCS significantly reduces pain levels in chronic pain patients (Vaseghi et al., 2014).
	HF rTMS and anodal M1-tDCS produce similar pain improvements and less adverse effects when compared to the FDA approved fibromyalgia pharmaceuticals.	
Limitations	High heterogeneity of the research protocols: differences with respect to the cortical targets for stimulation; differences in the number of stimulations per subject, with the presence of single and multiple-dose studies; application of low or high frequency stimulation (or differences in the current intensity); the type of pain disorder evaluated. Presence of adequate subject blinding during active and sham stimulation.	
Perspectives	The findings suggest that more clinical trials with rigorously designed protocols and larger sample sizes are still necessary to draw more accurate interpretations.	

tolerate their side effects (Marlow et al., 2013). Likewise, another meta-analysis supported that anodal M1-tDCS significantly reduces pain levels (represented by an average of nearly 15% pain reduction, measured with the visual analog scale—VAS of pain) in chronic pain patients (Vaseghi et al., 2014). Thus, despite the mounting evidence supporting the analgesic effects of non-invasive MCS, it is evident that additional clinical trials with standardized protocols and more robust data are needed to establish the extent to which tDCS and TMS can contribute to chronic pain management. Concurrently it is necessary to scrutinize the neurophysiological mechanisms as well as the neurochemical mediation associated with non-invasive brain stimulation.

HOW DO THEY ACT? PUTATIVE MECHANISMS OF NON-INVASIVE MOTOR CORTEX STIMULATION

Despite the large number of studies exploring the clinical efficacy of non-invasive methods of neuromodulation, their neurophysiological fundaments are largely unknown and numerous uncertainties remain. For example, is it possible to revert ingrained neuroplastic changes with MCS? Do non-invasive methods of neuromodulation elicit a significant placebo effect? What scientific evidence has been obtained from basic sciences and neuroimaging studies and what does this evidence indicate? Although some of these questions have been at least partially addressed, one of the most elementary and intriguing questions persists: How does the stimulation of the motor cortex grant significant chronic pain relief?

An indication of one possible role of the motor cortex in pain arose many years ago when in 1971 a published report revealed cortical removals of both postcentral and precentral facial representations resulted in facial pain relief (White and Sweet, 1969; Lende et al., 1971). Yet, the role of the motor cortex only truly started receiving special attention from clinicians and researchers after Tsubokawa's work with MCS in 1991 (Tsubokawa et al., 1991a,b). Afterwards, this cortical region became a common target for neuromodulation when intended to treat pain (Meyerson et al., 1993; Nguyen et al., 1997; García-Larrea et al., 1999; Saitoh et al., 2000, 2003). Interestingly, a study using navigation-guided rTMS examined if significant pain improvement could also be achieved by stimulating cortical areas other than the precentral gyrus (M1) in patients with intractable deafferentation pain (Hirayama et al., 2006). Specifically, the other areas evaluated were the postcentral gyrus, the supplementary motor area and the premotor cortex (Hirayama et al., 2006). Confirming previous works, results of the study found that M1 stimulation produced significant pain relief. Conversely, stimulation of the adjacent areas was not effective in the cohort evaluated, corroborating the prominent role of the primary motor cortex in pain relief, and more precisely the importance of stimulation over the anterior bank of the central sulcus for pain treatment. Similarly, an experimental study involving healthy subjects who volunteered to receive capsaicin application reported significantly higher analgesic effects of rTMS over M1 when compared to the stimulation of the DLPFC and occipital cortex (Sacco et al., 2014). In fact, it has been described that, at least with MCS, optimal analgesic effects can be accomplished when the electrodes are positioned over the somatotopic representation (within M1) of the painful territory. To this purpose, it is mandatory to work on a detailed functional and anatomical mapping of the cortical representation of the painful zone prior to the stimulation (Nguyen et al., 2011).

The neurobiological machinery activated when the motor cortex is stimulated is a matter of intense debate. The first studies investigating the mechanisms of MCS pointed to a decrease in chronic pain-induced thalamic hyperactivity related to the stimulation (Tsubokawa et al., 1991a, 1993), which led to the conclusion that antidromic modulation of thalamocortical pathways could play a role in the analgesia induced by M1 stimulation (Nguyen et al., 2011). In this regard, there are special features in the structural and functional organization of the motor cortex that determine the effects following its electrical stimulation (Amassian and Stewart, 2003). It seems that cathodal electrical stimulation applied directly to the motor cortex (MCS) is associated with a preferential activation of the interneurons that run parallel to the cortical surface and an indirect stimulation of the pyramidal tract, generating indirect waves (I-waves) at the spinal cord. On the other hand, anodal electrical cortical stimulation of the motor cortex would preferentially activate the pyramidal cell axons, represented by the fibers that run perpendicularly to the cortical surface, and thus result in a direct stimulation of the pyramidal tract, producing early direct waves (D-waves) (Amassian et al., 1987; Amassian and Stewart, 2003; Nguyen et al., 2011). It has been described that the activation of the axons that run parallel to the cortical surface and the indirect generation of I-waves, accomplished through cathodal precentral gyrus stimulation, optimizes MCS analgesic effects (Lefaucheur et al., 2010a; Nguyen et al., 2011). Studies have confirmed that the most effective MCS electrode configuration for pain control is the one that generates I-waves (Lefaucheur et al., 2010a). Such findings could indicate that that MCS acts though the activation of top-down controls associated with intracortical horizontal fibers, instead of direct stimulation of the pyramidal tract (Nguyen et al., 2011). The same fundament can be transposed to rTMS. Similar to cathodal electrical stimulation, rTMS produces I-waves, and significant pain decrease when its coil is positioned in an anteroposterior orientation, whereas D-waves are formed when its coil is positioned in a lateromedial orientation (André-Obadia et al., 2008; Lefaucheur et al., 2010a; Nguyen et al., 2011). It has been proposed that the activation of the fibers that run parallel to the cortical surface in the precentral gyrus would lead to both orthodromic activation of corticofugal pathways as well as antidromic activation of thalamocortical pathways. Thus, it would influence pathways and structures that are distant from the side of stimulation (Nguyen et al., 2011).

The general view that the analgesic effects observed with M1 stimulation derives from the activation of areas far beyond the cortical zone where the stimulus is applied has been confirmed by neuroimaging studies (García-Larrea et al., 1999; Peyron et al., 2007). Some of those studies proved the ability of MCS

to activate adjacent outer brain areas (e.g., orbitofrontal cortex—OFC, DLPFC) as well as remote inner brain structures, such as the insula and anterior, middle and posterior cingulate cortex, the putamen, the thalamus, and portions of the brainstem, including the periaqueductal gray matter (PAG) and the pons (García-Larrea et al., 1999; Peyron et al., 2007). Other studies have proved that rTMS can also influence the activity of a network that comprises cortical areas (M1, S1, supplementary motor cortex, dorsal premotor cortex, cingulate cortex, and insula), as well as the thalamus and basal ganglia (Strafella et al., 2003; Bestmann et al., 2004). It is important to highlight that all of those aforementioned elements of the human brain are largely recognized by their direct or indirect involvement in pain processing (Peyron et al., 2000; Zubieta et al., 2001). Remarkably, M1-rTMS consistently interferes with the activity of brain areas related to the emotional aspects of pain, including the cingulate cortex and insula, which explains the effects of M1 stimulation on the affective-emotional dimension of pain (Passard et al., 2007; Picarelli et al., 2010).

Changes in motor cortex excitability have also been explored for the purpose of understanding the neurophysiological aftereffects of M1 stimulation. Single- and paired-pulse TMS paradigms are important tools to assess motor cortex excitability parameters, including the resting motor threshold (RMT), the motor evoked potential (MEP) amplitude, the intracortical inhibition (ICI), the intracortical facilitation (ICF), and the electromyographic cortical silent period (CSP) (Ziemann et al., 1996; Sanger et al., 2001). It has been described that non-invasive MCS, achieved by tDCS or TMS, is associated with both immediate and long-lasting changes in motor cortex excitability (Wassermann et al., 1998; Nitsche and Paulus, 2000, 2001; Schambra et al., 2003; Jung et al., 2008). Noteworthy, it has been shown that changes in cortical excitability elicited by rTMS differ in healthy subjects (Wu et al., 2000; Romero et al., 2002) and chronic pain patients (Lefaucheur et al., 2006), suggesting that rTMS effects depend on the degree of cortical excitability present before the period of stimulation (Lefaucheur et al., 2006). Furthermore, previous studies have documented both increased (Schambra et al., 2003) and decreased (Wassermann et al., 1998) motor cortex excitability in the M1 contralateral to the stimulated side, which possibly indicates a role of TMS in the modulation of interhemispheric connections (Schambra et al., 2003).

Surprisingly, similar results could not be replicated with M1-tDCS. There is also evidence that tDCS does not act on glutamatergic transcallosal neurons, though it does influence the activity of ipsilateral inhibitory interneurons that receive transcallossal projections and that mediate transcallosal inhibition (Lang et al., 2004).

The results just presented suggest the functional effects of tDCS have a higher specificity, even though neuroimaging and computational modeling studies indicate conventional tDCS montages generate widespread electrical current that flows throughout outer brain regions and deeper structures (Faria et al., 2011; DaSilva et al., 2012; Neuling et al., 2012; Antal et al., 2014). In fact, it has been supported that reinforcement of both anatomical selectivity (e.g., guiding the electrical current to specific targets in the brain) and functional selectivity (e.g., activity and input selectivity) are required to promote a rational advancement of tDCS research (Bikson et al., 2013). In order to enhance the anatomical specificity and possibly its effectiveness in pain control, novel high-definition (HD)-tDCS montages that use ring instead of large electrodes have been tailored (DaSilva et al., 2015). In addition, the evaluation of the electrical current distribution through computational models have permitted the development of HD-tDCS montages (e.g., 2 × 2-HD) with the purpose of targeting specific areas of the motor cortex (e.g., head and face homuncular region of M1), thus reproducing the MCS parameters and principles (DaSilva et al., 2015; Donnell et al., 2015). However, further studies are necessary to establish the clinical relevance of enhancing anatomical specificity for tDCS-induced analgesia.

In addition to the mechanisms previously reported, the neurochemical mediation associated with the clinical outcomes of different neuromodulatory techniques has just started to be unveiled. The involvement of the endogenous opioid system, one of the most prominent analgesic mechanisms and target of the majority of opiates in this whole process, was initially indicated by a study that reported increased release of endogenous opioids in different pain-related brain areas after MCS (Maarrawi et al., 2007). Furthermore, it has been verified that the density of opioid receptor binding in the brain can predict the postoperative pain relief obtained with MCS in chronic pain patients (Maarrawi et al., 2013). Similarly, significant endogenous opioid release, confirmed by decreased binding potential of the selective μ-opioid receptor agonist [^{11}C]carfentanil in pain-related regions (e.g., precuneus, PAG, prefrontal cortex, thalamus, anterior cingulate cortex, and insula), has been associated with a single session of anodal M1-tDCS in both healthy subjects (DosSantos et al., 2014) and in a single case of postherpetic neuralgia (DosSantos et al., 2012). These findings clearly indicate the contribution of the endogenous opioid system, most likely exerted through activation of the μ-opioid neurotransmission, in the analgesic effects induced by non-invasive stimulation of the motor cortex. Supporting this concept, a TMS study reported that intravenous administration of the opioid receptor antagonist naloxone significantly reduces the analgesia achieved by M1-rTMS. Remarkably, in that particular study naloxone administration did not impact the analgesic effects of rTMS when applied to the DLFC (de Andrade et al., 2011), suggesting that specific neuromechanisms can be elicited when distinct cortical regions are stimulated. Nevertheless, this conclusion needs to be explored in depth since another study found naloxone treatment performed prior to TMS resulted in a significant decrease of DLFC rTMS-induced analgesia (Taylor et al., 2012). It is important to emphasize that both naloxone studies were performed in healthy volunteers and the inclusion of chronic pain patients might have produced different findings.

It has also been postulated that mechanisms other than the activation of opioid receptors might contribute to the pain relief observed with different methods of neuromodulation (Lefaucheur et al., 2006; Nguyen et al., 2011; Foerster et al., 2015). Those mechanisms can be associated with the activation of inhibitory (GABAergic) as well as excitatory (glutamatergic) pathways. Remarkably, both pathways can be examined through

the evaluation of some parameters of cortical excitability (e.g., ICI, ICF, and CSP) (Ziemann et al., 1996; Sanger et al., 2001). The scientific evidence currently available indicates that high frequency (10 Hz) rTMS can restore a defective ICI, which represents an impaired GABAergic neurotransmission present in chronic neuropathic pain patients (Lefaucheur et al., 2006). Moreover, according to the data available, the restoration of the defective ICI by rTMS correlates to the degree of pain relief (Lefaucheur et al., 2006).

One evidence that supports the involvement of the glutametergic neurotransmission in the analgesic effects driven by M1 stimulation is the focal release of dopamine in the putamen associated with M1-rTMS, an effect possibly induced by glutamatergic corticostriatal projections, originating in the stimulated motor cortex (Strafella et al., 2003). In fact, it has been described that the activation of descending mechanisms of pain control induced by M1 stimulation in experimental models of neuropathic pain presumably involves striatal dopamine D2 receptors (DRD2) (Viisanen et al., 2012). Additionally, it has been recently reported that the genetic regulation of DRD2 by 957C>T polymorphis affects the susceptibility for neuropathic pain and also pain modulation by rTMS (Jääskeläinen et al., 2014).

The participation of glutamate N-methyl-D-aspartate (NMDA) receptors in TMS-induced analgesia has also been explored. The establishment of this link has its origins in animal model studies (Ambriz-Tututi et al., 2012) and was confirmed in a study that showed a decrease in the analgesic effects induced by both M1 and DLPFC/PFC stimulation after the administration of the noncompetitive NMDA antagonist ketamine (Ciampi de Andrade et al., 2014). Such findings also point to the association between rTMS-induced analgesia and long-term potentiation- or long-term depression-like mechanisms, since NMDA exerts predominant control over synaptic plasticity and memory (Tsien, 2000; Li and Tsien, 2009). NMDA receptors could also be associated with tDCS-induced neuroplasticity (Liebetanz et al., 2002). The presence of long-term analgesic effects induced by rTMS (Lefaucheur et al., 2004) and its dependence on the frequency of stimulation (André-Obadia et al., 2006) support the presence of neuroplastic changes associated with rTMS. Indeed, the dependence on the frequency of stimulation used to induce synaptic plasticity and the duration exceeding the stimulation period, are characteristics of long-term potentiation and long-term depression (Cooke and Bliss, 2006). The ability of the NMDA-receptor antagonist dextromethorphan (DMO) to suppress the effects of both anodal and cathodal tDCS on cortical excitability also supports the contribution of NMDA receptors and synaptic plasticity to the tDCS effects (Liebetanz et al., 2002).

The results of clinical and experimental studies point to the participation of GABAergic mechanisms in the analgesia associated with MCS and M1-TMS (Bestmann et al., 2004; Lucas et al., 2011; Pagano et al., 2012; Cha et al., 2013). It has been proposed that such effect could be related to the thalamic modulation produced by M1 stimulation, which would act through GABA neurotransmission (Moisset et al., 2015). Moreover, the participation of the reticular formation components and monoaminergic projections in the analgesia induced by M1 stimulation has been examined. There is evidence from experimental models of neuropathic pain that the antinociception induced by the electrical stimulation of M1 possibly involves the rostroventromedial medulla as well as descending serotoninergic pathways (Viisanen and Pertovaara, 2010b). On the other hand, it has been reported that coeruleospinal noradrenergic pathways are not essential for this process (Viisanen and Pertovaara, 2010a). Nevertheless, considering the still limited scientific evidence, further studies will be necessary to expand the current knowledge regarding the neurotransmitters involved in MCS and M1 tDCS.

Recently, studies have also been explored the possible neurochemical actions of tDCS. Proton magnetic resonance spectroscopy (^{1}H-MRS) studies demonstrated increases in Glx, a combined marker of glutamine and glutamate, and N-acetylaspartate (NAA), which is considered to be a measure of neuronal integrity, in the parietal cortex underneath the anode (Clark et al., 2011). Another study reported a significant decrease in Glx levels in the anterior cingulate cortex, related to active M1-tDCS (when compared to sham stimulation), in a cohort of fibromyalgia patients. There was also a trend toward an increase of GABA levels in the anterior insula when comparing active tDCS to baseline. Interestingly, the same study found a significant increase in NAA in the posterior insula when comparing sham tDCS vs. baseline, suggesting the presence of a placebo effect associated with M1-tDCS (Foerster et al., 2015).

Placebo is a factor that must always be considered when analyzing the effects of chronic pain therapies. Although several clinical trials involving non-invasive brain stimulation for pain relief have found significant differences between active and sham stimulation (Fregni et al., 2006a,b; Lee et al., 2012), considering the major role of the placebo effect for analgesia (Zubieta and Stohler, 2009) it is certainly possible that placebo might also play a role in the benefits of M1 stimulation for chronic pain treatment. This hypothesis has been recently evaluated with TMS (André-Obadia et al., 2011). The results suggest that the relative timing of sham and active TMS is an important factor to the placebo effect driven by this method. It has been demonstrated that placebo rTMS produces significant analgesia when applied after a successful active TMS session. Nevertheless, when following an unsuccessful active TMS session, placebo TMS tends to worsen pain. Interestingly, pain scores remained unaltered only when placebo TMS was applied before an active TMS session. Taken together, those results could reflect an unconscious conditioned learning related to placebo TMS. Regarding tDCS, considering that conventional montages produce widespread electrical current flow, a reasonable hypothesis that has emerged is that tDCS could reinforce the same brain networks that are usually activated by the expectations of clinical improvements (Schambra et al., 2014). This hypothesis would provide an alternative explanation for the beneficial effects observed with tDCS in depression studies, especially when a concurrent training (e.g., cognitive behavioral therapy) was not adopted and therefore activity-specificity was absent (Brunoni et al., 2012, 2013). In a recent study, we were able to demonstrate the presence of changes in the μ-opioid neurotransmission during both active

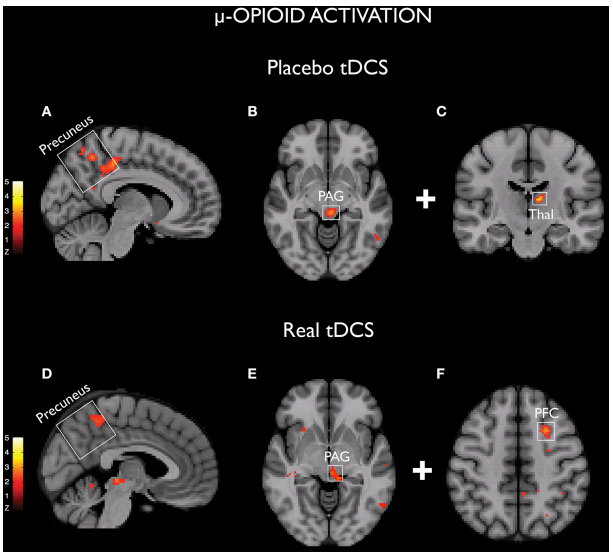


FIGURE 1 | Activation of μ-opioid receptors demonstrated with both sham (A–C) and real (D–F) tDCS (DosSantos et al., 2014).

and sham tDCS in humans. Surprisingly, we found concurrently (e.g., precuneus and PAG) as well as unrelated (e.g., PFC in active tDCS and thalamus during sham stimulation) μ-opioid activation (**Figure 1**), indicating that both shared and dissimilar mechanisms can drive the effects of sham and active tDCS in human subjects (DosSantos et al., 2014). These findings support the view that an earlier sham stimulation can build-up the effects of a subsequent active stimulation (DosSantos et al., 2014) and that heightening patients expectations with a placebo prior to active stimulation should also be considered (Schambra et al., 2014).

CONCLUDING REMARKS

Since the serendipitous observation that M1 stimulation produces significant clinical improvements in chronic neuropathic pain patients, this cortical region became the main target of several neuromodulatory techniques devoted to ameliorating chronic pain in human subjects. In fact, it has been reported that the stimulation of cortical regions adjacent to the primary motor cortex fail to produce similar analgesic effects, confirming the prominent role of M1 stimulation for pain control. Nevertheless, the intricate neurophysiological mechanisms that explain the clinical efficacy of M1 stimulation for pain relief are not completely understood. Evidence from MCS studies indicates that its analgesic mechanisms involve the activation of top-down controls related to the excitation of intracortical horizontal fibers. This concept can also be applied to TMS. However, results of neuroimaging studies also suggest that MCS and TMS act through modulation of deeper and remote brain structures related to pain, such as the insula, anterior, cingulate cortex, basal ganglia, thalamus, and brainstem. Interestingly, enhanced current flow in the same areas has also been demonstrated with tDCS. In addition, the neurochemical mediation driven by M1 stimulation has been recently unveiled in studies involving MCS, TMS, and tDCS. Opioidergic, glutamatergic, GABAergic and serotoninergic neurotransmissions are now considered components for the whole process. Nevertheless, there are still questions that must be answered, including those regarding the participation of other mechanisms of endogenous pain control, the clinical relevance of increasing anatomical and functional specificity in non-invasive procedures, and the presence and significance of a placebo effect. The answers to these questions are expected to be among the future perspectives of the field.

AUTHOR CONTRIBUTIONS

MD, NF, RT, AC and AD drafted the manuscript. All authors read and approved the current version of this article.

ACKNOWLEDGMENTS

AD was supported by MICHR Clinical Trial Planning Program/CTSA high-tech funding UL1RR024986, University of Michigan, National Institute of Health - National Institute of Neurological Disorders and Stroke-K23 NS062946, Dana Foundation's Brain and Immuno-Imaging Award, and the Migraine Research Foundation Research Grant Award.

REFERENCES

Amassian, V. E., and Stewart, M. (2003). Motor cortical and other cortical interneuronal networks that generate very high frequency waves. *Suppl. Clin. Neurophysiol.* 56, 119–142. doi: 10.1016/S1567-424X(09)70214-4

Amassian, V. E., Stewart, M., Quirk, G. J., and Rosenthal, J. L. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery* 20, 74–93. doi: 10.1097/00006123-198701000-00022

Ambriz-Tututi, M., Sánchez-González, V., and Drucker-Colín, R. (2012). Transcranial magnetic stimulation reduces nociceptive threshold in rats. *J. Neurosci. Res.* 90, 1085–1095. doi: 10.1002/jnr.22785

André-Obadia, N., Magnin, M., and Garcia-Larrea, L. (2011). On the importance of placebo timing in rTMS studies for pain relief. *Pain* 152, 1233–1237. doi: 10.1016/j.pain.2010.12.027

André-Obadia, N., Mertens, P., Gueguen, A., Peyron, R., and Garcia-Larrea, L. (2008). Pain relief by rTMS: differential effect of current flow but no specific action on pain subtypes. *Neurology* 71, 833–840. doi: 10.1212/01.wnl.0000325481.61471.f0

André-Obadia, N., Mertens, P., Lelekov-Boissard, T., Afif, A., Magnin, M., and Garcia-Larrea, L. (2014). Is Life better after motor cortex stimulation for pain control? Results at long-term and their prediction by preoperative rTMS. *Pain Physician* 17, 53–62.

André-Obadia, N., Peyron, R., Mertens, P., Mauguière, F., Laurent, B., and Garcia-Larrea, L. (2006). Transcranial magnetic stimulation for pain control. Double-blind study of different frequencies against placebo, and correlation with motor cortex stimulation efficacy. *Clin. Neurophysiol.* 117, 1536–1544. doi: 10.1016/j.clinph.2006.03.025

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., Kriener, N., Lang, N., Boros, K., and Paulus, W. (2011). Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. *Cephalalgia* 31, 820–828. doi: 10.1177/0333102411399349

Antal, A., Terney, D., Kühnl, S., and Paulus, W. (2010). Anodal transcranial direct current stimulation of the motor cortex ameliorates chronic pain and reduces short intracortical inhibition. *J. Pain Symptom Manage.* 39, 890–903. doi: 10.1016/j.jpainsymman.2009.09.023

Basbaum, A. I., Bautista, D. M., Scherrer, G., and Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284. doi: 10.1016/j.cell.2009.09.028

Bestmann, S., Baudewig, J., Siebner, H. R., Rothwell, J. C., and Frahm, J. (2004). Functional MRI of the immediate impact of transcranial magnetic stimulation on cortical and subcortical motor circuits. *Eur. J. Neurosci.* 19, 1950–1962. doi: 10.1111/j.1460-9568.2004.03277.x

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

Bolognini, N., Spandri, V., Ferraro, F., Salmaggi, A., Molinari, A. C., Fregni, F., et al. (2015). Immediate and sustained effects of 5-Day transcranial direct current stimulation of the motor cortex in phantom limb pain. *J. Pain* 16, 657–665. doi: 10.1016/j.jpain.2015.03.013

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur. J. Pain* 10, 287–333. doi: 10.1016/j.ejpain.2005.06.009

Brunoni, A. R., Ferrucci, R., Fregni, F., Boggio, P. S., and Priori, A. (2012). Transcranial direct current stimulation for the treatment of major depressive disorder: a summary of preclinical, clinical and translational findings. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 39, 9–16. doi: 10.1016/j.pnpbp.2012.05.016

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanão, T. A., de Oliveira, J. F., Goulart, A., et al. (2013). The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. *JAMA Psychiatry* 70, 383–391. doi: 10.1001/2013.jamapsychiatry.32

Cha, M., Ji, Y., and Masri, R. (2013). Motor cortex stimulation activates the incertothalamic pathway in an animal model of spinal cord injury. *J. Pain* 14, 260–269. doi: 10.1016/j.jpain.2012.11.007

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

Ciampi de Andrade, D., Mhalla, A., Adam, F., Texeira, M. J., and Bouhassira, D. (2014). Repetitive transcranial magnetic stimulation induced analgesia depends on N-methyl-D-aspartate glutamate receptors. *Pain* 155, 598–605. doi: 10.1016/j.pain.2013.12.022

Cooke, S. F., and Bliss, T. V. (2006). Plasticity in the human central nervous system. *Brain* 129, 1659–1673. doi: 10.1093/brain/awl082

DaSilva, A. F., Mendonca, M. E., Zaghi, S., Lopes, M., DosSantos, M. F., Spierings, E. L., et al. (2012). tDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. *Headache* 52, 1283–1295. doi: 10.1111/j.1526-4610.2012.02141.x

DaSilva, A. F., Truong, D. Q., DosSantos, M. F., Toback, R. L., Datta, A., and Bikson, M. (2015). State-of-art neuroanatomical target analysis of high-definition and conventional tDCS montages used for migraine and pain control. *Front. Neuroanat.* 9:89. doi: 10.3389/fnana.2015.00089

de Andrade, D. C., Mhalla, A., Adam, F., Texeira, M. J., and Bouhassira, D. (2011). Neuropharmacological basis of rTMS-induced analgesia: the role of endogenous opioids. *Pain* 152, 320–326. doi: 10.1016/j.pain.2010.10.032

Donnell, A. D., Nascimento, T., Lawrence, M., Gupta, V., Zieba, T., Truong, D. Q., et al. (2015). High-definition and non-invasive brain modulation of pain and motor dysfunction in chronic TMD. *Brain Stimul.* 8, 1085–1092. doi: 10.1016/j.brs.2015.06.008

DosSantos, M. F., Love, T. M., Martikainen, I. K., Nascimento, T. D., Fregni, F., Cummiford, C., et al. (2012). Immediate effects of tDCS on the μ-opioid system of a chronic pain patient. *Front. Psychiatry* 3:93. doi: 10.3389/fpsyt.2012.00093

DosSantos, M. F., Martikainen, I. K., Nascimento, T. D., Love, T. M., Deboer, M. D., Schambra, H. M., et al. (2014). Building up analgesia in humans via the endogenous μ-opioid system by combining placebo and active tDCS: a preliminary report. *PLoS ONE* 9:e102350. doi: 10.1371/journal.pone.0102350

Elliott, A. M., Smith, B. H., Hannaford, P. C., Smith, W. C., and Chambers, W. A. (2002). The course of chronic pain in the community: results of a 4-year follow-up study. *Pain* 99, 299–307. doi: 10.1016/S0304-3959(02)00138-0

Elliott, A. M., Smith, B. H., Penny, K. I., Smith, W. C., and Chambers, W. A. (1999). The epidemiology of chronic pain in the community. *Lancet* 354, 1248–1252. doi: 10.1016/S0140-6736(99)03057-3

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

Farina, S., Tinazzi, M., Le Pera, D., and Valeriani, M. (2003). Pain-related modulation of the human motor cortex. *Neurol. Res.* 25, 130–142. doi: 10.1179/016164103101201283

Fishman, S., Ballantyne, J., Rathmell, J. P., and Bonica, J. J. (2010). *Bonica's Management of Pain.* Philadelphia, PA: Lippincott, Williams & Wilkins.

Foerster, B. R., Nascimento, T. D., DeBoer, M., Bender, M. A., Rice, I. C., Truong, D. Q., et al. (2015). Excitatory and inhibitory brain metabolites as targets of motor cortex transcranial direct current stimulation therapy and predictors of its efficacy in fibromyalgia. *Arthritis Rheumatol.* 67, 576–581. doi: 10.1002/art.38945

Fontaine, D., Bruneto, J., El Fakir, H., Paquis, P., and Lanteri-Minet, M. (2009). Short-term restoration of facial sensory loss by motor cortex stimulation in peripheral post-traumatic neuropathic pain. *J. Headache Pain* 10, 203–206. doi: 10.1007/s10194-009-0115-5

Fregni, F., Boggio, P. S., Lima, M. C., Ferreira, M. J., Wagner, T., Rigonatti, S. P., et al. (2006a). A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. *Pain* 122, 197–209. doi: 10.1016/j.pain.2006.02.023

Fregni, F., Gimenes, R., Valle, A. C., Ferreira, M. J., Rocha, R. R., Natalle, L., et al. (2006b). A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis Rheum.* 54, 3988–3998. doi: 10.1002/art.22195

García-Larrea, L., Peyron, R., Mertens, P., Gregoire, M. C., Lavenne, F., Le Bars, D., et al. (1999). Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. *Pain* 83, 259–273. doi: 10.1016/S0304-3959(99)00114-1

Hagenacker, T., Bude, V., Naegel, S., Holle, D., Katsarava, Z., Diener, H. C., et al. (2014). Patient-conducted anodal transcranial direct current stimulation of the motor cortex alleviates pain in trigeminal neuralgia. *J. Headache Pain* 15, 78. doi: 10.1186/1129-2377-15-78

Hasan, M., Whiteley, J., Bresnahan, R., Maciver, K., Sacco, P., Das, K., et al. (2014). Somatosensory change and pain relief induced by repetitive transcranial magnetic stimulation in patients with central poststroke pain. *Neuromodulation* 17, 731–736. discussion: 736. doi: 10.1111/ner.12198

Hirayama, A., Saitoh, Y., Kishima, H., Shimokawa, T., Oshino, S., Hirata, M., et al. (2006). Reduction of intractable deafferentation pain by navigation-guided repetitive transcranial magnetic stimulation of the primary motor cortex. *Pain* 122, 22–27. doi: 10.1016/j.pain.2005.12.001

Hodaj, H., Alibeu, J. P., Payen, J. F., and Lefaucheur, J. P. (2015). Treatment of chronic facial pain including cluster headache by repetitive transcranial magnetic stimulation of the motor cortex with maintenance sessions: a naturalistic study. *Brain Stimul.* 8, 801–807. doi: 10.1016/j.brs.2015.01.416

Hosomi, K., Saitoh, Y., Kishima, H., Oshino, S., Hirata, M., Tani, N., et al. (2008). Electrical stimulation of primary motor cortex within the central sulcus for intractable neuropathic pain. *Clin. Neurophysiol.* 119, 993–1001. doi: 10.1016/j.clinph.2007.12.022

Hosomi, K., Shimokawa, T., Ikoma, K., Nakamura, Y., Sugiyama, K., Ugawa, Y., et al. (2013). Daily repetitive transcranial magnetic stimulation of primary motor cortex for neuropathic pain: a randomized, multicenter, double-blind, crossover, sham-controlled trial. *Pain* 154, 1065–1072. doi: 10.1016/j.pain.2013.03.016

Jääskeläinen, S. K., Lindholm, P., Valmunen, T., Pesonen, U., Taiminen, T., Virtanen, A., et al. (2014). Variation in the dopamine D2 receptor gene plays a key role in human pain and its modulation by transcranial magnetic stimulation. *Pain* 155, 2180–2187. doi: 10.1016/j.pain.2014.08.029

Jensen, M. P., Sherlin, L. H., Askew, R. L., Fregni, F., Witkop, G., Gianas, A., et al. (2013). Effects of non-pharmacological pain treatments on brain states. *Clin. Neurophysiol.* 124, 2016–2024. doi: 10.1016/j.clinph.2013.04.009

Jung, S. H., Shin, J. E., Jeong, Y. S., and Shin, H. I. (2008). Changes in motor cortical excitability induced by high-frequency repetitive transcranial magnetic stimulation of different stimulation durations. *Clin. Neurophysiol.* 119, 71–79. doi: 10.1016/j.clinph.2007.09.124

Kim, Y. J., Ku, J., Kim, H. J., Im, D. J., Lee, H. S., Han, K. A., et al. (2013). Randomized, sham controlled trial of transcranial direct current stimulation for painful diabetic polyneuropathy. *Ann. Rehabil. Med.* 37, 766–776. doi: 10.5535/arm.2013.37.6.766

Klein, M. M., Treister, R., Raij, T., Pascual-Leone, A., Park, L., Nurmikko, T., et al. (2015). Transcranial magnetic stimulation of the brain: guidelines for pain treatment research. *Pain* 156, 1601–1614. doi: 10.1097/j.pain.0000000000000210

Kumar, K., and Rizvi, S. (2014). Historical and present state of neuromodulation in chronic pain. *Curr. Pain Headache Rep.* 18, 387. doi: 10.1007/s11916-013-0387-y

Lang, N., Nitsche, M. A., Paulus, W., Rothwell, J., and Lemon, R. (2004). 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

Lee, S. J., Kim, D. Y., Chun, M. H., and Kim, Y. G. (2012). The effect of repetitive transcranial magnetic stimulation on fibromyalgia: a randomized sham-controlled trial with 1-mo follow-up. *Am. J. Phys. Med. Rehabil.* 91, 1077–1085. doi: 10.1097/PHM.0b013e3182745a04

Lefaucheur, J., Drouot, X., Cunin, P., Bruckert, R., Lepetit, H., Créange, A., et al. (2009). Motor cortex stimulation for the treatment of refractory peripheral neuropathic pain. *Brain* 132, 1463–1471. doi: 10.1093/brain/awp035

Lefaucheur, J. P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., et al. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin. Neurophysiol.* 125, 2150–2206. doi: 10.1016/j.clinph.2014.05.021

Lefaucheur, J. P., Drouot, X., Keravel, Y., and Nguyen, J. P. (2001). Pain relief induced by repetitive transcranial magnetic stimulation of precentral cortex. *Neuroreport* 12, 2963–2965. doi: 10.1097/00001756-200109170-00041

Lefaucheur, J. P., Drouot, X., Ménard-Lefaucheur, I., Keravel, Y., and Nguyen, J. P. (2006). Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. *Neurology* 67, 1568–1574. doi: 10.1212/01.wnl.0000242731.10074.3c

Lefaucheur, J. P., Drouot, X., Ménard-Lefaucheur, I., and Nguyen, J. P. (2004). Neuropathic pain controlled for more than a year by monthly sessions of repetitive transcranial magnetic stimulation of the motor cortex. *Neurophysiol. Clin.* 34, 91–95. doi: 10.1016/j.neucli.2004.02.001

Lefaucheur, J. P., Holsheimer, J., Goujon, C., Keravel, Y., and Nguyen, J. P. (2010a). Descending volleys generated by efficacious epidural motor cortex stimulation in patients with chronic neuropathic pain. *Exp. Neurol.* 223, 609–614. doi: 10.1016/j.expneurol.2010.02.008

Lefaucheur, J. P., Jarry, G., Drouot, X., Ménard-Lefaucheur, I., Keravel, Y., and Nguyen, J. P. (2010b). Motor cortex rTMS reduces acute pain provoked by laser stimulation in patients with chronic neuropathic pain. *Clin. Neurophysiol.* 121, 895–901. doi: 10.1016/j.clinph.2009.12.028

Lefaucheur, J. P., Ménard-Lefaucheur, I., Goujon, C., Keravel, Y., and Nguyen, J. P. (2011). Predictive value of rTMS in the identification of responders to epidural motor cortex stimulation therapy for pain. *J. Pain* 12, 1102–1111. doi: 10.1016/j.jpain.2011.05.004

Lende, R. A., Kirsch, W. M., and Druckman, R. (1971). Relief of facial pain after combined removal of precentral and postcentral cortex. *J. Neurosurg.* 34, 537–543. doi: 10.3171/jns.1971.34.4.0537

Li, F., and Tsien, J. Z. (2009). Memory and the NMDA receptors. *N. Engl. J. Med.* 361, 302–303. doi: 10.1056/NEJMcibr0902052

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

Lucas, J. M., Ji, Y., and Masri, R. (2011). Motor cortex stimulation reduces hyperalgesia in an animal model of central pain. *Pain* 152, 1398–1407. doi: 10.1016/j.pain.2011.02.025

Maarrawi, J., Peyron, R., Mertens, P., Costes, N., Magnin, M., Sindou, M., et al. (2007). Motor cortex stimulation for pain control induces changes in the endogenous opioid system. *Neurology* 69, 827–834. doi: 10.1212/01.wnl.0000269783.86997.37

Maarrawi, J., Peyron, R., Mertens, P., Costes, N., Magnin, M., Sindou, M., et al. (2013). Brain opioid receptor density predicts motor cortex stimulation efficacy for chronic pain. *Pain* 154, 2563–2568. doi: 10.1016/j.pain.2013.07.042

Marlow, N. M., Bonilha, H. S., and Short, E. B. (2013). Efficacy of transcranial direct current stimulation and repetitive transcranial magnetic stimulation for treating fibromyalgia syndrome: a systematic review. *Pain Pract.* 13, 131–145. doi: 10.1111/j.1533-2500.2012.00562.x

McMahon, S. B. (2013). *Wall and Melzack's Textbook of Pain.* Philadelphia, PA: Elsevier/Saunders.

Melzack, R., and Casey, K. L. (1968). "Sensory, motivational, and central control determinants of pain: a new conceptual model," in *The Skin Senses,* ed D. Kenshalo (Springfield, IL: Charles C Thomas), 423–439.

Mendonca, M. E., Santana, M. B., Baptista, A. F., Datta, A., Bikson, M., Fregni, F., et al. (2011). Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. *J. Pain* 12, 610–617. doi: 10.1016/j.jpain.2010.12.015

Merskey, H., Bogduk, N., and International Association for the Study of Pain (1994). *Classification of Chronic Pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms.* Seattle, WA: IASP Press.

Meyerson, B. A., Lindblom, U., Linderoth, B., Lind, G., and Herregodts, P. (1993). Motor cortex stimulation as treatment of trigeminal neuropathic pain. *Acta Neurochir. Suppl. (Wien)* 58, 150–153. doi: 10.1007/978-3-7091-9297-9_34

Mhalla, A., Baudic, S., Ciampi de Andrade, D., Gautron, M., Perrot, S., Teixeira, M. J., et al. (2011). Long-term maintenance of the analgesic effects of transcranial magnetic stimulation in fibromyalgia. *Pain* 152, 1478–1485. doi: 10.1016/j.pain.2011.01.034

Moisset, X., de Andrade, D. C., and Bouhassira, D. (2015). From pulses to pain relief: an update on the mechanisms of rTMS-induced analgesic effects. *Eur. J. Pain*. doi: 10.1002/ejp.811. [Epub ahead of print].

Mouraux, A., Diukova, A., Lee, M. C., Wise, R. G., and Iannetti, G. D. (2011). A multisensory investigation of the functional significance of the "pain matrix". *Neuroimage* 54, 2237–2249. doi: 10.1016/j.neuroimage.2010.09.084

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

Ngernyam, N., Jensen, M. P., Arayawichanon, P., Auvichayapat, N., Tiamkao, S., Janjarasjitt, S., et al. (2015). The effects of transcranial direct current stimulation in patients with neuropathic pain from spinal cord injury. *Clin. Neurophysiol.* 126, 382–390. doi: 10.1016/j.clinph.2014.05.034

Nguyen, J. P., Keravel, Y., Feve, A., Uchiyama, T., Cesaro, P., Le Guerinel, C., et al. (1997). Treatment of deafferentation pain by chronic stimulation of the motor cortex: report of a series of 20 cases. *Acta Neurochir. Suppl.* 68, 54–60. doi: 10.1007/978-3-7091-6513-3_10

Nguyen, J. P., Lefaucheur, J. P., Decq, P., Uchiyama, T., Carpentier, A., Fontaine, D., et al. (1999). Chronic motor cortex stimulation in the treatment of central and neuropathic pain. Correlations between clinical, electrophysiological and anatomical data. *Pain* 82, 245–251. doi: 10.1016/S0304-3959(99)00062-7

Nguyen, J. P., Lefaucheur, J. P., Raoul, S., Roualdes, V., Péréon, Y., and Keravel, Y. (2009). Treatment of trigeminal neuropathic pain by motor cortex stimulation. *Neurochirurgie* 55, 226–230. doi: 10.1016/j.neuchi.2009.02.008

Nguyen, J. P., Nizard, J., Keravel, Y., and Lefaucheur, J. P. (2011). Invasive brain stimulation for the treatment of neuropathic pain. *Nat. Rev. Neurol.* 7, 699–709. doi: 10.1038/nrneurol.2011.138

Nguyen, J. P., Velasco, F., Brugières, P., Velasco, M., Keravel, Y., Boleaga, B., et al. (2008). Treatment of chronic neuropathic pain by motor cortex stimulation:

results of a bicentric controlled crossover trial. *Brain Stimul.* 1, 89–96. doi: 10.1016/j.brs.2008.03.007

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

Nuti, C., Peyron, R., Garcia-Larrea, L., Brunon, J., Laurent, B., Sindou, M., et al. (2005). Motor cortex stimulation for refractory neuropathic pain: four year outcome and predictors of efficacy. *Pain* 118, 43–52. doi: 10.1016/j.pain.2005.07.020

O'connell, N. E., Wand, B. M., Marston, L., Spencer, S., and Desouza, L. H. (2014). Non-invasive brain stimulation techniques for chronic pain. *Cochrane Database Syst. Rev.* 4, CD008208. doi: 10.1002/14651858.CD008208.pub3

Pagano, R. L., Fonoff, E. T., Dale, C. S., Ballester, G., Teixeira, M. J., and Britto, L. R. (2012). Motor cortex stimulation inhibits thalamic sensory neurons and enhances activity of PAG neurons: possible pathways for antinociception. *Pain* 153, 2359–2369. doi: 10.1016/j.pain.2012.08.002

Passard, A., Attal, N., Benadhira, R., Brasseur, L., Saba, G., Sichere, P., et al. (2007). Effects of unilateral repetitive transcranial magnetic stimulation of the motor cortex on chronic widespread pain in fibromyalgia. *Brain* 130(Pt 10), 2661–26670. doi: 10.1093/brain/awm189

Patel, A. S., Farquharson, R., Carroll, D., Moore, A., Phillips, C. J., Taylor, R. S., et al. (2012). The impact and burden of chronic pain in the workplace: a qualitative systematic review. *Pain Pract.* 12, 578–589. doi: 10.1111/j.1533-2500.2012.00547.x

Peyron, R., Faillenot, I., Mertens, P., Laurent, B., and Garcia-Larrea, L. (2007). Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. *Neuroimage* 34, 310–321. doi: 10.1016/j.neuroimage.2006.08.037

Peyron, R., Laurent, B., and García-Larrea, L. (2000). Functional imaging of brain responses to pain. A review and meta-analysis. *Neurophysiol. Clin.* 30, 263–288. doi: 10.1016/S0987-7053(00)00227-6

Picarelli, H., Teixeira, M. J., de Andrade, D. C., Myczkowski, M. L., Luvisotto, T. B., Yeng, L. T., et al. (2010). Repetitive transcranial magnetic stimulation is efficacious as an add-on to pharmacological therapy in complex regional pain syndrome (CRPS) type I. *J. Pain* 11, 1203–1210. doi: 10.1016/j.jpain.2010.02.006

Radic, J. A., Beauprie, I., Chiasson, P., Kiss, Z. H., and Brownstone, R. M. (2015). Motor cortex stimulation for neuropathic pain: a randomized cross-over trial. *Can. J. Neurol. Sci.* 42, 401–409. doi: 10.1017/cjn.2015.292

Rasche, D., Ruppolt, M., Stippich, C., Unterberg, A., and Tronnier, V. M. (2006). Motor cortex stimulation for long-term relief of chronic neuropathic pain: a 10 year experience. *Pain* 121, 43–52. doi: 10.1016/j.pain.2005.12.006

Romero, J. R., Anschel, D., Sparing, R., Gangitano, M., and Pascual-Leone, A. (2002). Subthreshold low frequency repetitive transcranial magnetic stimulation selectively decreases facilitation in the motor cortex. *Clin. Neurophysiol.* 113, 101–107. doi: 10.1016/S1388-2457(01)00693-9

Sacco, P., Prior, M., Poole, H., and Nurmikko, T. (2014). Repetitive transcranial magnetic stimulation over primary motor vs non-motor cortical targets; effects on experimental hyperalgesia in healthy subjects. *BMC Neurol.* 14:166. doi: 10.1186/s12883-014-0166-3

Saitoh, Y., Kato, A., Ninomiya, H., Baba, T., Shibata, M., Mashimo, T., et al. (2003). Primary motor cortex stimulation within the central sulcus for treating deafferentation pain. *Acta Neurochir. Suppl.* 87, 149–152. doi: 10.1007/978-3-7091-6081-7_31

Saitoh, Y., Shibata, M., Hirano, S., Hirata, M., Mashimo, T., and Yoshimine, T. (2000). Motor cortex stimulation for central and peripheral deafferentation pain. Report of eight cases. *J. Neurosurg.* 92, 150–155. doi: 10.3171/jns.2000.92.1.0150

Sanger, T. D., Garg, R. R., and Chen, R. (2001). Interactions between two different inhibitory systems in the human motor cortex. *J. Physiol.* 530, 307–317. doi: 10.1111/j.1469-7793.2001.0307l.x

Schabrun, S. M., Jones, E., Elgueta Cancino, E. L., and Hodges, P. W. (2014). Targeting chronic recurrent low back pain from the top-down and the bottom-up: a combined transcranial direct current stimulation and peripheral electrical stimulation intervention. *Brain Stimul.* 7, 451–459. doi: 10.1016/j.brs.2014.01.058

Schambra, H. M., Bikson, M., Wager, T. D., DosSantos, M. F., and DaSilva, A. F. (2014). It's all in your head: reinforcing the placebo response with tDCS. *Brain Stimul.* 7, 623–624. doi: 10.1016/j.brs.2014.04.002

Schambra, H. M., Sawaki, L., and Cohen, L. G. (2003). Modulation of excitability of human motor cortex (M1) by 1 Hz transcranial magnetic stimulation of the contralateral M1. *Clin. Neurophysiol.* 114, 130–133. doi: 10.1016/S1388-2457(02)00342-5

Strafella, A. P., Paus, T., Fraraccio, M., and Dagher, A. (2003). Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. *Brain* 126, 2609–2615. doi: 10.1093/brain/awg268

Taylor, J. J., Borckardt, J. J., and George, M. S. (2012). Endogenous opioids mediate left dorsolateral prefrontal cortex rTMS-induced analgesia. *Pain* 153, 1219–1225. doi: 10.1016/j.pain.2012.02.030

Tsien, J. Z. (2000). Building a brainier mouse. *Sci. Am.* 282, 62–68. doi: 10.1038/scientificamerican0400-62

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., and Koyama, S. (1991a). Chronic motor cortex stimulation for the treatment of central pain. *Acta Neurochir. Suppl. (Wien)* 52, 137–139. doi: 10.1007/978-3-7091-9160-6_37

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., and Koyama, S. (1991b). Treatment of thalamic pain by chronic motor cortex stimulation. *Pacing Clin. Electrophysiol.* 14, 131–134. doi: 10.1111/j.1540-8159.1991.tb04058.x

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., and Koyama, S. (1993). Chronic motor cortex stimulation in patients with thalamic pain. *J. Neurosurg.* 78, 393–401. doi: 10.3171/jns.1993.78.3.0393

Tzabazis, A., Aparici, C. M., Rowbotham, M. C., Schneider, M. B., Etkin, A., and Yeomans, D. C. (2013). Shaped magnetic field pulses by multi-coil repetitive transcranial magnetic stimulation (rTMS) differentially modulate anterior cingulate cortex responses and pain in volunteers and fibromyalgia patients. *Mol. Pain* 9, 33. doi: 10.1186/1744-8069-9-33

Vaseghi, B., Zoghi, M., and Jaberzadeh, S. (2014). Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clin. Neurophysiol.* 125, 1847–1858. doi: 10.1016/j.clinph.2014.01.020

Velasco, F., Argüelles, C., Carrillo-Ruiz, J. D., Castro, G., Velasco, A. L., Jiménez, F., et al. (2008). Efficacy of motor cortex stimulation in the treatment of neuropathic pain: a randomized double-blind trial. *J. Neurosurg.* 108, 698–706. doi: 10.3171/JNS/2008/108/4/0698

Verhaak, P. F., Kerssens, J. J., Dekker, J., Sorbi, M. J., and Bensing, J. M. (1998). Prevalence of chronic benign pain disorder among adults: a review of the literature. *Pain* 77, 231–239. doi: 10.1016/S0304-3959(98)00117-1

Viganò, A., D'elia, T. S., Sava, S. L., Auvé, M., De Pasqua, V., Colosimo, A., et al. (2013). Transcranial Direct Current Stimulation (tDCS) of the visual cortex: a proof-of-concept study based on interictal electrophysiological abnormalities in migraine. *J. Headache Pain* 14:23. doi: 10.1186/1129-2377-14-23

Viisanen, H., and Pertovaara, A. (2010a). Antinociception by motor cortex stimulation in the neuropathic rat: does the locus coeruleus play a role? *Exp. Brain Res.* 201, 283–296. doi: 10.1007/s00221-009-2038-4

Viisanen, H., and Pertovaara, A. (2010b). Roles of the rostroventromedial medulla and the spinal 5-HT(1A) receptor in descending antinociception induced by motor cortex stimulation in the neuropathic rat. *Neurosci. Lett.* 476, 133–137. doi: 10.1016/j.neulet.2010.04.014

Viisanen, H., Ansah, O. B., and Pertovaara, A. (2012). The role of the dopamine D2 receptor in descending control of pain induced by motor cortex stimulation in the neuropathic rat. *Brain Res. Bull.* 89, 133–143. doi: 10.1016/j.brainresbull.2012.08.002

Villamar, M. F., Wivatvongvana, P., Patumanond, J., Bikson, M., Truong, D. Q., Datta, A., et al. (2013). Focal modulation of the primary motor cortex in fibromyalgia using 4×1-ring high-definition transcranial direct current stimulation (HD-tDCS): immediate and delayed analgesic effects of cathodal and anodal stimulation. *J. Pain* 14, 371–383. doi: 10.1016/j.jpain.2012.12.007

Wassermann, E. M., Wedegaertner, F. R., Ziemann, U., George, M. S., and Chen, R. (1998). Crossed reduction of human motor cortex excitability by 1-Hz transcranial magnetic stimulation. *Neurosci. Lett.* 250, 141–144. doi: 10.1016/S0304-3940(98)00437-6

White, J. C., and Sweet, W. H. (1969). *Pain and the Neurosurgeon; a Forty-Year Experience*. Springfield, IL: C. C. Thomas.

Wrigley, P. J., Gustin, S. M., McIndoe, L. N., Chakiath, R. J., Henderson, L. A., and Siddall, P. J. (2013). Longstanding neuropathic pain after spinal cord injury is refractory to transcranial direct current stimulation: a randomized controlled trial. *Pain* 154, 2178–2184. doi: 10.1016/j.pain.2013.06.045

Wu, T., Sommer, M., Tergau, F., and Paulus, W. (2000). Lasting influence of repetitive transcranial magnetic stimulation on intracortical excitability in human subjects. *Neurosci. Lett.* 287, 37–40. doi: 10.1016/S0304-3940(00)01132-0

Ziemann, U., Rothwell, J. C., and Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.* 496(Pt 3), 873–881. doi: 10.1113/jphysiol.1996.sp021734

Zubieta, J. K., and Stohler, C. S. (2009). Neurobiological mechanisms of placebo responses. *Ann. N.Y. Acad. Sci.* 1156, 198–210. doi: 10.1111/j.1749-6632.2009.04424.x

Zubieta, J. K, Smith, Y. R., Bueller, J. A., Xu, Y., Kilbourn, M. R., Jewett, D. M., et al. (2001). Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* 293, 311–315. doi: 10.1126/science.1060952

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 Neuroscience

REVIEW
published: 07 January 2016
doi: 10.3389/fnins.2015.00486

The Effectiveness of Transcranial Brain Stimulation in Improving Clinical Signs of Hyperkinetic Movement Disorders

Ignacio Obeso[1,2]*, *Antonio Cerasa*[3] and *Aldo Quattrone*[3,4]

[1] Centro Integral en Neurociencias A. C. (CINAC), HM Hospitales – Puerta del Sur. Móstoles, Madrid, Spain, [2] Center for Networked Biomedical Research on Neurodegenerative Diseases, Madrid, Spain, [3] Neuroimaging Research Unit, Institute of Molecular Bioimaging and Physiology - National Research Council, Germaneto, Italy, [4] Neurology Unit, Institute of Neurology, University "Magna Graecia", Catanzaro, Italy

Repetitive transcranial magnetic stimulation (rTMS) is a safe and painless method for stimulating cortical neurons. In neurological realm, rTMS has prevalently been applied to understand pathophysiological mechanisms underlying movement disorders. However, this tool has also the potential to be translated into a clinically applicable therapeutic use. Several available studies supported this hypothesis, but differences in protocols, clinical enrollment, and variability of rTMS effects across individuals complicate better understanding of efficient clinical protocols. The aim of this present review is to discuss to what extent the evidence provided by the therapeutic use of rTMS may be generalized. In particular, we attempted to define optimal cortical regions and stimulation protocols that have been demonstrated to maximize the effectiveness seen in the actual literature for the three most prevalent hyperkinetic movement disorders: Parkinson's disease (PD) with levodopa-induced dyskinesias (LIDs), essential tremor (ET) and dystonia. A total of 28 rTMS studies met our search criteria. Despite clinical and methodological differences, overall these studies demonstrated that therapeutic applications of rTMS to "normalize" pathologically decreased or increased levels of cortical activity have given moderate progress in patient's quality of life. Moreover, the present literature suggests that altered pathophysiology in hyperkinetic movement disorders establishes motor, premotor or cerebellar structures as candidate regions to reset cortico-subcortical pathways back to normal. Although rTMS has the potential to become a powerful tool for ameliorating the clinical outcome of hyperkinetic neurological patients, until now there is not a clear consensus on optimal protocols for these motor disorders. Well-controlled multicenter randomized clinical trials with high numbers of patients are urgently required.

Keywords: rTMS, Parkinson's disease, levodopa-induced dyskinesias, essential tremor, dystonia

OPEN ACCESS

Edited by:
Wendy Noble,
King's College London, UK

Reviewed by:
Carmelo Mario Vicario,
Bangor University, UK
Chrystalina A. Antoniades,
University of Oxford, UK

***Correspondence:**
Ignacio Obeso
i.obesomartin@gmail.com

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

Received: *28 September 2015*
Accepted: *07 December 2015*
Published: *07 January 2016*

Citation:
Obeso I, Cerasa A and Quattrone A (2016) The Effectiveness of Transcranial Brain Stimulation in Improving Clinical Signs of Hyperkinetic Movement Disorders. Front. Neurosci. 9:486. doi: 10.3389/fnins.2015.00486

INTRODUCTION

Alteration in dynamics of neural connectivity is the hallmark of motor and behavioral disease in humans. Brain connectivity affected by functional deficits will either produce exacerbated or reduced brain signal and thus the observed clinical symptomatology. In the motor domain, presence of hyperkinetic movement disorders is typically manifested as increased muscular activity

that leads to involuntary and unwanted movements, abnormal postures or combination of both. These are present in several neurological disorders, such as essential tremor (ET), dystonia, and Parkinson's disease (PD). In contrast, hypokinetic movement disorders represent loss of vigor and movement that produces rigidity and the inability to initiate and terminate actions efficiently, present in bradykinesia or freezing of gait in PD. Current treatments are mainly pharmacological, but recently functional surgery has made progress in remediation of uncontrolled and unwanted motor disorders (Fasano and Lozano, 2015).

The basal ganglia are considered the main neurodegenerative site of hyper- and hypo-kinetic movement (Middleton and Strick, 2000; Hamani et al., 2004). Due to its strict relationship with several brain regions, the basal ganglia are considered the principal hub of the neural pathways involved in motor control, which included other regions such as the subthalamic nucleus (STN), globus pallidum (GP), thalamus, together with the supplementary motor area (SMA), motor cortex (M1), and frontal regions (Alexander et al., 1986; Kehagia et al., 2013). In the last few years, advances in the neurophysiological and neuroimaging fields have provided alternative scenarios for understanding the neurobiological mechanisms of motor disorders. Indeed, several lines of evidence support the notion that others structures, outside traditional striato-thalamo-cortical pathways, are strongly involved. In particular, the cerebello-thalamic circuitry (Pinto et al., 2003; Lehéricy et al., 2013) as well as intra-cortical connections between the premotor cortex and the inferior frontal cortex (IFC), would seem to play a key role in the dysfunctional pathophysiological model of some hyperkinetic motor disorders (Herz et al., 2014; Cerasa et al., 2015).

When traditional treatments fail or do not reach the expected motor benefit, it is now possible to modulate the pathological level of cortical activity using invasive methods such as deep brain stimulation (Diamond and Jankovic, 2005). However, considerable effort is being made on applying other methods that are non-invasive, less costly, and capable of producing beneficial effects in the long-term. The increasing number of research and clinical protocols using non-invasive brain stimulation protocols in patients with neurological conditions show intermixed effects and reports. To date, therapeutic trials using repetitive transcranial magnetic stimulation (rTMS) in PD, ET or dystonia have reported some controversial findings. The use of inhibitory brain stimulation to reduce excessive and abnormal cortical activity in hyperkinetic motor disorders is a potential tool to remediate motor control, posture, muscle tone, and cognitive problems, but considerable effort is needed to test the multiple available protocols when using brain stimulation tools in neurological patients (Ridding and Rothwell, 2007; Elahi et al., 2009).

The present review aims to focus on studies using transcranial brain stimulation protocols to modulate hyperkinetic neurological disorders aimed at clarifying the optimal conditions in which non-invasive stimulation may be used in movement disorders. We selected studies with constrained search in PubMed and Medline using as search terms: dyskinesias, dystonia, and ET in combination with widely used brain stimulation terms: TMS, rTMS, and TBS, from inception to September 25, 2015. Publication lists of relevant studies were later scanned for potential eligible articles. We summarize key technical aspects of rTMS with effective results for PD, ET, and dystonia to propose focused research plans to increase the positive impact of non-invasive brain stimulation in clinical practice.

rTMS Protocol for Therapeutic Purpose

rTMS has effects on the brain and behavior that outlast the period of stimulation due to plastic changes of long-term potentiation or depression in synaptic connections amongst cortical networks. Regions or networks with suboptimal functioning after brain damage or neurodegenerative disease are potential candidates for neuromodulation therapy. So far, the therapeutic use of rTMS has been proved effective in patients with major depression refractory to regular treatment (George et al., 2013). In neurological realm, movement disorders has received much attention with regard to rTMS therapeutic studies. However, experiments in healthy subjects suggest that rTMS protocols have short-lived after-effects. Hence, clinical neuroscience encounters a challenge with aim boosting longer time-periods of beneficial effects in patient's quality of life.

Despite illness, several rTMS protocols may be used for therapeutic purpose (for review see Ridding and Rothwell, 2007). The key aspect to consider is how to prolong rTMS positive effects in clinical conditions and quality of life. Current rTMS protocols apply *low frequency* (<1 Hz) or *high frequency* (>1 Hz), as well as *single* rTMS or *multiple* rTMS sessions. Generally, high frequency stimulation induces an increase in cortical excitability and low frequency stimulation causes a decrease in cortical excitability. To benefit plastic and long-term rTMS potentiation, multiple sessions tend to show stronger and cumulative effects in clinical and behavioral measures. An alternative use of rTMS is theta burst stimulation (TBS), consisting of short, repeated bursts of TMS pulses at 50 Hz (Huang et al., 2005). Again, the use of TBS allows decrease (using continuous TBS) or increase (using intermittent TBS) of cortical excitability using different sets of magnetic trains.

The fact that cortical baseline activity may be either hyperexcitable or hypoexcitable has formed the idea of using low-frequency rTMS to treat disorders with marked cortical hyperexcitability, while using high frequency rTMS in conditions with low cortical excitability. For this reason, in hyperkinetic motor disorders the rationale behind the application of rTMS protocol is to reduce abnormal cortical hyperexcitability, although this is not true in all the circumstances as it depends on several methodological and clinical factors, discussed in the present review.

THERAPEUTIC POTENTIAL OF rTMS IN PARKINSON'S DISEASE PATIENTS

PD is primarily a disorder of response initiation characterized by an excessive motor inhibition. In particular, bradykinesia (slowness of voluntary movements), tremor, rigidity, and gait problems are cardinal motor signs in PD, greatly improved by

treatments with dopamine replacement therapy. However, PD-related neurochemical changes are long-lasting and difficult to contrast by pharmacological interventions. For this reason, new treatment strategies have been proposed. rTMS has been studied as an intervention to ameliorate motor symptoms (Edwards et al., 2008; Elahi et al., 2009), including rigidity and bradykinesia, motor complications of therapy (e.g., dyskinesias) and non-motor symptoms, mainly depression and speech (Lefaucheur et al., 2004). Despite a large heterogeneity among these studies (Koch, 2013), it was proposed that high frequency rTMS (i.e., 5 Hz) applied over M1 could turn as a gold-standard use in PD to significantly reduce motor signs as measured by UPDRS-III (Elahi et al., 2009). Moreover, the diverse results provided by the literature indicate updating in future interventions, which will necessitate separation of PD motor signs in an attempt to separate the diverse pathophysiology present in tremor, bradykinesia, rigidity, and gait problems. In case where such separation turns successful, perhaps we could foresee new ways of understanding and treating PD symptoms alternatively.

rTMS in Parkinson's Disease Patients with Levodopa-Induced Dyskinesias

Nowadays, treating secondary motor signs related to PD treatments is a possibility based on clear pathophysiological models to reach effective targets. Despite pharmacological or non-pharmacological interventions, after 4–6 years of levodopa therapy, a significant proportion of patients exhibit a decline in the therapeutic efficacy of levodopa and develop disabling motor symptoms, termed levodopa-induced dyskinesias (LIDs). The time-to-onset and severity of this motor complication show large individual variability thus limiting the long-term use of levodopa and clinical strategies aimed at reducing LIDs manifestation.

In the last few years, a considerable effort has been made to understand the neurobiological basis of this motor complication. LIDs are classically ascribed to the degree of nigrostriatal neurodegeneration and striatal changes associated with chronic levodopa therapy (Obeso et al., 2000). These interact to induce maladaptive striatal plasticity, which has the effect of altering neuronal activity in striato-pallidal circuits. The pioneering works of Rascol et al. (1998) and Brooks et al. (2000) demonstrated *in vivo* that these abnormal neuronal firing patterns extended on the brain cortex mainly including the sensorimotor areas of the cortico-basal ganglia loop.

After these first functional neuroimaging studies, for a long time no additional neuroimaging investigations have been performed on LIDs patients. From 2010 to date, new functional and structural neuroimaging studies have shed new light on the pathophysiological mechanisms underlying LIDs suggesting that that LIDs-related symptoms may originate in brain network beyond the "classical" basal ganglia dysfunctional model, including cortical regions strongly involved in motor inhibition processes. Indeed, what has clearly been demonstrated was that PD patients with LIDs are characterized by dysfunctional coupling between the prefrontal cortex, including the right IFC and the SMA and basal ganglia measured at rest (Cerasa et al., 2015), during a simple finger-tapping task (Cerasa et al., 2012) or during a GoNo-Go task (Herz et al., 2014, 2015). Moreover, these functional abnormalities in LIDs patients were also mirrored by abnormal anatomical changes detected in the SMA and IFC (Cerasa et al., 2011, 2013a,b). These findings have already raised an interesting scientific debate on the toxic effects of levodopa on brain morphometry (Vernon and Modo, 2012; Cerasa et al., 2014) and on the hypothetical role of the prefrontal cortex as a new target for brain stimulation useful to decrease the severity of LIDs (Cerasa and Quattrone, 2014a,b; Obeso and Strafella, 2014a,b; Rothwell and Obeso, 2015), seen to improve motor inhibition due to compensatory processes of interconnected regions (Obeso et al., 2013; Zandbelt et al., 2013).

Indeed, to treat secondary effects of principal treatments in PD such as LIDs is also an actual necessity and priority. Guided by imaging results, rTMS over regions showing functional overactivity in LIDs was reported either over the SMA (Koch et al., 2005; Brusa et al., 2006) or over the IFC (Cerasa et al., 2015) (**Table 1**). Otherwise, no significant or moderate effects emerged when TMS protocol was applied over the primary motor cortex (Wagle-Shukla et al., 2007; Filipovic et al., 2009; Kodama et al., 2011; Filipović et al., 2013; Cerasa et al., 2015). In particular, the Koch's group was the first in using rTMS approach with therapeutical purpose (Koch et al., 2005). In 2005, they demonstrated that one single session of rTMS at low frequency (1 Hz) over the SMA produced significant motor improvements in eight patients with LIDs. The rationale behind the choice to stimulate SMA is based either on previous neuroimaging findings describing functional overactivity in this region (Rascol et al., 1998; Brooks et al., 2000) or on the notion that repeated sessions of premotor cortex stimulation induces cumulative changes in the excitability over the primary motor cortex (Bäumer et al., 2003). With this in mind, Brusa et al. (2006) tried to translate this single TMS protocol in a prolonged therapeutic session (5 days), failing to demonstrate a clear beneficial effect. Contrarily, prolonged session (2 weeks) applied on the bilateral cerebellar cortex using high frequency (50 Hz) cTBS, showed persistent clinical beneficial effects in LIDs patients for up to 4 weeks (Koch et al., 2009). To explain this discrepancy, these authors proposed that this might be dependent upon the fact that the cerebellum has greater plastic mechanisms involved in motor learning (Ito, 2008) compared to SMA and therefore could be susceptible to more sustained rTMS-induced changes, thus leading to marked clinical beneficial effects. Moreover, recent evidence suggested a causal role of the effective cerebello-cortical connectivity in motor inhibition (Picazio and Koch, 2015), a cognitive domain strongly involved in the pathophysiological mechanisms of LIDs (Cerasa et al., 2015). The intimate link between motor inhibition and LIDs has also been confirmed in a recent study (Cerasa et al., 2015) where it was demonstrated that a single session of continuous but not intermittent or sham TBS applied over the right IFC was able to significantly reduce the amount of dyskinesias as measured by the conventional abnormal involuntary movement scale (AIMS).

The primary goal of the motor inhibition system (mainly composed by STN, basal ganglia, SMA, and IFC) is to control/modulate the primary motor output pathway. Idiopathic PD is primarily a disorder of response initiation characterized by an excessive motor inhibition (i.e., akinesia, bradykinesia),

TABLE 1 | rTMS application on PD with LIDs.

References	Sample	TMS protocol	Anatomical localization	Main findings
Koch et al., 2005	8 Dyskinetic PD	Single Session rTMS train at 1 Hz or 5 Hz	SMA	Single Session Low frequency (1 Hz): reduced AIMS after 15 min Single Session High frequency (5 Hz): induced a slight but not significant effect
Brusa et al., 2006	10 Dyskinetic PD	Single and Prolonged (5 days) sessions rTMS train at 1 Hz	SMA	Single Session Low frequency (1 Hz): reduced AIMS and improved UPDRS scores after 15 min Prolonged Session Low frequency (1 Hz): failed to enhance beneficial effects
Wagle-Shukla et al., 2007	6 Dyskinetic PD	Prolonged (2 weeks) sessions rTMS train at 1 Hz	M1	Prolonged Session Low frequency (1 Hz): induced a slight but not significant effect
Filipovic et al., 2009	10 Dyskinetic PD	Prolonged (4 days) sessions rTMS train at 1 Hz	M1	Prolonged Session Low frequency (1 Hz): induced a modest beneficial effect
Kodama et al., 2011	Case Report PD with painful off-period dystonia	Single Session rTMS train at 0.9 Hz	M1 SMA	Single Session Low frequency over M1: reduced painful dystonia and walking disturbances Single Session Low frequency over SMA: induced no significant effects
Filipović et al., 2013	Case Report PD with diphasic dyskinesia	Prolonged (4 days) sessions rTMS train at 1 Hz	M1	Prolonged Session Low frequency (1 Hz): yielded beneficial effects in the upper limb
Koch et al., 2009	10 Dyskinetic PD	Prolonged (2 weeks) sessions cTBS 3 pulse bursts at 50 Hz	Cerebellum	Prolonged Session High frequency (50 Hz): yielded beneficial effects
Cerasa et al., 2015	11 Dyskinetic PD	Single Session cTBS 3 pulse bursts at 50 Hz	Right Inferior Frontal Cortex M1	Single Session High frequency (50 Hz): reduced AIMS after 45 min Single Session High frequency (50 Hz): failed to enhance beneficial effects

PD, Parkinson's disease; LIDs, Levodopa-Induced Dyskinesias; SMA, Supplementary Motor Area; M1, Primary motor cortex; rTMS, repetitive Transcranial Magnetic Stimulation; iTBS, intermittent theta burst Transcranial Magnetic Stimulation; cTBS, continuous theta burst Transcranial Magnetic Stimulation; AIMS, Abnormal Involuntary Movement Scale.

whereas LIDs are clearly a clinical expression of disinhibition of movement. For this reason, the recent neuroimaging evidence strongly supports the idea that dysfunctions of the primary motor system in LIDs patients are related to that of motor inhibition pathway. However, it remains to be clarified why clinical beneficial effects are evident after rTMS over the cortical regions involved in the motor inhibition system (SMA and IFC), whereas brain stimulation on the primary motor cortex produced conflicting results (Wagle-Shukla et al., 2007). Indeed, Wagle-Shukla et al. (2007), using a prolonged session (2 weeks) of low frequency (1 HZ) rTMS over the primary motor cortex, did not report evident clinical improvements in 6 PD patients with LIDs. This preliminary evidence has also been confirmed in a recent study (Cerasa et al., 2015), despite the employment of a different TMS protocol [single session high-frequency (50 Hz) cTBS]. Three additional studies, otherwise, reported moderate evidence about the role of the primary motor cortex as potential stimulation site for LID treatment. First, Filipovic et al. (2009), using low-frequency rTMS (1 Hz) for 4 consecutive days in 10 PD patients with LIDs, reported residual beneficial clinical effects in dyskinesia severity. With the same TMS protocol, these authors found an increased beneficial effect also in one PD patient with diphasic dyskinesia, which is far less studied than more common peak-of-dose dyskinesias (Filipović et al., 2013). Finally, in another case report, 0.9 Hz rTMS over primary motor area significantly reduced the painful dystonia and walking disturbances in one dyskinetic patient with painful off-period dystonia (Kodama et al., 2011).

To sum up, the current literature on therapeutic trials of rTMS in PD patients with LIDs is in its relative infancy, and nowadays there is insufficient information to support evidence-based clinical protocols. However, the search for the most effective protocol leads us to the conclusion that brain stimulation on cortical regions part of the motor inhibition network (IFC, SMA, and cerebellum) might be highly promising as therapeutical sites for treatment of LID. Otherwise, evidence provided by rTMS over the primary motor cortex requires further confirmation. Indeed, while in idiopathic PD a plethora of studies demonstrated the beneficial effects on motor symptoms after high-frequency stimulation of the primary motor cortex (Edwards et al., 2008), in dyskinetic patients the high clinical heterogeneity, as well as variability in TMS protocols prevents us from making a general

conclusion about these findings. The lack of consistency is also dependent upon the fact that advanced neuroimaging has not yet clarified how levodopa influences neurofunctional activity in the motor cortex.

THE POTENTIAL USE OF rTMS TO TREAT DYSTONIA

Dystonia is a hyperkinetic movement disorder mainly characterized by excessive and painful muscle contraction producing muscle twists, abnormal posture, and inefficient moves. Body limbs involved in such muscles alteration classify the diverse types of dystonia. Focal dystonia are those where abnormal participation of muscles and gestures give raise to painful postures within an isolated body region. Meanwhile, segmental dystonia must involve two or more adjacent body regions and generalized dystonia, which affects upper and lower limbs of the body (Marsden, 1976). According to its etiology, dystonia can be divided into primary dystonia, dystonia plus syndrome or secondary dystonia (Marsden, 1976). Primary dystonia corresponds to those patients showing no brain lesions as revealed by structural MRI scans. It is well known that primary dystonia can be task-specific, altering movements involved in fine motor control (such as writer's cramp), speaking (dysphonia), playing piano, or running (Breakefield et al., 2008). This dystonia form may be idiopathic or genetic, based on a variety of more than 30 genes involved in the disease (Bragg et al., 2011). Secondary dystonia results from stroke or traumatic brain injury or induced by certain treatments thus has a certain origin. However, the causes of most dystonia are unknown but some monogenic subtype alterations (in DYT1, DYT6, or DYT13) are considered potentially relevant in developing dystonic motor symptoms (Bragg et al., 2011).

Considering the etiology heterogeneity in dystonia, its pathophysiological model may vary across dystonia subtypes. Based on clinico-pathological studies in patients with symptomatic dystonia (Marsden et al., 1985) and intracranial recordings from the GPi and thalamus (Vitek et al., 1999; Zhuang et al., 2004), dystonia is considered a basal ganglia disorder (Berardelli et al., 1998; Zheng et al., 2012). Indeed, DBS produces a significant positive response over the GPi (Vidailhet et al., 2005) and reduces metabolic activity over important cortical regions part of fronto-striatal loops [i.e., the dorsolateral prefrontal cortex (DLPFC) or the orbitofrontal cortex (OFC); Detante et al., 2004]. Recent findings from neuropathological data show in a large cohort of adult and child dystonia significant reductions of substantia nigra neurons as compared to controls (Iacono et al., 2015). This evidence has also been confirmed by positron emission tomography (PET) studies. Indeed, using dopaminergic markers at rest, some groups have pinpointed cell loss over striatal and cortical regions in primary dystonia (Otsuka et al., 1992; Berman et al., 2013). Moreover, increased glucose metabolism over the lentiform nucleus and cortical motor regions including SMA, lateral premotor cortex, anterior cingulate cortex (ACC), and DLPFC have also been reported in primary dystonia (Eidelberg et al., 1995; Odergren et al., 1998; Ibáñez et al., 1999; Pujol et al., 2000; Oga et al., 2002; Butterworth et al., 2003; Lerner et al., 2004). Functional alterations in dystonic patients were also coupled by underlying anatomical brain abnormalities. Indeed, patients with cervical dystonia, blepharospasm, or writer's cramp are characterized by anatomical changes in the basal ganglia, motor and premotor cortices, cerebellum and SMA (Eidelberg et al., 1995; Berardelli et al., 1998; Draganski et al., 2003; Zheng et al., 2012).

However, dystonia is not only considered to be dependent upon the basal ganglia-thalamo-cortical pathway (Breakefield et al., 2008), but recent evidence strongly highlights the involvement of the cerebellar cortex and its direct connections with the motor cortex (Lehéricy et al., 2013; Neumann et al., 2015). Cerebellar modulation over motor cortex seems to be compromised in dystonia patients and M1 excitability (i.e., intra-cortical facilitation) seems responsive to cerebellar rTMS (Brighina et al., 2009). However, it should bear in mind that although dystonic patients are not characterized by evident cerebellar motor signs (i.e., loss of balance or frequent falling), it has been proposed that the cerebellum in dystonia patients might be involved in compensatory modulation of the abnormal activity detected in the motor cortex, or as a potential effective input to modulate basal ganglia dysfunctional state (Wu and Hallett, 2013). Moreover, previous evidence points to altered cerebellar activation along the inhibitory motor circuits in dystonia (Huang et al., 2011; Koch et al., 2014), thus increasing the probability of such loops as potential candidate for neuromodulation.

The role of cortico-striatal and cerebellar-thalamo-cortical loops in dystonia, thus support two open accesses to cortical neuromodulation over motor, premotor, or cerebellar targets. The target location problem in dystonia seems rather straightforward based on current pathophysiological knowledge. So far, studies using rTMS to treat dystonia motor signs have reported beneficial clinical effects when targeting stimulation to motor (Odergren et al., 1998; Ibáñez et al., 1999; Pujol et al., 2000; Oga et al., 2002; Butterworth et al., 2003; Lerner et al., 2004; Murase et al., 2005; Allam et al., 2007; Angelakis et al., 2013; Berman et al., 2013) or somatosensory regions (Borich et al., 2009; Havrankova et al., 2010), but less clinical beneficial effects after cerebellar stimulation (Koch et al., 2014; Sadnicka et al., 2014) (see **Table 2**). Positive and acute effects after cerebellar stimulation in one study (Koch et al., 2014) offer new insights to further assess stimulation protocols with aim maintenance of prolonged positive effects (although not every study assessed long-term effects, **Table 2**). However, the gold-standard in dystonia seems to be targeting motor regions that produce functional changes over basal ganglia (Bharath et al., 2015).

The apparent efficient parameters to find positive results in dystonia seem to be closely associated to the number of stimulation sessions. Some single session studies have shown effective results (Murase et al., 2005; Tyvaert et al., 2006; Furuya et al., 2014) but are less persistent across time. This single session protocols stimulating premotor regions (at low frequencies) reported motor improvement (hand writing) in focal hand dystonia patients (Siebner et al., 1999; Lefaucheur et al., 2004;

TABLE 2 | rTMS application on dystonia.

References	Sample	TMS Protocol	Anatomical localization	Main findings
Siebner et al., 1999	16 WC	Single session rTMS at 1 Hz, placebo controlled	M1	Single session yielded positive results as measured by pen pressure reductions and self-reported improvement
Lefaucheur et al., 2004	3 secondary dystonia	Prolonged sessions (5 consecutive days) rTMS at 1 Hz	Premotor	Prolonged session yielded positive results in movement rating scale and decrease in painful axial spams
Murase et al., 2005	9 WC	Single session (1 day) rTMS at 0.2 Hz	Premotor SMA M1	Single session yielded positive results over premotor site, in decrease contraction and pen pressure
Tyvaert et al., 2006	8 WC	Single session (1 day) rTMS at 1 Hz	Premotor	Single session yielded positive results in handwriting velocity and decreased discomfort
Allam et al., 2007	1 cervical dyst./WC	Prolonged sessions (5 consecutive days) rTMS at 1 Hz	Premotor	Prolonged session yielded positive results in a single case study in cervical dystonia
Borich et al., 2009	6 FHD 9 HC	Prolonged sessions (5 consecutive days) rTMS at 1 Hz	Premotor	Prolonged session rTMS yielded reduced cortical excitability and improved handwriting performance were observed and maintained at least 10 days
Havrankova et al., 2010	20 WC	Prolonged sessions (5 consecutive days) rTMS at 1 Hz	Somatosensory	Prolonged sessions yielded positive results in subjective and objective writing maintained for 3-week time period
Schneider et al., 2010	5 WC 5 HC	Single session (1 day) rTMS train at 5 Hz fMRI pre vs. post rTMS	Somatosensory	Single session no effects in frequency discrimination task in patients linked to decrease in GPi
Benninger et al., 2011	12 FHD (6 sham)	Prolonged sessions (3 in 1 week) Cathodal tDCS	M1 contralateral to FHD	Prolonged sessions of tDCS yielded no positive effects in clinical measures nor handwriting and cortical excitability
Kimberley et al., 2013	12 FHD	Prolonged session (5 days) at 1 Hz rTMS	Dorsal premotor	Prolonged sessions yielded beneficial effects in pen force at day 1 and 5
Furuya et al., 2014	10 FHC (pianists) 10 HC	Single session of tDCS (cathodal or anodal over affected or unaffected side)	M1	Single session yielded rhythm sequence improvement using cathodal tDCS over affected cortex
Sadnicka et al., 2014	10 WC	Single session anodal tDCS (sham controlled)	Cerebellum	Single session tDCS revealed no positive effects in clinical measures
Koch et al., 2014	18 cervical dystonia	Prolonged sessions (2 weeks) cTBS	Bilateral cerebellum	Prolonged sessions yielded positive acute results (immediate effect after 2-week cTBS) in clinical scales
Bharath et al., 2015	19 WC 20 HC	Single session (1 day) rTMS train at 1 Hz; fMRI pre vs. post	Premotor	Single session reduction in left cerebellum, thalamus, globus pallidus, putamen, bilateral supplementary motor area, medial prefrontal lobe

WC, writer's cramp; HC, healthy controls; FHD, focal hand dystonia; ICD, Idiopathic cervical dystonia; CD, cerebellar dystonia; cTBS, continuous theta burst stimulation; rTMS, repetitive transcranial magnetic stimulation; tACS, transcranial alternating current stimulation; AMT, active motor threshold; tDCS, transcranial direct current stimulation; M1, primary motor cortex; SMA, supplementary motor area.

Murase et al., 2005). Others reported beneficial clinical effects, as measured by subjective clinical evaluations using 1 Hz rTMS (Murase et al., 2005; Tyvaert et al., 2006), but not always single session turns useful in dystonia (using 5 Hz rTMS; Schneider et al., 2010). Moreover, single sessions are influenced by patient's expectancy or state-dependent effects. Studies that opted for multiple sessions (5 consecutive days) however provide positive and promising results in clinical terms (Lefaucheur et al., 2004; Borich et al., 2009; Angelakis et al., 2013; Kimberley et al., 2013; Koch et al., 2014). Following multiple sessions rTMS,

TABLE 3 | rTMS application on essential tremor.

References	Sample	TMS protocol	Anatomical localization	Main findings
Gironell et al., 2002	10 ET	Single sessions (2 days) Active vs. sham rTMS train at 1 Hz	Cerebellum	Single session acute rTMS beneficial effects on tremor, dissipated in 1 h
Avanzino et al., 2009	15 ET 11 HC	Single session rTMS train at 1 Hz	Right cerebellum	Single session rTMS yielded beneficial effects on tremor
Hellriegel et al., 2012	10 ET 10 HC	Single sessions (2 days) cTBS 3 pulse bursts at 50 Hz 80 vs. 30% AMT	Left M1	Single session cTBS M1 produced subclinical beneficial effects
Popa et al., 2013	11 ET	Prolonged sessions (5 consecutive days) rTMS train at 1 Hz	Bilateral cerebellum	Prolonged Session rTMS yielded beneficial effects on tremor during 3 weeks
Chuang et al., 2014	13 ET 18 HC	Single sessions (3 days) cTBS 3 pulse bursts at 50 Hz	M1, premotor and sham	Single session cTBS modulated cortical excitability for shorter duration in ET patients

ET, essential tremor; HC, healthy controls; cTBS, continuous theta burst stimulation; rTMS, repetitive transcranial magnetic stimulation; AMT, active motor threshold; M1, primary motor cortex.

plastic changes in dystonic patients lasted 10 days post-treatment (Borich et al., 2009) and importantly, subjective perception of well-being was maintained for a 3-week period (Havrankova et al., 2010). Others reported only acute amelioration of dystonic signs after cTBS, however after cerebellar stimulation (Koch et al., 2014) thus suggesting premotor regions as responsive for multiple neuromodulation sessions in dystonia.

Regarding rTMS stimulation protocols, the disparity in frequency of stimulation (i.e., low vs. high frequencies) is a solid factor of variability in the current literature. Most of the available literature reports low frequencies (see **Table 3**), although higher ones, i.e., cTBS, do produce enhanced clinical effects. In Koch et al. (2014), 2-week of TBS applied bilaterally over the cerebellum was compared against a sham TBS condition. Patients under the active stimulation showed ameliorated clinical conditions acutely, but not persistently, with a marked decrease in muscle contraction evaluated by a blinded neurologist. In a similar protocol, following 1 Hz rTMS applied over the left somatosensory parietal region in WC patients (Havrankova et al., 2010), patients showed subjective and objective improvements in writing quality during a 3-week time period. Similarly, using 1 Hz rTMS over dorsal premotor area produced positive results in pen force use and general patients mobility (Kimberley et al., 2013). Such increment in patients response to rTMS may be driven by the fact that low frequency rTMS seems to modulate somatosensory integration in patients with dystonia and WC (Bäumer et al., 2007). Thus, the working hypothesis is that use of repeated sessions may induce cortical plasticity that induces facilitation of sensory outputs or facilitation of contralateral hemisphere (Bharath et al., 2015) to control motor functions. Further evidence is urgently needed to confirm the use of multiple rTMS sessions and to determine ways of prolonging its duration.

Stimulation over cortical premotor and motor regions connecting with basal ganglia renders a potential treatment in dystonia characterized by functional and compensatory changes in the subcortical regions. Still, greater accuracy in the protocols used to induce subcortical changes are needed. TMS studies trying to induce enhancement of dystonic signs have mostly tackled regions part of the basal ganglia motor loops, i.e., motor, premotor, SMA, and somatosensory regions. The necessity to test alternative TMS protocols under different dystonic symptoms or use stimulation techniques in combination with medication or rehabilitation is obvious.

TURNING DOWN HYPERACTIVE CEREBELLO-THALAMIC LOOPS IN ESSENTIAL TREMOR WITH rTMS

ET is a hyperkinetic motor disorder that affects one or more body parts by inducing involuntary and rhythmic movements. This may occur in a single limb or at any body part, such as a chin or head with larger prevalence in upper limbs (Helmich et al., 2013). Typically, is presented while moving, bilaterally or kinetic tremor that is visible and persistent. Today, the use of pharmacological in treatment of ET remains poor and unsatisfactory (Louis, 2015). In contrast, surgical treatment is effective in reducing hand tremor in 95% of patients and improved function in 74%, however with added potential risks being an invasive approach (Sandvik et al., 2012).

ET has been associated with altered oscillatory activity in the motor loop involving the cerebello-thalamo-cortical network (Pinto et al., 2003). Several imaging and animal evidence are in keeping with this view of the disease. Indeed, dysfunctional activities (measured as fMRI or PET) and anatomical changes

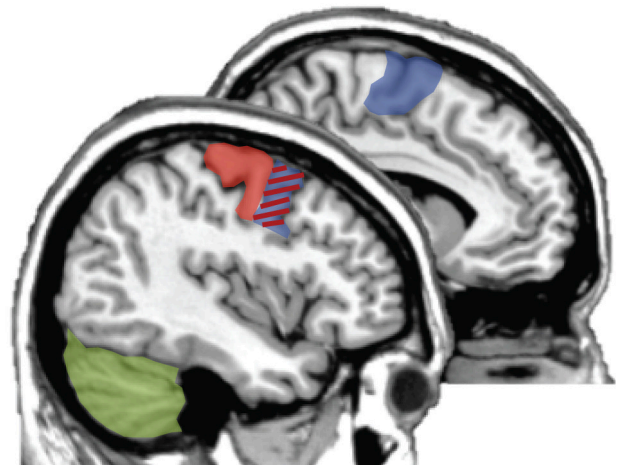


FIGURE 1 | Optimal brain targets of stimulation for therapeutic purposes. rTMS targeting the premotor regions and supplementary motor area (SMA, colored in blue) has been demonstrated as the most plausible site of stimulation for reducing hyperkinetic motor disorders in PD patients with levodopa-induced dyskinesias. Moreover, either the premotor or the primary motor cortices (colored in red) are the most frequently used cortical targets for dystonic patients. Finally, based on the literature, the cerebellum (colored in green) has been proposed as the best target for maximizing the effectiveness of rTMS in patients with essential tremor. Of interest, the premotor region is an effective region for two hyperkinetic disorders: dyskinesias and dystonia (colored in red/blue). Figure summarizes (Koch et al., 2005; Brusa et al., 2006; Tyvaert et al., 2006; Popa et al., 2013).

(gray and white matter atrophies) have been found in the well-known *tremor network* (Hallett, 2014), as well as in a plethora of other brain regions involving M1, GPi, thalamus, or cerebellum (Passamonti et al., 2011). However, both functional and structural imaging studies reported convergent findings about the role of the cerebellum as the most consistent area of pathology in ET. This hypothesis has also been confirmed by recent post-mortem studies (Louis et al., 2007, 2011) where it was demonstrated that the average amount of cerebellar Purkinje cells is reduced 25% in tremor patients compared to controls.

Either motor or cerebellum regions are the main target regions to use in neuromodulation for ET. This is mainly guided following results from cortical infarction over motor regions, in which ET motor signs disappeared (Le Pira et al., 2004; Kim et al., 2006). Similarly, single magnetic pulses over M1 seemed to modulate postural tremor in PD patients (Pascual-Leone et al., 1994). These results are in part explained by the correlated frequencies in hand tremor and cortical activity (Hellwig et al., 2001). Yet, rTMS studies trying to apply long-lasting modulatory stimulation in ET have focused over cerebellum and M1 (Gironell et al., 2002, 2014; Avanzino et al., 2009; Hellriegel et al., 2012; Popa et al., 2013; Chuang et al., 2014).

Overall, four studies reported positive anti-tremoric effects using prolonged sessions of rTMS, for a time-period of 3 weeks in ET patients refractory to medical treatment (see **Table 3**). Beneficial clinical effects assessed by a tremor rating scale (Fahn-Tolosa-Marin tremor scale) were shown as acute and lasting reductions in tremor amplitude and substantial improvement in functional disability (drawing, writing) after rTMS (Popa et al., 2013). Moreover, baseline functional connectivity showed impaired activity in the cerebello-thalamic-cortical loop, a dysfunction that was reset back to near normal levels after rTMS (Popa et al., 2013). The lack of a sham group leaves their results as pending to rule out possible placebo effects.

Historically, the first rTMS application on ET patients was performed by Gironell et al. (2002), who reported acute positive effects after a single session of 1 Hz rTMS over the cerebellum. The study was double blind, crossover, and placebo-controlled design. Their results were significant just in acute evaluation on subjective assessments performed by patients. The nature of their study was exploratory with limited sample size and makes results hard to interpret due to its moderate and transient effects. Next, a second study (Avanzino et al., 2009) using a single session of unilateral 1 Hz cerebellar TMS stimulation, also reported a transient improvement in motor scores evaluated using a tapping task. However, no translational results into clinical scores were found. By contrast, inhibitory cTBS of the left primary motor hand area for 2 consecutive days yielded significant motor benefits by reducing tremor total power, assessed with an accelerometer (Hellriegel et al., 2012). Similarly, Chuang et al. (2014) were interested to alter motor cortical dysfunction in ET by applying cTBS over the primary motor and premotor cortices. They found that cTBS was capable of producing a suppressive effect on motor cortical excitability in ET patients, but the effects lasted for a significantly shorter time compared with the effect produced in healthy individuals. Clinically speaking, tremor amplitude was decreased significantly after cTBS but the tremor frequency remained unchanged. The authors concluded that inhibitory circuits within the motor cortex are aberrant and less modifiable in ET patients.

Mechanistically, cerebellar rTMS seems to turn back-to-normal the altered activity in tremor by re-establishing an appropriate synaptic plasticity involved in programming of motor plans (Ito, 2008), resulting in the most plausible candidate target for ET. It turns, thus, possible to boost tremor sign reduction using rTMS protocols with bilateral cerebellar stimulation and low frequency types under multiple rTMS sessions.

CONCLUSIONS

The results of this systematic review show that there was not a clear consensus on optimal protocols to be used for these motor disorders. Future studies are key to consolidate the use of rTMS in this clinical context in order to reduce hyperkinetic brain dysfunctions. However, some positive results give clinical researchers hints of effective neuromodulatory paradigms and uses. Beneficial effects will most likely be boosted if: (i) prolonged sessions are possible, (ii) the use of low frequency rTMS (i.e., 1 Hz), (iii) samples selection restricted to those patients refractory to regular medical treatment, and (iv) choosing the adequate target based on known cortical regions altered in pathophysiological models. **Figure 1** represents a summary of candidate regions for treating hyperkinetic dysfunction based on

the present literature. Optimal cortical regions that have been demonstrated to maximize the effectiveness of rTMS protocols are: (i) the premotor cortex and SMA for dyskinesias in PD; (ii) the motor and premotor cortices for dystonic patients; and (iii) the cerebellum for patients with ET.

Still, basic procedures or techniques, such as cTBS in tremor or combined therapies (different motor rehabilitation programs and rTMS) have not yet been applied in these disorders. Also, if rTMS protocols are used with patient samples grouped by disease onset and symptom type we may expand knowledge on patient-dependent states and how TMS may modulate differently at each disease stage or symptomatology.

The reader should also note that in this review we neglected some other important hyperkinetic movement disorders such as, Huntington's disease and Tourette syndrome. With respect to Huntington's disease where the application of rTMS for therapeutic purpose is in its relative infancy (Berardelli and Suppa, 2013; Philpott et al., 2013), the large amount of works in psychiatric realm supported the notion that non-invasive brain stimulation is widely recognized as a alternative non-pharmacological approach for decreasing the frequency and intensity of tics in patients with Tourette syndrome (Bloch et al., 2014).

To sum up, the current literature on therapeutic trials of rTMS in hyperkinetic movement disorders patients is still ambiguous, and there is need of well-controlled multicenter randomized clinical trials to define the most effective protocol. However, advancements in technology, as well as, in pathophysiological understanding will improve the effectiveness of this safe and potentially therapeutic option in hyperkinetic movement disorder patients.

AUTHOR CONTRIBUTIONS

IO designed and wrote the manuscript. AC designed and wrote the manuscript. AQ wrote the manuscript

REFERENCES

Alexander, G. E., DeLong, M. R., and Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking the basal ganglia and cortex. *Ann. Rev. Neurosci.* 9, 357–381. doi: 10.1146/annurev.ne.09.030186.002041

Allam, N., Brasil-Neto, J. P., Brandão, P., Weiler, F., Barros Filho, J. d., and Tomaz, C. (2007). Relief of primary cervical dystonia symptoms by low frequency transcranial magnetic stimulation of the premotor cortex: case report. *Arq. Neuropsiquiatr.* 65, 697–699. doi: 10.1590/S0004-282X2007000400030

Angelakis, E., Liouta, E., Andreadis, N., Leonardos, A., Ktonas, P., Stavrinou, L. C., et al. (2013). Transcranial alternating current stimulation reduces symptoms in intractable idiopathic cervical dystonia: a case study. *Neurosci. Lett.* 533, 39–43. doi: 10.1016/j.neulet.2012.11.007

Avanzino, L., Bove, M., Tacchino, A., Ruggeri, P., Giannini, A., Trompetto, C., et al. (2009). Cerebellar involvement in timing accuracy of rhythmic finger movements in essential tremor. *Eur. J. Neurosci.* 30, 1971–1979. doi: 10.1111/j.1460-9568.2009.06984.x

Bäumer, T., Demiralay, C., Hidding, U., Bikmullina, R., Helmich, R. C., Wunderlich, S., et al. (2007). Abnormal plasticity of the sensorimotor cortex to slow repetitive transcranial magnetic stimulation in patients with writer's cramp. *Mov. Disord.* 22, 81–90. doi: 10.1002/mds.21219

Bäumer, 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

Benninger, D. H., Lomarev, M., Lopez, G., Pal, N., Luckenbaugh, D. A., and Hallett, M. (2011). Transcranial direct current stimulation for the treatment of focal hand dystonia. *Mov. Disord.* 26, 1698–1702. doi: 10.1002/mds.23691

Berardelli, A., Rothwell, J. C., Hallett, M., Thompson, P. D., Manfredi, M., and Marsden, C. D. (1998). The pathophysiology of primary dystonia. *Brain* 121(Pt 7), 1195–1212. doi: 10.1093/brain/121.7.1195

Berardelli, A., and Suppa, A. (2013). Noninvasive brain stimulation in Huntington's disease. *Handb. Clin. Neurol.* 116, 555–560. doi: 10.1016/B978-0-444-53497-2.00044-9

Berman, B. D., Hallett, M., Herscovitch, P., and Simonyan, K. (2013). Striatal dopaminergic dysfunction at rest and during task performance in writer's cramp. *Brain* 136, 3645–3658. doi: 10.1093/brain/awt282

Bharath, R. D., Biswal, B. B., Bhaskar, M. V., Gohel, S., Jhunjhunwala, K., Panda, R., et al. (2015). Repetitive transcranial magnetic stimulation induced modulations of resting state motor connectivity in writer's cramp. *Eur. J. Neurol.* 22, 796–805; e53–e54. doi: 10.1111/ene.12653

Bloch, Y., Arad, S., and Levkovitz, Y. (2014). Deep TMS add-on treatment for intractable tourette syndrome: a feasibility study. *World J. Biol. Psychiatry* 24, 1–5. doi: 10.3109/15622975.2014.964767

Borich, M., Arora, S., and Kimberley, T. J. (2009). Lasting effects of repeated rTMS application in focal hand dystonia. *Restor. Neurol. Neurosci.* 27, 55–65. doi: 10.3233/RNN-2009-0461

Bragg, D. C., Armata, I. A., Nery, F. C., Breakefield, X. O., and Sharma, N. (2011). Molecular pathways in dystonia. *Neurobiol. Dis.* 42, 136–147. doi: 10.1016/j.nbd.2010.11.015

Breakefield, X. O., Blood, A. J., Li, Y., Hallett, M., Hanson, P. I., and Standaert, D. G. (2008). The pathophysiological basis of dystonias. *Nat. Rev. Neurosci.* 9, 222–234. doi: 10.1038/nrn2337

Brighina, F., Romano, M., Giglia, G., Saia, V., Puma, A., Giglia, F., et al. (2009). Effects of cerebellar TMS on motor cortex of patients with focal dystonia: a preliminary report. *Exp. Brain Res.* 192, 651–656. doi: 10.1007/s00221-008-1572-9

Brooks, D. J., Piccini, P., Turjanski, N., and Samuel, M. (2000). Neuroimaging of dyskinesia. *Ann. Neurol.* 47, S154–S158. discussion: S158–S159.

Brusa, L., Versace, V., Koch, G., Iani, C., Stanzione, P., Bernardi, G., et al. (2006). Low frequency rTMS of the SMA transiently ameliorates peak-dose LID in Parkinson's disease. *Clin. Neurophysiol.* 117, 1917–1921. doi: 10.1016/j.clinph.2006.03.033

Butterworth, S., Francis, S., Kelly, E., McGlone, F., Bowtell, R., and Sawle, G., V (2003). Abnormal cortical sensory activation in dystonia: an fMRI study. *Mov. Disord.* 18, 673–682. doi: 10.1002/mds.10416

Cerasa, A., Fasano, A., Morgante, F., Koch, G., and Quattrone, A. (2014). Maladaptive plasticity in levodopa-induced dyskinesias and tardive dyskinesias: old and new insights on the effects of dopamine receptor pharmacology. *Front. Neurol.* 5:49. doi: 10.3389/fneur.2014.00049

Cerasa, A., Koch, G., Donzuso, G., Mangone, G., Morelli, M., Brusa, L., et al. (2015). A network centred on the inferior frontal cortex is critically involved in levodopa-induced dyskinesias. *Brain* 138, 414–427. doi: 10.1093/brain/awu329

Cerasa, A., Messina, D., Pugliese, P., Morelli, M., Lanza, P., Salsone, M., et al. (2011). Increased prefrontal volume in PD with levodopa-induced dyskinesias: a voxel-based morphometry study. *Mov. Disord.* 26, 807–812. doi: 10.1002/mds.23660

Cerasa, A., Morelli, M., Augimeri, A., Salsone, M., Novellino, F., Gioia, M. C., et al. (2013a). Prefrontal thickening in PD with levodopa-induced dyskinesias: new evidence from cortical thickness measurement. *Parkinsonism Relat. Disord.* 19, 123–125. doi: 10.1016/j.parkreldis.2012.06.003

Cerasa, A., Pugliese, P., Messina, D., Morelli, M., Gioia, M. C., Salsone, M., et al. (2012). Prefrontal alterations in Parkinson's disease with levodopa-induced dyskinesia during fMRI motor task. *Mov. Disord.* 27, 364–371. doi: 10.1002/mds.24017

Cerasa, A., and Quattrone, A. (2014a). May hyperdirect pathway be a plausible neural substrate for understanding the rTMS-related effects on PD patients with levodopa-induced dyskinesias? *Brain Stimul.* 7, 488–489. doi: 10.1016/j.brs.2014.01.007

Cerasa, A., and Quattrone, A. (2014b). May stimulation of the pre-SMA become a new therapeutic target for PD patients with levodopa-induced dyskinesias? *Brain Stimul.* 7, 335–336. doi: 10.1016/j.brs.2013.12.003

Cerasa, A., Salsone, M., Morelli, M., Pugliese, P., Arabia, G., Gioia, C. M., et al. (2013b). Age at onset influences neurodegenerative processes underlying PD with levodopa-induced dyskinesias. *Parkinsonism Relat. Disord.* 19, 883–888. doi: 10.1016/j.parkreldis.2013.05.015

Chuang, W.-L., Huang, Y.-Z., Lu, C.-S., and Chen, R.-S. (2014). Reduced cortical plasticity and GABAergic modulation in essential tremor. *Mov. Disord.* 29, 501–507. doi: 10.1002/mds.25809

Detante, O., Vercueil, L., Thobois, S., Broussolle, E., Costes, N., Lavenne, F., et al. (2004). Globus pallidus internus stimulation in primary generalized dystonia: a H215O PET study. *Brain* 127, 1899–1908. doi: 10.1093/brain/awh213

Diamond, A., and Jankovic, J. (2005). The effect of deep brain stimulation on quality of life in movement disorders. *J. Neurol. Neurosurg. Psychiatry* 76, 1188–1193. doi: 10.1136/jnnp.2005.065334

Draganski, B., Thun-Hohenstein, C., Bogdahn, U., Winkler, J., and May, A. (2003). "Motor circuit" gray matter changes in idiopathic cervical dystonia. *Neurology* 61, 1228–1231. doi: 10.1212/01.WNL.0000094240.93745.83

Edwards, M. J., Talelli, P., and Rothwell, J. C. (2008). Clinical applications of transcranial magnetic stimulation in patients with movement disorders. *Lancet Neurol.* 7, 827–840. doi: 10.1016/S1474-4422(08)70190-X

Eidelberg, D., Moeller, J. R., Ishikawa, T., Dhawan, V., Spetsieris, P., Przedborski, S., et al. (1995). The metabolic topography of idiopathic torsion dystonia. *Brain* 118(Pt 6), 1473–1484. doi: 10.1093/brain/118.6.1473

Elahi, B., Elahi, B., and Chen, R. (2009). Effect of transcranial magnetic stimulation on Parkinson motor function–systematic review of controlled clinical trials. *Mov. Disord.* 24, 357–363. doi: 10.1002/mds.22364

Fasano, A., and Lozano, A. M. (2015). Deep brain stimulation for movement disorders: 2015 and beyond. *Curr. Opin. Neurol.* 28, 423–436. doi: 10.1097/WCO.0000000000000226

Filipović, S. R., Bhatia, K. P., and Rothwell, J. C. (2013). 1-Hz repetitive transcranial magnetic stimulation and diphasic dyskinesia in Parkinson's disease. *Mov. Disord.* 28, 245–246. doi: 10.1002/mds.25261

Filipovic, S. R., Rothwell, J. C., van de Warrenburg, B. P., and Bhatia, K. (2009). Repetitive transcranial magnetic stimulation for levodopa-induced dyskinesias in Parkinson's disease. *Mov. Disord.* 24, 246–253. doi: 10.1002/mds.22348

Furuya, S., Nitsche, M. A., Paulus, W., and Altenmüller, E. (2014). Surmounting retraining limits in Musicians' dystonia by transcranial stimulation. *Ann. Neurol.* 75, 700–707. doi: 10.1002/ana.24151

George, M. S., Taylor, J. J., and Short, E. B. (2013). The expanding evidence base for rTMS treatment of depression. *Curr. Opin. Psychiatry* 26, 13–18. doi: 10.1097/YCO.0b013e32835ab46d

Gironell, A., Aguilar, S., Torres, V., and Pagonabarraga, J. (2014). Transcranial direct current stimulation of the cerebellum in essential tremor?: a controlled study deep transcranial magnetic stimulation in a woman with chronic tinnitus?: clinical and fMRI findings. seeking relief from a symptom and finding vivid memor. *BRS* 7, 491–492. doi: 10.1016/j.brs.2014.02.001

Gironell, A., Kulisevsky, J., Lorenzo, J., Barbanoj, M., Pascual-Sedano, B., and Otermin, P. (2002). Transcranial magnetic stimulation of the cerebellum in essential tremor: a controlled study. *Arch. Neurol.* 59, 413–417. doi: 10.1001/archneur.59.3.413

Hallett, M. (2014). Tremor: pathophysiology. *Parkinsonism Relat. Disord.* 20(Suppl. 1), S118–S122. doi: 10.1016/S1353-8020(13)70029-4

Hamani, C., Saint-Cyr, J. A., Fraser, J., Kaplitt, M., and Lozano, A. M. (2004). The subthalamic nucleus in the context of movement disorders. *Brain* 127, 4–20. doi: 10.1093/brain/awh029

Havrankova, P., Jech, R., Walker, N. D., Operto, G., Tauchmanova, J., Vymazal, J., et al. (2010). Repetitive TMS of the somatosensory cortex improves writer's cramp and enhances cortical activity. *Neuro Endocrinol. Lett.* 31, 73–86.

Hellriegel, H., Schulz, E. M., Siebner, H. R., Deuschl, G., and Raethjen, J. H. (2012). Continuous theta-burst stimulation of the primary motor cortex in essential tremor. *Clin. Neurophysiol.* 123, 1010–1015. doi: 10.1016/j.clinph.2011.08.033

Hellwig, B., Häussler, S., Schelter, B., Lauk, M., Guschlbauer, B., Timmer, J., et al. (2001). Tremor-correlated cortical activity in essential tremor. *Lancet* 357, 519–523. doi: 10.1016/S0140-6736(00)04044-7

Helmich, R. C., Toni, I., Deuschl, G., and Bloem, B. R. (2013). The pathophysiology of essential tremor and parkinson's tremor. *Curr. Neurol. Neurosci. Rep.* 13:378. doi: 10.1007/s11910-013-0378-8

Herz, D. M., Haagensen, B. N., Christensen, M. S., Madsen, K. H., Rowe, J. B., Løkkegaard, A., et al. (2014). The acute brain response to levodopa heralds dyskinesias in Parkinson disease. *Ann. Neurol.* 75, 829–836. doi: 10.1002/ana.24138

Herz, D. M., Haagensen, B. N., Christensen, M. S., Madsen, K. H., Rowe, J. B., Løkkegaard, A., et al. (2015). Abnormal dopaminergic modulation of striato-cortical networks underlies levodopa-induced dyskinesias in humans. *Brain* 138, 1658–1666. doi: 10.1093/brain/awv096

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

Huang, Y. Z., Rothwell, J. C., Lu, C. S., Wang, J., and Chen, R. S. (2011). Restoration of motor inhibition through an abnormal premotor-motor connection in dystonia. *Mov. Disord.* 25, 696–703. doi: 10.1002/mds.22814

Iacono, D., Geraci-Erck, M., Peng, H., Rabin, M. L., and Kurlan, R. (2015). Reduced number of pigmented neurons in the substantia nigra of dystonia patients? Findings from extensive neuropathologic, immunohistochemistry, and quantitative analyses. *Tremor Other Hyperkinet. Mov. (N.Y).* 5:tre-5-301. doi: 10.7916/D8T72G9G

Ibáñez, V., Sadato, N., Karp, B., Deiber, M. P., and Hallett, M. (1999). Deficient activation of the motor cortical network in patients with writer's cramp. *Neurology* 53, 96–105.

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nat. Rev. Neurosci.* 9, 304–313. doi: 10.1038/nrn2332

Kehagia, A. A., Barker, R. A., and Robbins, T. W. (2013). Cognitive impairment in Parkinson's disease: the dual syndrome hypothesis. *Neurodegener. Dis.* 11, 79–92. doi: 10.1159/000341998

Kim, J.-S., Park, J.-W., Kim, W.-J., Kim, H.-T., Kim, Y.-I., and Lee, K.-S. (2006). Disappearance of essential tremor after frontal cortical infarct. *Mov. Disord.* 21, 1284–1285. doi: 10.1002/mds.20894

Kimberley, T. J., Borich, M. R., Arora, S., and Siebner, H. R. (2013). Multiple sessions of low-frequency repetitive transcranial magnetic stimulation in focal hand dystonia: Clinical and physiological effects. *Restor. Neurol. Neurosci.* 31, 533–542. doi: 10.3233/RNN-120259

Koch, G. (2013). Do studies on cortical plasticity provide a rationale for using non-invasive brain stimulation as a treatment for Parkinson's disease patients? *Front. Neurol.* 4:180. doi: 10.3389/fneur.2013.00180

Koch, G., Brusa, L., Caltagirone, C., Peppe, A., Oliveri, M., Stanzione, P., et al. (2005). rTMS of supplementary motor area modulates therapy-induced dyskinesias in Parkinson disease. *Neurology* 65, 623–625. doi: 10.1212/01.wnl.0000172861.36430.95

Koch, G., Brusa, L., Carrillo, F., Lo Gerfo, E., Torriero, S., Oliveri, M., et al. (2009). Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. *Neurology* 73, 113–119. doi: 10.1212/WNL.0b013e3181ad5387

Koch, G., Porcacchia, P., Ponzo, V., Carrillo, F., Cáceres-Redondo, M. T., Brusa, L., et al. (2014). Effects of two weeks of cerebellar theta burst stimulation in cervical dystonia patients. *Brain Stimul.* 7, 564–572. doi: 10.1016/j.brs.2014.05.002

Kodama, M., Kasahara, T., Hyodo, M., Aono, K., Sugaya, M., Koyama, Y., et al. (2011). Effect of low-frequency repetitive transcranial magnetic stimulation combined with physical therapy on L-dopa-induced painful off-period dystonia in Parkinson's disease. *Am. J. Phys. Med. Rehabil.* 90, 150–155. doi: 10.1097/PHM.0b013e3181fc7ccd

Le Pira, F., Giuffrida, S., Panetta, M. R., Lo Bartolo, M. L., and Politi, G. (2004). Selective disappearance of essential tremor after ischaemic stroke. *Eur. J. Neurol.* 11, 422–423. doi: 10.1111/j.1468-1331.2004.00824.x

Lefaucheur, J. P., Drouot, X., Von Raison, F., Ménard-Lefaucheur, I., Cesaro, P., and Nguyen, J. P. (2004). Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's disease. *Clin. Neurophysiol.* 115, 2530–2541. doi: 10.1016/j.clinph.2004.05.025

Lehéricy, S., Tijssen, M. A. J., Vidailhet, M., Kaji, R., and Meunier, S. (2013). The anatomical basis of dystonia: current view using neuroimaging. *Mov. Disord.* 28, 944–957. doi: 10.1002/mds.25527

Lerner, A., Shill, H., Hanakawa, T., Bushara, K., Goldfine, A., and Hallett, M. (2004). Regional cerebral blood flow correlates of the severity of writer's cramp symptoms. *Neuroimage* 21, 904–913. doi: 10.1016/j.neuroimage.2003.10.019

Louis, E. D. (2015). Medication non-adherence in essential tremor. *Parkinsonism Relat. Disord.* 21, 138–141. doi: 10.1016/j.parkreldis.2014.12.001

Louis, E. D., Asabere, N., Agnew, A., Moskowitz, C. B., Lawton, A., Cortes, E., et al. (2011). Rest tremor in advanced essential tremor: a post-mortem study of nine cases. *J. Neurol. Neurosurg. Psychiatry* 82, 261–265. doi: 10.1136/jnnp.2010.215681

Louis, E. D., Faust, P. L., Vonsattel, J.-P. G., Honig, L. S., Rajput, A., Robinson, C. A., et al. (2007). Neuropathological changes in essential tremor: 33 cases compared with 21 controls. *Brain* 130, 3297–3307. doi: 10.1093/brain/awm266

Marsden, C. D. (1976). Dystonia: the spectrum of the disease. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* 55, 351–367.

Marsden, C. D., Obeso, J. A., Zarranz, J. J., and Lang, A. E. (1985). The anatomical basis of symptomatic hemidystonia. *Brain* 108(Pt 2), 463–483. doi: 10.1093/brain/108.2.463

Middleton, F. A., and Strick, P. L. (2000). Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies. *Brain Cogn.* 42, 183–200. doi: 10.1006/brcg.1999.1099

Murase, N., Rothwell, J. C., Kaji, R., Urushihara, R., Nakamura, K., Murayama, N., et al. (2005). Subthreshold low-frequency repetitive transcranial magnetic stimulation over the premotor cortex modulates writer's cramp. *Brain* 128, 104–115. doi: 10.1093/brain/awh315

Neumann, W.-J., Jha, A., Bock, A., Huebl, J., Horn, A., Schneider, G.-H., et al. (2015). Cortico-pallidal oscillatory connectivity in patients with dystonia. *Brain* 138, 1894–1906. doi: 10.1093/brain/awv109

Obeso, I., Cho, S. S., Antonelli, F., Houle, S., Jahanshahi, M., Ko, J. H., et al. (2013). Stimulation of the pre-SMA influences cerebral blood flow in frontal areas involved with inhibitory control of action. *Brain Stimul.* 6, 769–776. doi: 10.1016/j.brs.2013.02.002

Obeso, I., and Strafella, A. P. (2014a). Boosting response inhibition neural network with rTMS may improve dyskinesias in Parkinson's disease. *Brain Stimul.* 7, 336–337. doi: 10.1016/j.brs.2013.12.004

Obeso, I., and Strafella, A. P. (2014b). Top-down control of dyskinesias in PD using brain stimulation. *Brain Stimul.* 7, 488. doi: 10.1016/j.brs.2014.01.005

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., DeLong, M. R., and Olanow, C. W. (2000). Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Ann. Neurol.* 47(4 Suppl.), S22–S32. discussion: S32-S34.

Odergren, T., Stone-Elander, S., and Ingvar, M. (1998). Cerebral and cerebellar activation in correlation to the action-induced dystonia in writer's cramp. *Mov. Disord.* 13, 497–508. doi: 10.1002/mds.870130321

Oga, T., Honda, M., Toma, K., Murase, N., Okada, T., Hanakawa, T., et al. (2002). Abnormal cortical mechanisms of voluntary muscle relaxation in patients with writer's cramp: an fMRI study. *Brain* 125, 895–903. doi: 10.1093/brain/awf083

Otsuka, M., Ichiya, Y., Shima, F., Kuwabara, Y., Sasaki, M., Fukumura, T., et al. (1992). Increased striatal 18F-dopa uptake and normal glucose metabolism in idiopathic dystonia syndrome. *J. Neurol. Sci.* 111, 195–199. doi: 10.1016/0022-510X(92)90068-V

Pascual-Leone, A., Valls-Solé, J., Toro, C., Wassermann, E. M., and Hallett, M. (1994). Resetting of essential tremor and postural tremor in Parkinson's disease with transcranial magnetic stimulation. *Muscle Nerve* 17, 800–807. doi: 10.1002/mus.880170716

Passamonti, L., Novellino, F., Cerasa, A., Chiriaco, C., Rocca, F., Matina, M. S., et al. (2011). Altered cortical-cerebellar circuits during verbal working memory in essential tremor. *Brain* 134, 2274–2286. doi: 10.1093/brain/awr164

Philpott, A. L., Fitzgerald, P. B., Cummins, T. D., and Georgiou-Karistianis, N. (2013). Transcranial magnetic stimulation as a tool for understanding neurophysiology in Huntington's disease: a review. *Neurosci. Biobehav. Rev.* 37, 1420–1433. doi: 10.1016/j.neubiorev.2013.05.009

Picazio, S., and Koch, G. (2015). Is motor inhibition mediated by cerebello-cortical interactions? *Cerebellum* 14, 47–49. doi: 10.1007/s12311-014-0609-9

Pinto, A. D., Lang, A. E., and Chen, R. (2003). The cerebellothalamocortical pathway in essential tremor. *Neurology* 60, 1985–1987. doi: 10.1212/01.WNL.0000065890.75790.29

Popa, T., Russo, M., Vidailhet, M., Roze, E., and Lehéricy, S. (2013). Brain Stimulation Cerebellar rTMS stimulation may induce prolonged clinical bene fi ts in essential tremor, and subjacent changes in functional connectivity?: an open label trial. *Brain Stimul.* 6, 175–179. doi: 10.1016/j.brs.2012.04.009

Pujol, J., Roset-Llobet, J., Rosinés-Cubells, D., Deus, J., Narberhaus, B., Valls-Solé, J., et al. (2000). Brain cortical activation during guitar-induced hand dystonia studied by functional MRI. *Neuroimage* 12, 257–267. doi: 10.1006/nimg.2000.0615

Rascol, O., Sabatini, U., Brefel, C., Fabre, N., Rai, S., Senard, J. M., et al. (1998). Cortical motor overactivation in parkinsonian patients with L-dopa-induced peak-dose dyskinesia. *Brain* 121(Pt 3), 527–533. doi: 10.1093/brain/121.3.527

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

Rothwell, J. C., and Obeso, J. A. (2015). Can levodopa-induced dyskinesias go beyond the motor circuit? *Brain* 138, 242–244. doi: 10.1093/brain/awu365

Sadnicka, A., Hamada, M., Bhatia, K. P., Rothwell, J. C., and Edwards, M. J. (2014). Cerebellar stimulation fails to modulate motor cortex plasticity in writing dystonia. *Mov. Disord.* 29, 1304–1307. doi: 10.1002/mds.25881

Sandvik, U., Koskinen, L.-O., Lundquist, A., and Blomstedt, P. (2012). Thalamic and subthalamic deep brain stimulation for essential tremor: where is the optimal target? *Neurosurgery* 70, 840–5. discussion: 845–846. doi: 10.1227/NEU.0b013e318236a809

Schneider, S. A., Pleger, B., Draganski, B., Cordivari, C., Rothwell, J. C., Bhatia, K. P., et al. (2010). Modulatory effects of 5Hz rTMS over the primary somatosensory cortex in focal dystonia - An fMRI-TMS Study. *Mov. Disord.* 25, 76–83. doi: 10.1002/mds.22825

Siebner, H. R., Tormos, J. M., Ceballos-Baumann, A. O., Auer, C., Catala, M. D., Conrad, B., et al. (1999). Low-frequency repetitive transcranial magnetic stimulation of the motor cortex in writer's cramp. *Neurology* 52, 529–537. doi: 10.1212/WNL.52.3.529

Tyvaert, L., Houdayer, E., Devanne, H., Monaca, C., Cassim, F., and Derambure, P. (2006). The effect of repetitive transcranial magnetic stimulation on dystonia: a clinical and pathophysiological approach. *Neurophysiol. Clin.* 36, 135–143. doi: 10.1016/j.neucli.2006.08.007

Vernon, A. C., and Modo, M. (2012). Do levodopa treatments modify the morphology of the parkinsonian brain? *Mov. Disord.* 27, 166–167. doi: 10.1002/mds.24018

Vidailhet, M., Vercueil, L., Houeto, J.-L., Krystkowiak, P., Benabid, A.-L., Cornu, P., et al. (2005). Bilateral deep-brain stimulation of the globus pallidus in primary generalized dystonia. *N. Engl. J. Med.* 352, 459–467. doi: 10.1056/NEJMoa042187

Vitek, J. L., Chockkan, V., Zhang, J. Y., Kaneoke, Y., Evatt, M., DeLong, M. R., et al. (1999). Neuronal activity in the basal ganglia in patients with generalized dystonia and hemiballismus. *Ann. Neurol.* 46, 22–35.

Wagle-Shukla, A., Angel, M. J., Zadikoff, C., Enjati, M., Gunraj, C., Lang, A. E., et al. (2007). Low-frequency repetitive transcranial magnetic stimulation for treatment of levodopa-induced dyskinesias. *Neurology* 68, 704–705. doi: 10.1212/01.wnl.0000256036.20927.a5

Wu, T., and Hallett, M. (2013). Reply: the cerebellum in Parkinson's disease and parkinsonism in cerebellar disorders. *Brain* 136:e249. doi: 10.1093/brain/aws360

Zandbelt, B. B., Bloemendaal, M., Hoogendam, J. M., Kahn, R. S., and Vink, M. (2013). Transcranial magnetic stimulation and functional MRI reveal cortical and subcortical interactions during stop-signal response inhibition. *J. Cogn. Neurosci.* 25, 157–174. doi: 10.1162/jocn_a_00309

Zheng, Z., Pan, P., Wang, W., and Shang, H. (2012). Neural network of primary focal dystonia by an anatomic likelihood estimation meta-analysis of gray matter abnormalities. *J. Neurol. Sci.* 316, 51–55. doi: 10.1016/j.jns.2012.01.032

Zhuang, P., Li, Y., and Hallett, M. (2004). Neuronal activity in the basal ganglia and thalamus in patients with dystonia. *Clin. Neurophysiol.* 115, 2542–2557. doi: 10.1016/j.clinph.2004.06.006

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 Neuroscience

ORIGINAL RESEARCH
published: 31 March 2016
doi: 10.3389/fnins.2016.00134

Transcranial Direct Current Stimulation Modulates Cortical Neuronal Activity in Alzheimer's Disease

Sara Marceglia[1,2†], Simona Mrakic-Sposta[1,3†], Manuela Rosa[1†], Roberta Ferrucci[1], Francesca Mameli[1], Maurizio Vergari[1], Mattia Arlotti[1,4], Fabiana Ruggiero[1], Elio Scarpini[5], Daniela Galimberti[5], Sergio Barbieri[1] and Alberto Priori[1,6]*

[1] Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Clinical Center for Neurostimulation, Neurotechnology, and Movement Disorders, Milano, Italia, [2] Dipartimento di Ingegneria e Architettura, Università degli Studi di Trieste, Trieste, Italia, [3] Istituto di Bioimmagini e di Fisiologia Molecolare, Consiglio Nazionale delle Ricerche, Segrate, Italia, [4] Dipartimento di Ingegneria Elettrica e dell'Informazione "Guglielmo Marconi," Università di Bologna, Cesena, Italia, [5] Unità di Neurologia, Dipartimento di Fisiologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italia, [6] Dipartimento di Scienze della Salute, Università degli Studi di Milano, Polo Ospedaliero San Paolo, Milano, Italia

OPEN ACCESS

Edited by:
Ignacio Obeso,
Hospitales Madrid and CEU-San Pablo University, Spain

Reviewed by:
Merce Boada,
Institut Català de Neurociències Aplicades, Spain
David Bartrés-Faz,
University of Barcelona, Spain
Juan Antonio Barios Heredero,
Universidad Politecnica de Madrid, Spain

***Correspondence:**
Alberto Priori
alberto.priori@unimi.it

[†] These authors have contributed equally to this work and are listed in alphabetical order.

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

Received: 16 October 2015
Accepted: 16 March 2016
Published: 31 March 2016

Citation:
Marceglia S, Mrakic-Sposta S, Rosa M, Ferrucci R, Mameli F, Vergari M, Arlotti M, Ruggiero F, Scarpini E, Galimberti D, Barbieri S and Priori A (2016) Transcranial Direct Current Stimulation Modulates Cortical Neuronal Activity in Alzheimer's Disease. Front. Neurosci. 10:134. doi: 10.3389/fnins.2016.00134

Quantitative electroencephalography (qEEG) showed that Alzheimer's disease (AD) is characterized by increased theta power, decreased alpha and beta power, and decreased coherence in the alpha and theta band in posterior regions. These abnormalities are thought to be associated with functional disconnections among cortical areas, death of cortical neurons, axonal pathology, and cholinergic deficits. Since transcranial Direct Current Stimulation (tDCS) over the temporo-parietal area is thought to have beneficial effects in patients with AD, in this study we aimed to investigate whether tDCS benefits are related to tDCS-induced changes in cortical activity, as represented by qEEG. A weak anodal current (1.5 mA, 15 min) was delivered bilaterally over the temporal-parietal lobe to seven subjects with probable AD (Mini-Mental State Examination, MMSE score >20). EEG (21 electrodes, 10–20 international system) was recorded for 5 min with eyes closed before (baseline, t0) and 30 min after anodal and cathodal tDCS ended (t1). At the same time points, patients performed a Word Recognition Task (WRT) to assess working memory functions. The spectral power and the inter- and intra-hemispheric EEG coherence in different frequency bands (e.g., low frequencies, including delta and theta; high frequencies, including alpha and beta) were calculated for each subject at t0 and t1. tDCS-induced changes in EEG neurophysiological markers were correlated with the performance of patients at the WRT. At baseline, qEEG features in AD patients confirmed that the decreased high frequency power was correlated with lower MMSE. After anodal tDCS, we observed an increase in the high-frequency power in the temporo-parietal area and an increase in the temporo-parieto-occipital coherence that correlated with the improvement at the WRT. In addition, cathodal tDCS produced a non-specific effect of decreased theta power all over the scalp that was not correlated with the clinical observation at the WRT. Our findings disclosed that tDCS induces significant modulations in the cortical EEG activity in AD patients. The abnormal pattern of EEG activity observed in AD during memory processing is partially reversed by applying anodal

tDCS, suggesting that anodal tDCS benefits in AD patients during working memory tasks are supported by the modulation of cortical activity.

Keywords: transcranial Direct Current Stimulation (tDCS), neuromodulation, Alzheimer's disease, quantitative EEG, coherence, spectral analysis

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder characterized by memory loss, severe intellectual impairment, and widespread cortical atrophy mainly localized in temporal-parietal (TP) lobe (Guze et al., 1991; Scarpini and Cogiamanian, 2003; Migliaccio et al., 2012). Morphological and functional data point out an early involvement of the temporal mesial areas followed by a progressive spread to the fronto-temporo-parietal areas with relative maintenance of the primary motor cortex (Kesslak et al., 1991; Braak et al., 1996; Karas et al., 2003). Functional neuroimaging studies showed a decreased metabolic activity in these areas (Haxby et al., 1987; Biegon et al., 1994; De Santi et al., 2001; Ewers et al., 2011). Brain tissue in AD patients is characterized by an increase of oxidative stress (OxS), with damage to proteins, lipids, and DNA oxidation/glycoxidation processes (Feng and Wang, 2012). OxS is generally an imbalance in production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) vs. the antioxidant defense system. OxS caused by excessive production of ROS, primarily superoxide anion, is considered the most important mechanism by which risk factors deprive the endothelium of Nitric Oxide (NO) (Alusik et al., 2008). AD is hence characterized by a decreased concentration of NO (Selley, 2003; Guix et al., 2005) that is thought to contribute to cognitive decline (Katusic and Austin, 2014).

The application of transcranial Direct Current Stimulation (tDCS), a non-invasive technique for focal modulation of brain and nerve function (Nitsche and Paulus, 2000, 2001; Priori, 2003; Paulus, 2004; Wassermann and Grafman, 2005), over the TP brain areas provided encouraging results on memory improvement in patients with AD and was proposed as adjuvant therapy for AD (Ferrucci et al., 2008; Boggio et al., 2012). The facilitating effect of anodal tDCS is believed to improve the TP hypoactivation in AD patients, thus enhancing memory performances (Ferrucci et al., 2008). However, neither our previous work nor the other literature on tDCS in AD assessed tDCS effects at the neurophysiological level.

Quantitative electroencephalography (qEEG) consists in the application of mathematical algorithms to the EEG signal, aimed at highlighting "quantitative" information not available in "qualitative" (or paper-based) EEG analysis. In particular, EEG analysis in the frequency domain (power spectral analysis) provides information about the presence of different oscillations in the EEG that reflect the general arousal levels in the brain. Coherence analysis can be used to evaluate cortical connections and to provide additional sources of information about the topography of synchronous oscillatory activity (Locatelli et al., 1998; Anghinah et al., 2000; Stevens et al., 2001; Fonseca et al., 2015). qEEG is now well established for assessing the functional state of the brain (Gudmundsson et al., 2007), and for supporting the discrimination of different pathologies (Koberda et al., 2013).

In children with autism, the application of tDCS induced an improvement in the health/behavior and social domains as measured by the autism treatment evaluation checklist (ATEC), that was reflected in an improvement of the cortical activity pattern measured by EEG (Amatachaya et al., 2015). This study suggests that EEG analysis can provide a significant contribution for understanding tDCS-induced neurophysiological changes correlated to tDCS-induced clinical changes.

The neurophysiological cortical pattern of AD was studied since 1980s (Klimesch, 1999). Whereas, theta (2.5–7 Hz) oscillations (i.e., low-frequency activity) appear to be higher in AD patients than in normal subjects in TP areas, alpha (8–12 Hz) and beta (13–25 Hz) oscillations (i.e., high-frequency activity) are lower in AD patients in frontal and TP brain areas (Duffy et al., 1984; Chiaramonti et al., 1997; Jelic et al., 2000; Kramer et al., 2007; Koberda et al., 2013; Fonseca et al., 2015). Even though the definition of frequency band limits may vary according to the subject population (Klimesch, 1999), the alpha band power was positively associated with the search and retrieval mechanisms in long term memory whereas the theta band power was negatively associated with the information encoding in the hippocampo-cortical loops (Klimesch, 1999). In addition, a decreased alpha coherence was found with bipolar recordings in AD (Leuchter et al., 1992; Wang et al., 2014) particularly in the inter-hemispheric alpha coherence between occipital sites (Anghinah et al., 2000) and in temporo-parieto-occipital areas (Locatelli et al., 1998; Stevens et al., 2001; Jeong, 2004; Wang et al., 2014) thus suggesting that the alpha coherence decrease could be related to the lack of influence of subcortical cholinergic structures on cortical electrical activity. Also, Locatelli et al. (1998) reported an increase in delta coherence between frontal and posterior regions in AD patients, but only in a few patients, whereas others reported decreased theta coherence in the fronto and parieto-occipital areas (Wang et al., 2014). The decreased inter-hemispheric theta coherence correlates with lower Quality of Life indicators in AD patients than in controls (Fonseca et al., 2015).

Interestingly, the higher density of sources of theta, alpha, and beta activity were localized in the TP areas in AD patients whereas the source of these activities were more distributed in healthy controls (Vecchio et al., 2013). This suggests that applying tDCS over TP areas may have an effect also on the EEG pattern. Also, direct electric fields applied in endothelial cells culture were shown to increase NO production (Trivedi et al., 2013) thus suggesting that tDCS may change NO levels. In fact, models of the electric properties of the brain suggest 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.

Because AD is characterized by impaired EEG pattern and decreased NO levels (Guix et al., 2005; Zhu et al., 2007; Katusic and Austin, 2014) and since tDCS is thought to affect both EEG and NO, in this work we investigated whether the effects of tDCS on memory functions in AD patients were consistent with tDCS-induced changes in EEG and NO levels, by analyzing a population of AD patients in which, in a previous work, we showed that anodal tDCS improved memory functions (Ferrucci et al., 2008).

METHODS

Patients

We studied a subset of seven subjects (5 women and 2 men; mean ± SD age 75.4 ± 7.2 years; years of education 11.4 ± 5.5), from the patient set already considered in Ferrucci et al. (2008). The diagnosis of probable AD was based on the criteria of NINCDS-ADRDA (McKhann et al., 1984) and the DSM-IV. All patients were under treatment with cholinesterase inhibitors (ChEI). The Mini-Mental State Examination (MMSE) score was above 20 (22.4 ± 1.39). The study was carried out in accordance with the recommendations of the Ethical Committee of the Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

EEG Recordings and Analysis

EEG was recorded in a quiet room, with the subject awake, seated on a comfortable high-backed chair and with closed eyes, under healthcare personnel continuous control. Twenty-one electrodes (Ag/AgCl) were positioned according to the 10–20 International System using the EBNeuro Mizar-Light system (EBNeuro, Florence, IT). The sampling frequency was 1024 Hz with a bandpass of 0.5–500 Hz and a sensibility of 7 μV/mm. EEG was recorded for 5 min with eyes closed at baseline (t0) and 30 min (t1) after anodal tDCS (AtDCS) and cathodal tDCS (CtDCS) (**Figure 1**).

The software toolbox EEGLAB (Delorme and Makeig, 2004), running under the cross-platform MATLAB environment (The Math-Works 7.0, Inc.) was used for data processing. Preprocessing procedures included artifact rejection and filtering. EEG was analyzed in the frequency domain through power spectrum estimation. Power spectra were calculated with the Welch's averaged modified periodogram method (Welch, 1967) with a resolution of 1 Hz. Spectral power in the bands that were identified as neurophysiological biomarkers of AD were calculated for each subject before and after A- and CtDCS, namely delta (1–3 Hz), theta (4–7 Hz), alpha (8–12 Hz), and beta (13–35 Hz). The band definition (in terms of frequency interval) followed the classical EEG analysis. Spectral power was calculated on each electrode.

As a measure of synchronization between brain areas, coherence was estimated as:

$$C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)}$$

Where x and y are two EEG signals from two different electrodes, $P_{xx}(f)$ and $P_{yy}(f)$ are the power spectral densities of x and y, and $P_{xy}(f)$ is their cross-spectral density.

In particular, inter-hemispheric EEG coherence in the frontal and antero-temporal (F-A-T) regions (Fp1-F7; Fp2-F8; Fp1-F3; Fp2-F4; Fp1-C3; Fp2-C4; F7-C3; F8-C4; F3-C3; F4-C4) and in the temporo-parieto-occipital (TP-O) regions (O1-P3; O2-P4; O1-T5; O2-T6; O1-C3; O2-C4; P3-C3; P4-C4; T5-C3; T6-C4) were calculated in the same frequency bands.

tDCS and Memory Task

The full stimulation protocol is described in Ferrucci et al. (2008). tDCS was delivered at 1.5 mA intensity for 15 min over bilateral TP areas (above P3-T5 left side and P6-T4 right side according to the international 10–20 electrode placement system) through a commercial DC stimulator, connected to thick (0.3 mm) saline-soaked sponge electrodes, two placed over the scalp (active electrodes) and the other one (reference electrode) over the right deltoid muscle (for all the details, see Ferrucci et al., 2008). Each patient underwent two tDCS sessions, one for AtDCS and one for CtDCS stimulation, in a randomized order, with at least 1-week interval between the two sessions. tDCS polarity referred to the active electrodes over the scalp. The wide electrode surface (scalp electrode 25 cm^2; deltoid electrode 64 cm^2) avoided the possible harmful effects of high current density. To guarantee safety we applied to each stimulation site current at a density of 0.06 mA/cm^2 and delivered a total charge of 0.054 C/cm^2. These intensities are below the threshold for tissue damage (Liebetanz et al., 2009).

Before and after tDCS, recognition memory function was assessed by a pencil-and-paper word recognition task (WRT) over a set of 24 words (12 previously seen by the patients, and 12 randomly chosen from a word set), as fully described in our previous paper (Ferrucci et al., 2008). The difference between the words correctly recognized as previously seen (true positive) and those incorrectly recognized as previously seen (false positive) was considered for the analysis.

Blood Sample Collection

A blood sample was collected to determine plasma levels of nitrite and nitrate (NO^2 + NO^3) both at baseline (t0) and 30 min (t1) after anodal and cathodal tDCS (**Figure 1**). Venous blood was drawn from the antecubital vein into a 10-mL EDTA vacutainer tube (Vacutainer, Becton Dickinson, USA). Plasma was immediately separated by centrifuge (5702R, Eppendorf, Germany) at 1000 × g for 10 min at 4°C. Total NO level (NOx) determination was performed using the Griess method with a commercial assay kit: Nitric Oxide (NO^{2-}/NO^{3-}) detection kit (Fisher Scientific, USA).

Samples were read by the addition of Griess reagents at 545 nm by a microplate reader spectrophotometer (Infinite M200, Tecan, Austria). The results were expressed in umol/L. All samples were determined in duplicate and the inter-assay coefficient of variation was in the range indicated by the manufacturer.

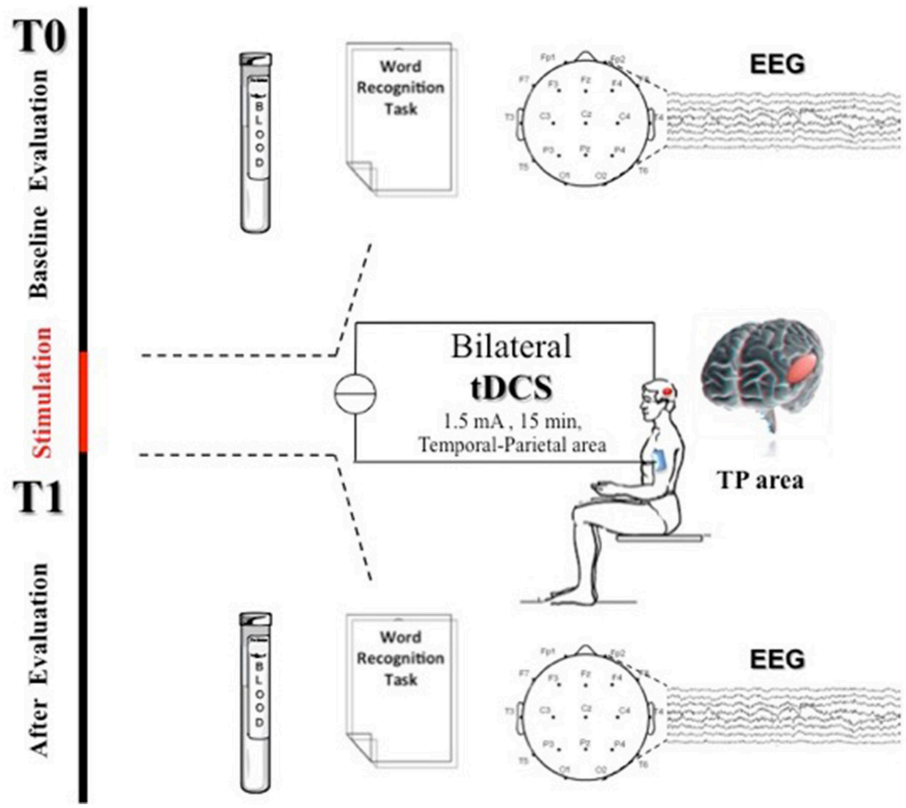


FIGURE 1 | Experimental protocol. Transcranial Direct Current Stimulation (tDCS) was applied bilaterally for 15 min over the scalp in the TP areas (above P3-T5 left side and P6-T4 right side in according to the international 10–20 electrode placement system) at 1.5 mA. At baseline (t0) and 30 min after tDCS end (t1) patients were assessed through a word recognition task (WRT), EEG recording, and blood sample collection for NOx analysis.

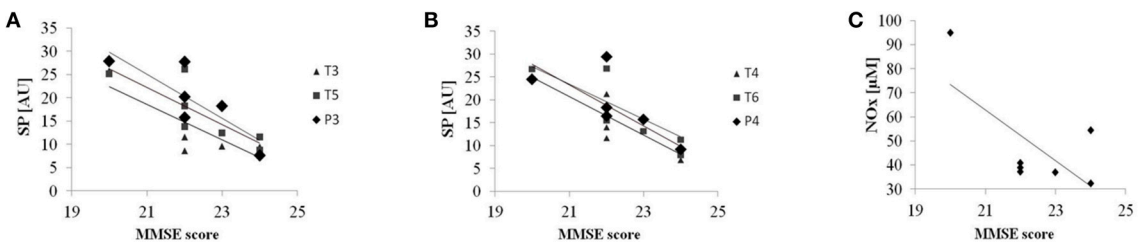


FIGURE 2 | Baseline correlations. Correlation between Mini-Mental State Examination (MMSE score) and EEG spectral powers (SP) in the HF at baseline under the electrodes in left **(A)** and right **(B)** temporo-parietal areas. **(C)** Correlation between Mini-Mental State Examination (MMSE score) and NOx concentration (uM). Solid lines represent the linear regression fit.

Data Analysis

As a first step, to establish the relationship between patient's cognitive condition and EEG measures, we verified whether baseline (t0) spectral powers in the area below the tDCS electrodes correlated with the patient's MMSE. We did the same with the NOx concentrations at baseline. Also, we verified the effect of AtDCS and CtDCS on the WRT task in our patients that were a subset of those described by Ferrucci et al. (2008).

We then assessed the effect of tDCS on both EEG spectral powers of the selected frequency bands (spectral power in a certain frequency band over a certain electrode) and coherences (coherence in a certain frequency band between two electrodes) by calculating the percentage changes of each variable between t0 (baseline) and t1 (30 min after tDCS) as:

$$\text{Delta} = (v(t1) - v(t0))/v(t0) \quad (1)$$

Where v(t) is the value of the spectral or coherence variable at t0 and at t1.

For spectral powers, because the limits of the frequency bands can vary from patient to patient (Klimesch, 1999), while

we calculated the spectral power in the classical EEG bands, we did not consider the bands as completely independent variables: we considered separately the "low-frequencies" (LF) (delta and theta) to cover the whole 2–7 Hz band, and the "high-frequencies" (HF) (alpha and beta), to cover the whole 8–25 Hz band. Also, we considered separately the electrodes over the frontal (Fp1, Fp2, F3, F4, F7, F8), TP (P3, P4, T3, T4, T5, T6), central (C3, C4, Cz), and occipital (O1, O2) areas.

Spectral powers and coherences were normally distributed (single-sample Kolmogorov–Smirnov tests $p > 0.05$), and therefore mean, standard error of the means, and parametric statistical analyses are presented. Hence, we used a four-way ANOVA with factors "electrode" (one level for each of the electrodes in the area), "side" (right and left), "band" (delta and theta for LF and alpha and beta for HF), and "stimulation" (AtDCS and CtDCS).

For coherences, we considered separately F-A-T and TP-O coherences and we run a two-way ANOVA with factors "electrode pair" (one level for each of the electrode pairs in the area) and "stimulation" (AtDCS and CtDCS).

Tukey's honest tests were used for *post-hoc* analysis. Probability levels of $p < 0.05$ were considered significant.

Finally, we assessed the correlation of tDCS-induced memory function improvement with the tDCS-induced EEG changes that resulted significant. To do so, Spearman's correlation coefficient ($p < 0.05$) was calculated between spectral powers or coherences that displayed significant tDCS-induced changes at t0 and t1 and the corresponding WRT result at t0 and t1 (after AtDCS or CtDCS).

Finally, to disclose the correlation between significant EEG changes and NOx changes, we calculated the Spearman's correlation coefficient ($p < 0.05$) between spectral power at t0 and t1 and the corresponding NO level at t0 and t1 (after AtDCS or CtDCS).

Throughout the text, values are expressed as mean ± standard errors of the mean (SE).

RESULTS

Baseline Evaluation and tDCS Effect on Memory Functions

Baseline patient's characteristics are reported in Ferrucci et al. (2008). We found an inverse correlation between MMSE scores and spectral powers in the HF under the electrodes in both the left (T3: $R^2 = 0.67$, $p = 0.024$; T5: $R^2 = 0.67$, $p = 0.023$; P3: $R^2 = 0.74$, $p = 0.012$) (**Figure 2A**) and the right TP areas (T4: $R^2 = 0.75$, $p = 0.011$; T6: $R^2 = 0.72$, $p = 0.015$; P4: $R^2 = 0.62$, $p = 0.035$) (**Figure 2B**). Even though not reaching significance ($R^2 = 0.46$, $p = 0.095$), NOx baseline concentrations showed a similar trend: lower NOx levels are associated with higher MMSE scores (**Figure 2C**).

As previously reported on the full population (Ferrucci et al., 2008), AtDCS improved WRT results in all subjects (t0 vs. t1: 3.1 ± 0.8 vs. 5.6 ± 1.1, $p = 0.015$), whereas CtDCS tended to worsen it (t0 vs. t1: 4.5 ± 0.9 vs. 2.6 ± 1.1, $p = 0.08$). **Table 1** reports individual WRT scores and NO concentrations. All the patients tolerated the procedure well, and did not experience adverse effects. None of them was able to distinguish AtDCS from CtDCS.

TABLE 1 | Individual results at the Word Recognition Task (WRT) and NO levels before (t0) and after (t1) AtDCS and CtDCS.

Subject	AtDCS				CtDCS			
	WRT		NO_x		WRT		NO_x	
	t0	t1	t0	t1	t0	t1	t0	t1
1	2.0	2.0	95.0	105.3	3.0	2.0	77.2	74.9
2	1.0	4.0	36.8	36.2	0.0	0.0	38.1	35.5
3	4.0	10.0	39.5	39.5	6.0	5.0	61.0	65.6
4	1.0	3.0	38.9	39.5	7.0	6.0	28.7	31.9
5	2.0	4.0	54.4	53.8	7.0	0.0	47.7	44.0
6	5.0	8.0	38.8	28.4	5.0	5.0	39.1	34.4
7	7.0	8.0	32.4	34.1	4.0	0.0	50.6	52.7

tDCS Effects on EEG Rhythms

In frontal areas, tDCS had no effects on EEG power neither in the LF nor in the HF. In TP areas, CtDCS significantly decreased LF below tDCS electrodes (P3 and P4, AtDCS vs. CtDCS: 3.7 ± 7.2% vs. −31.8 ± 4.3%, $p = 0.03$, **Figure 3A** upper panel), whereas AtDCS significantly increased HF (T3 and T4, AtDCS vs. CtDCS: 19.2 ± 7.4% vs. −5.2 ± 3.9%, $p = 0.02$, **Figure 3A** lower panel). In central areas, CtDCS significantly decreases LF below all electrodes (C3, AtDCS vs. CtDCS: 15.7 ± 7.3% vs. −21.9 ± 5.9%, $p < 0.0001$; C4, AtDCS vs. CtDCS: 1.04 ± 8.7% vs. −34.7 ± 3.8%, $p < 0.0001$; Cz, AtDCS vs. CtDCS: 18.6 ± 8.6% vs. −8.1 ± 8.3%, $p < 0.0001$; **Figure 3B**, upper panel). Conversely, AtDCS increased HF oscillations below C3, whereas CtDCS decreased them below C4 (C3, AtDCS vs. CtDCS: 13.3 ± 3.9% vs. −7.8 ± 1.9%, $p = 0.0005$; C4, AtDCS vs. CtDCS: −4.8 ± 5.2% vs. −19.4 ± 4.2%, $p = 0.007$; **Figure 3B**, lower panel). As well as in the other areas, CtDCS increased LF in the whole occipital area (O1, AtDCS vs. CtDCS: 0.22 ± 6.5% vs. −31.9 ± 4.7%, $p < 0.0001$; O2, AtDCS vs. CtDCS: −3.43 ± 6.0% vs. −25.5 ± 5.6%, $p < 0.0001$; **Figure 3C**), whereas no effect was observed on HF.

tDCS Effects on EEG Coherences

We observed a significant effect of tDCS on the fronto-antero-temporal (**Figure 4**) and the temporo-parieto-occipital (**Figure 5**) coherences. AtDCS significantly increased the fronto-antero-temporal coherence in the LF oscillation (Fp1-C3, AtDCS vs. CtDCS: 36.8 ± 38.5% vs. −5.0 ± 42.6%; F7-C3, AtDCS vs. CtDCS: 54.2 ± 18.4% vs. 20.7 ± 19.6%; F3-C3 AtDCS vs. CtDCS 9.26 ± 6.6% vs. −3.2 ± 5.8%, $p = 0.020$%; **Figure 4**). Similarly, in the temporo-parieto-occipital area, AtDCS significantly increased both LF and HF coherences, whereas CtDCS decreased them. More specifically, AtDCS increased LF T5-C3 and O1-C3 cohenrences (T5-C3, AtDCS vs. CtDCS: 4.0 ± 7.9% vs. −7.1 ± 10.8%; $p = 0.044$; **Figure 5A**, upper panel; O1-C3, AtDCS vs. CtDCS: 0.9 ± 5.6% vs. −8.6 ± 4.6%; $p = 0.034$; **Figure 5B**) and it increased HF T5-C3 and O2-C4 coherences (T5-C3, AtDCS vs.

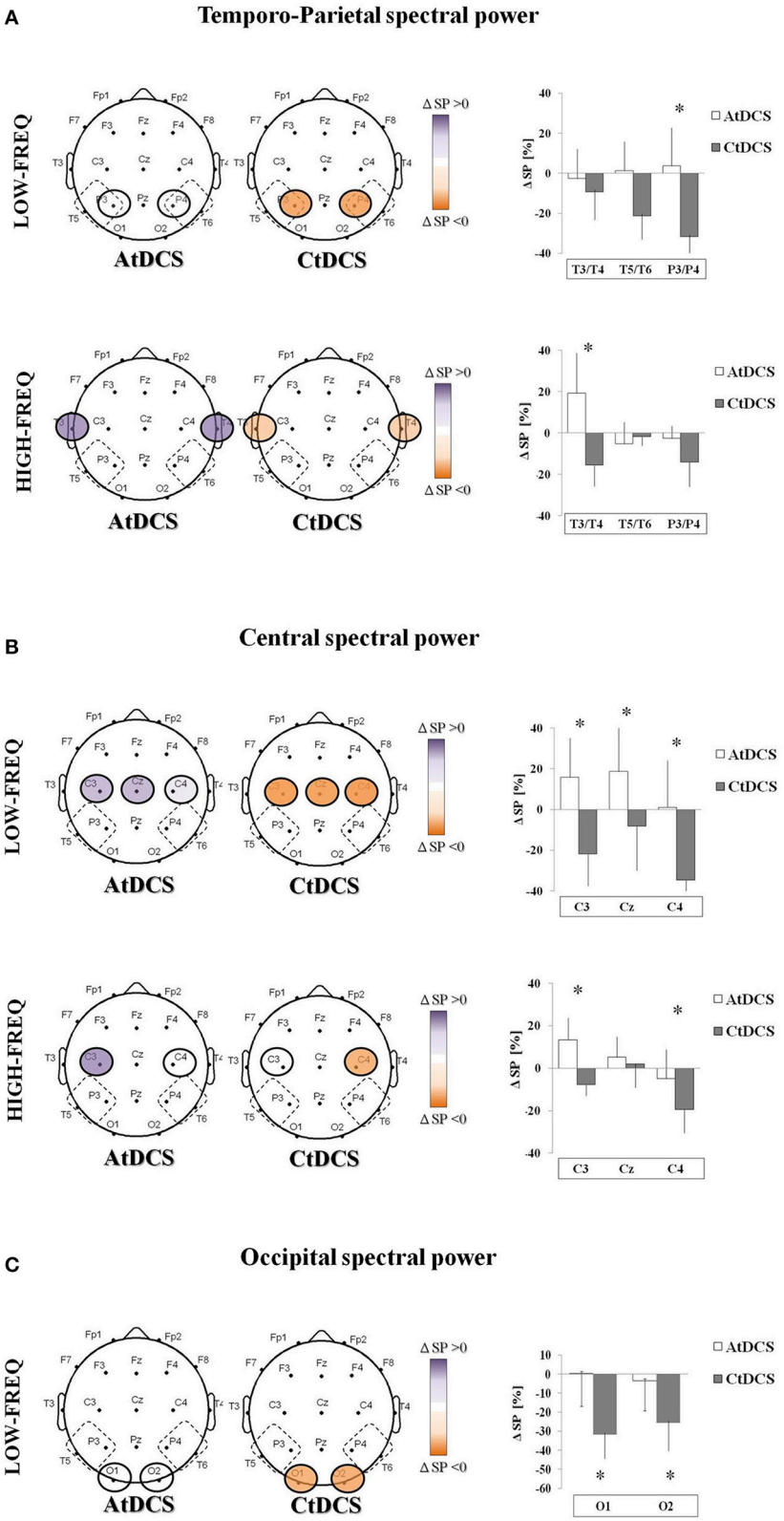


FIGURE 3 | EEG spectral power (SP) changes induced by Anodal tDCS (AtDCS) and Cathodal tDCS (CtDCS) from statistical analysis (repeated measures analysis of variance ANOVA; *$p < 0.05$). The graphical representation of the scalps displays the EEG channels with significant SP changes from *post-hoc* test. Violet represents SP increase and orange SP decrease from baseline. The histograms show the mean SP changes ($n = 7$) after AtDCS (white) and

(Continued)

FIGURE 3 | Continued
CtDCS (gray). On the y-axis is represented the SP percentage change from baseline and on the x-axis the EEG channels. Results are expressed as mean ± standard error (SE). **(A)** EEG SP changes induced by AtDCS and CtDCS in the Temporo-Parietal area in the low-frequency (delta and theta, upper panel) and high-frequency (alpha and beta, lower panel) bands: T3, T5, P3: left side; T4, T6, P4: right side. **(B)** EEG SP changes induced by AtDCS and CtDCS in the Central area in the low-frequency (delta and theta, upper panel) and high-frequency (alpha and beta, lower panel) bands: C3, Cz, C4. **(C)** EEG SP changes induced by AtDCS and CtDCS in the Occipital area in the low-frequency (delta and theta, upper panel) and high-frequency (alpha and beta, lower panel) bands: O1, O2.

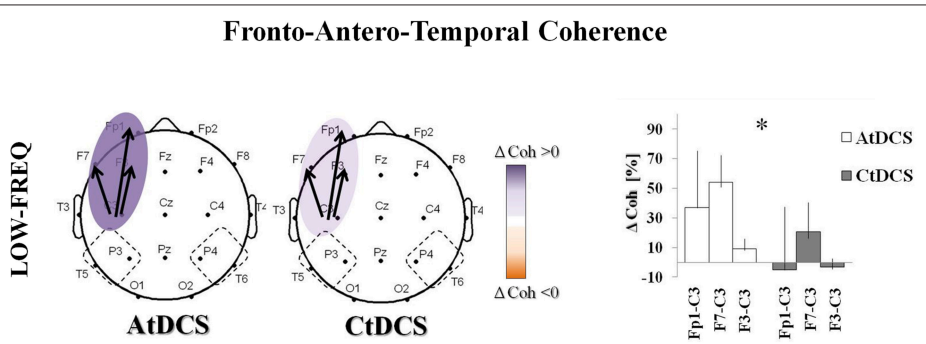


FIGURE 4 | EEG coherence (Coh) changes induced by Anodal tDCS (AtDCS) and Cathodal tDCS (CtDCS) in the Fronto-Antero-Temporalarea. The panel shows the significant differences on EEG Coh (two-way repeated measure analysis of variance ANOVA; *$p < 0.05$) for low-frequency (delta): Fp1-C3, F7-C3, F3-C3. The graphical representation of the scalps displays the significant difference on EEG Coh (Violet represents Coh increase and orange Coh decrease from baseline). The bold arrows show the significant differences at *post-hoc* test. The histograms represent the mean Coh changes ($n = 7$) after AtDCS (white) and CtDCS (gray). On the y-axis is represented the Coh percentage change from baseline and on the x-axis the EEG channels. Results are expressed as mean ± standard errors (SE).

CtDCS: 6.3 ± 7.1% vs. −3.6 ± 6.9%; $p = 0.050$; **Figure 5A**, lower panel; O2-C4, AtDCS vs. CtDCS: 4.7 ± 10.9% vs. −14.3 ± 6.9%; $p = 0.009$; **Figure 5C**).

Correlation between tDCS Effects on EEG and Cognitive Performance

The boosting effect of AtDCS on LF coherences significantly correlated with the cognitive performance at the WRT task (F7-C3: $R^2 = 0.31$, $p = 0.037$; O1-T5: $R^2 = 0.49$, $p = 0.012$) (**Figure 6A**). Although not significant, we also observed a trend toward correlation between the WRT task performance and the increase of HF power in the TP area (P3: $R^2 = 0.41$, $p = 0.10$) (**Figure 6B**).

In addition, the HF power increase after AtDCS in the TP areas was directly correlated with an increase in the NOx levels observed in patients after AtDCS (T3: $R^2 = 0.30$; $p = 0.002$; T4: $R^2 = 0.56$; $p = 0.012$) (**Figure 7**).

DISCUSSION

In this study, we investigated whether tDCS has an effect on EEG rhythms and coherences, and whether these changes could provide insights on the positive effects of tDCS on memory functions in AD. Our results showed that both A- and CtDCS are able to modulate cortical electrical activity as measured by qEEG and that the tDCS-induced modulations in EEG are consistent with the clinical effects of tDCS on memory in AD patients (Ferrucci et al., 2008; Boggio et al., 2009, 2012). More specifically, even though in a limited number of subjects, we observed that tDCS improves EEG patterns (**Figure 8**), both acting on the LF (delta and theta) and the HF (alpha and beta) oscillations. Whereas, CtDCS produces an unspecific positive decrease in the LF oscillations in the central-temporal-parietal-occipital areas, AtDCS has a more specific effect in the stimulation area, by increasing HF oscillations and coherences. Also, the effects of AtDCS on spectral powers and coherences correlate both with the improved clinical performance of the subjects at the WRT task and with the increased level of NOx following stimulation. These results suggest that the increased HF power and LF/HF coherences following AtDCS might be involved in the improved performance of AD patients at the memory task. The effect of CtDCS, despite being positive for the EEG pattern, has no correlation with the performance at the WRT task.

In fact, the EEG pattern of AD patients described in the literature suggests that decreased HF spectral powers in the frontal and TP areas can be involved in the long-term memory search and retrieval mechanisms (Klimesch, 1999; Koberda et al., 2013). This is consistent with our findings on the inverse correlation between patient's MMSE and basal HF spectral powers as well as on the direct correlation between HF increase and WRT improvement after AtDCS. Also, the literature shows that AD is characterized by abnormal decrease of inter- and intra- hemispheric EEG coherences that can be representative of AD widespread cerebral degeneration (Jiang, 2005), and may indicate an abnormal connectivity between cortical and subcortical structures (Locatelli et al., 1998; Vecchio et al., 2016). An increased demand of HF power and coherence in the temporal areas was observed in AD patients compared to controls during working memory workload (Hogan et al., 2003), possibly reflecting an enhanced efforts in patients than

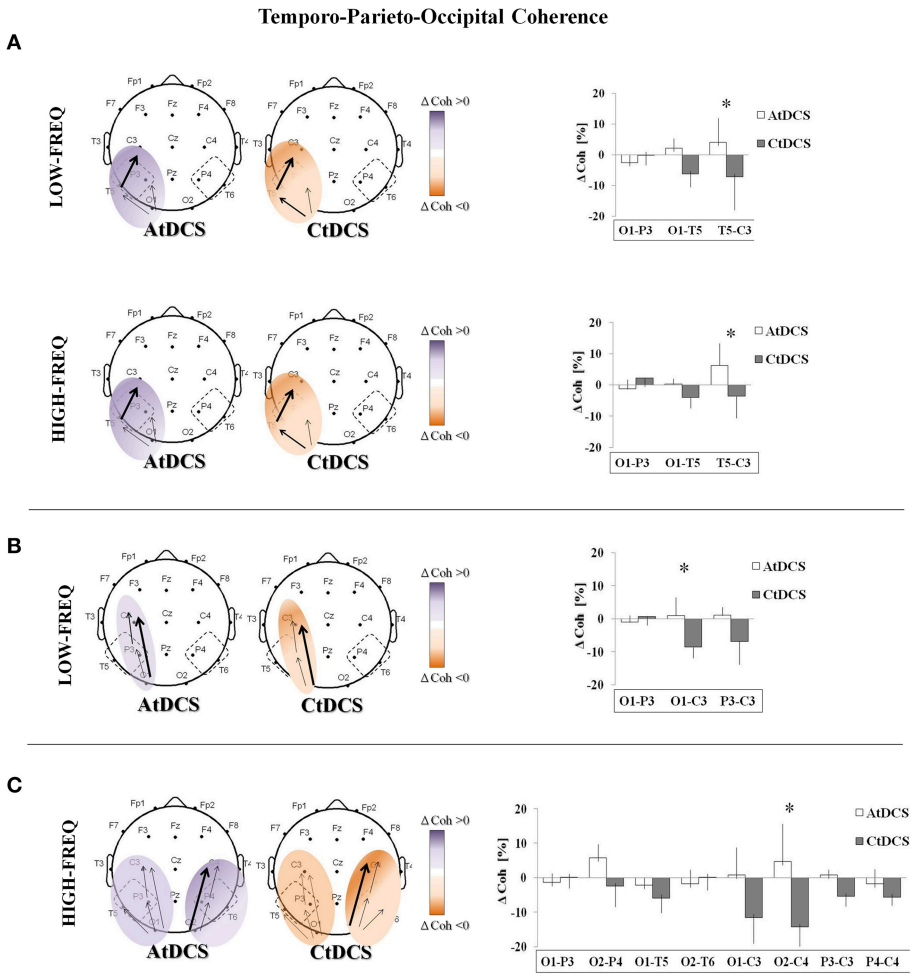


FIGURE 5 | EEG coherence (Coh) changes induced by Anodal tDCS (AtDCS) and Cathodal tDCS (CtDCS) in the Temporo-Parieto-Occipital area. The panel shows the significant differences on EEG Coh (two-way repeated measure analysis of variance ANOVA; $*p < 0.05$) for low-frequency (delta and theta, upper panel) and high-frequency (alpha and beta, lower panel): O1-P3, O1-T5, T5-C3 **(A)**; low-frequency (delta, theta): O1-P3, O1-C3, P3-C3 **(B)**; high-frequency (alpha, beta): O1-P3, O2-P4, O1-T5, O2-T6, O1-C3, O2-C4, P3-C3, P4-C4 **(C)**. The graphical representation of the scalps displays the significant difference on EEG Coh (Violet represents Coh increase and orange Coh decrease from baseline). The bold arrows show the significant differences at *post-hoc* test. The histograms represent the mean Coh changes ($n = 7$) after AtDCS (white) and CtDCS (gray). On the y-axis is represented the Coh percentage change from baseline and on the x-axis the EEG channels. Results are expressed as mean ± standard errors (SE).

in controls. Hence, AtDCS, by increasing the HF power level and coherence in the TP areas, could respond to the increased demand in AD, thus improving WRT performance.

AtDCS, in our findings, also had an increasing effect on LF coherence, that was positively correlated with better performances at the WRT. These results complement previous observations that associated lower theta coherence with poorer quality of life indicators in AD patients than in controls (Fonseca et al., 2015).

On the other hand, our data showed that CtDCS has a widespread decreasing effect on the LF oscillation power not correlated to the WRT performance. However, the role of theta band in humans is still to be clarified: the increased theta power is not specifically associated with AD, but it was observed also in attention deficit disorders and in traumatic brain injuries (Koberda et al., 2013; Ulam et al., 2015). Even though the AD EEG pattern is characterized by an increased activity in the theta oscillation (Klimesch, 1999; Koberda et al., 2013), this pattern is not directly related to working memory processing (Hogan et al., 2003), thus possibly explaining why we did not find a correlation between the WRT performance and the decrease of theta power.

Our results are in agreement with previous findings on patients with traumatic brain injury, showing that tDCS-induced normalization of the EEG pattern correlates with better performances at neuropsychological tests (Ulam et al., 2015). In particular, authors report a decreased theta power and increased alpha power in frontal areas after AtDCS and suggest that the cumulative effect of consecutive tDCS sessions may regulate cortical excitability by normalizing frontal EEG pattern.

In addition to EEG features, we provided preliminary data on the correlation between neurobiological markers and the memory state: even though observed in the acute stage, higher

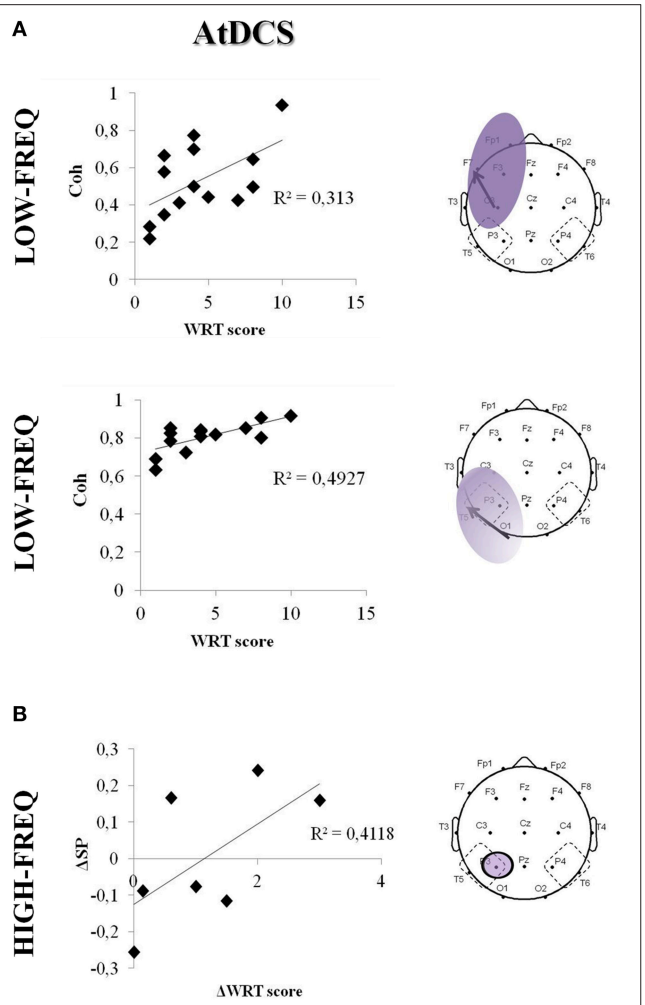


FIGURE 6 | Correlation between cognitive task performance (ΔWRT score) and EEG coherence (Coh) in the LF (A) and HF (B) in the fronto-central area/temporo-parieto-occipital after AtDCS. The bold arrows represent the coherences under examination. Single data are represented as diamonds. Solid lines represent the linear regression fit. The correlation coefficient (r^2) is reported in each panel.

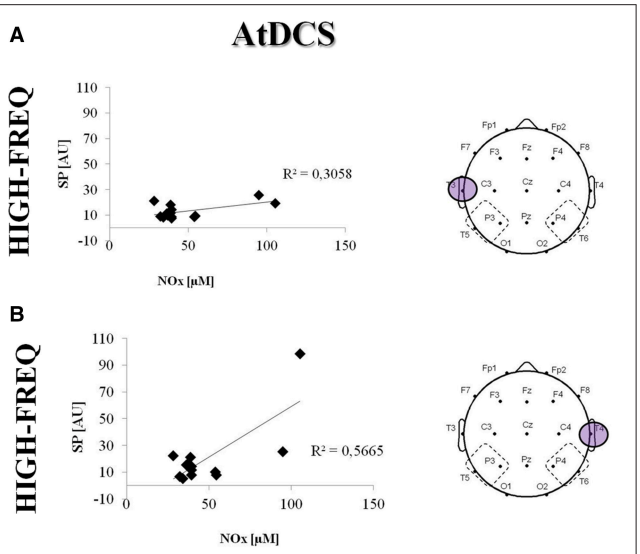


FIGURE 7 | Correlation between NOx concentration (uM) and Spectral Powers (SP, AU) in the HF under the electrodes T3 (A) and T4 (B) after AtDCS. Single data are represented as diamonds. Solid lines represent the linear regression fit. The correlation coefficient (R^2) is reported in each panel.

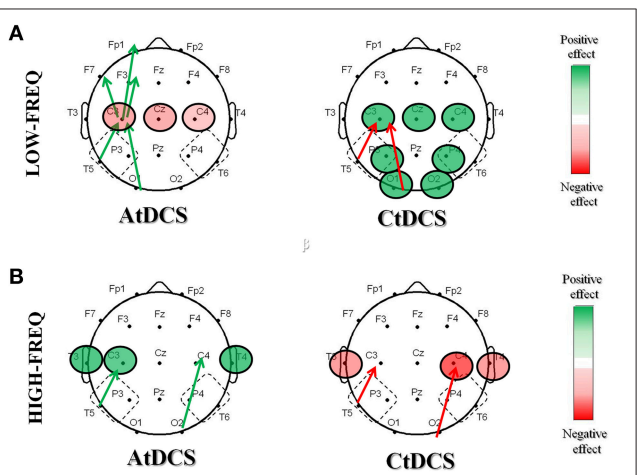


FIGURE 8 | Summary of the significant effects induced by AtDCS and CtDCS on the EEG pattern in the low frequency (A) and the high frequency (B) oscillations. The graphical representation of the scalps displays the significant positive (green) and negative (red) effects on EEG spectral powers (circles) and coherences (arrows).

NOx levels after tDCS correlated with both the positive effects of AtDCS on HF and on LF. Recent findings showed that tDCS has a role in the neurogenic control of the cerebral blood flow (Pulgar, 2015) that is directly related to the development of neurodegenerative diseases (Farkas and Luiten, 2001). Low electrical fields applied to endothelial cells produced increased NO levels (Trivedi et al., 2013), and, in turn, produce vasodilatation. These findings suggest that tDCS may act on NO to increase brain perfusion and improve memory performance.

Despite promising, our results suffer from the limited number of subjects treated with tDCS that claims for a study on a larger sample of AD patients. This implied that some trends in EEG and neurobiological markers did not reach statistical significance. Also, since the exact definition of EEG band limits in AD is variable across subjects (Klimesch, 1999), in our study, we decided to refer to LF oscillations, including delta and theta bands, and HF oscillations, including alpha and beta bands. Finally, in our subgroup of patients, to avoid subjecting participants to another long experimental session, we decided not to record sham EEG. This was in line with our aim, because we only wanted to investigate whether the effects of tDCS on memory were reflected by EEG pattern changes. Our results showed that A- and CtDCS have different effects on the electrical activity, thus ruling out the possibility that modifications in the EEG could be observed in any group after tDCS (i.e., the second time that EEG is measured). Our results are also supported by other findings proposing that each tDCS polarity

can be considered as the best possible control for the other (Cogiamanian et al., 2008; Truini et al., 2011; Lamy and Boakye, 2013; Bocci et al., 2015).

In conclusion our results provided evidence that tDCS induces significant modulations in the cortical EEG activity in AD patients. The abnormal pattern of EEG activity observed in AD during memory processing is partially reversed by applying AtDCS, suggesting that AtDCS benefits in AD patients during working memory tasks are supported by the modulation of neuronal cortical activity.

AUTHOR CONTRIBUTIONS

SM, SM-S, and MR designed and conducted the experiment, analyzed the data, interpreted the results, and drafted the manuscript. AP: designed the experiment, interpreted the results, and reviewed the manuscript. MA analyzed the data and reviewed the manuscript. RF, FM, FR conducted the experiment, interpreted the results, and reviewed the manuscript. MV, DG conducted the experiment, and reviewed the manuscript. ES, SB interpreted the results, and reviewed the manuscript. All the authors approved the final version of the manuscript.

FUNDING

The study was supported by internal funds of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and by Ministry of Health Young Researcher grant no. GR-2011-0235287.

REFERENCES

Alusik, S., Jedlickova, V., Paluch, Z., and Zecova, S. (2008). Plasma levels of nitrite/nitrate and inflammation markers in elderly individuals. *Bratisl. Lek. Listy* 109, 289–292.

Amatachaya, A., Jensen, M. P., Patjanasoontorn, N., Auvichayapat, N., Suphakunpinyo, C., Janjarasjitt, S., et al. (2015). The short-term effects of transcranial direct current stimulation on electroencephalography in children with autism: a randomized crossover controlled trial. *Behav. Neurol.* 2015, 1–11. doi: 10.1155/2015/928631

Anghinah, R., Kanda, P. A., Jorge, M. S., Lima, E. E., Pascuzzi, L., and Melo, A. C. (2000). [Alpha band coherence analysis of EEG in healthy adult's and Alzheimer's type dementia patients]. *Arq. Neuropsiquiatr.* 58, 272–275. doi: 10.1590/S0004-282X2000000200011

Biegon, A., Eberling, J. L., Richardson, B. C., Roos, M. S., Wong, S. T., Reed, B. R., et al. (1994). Human corpus callosum in aging and Alzheimer's disease: a magnetic resonance imaging study. *Neurobiol. Aging* 15, 393–397. doi: 10.1016/0197-4580(94)90070-1

Bocci, T., Marceglia, S., Vergari, M., Cognetto, V., Cogiamanian, F., Sartucci, F., et al. (2015). Transcutaneous spinal direct current stimulation modulates human corticospinal system excitability. *J. Neurophysiol.* 114, 440–446. doi: 10.1152/jn.00490.2014

Boggio, P. S., Ferrucci, R., Mameli, F., Martins, D., Martins, O., Vergari, M., et al. (2012). Prolonged visual memory enhancement after direct current stimulation in Alzheimer's disease. *Brain Stimulat.* 5, 223–230. doi: 10.1016/j.brs.2011.06.006

Boggio, P. S., Khoury, L. P., Martins, D. C. S., Martins, O. E. M. S., 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. Psychiatry* 80, 444–447. doi: 10.1136/jnnp.2007.141853

Braak, H., Braak, E., Yilmazer, D., de Vos, R. A., Jansen, E. N., and Bohl, J. (1996). New aspects of pathology in Parkinson's disease with concomitant incipient Alzheimer's disease. *J. Neural Transm. Suppl.* 48, 1–6. doi: 10.1007/978-3-7091-7494-4_1

Chiaramonti, R., Muscas, G. C., Paganini, M., Müller, T. J., Fallgatter, A. J., Versari, A., et al. (1997). Correlations of topographical EEG features with clinical severity in mild and moderate dementia of Alzheimer type. *Neuropsychobiology* 36, 153–158. doi: 10.1159/000119375

Cogiamanian, F., Vergari, M., Pulecchi, F., Marceglia, S., and Priori, A. (2008). Effect of spinal transcutaneous direct current stimulation on somatosensory evoked potentials in humans. *Clin. Neurophysiol.* 119, 2636–2640. doi: 10.1016/j.clinph.2008.07.249

De Santi, S., de Leon, M. J., Rusinek, H., Convit, A., Tarshish, C. Y., Roche, A., et al. (2001). Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol. Aging* 22, 529–539. doi: 10.1016/S0197-4580(01)00230-5

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

Duffy, F. H., Albert, M. S., and McAnulty, G. (1984). Brain electrical activity in patients with presenile and senile dementia of the Alzheimer type. *Ann. Neurol.* 16, 439–448. doi: 10.1002/ana.410160404

Ewers, M., Frisoni, G. B., Teipel, S. J., Grinberg, L. T., Amaro, E. Jr., Heinsen, H., et al. (2011). Staging Alzheimer's disease progression with multimodality neuroimaging. *Prog. Neurobiol.* 95, 535–546. doi: 10.1016/j.pneurobio.2011.06.004

Farkas, E., and Luiten, P. G. (2001). Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog. Neurobiol.* 64, 575–611. doi: 10.1016/S0301-0082(00)00068-X

Feng, Y., and Wang, X. (2012). Antioxidant therapies for Alzheimer's disease. *Oxid. Med. Cell. Longev.* 2012:472932. doi: 10.1155/2012/472932.

Ferrucci, R., Mameli, F., Guidi, I., Mrakic-Sposta, S., Vergari, M., Marceglia, S., et al. (2008). Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology* 71, 493–498. doi: 10.1212/01.wnl.0000317060.43722.a3

Fonseca, L. C., Tedrus, G. M. A. S., Rezende, A. L. R. A., and Giordano, H. F. (2015). Coherence of brain electrical activity: a quality of life indicator in Alzheimer's disease? Coerência da atividade elétrica cerebral: indicador da qualidade de vida na doença de Alzheimer? *Arq. Neuropsiquiatr.* 73, 396–401. doi: 10.1590/0004-282X20150035

Gudmundsson, S., Runarsson, T. P., Sigurdsson, S., Eiriksdottir, G., and Johnsen, K. (2007). Reliability of quantitative EEG features. *Clin. Neurophysiol.* 118, 2162–2171. doi: 10.1016/j.clinph.2007.06.018

Guix, F. X., Uribesalgo, I., Coma, M., and Muñoz, F. J. (2005). The physiology and pathophysiology of nitric oxide in the brain. *Prog. Neurobiol.* 76, 126–152. doi: 10.1016/j.pneurobio.2005.06.001

Guze, B. H., Hoffman, J. M., Baxter, L. R., Mazziotta, J. C., and Phelps, M. E. (1991). Functional brain imaging and Alzheimer-type dementia. *Alzheimer Dis. Assoc. Disord.* 5, 215–230. doi: 10.1097/00002093-199100540-00001

Haxby, J. V., Grady, C. L., Friedland, R. P., and Rapoport, S. I. (1987). Neocortical metabolic abnormalities precede nonmemory cognitive impairments in early dementia of the Alzheimer type: longitudinal confirmation. *J. Neural Transm. Suppl.* 24, 49–53.

Hogan, M. J., Swanwick, G. R. J., Kaiser, J., Rowan, M., and Lawlor, B. (2003). Memory-related EEG power and coherence reductions in mild Alzheimer's disease. *Int. J. Psychophysiol.* 49, 147–163. doi: 10.1016/S0167-8760(03)00118-1

Jelic, V., Johansson, S. E., Almkvist, O., Shigeta, M., Julin, P., Nordberg, A., et al. (2000). Quantitative electroencephalography in mild cognitive impairment: longitudinal changes and possible prediction of Alzheimer's disease. *Neurobiol. Aging* 21, 533–540. doi: 10.1016/S0197-4580(00)00153-6

Jeong, J. (2004). EEG dynamics in patients with Alzheimer's disease. *Clin. Neurophysiol.* 115, 1490–1505. doi: 10.1016/j.clinph.2004.01.001

Jiang, Z. (2005). Abnormal cortical functional connections in Alzheimer's disease: analysis of inter- and intra-hemispheric EEG coherence. *J. Zhejiang Univ. Sci. B* 6, 259–264. doi: 10.1631/jzus.2005.B0259

Karas, G. B., Burton, E. J., Rombouts, S. A. R. B., van Schijndel, R. A., O'Brien, J. T., Scheltens, P. H., et al. (2003). A comprehensive study of gray matter loss in patients with Alzheimer's disease using optimized voxel-based morphometry. *Neuroimage* 18, 895–907. doi: 10.1016/S1053-8119(03)00041-7

Katusic, Z. S., and Austin, S. A. (2014). Endothelial nitric oxide: protector of a healthy mind. *Eur. Heart J.* 35, 888–894. doi: 10.1093/eurheartj/eht544

Kesslak, J. P., Nalcioglu, O., and Cotman, C. W. (1991). Quantification of magnetic resonance scans for hippocampal and parahippocampal atrophy in Alzheimer's disease. *Neurology* 41, 51–54. doi: 10.1212/WNL.41.1.51

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

Koberda, J. L., Moses, A., Koberda, P., and Koberda, L. (2013). Clinical advantages of quantitative electroencephalogram (QEEG)-electrical neuroimaging application in general neurology practice. *Clin. EEG Neurosci.* 44, 273–285. doi: 10.1177/1550059412475291

Kramer, M. A., Chang, F. L., Cohen, M. E., Hudson, D., and Szeri, A. J. (2007). Synchronization measures of the scalp electroencephalogram can discriminate healthy from Alzheimer's subjects. *Int. J. Neural Syst.* 17, 61–69. doi: 10.1142/S0129065707000932

Lamy, J. C., and Boakye, M. (2013). BDNF Val66Met polymorphism alters spinal DC stimulation-induced plasticity in humans. *J. Neurophysiol.* 110, 109–116. doi: 10.1152/jn.00116.2013

Leuchter, A. F., Newton, T. F., Cook, I. A., Walter, D. O., Rosenberg-Thompson, S., and Lachenbruch, P. A. (1992). Changes in brain functional connectivity in Alzheimer-type and multi-infarct dementia. *Brain J. Neurol.* 115(Pt 5), 1543–1561. doi: 10.1093/brain/115.5.1543

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

Locatelli, T., Cursi, M., Liberati, D., Franceschi, M., and Comi, G. (1998). EEG coherence in Alzheimer's disease. *Electroencephalogr. Clin. Neurophysiol.* 106, 229–237. doi: 10.1016/S0013-4694(97)00129-6

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34, 939–944. doi: 10.1212/WNL.34.7.939

Migliaccio, R., Agosta, F., Possin, K. L., Rabinovici, G. D., Miller, B. L., and Gorno-Tempini, M. L. (2012). White matter atrophy in Alzheimer's disease variants. *Alzheimers Dement.* 8(5 Suppl.), S78–S87.e1-2. doi: 10.1016/j.jalz.2012.04.010

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., 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

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

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

Pulgar, V. M. (2015). Direct electric stimulation to increase cerebrovascular function. *Front. Syst. Neurosci.* 9:54. doi: 10.3389/fnsys.2015.00054

Scarpini, E., and Cogiamanian, F. (2003). Alzheimer's disease: from molecular pathogenesis to innovative therapies. *Expert Rev. Neurother.* 3, 619–630. doi: 10.1586/14737175.3.5.619

Selley, M. L. (2003). Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. *Neurobiol. Aging* 24, 903–907. doi: 10.1016/S0197-4580(03)00007-1

Stevens, A., Kircher, T., Nickola, M., Bartels, M., Rosellen, N., and Wormstall, H. (2001). Dynamic regulation of EEG power and coherence is lost early and globally in probable DAT. *Eur. Arch. Psychiatry Clin. Neurosci.* 251, 199–204. doi: 10.1007/s004060170027

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

Truini, A., Vergari, M., Biasiotta, A., La Cesa, S., Gabriele, M., Di Stefano, G., et al. (2011). Transcutaneous spinal direct current stimulation inhibits nociceptive spinal pathway conduction and increases pain tolerance in humans. *Eur. J. Pain* 15, 1023–1027. doi: 10.1016/j.ejpain.2011.04.009

Ulam, F., Shelton, C., Richards, L., Davis, L., Hunter, B., and Fregni, F. (2015). Cumulative effects of transcranial direct current stimulation on EEG oscillations and attention/working memory during subacute neurorehabilitation of traumatic brain injury. *Clin. Neurophysiol.* 126, 486–496. doi: 10.1016/j.clinph.2014.05.015

Vecchio, F., Babiloni, C., Lizio, R., Fallani, F. D. V., Blinowska, K., Verrienti, G., et al. (2013). Resting state cortical EEG rhythms in Alzheimer's disease: toward EEG markers for clinical applications: a review. *Suppl. Clin. Neurophysiol.* 62, 223–236. doi: 10.1016/B978-0-7020-5307-8.00015-6

Vecchio, F., Miraglia, F., Quaranta, D., Granata, G., Romanello, R., Marra, C., et al. (2016). Cortical connectivity and memory performance in cognitive decline: A study via graph theory from EEG data. *Neuroscience* 316, 143–150. doi: 10.1016/j.neuroscience.2015.12.036

Wang, R., Wang, J., Yu, H., Wei, X., Yang, C., and Deng, B. (2014). Decreased coherence and functional connectivity of electroencephalograph in Alzheimer's disease. *Chaos* 24, 033136. doi: 10.1063/1.4896095

Wassermann, E. M., and Grafman, J. (2005). Recharging cognition with DC brain polarization. *Trends Cogn. Sci.* 9, 503–505. doi: 10.1016/j.tics.2005.09.001

Welch, P. (1967). The use of fast Fourier transform for the estimation of power spectra: A method based on time averaging over short, modified periodograms. *IEEE Trans. Audio Electroacoustics* 15, 70–73. doi: 10.1109/TAU.1967.1161901

Zhu, X., Smith, M. A., Honda, K., Aliev, G., Moreira, P. I., Nunomura, A., et al. (2007). Vascular oxidative stress in Alzheimer disease. *J. Neurol. Sci.* 257, 240–246. doi: 10.1016/j.jns.2007.01.039

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.

SM, SM-S, MV, RF, FM, SB, and AP are founders and shareholders of Newronika srl, Milan, Italy, a spin-off company of the Fondazione IRCCS Ca'; Granda Ospedale Maggiore Policlinico and of the University of Milan, Milan, Italy.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 31 March 2016
doi: 10.3389/fnins.2016.00128

Transcranial Direct Current Stimulation Over the Primary and Secondary Somatosensory Cortices Transiently Improves Tactile Spatial Discrimination in Stroke Patients

Shuhei Fujimoto[1,2,3,4], *Noriko Kon*[3], *Yohei Otaka*[1,5], *Tomofumi Yamaguchi*[1,5], *Takeo Nakayama*[3], *Kunitsugu Kondo*[1], *Patrick Ragert*[6,7] *and Satoshi Tanaka*[2]*

[1] Tokyo Bay Rehabilitation Hospital, Chiba, Japan, [2] Laboratory of Psychology, Hamamatsu University School of Medicine, Shizuoka, Japan, [3] Department of Public Health, Kyoto University Graduate School of Medicine, Kyoto, Japan, [4] Medley, Inc., Tokyo, Japan, [5] Department of Rehabilitation Medicine, Keio University School of Medicine, Tokyo, Japan, [6] Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, [7] Faculty of Sport Science, Institute for General Kinesiology and Exercise Science, University of Leipzig, Leipzig, Germany

In healthy subjects, dual hemisphere transcranial direct current stimulation (tDCS) over the primary (S1) and secondary somatosensory cortices (S2) has been found to transiently enhance tactile performance. However, the effect of dual hemisphere tDCS on tactile performance in stroke patients with sensory deficits remains unknown. The purpose of this study was to investigate whether dual hemisphere tDCS over S1 and S2 could enhance tactile discrimination in stroke patients. We employed a double-blind, crossover, sham-controlled experimental design. Eight chronic stroke patients with sensory deficits participated in this study. We used a grating orientation task (GOT) to measure the tactile discriminative threshold of the affected and non-affected index fingers before, during, and 10 min after four tDCS conditions. For both the S1 and S2 conditions, we placed an anodal electrode over the lesioned hemisphere and a cathodal electrode over the opposite hemisphere. We applied tDCS at an intensity of 2 mA for 15 min in both S1 and S2 conditions. We included two sham conditions in which the positions of the electrodes and the current intensity were identical to that in the S1 and S2 conditions except that current was delivered for the initial 15 s only. We found that GOT thresholds for the affected index finger during and 10 min after the S1 and S2 conditions were significantly lower compared with each sham condition. GOT thresholds were not significantly different between the S1 and S2 conditions at any time point. We concluded that dual-hemisphere tDCS over S1 and S2 can transiently enhance tactile discriminative task performance in chronic stroke patients with sensory dysfunction.

Keywords: cortical plasticity, inter-hemispheric inhibition (IHI), palsy, grating orientation, transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS)

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales Madrid and CEU-San Pablo University, Spain

Reviewed by:
Hubert R. Dinse, Ruhr-University Bochum, Germany
Elena M. Marrón, Universitat Oberta de Catalunya, Spain
Juan Pablo Romero, Universidad Francisco de Vitoria, Spain

***Correspondence:**
Satoshi Tanaka
tanakas@hama-med.ac.jp

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

Received: *14 December 2015*
Accepted: *14 March 2016*
Published: *31 March 2016*

Citation:
Fujimoto S, Kon N, Otaka Y, Yamaguchi T, Nakayama T, Kondo K, Ragert P and Tanaka S (2016) Transcranial Direct Current Stimulation Over the Primary and Secondary Somatosensory Cortices Transiently Improves Tactile Spatial Discrimination in Stroke Patients. Front. Neurosci. 10:128. doi: 10.3389/fnins.2016.00128

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a process by which a weak direct current is passed through the skull, stimulating specific brain regions (Priori et al., 1998; Nitsche and Paulus, 2000, 2001; Furubayashi et al., 2008; Tatemoto et al., 2013). In stroke patients, tDCS over primary motor cortex (M1) has been found to improve motor performance in the affected upper/lower extremity (Tanaka et al., 2011; Elsner et al., 2013; Lüdemann-Podubecká et al., 2014; Pollock et al., 2014; Kang et al., 2015), as well as heighten muscle strength, motor learning, gait, and activities of daily living. Furthermore, tDCS has led to improvements in language and other cognitive functions in stroke patients (Elsner et al., 2013, 2015; Otal et al., 2015; Yang et al., 2015). However, the effect of tDCS on sensory dysfunctions in stroke patients remains unknown.

Previous studies have shown that tDCS can modulate somatosensory evoked potentials (SEP) and somatosensory processing (Matsunaga et al., 2004; Dieckhöfer et al., 2006; Boggio et al., 2008; Song et al., 2011; Costa et al., 2015; Sugawara et al., 2015; Wang et al., 2015; Nakagawa et al., 2016). For example, anodal tDCS over M1 led to increased SEP amplitude (Matsunaga et al., 2004), whereas cathodal tDCS over the primary somatosensory cortex (S1) led to decreased SEP amplitude (Dieckhöfer et al., 2006). Behaviorally, cathodal tDCS over S1 decreased participant performance on a tactile frequency discrimination task (Rogalewski et al., 2004), while anodal tDCS over S1 improved tactile spatial discrimination task performance (Ragert et al., 2008). A recent study reported that the repeated application of tDCS over S1 improved spatial tactile sensation in individuals with multiple sclerosis (MS) (Mori et al., 2012). These findings imply that tDCS may modulate somatosensory function, making it a potentially useful treatment for patients with somatosensory dysfunction (Song et al., 2011).

Dual-hemisphere tDCS, in which one hemisphere is excited while the other is inhibited, can have a powerful effect on behavioral performance (Vines et al., 2008; Kasahara et al., 2013; Fujimoto et al., 2014a; Sakai et al., 2014; Koyama et al., 2015). This improved performance appears to be the combined consequence of increased excitability in one hemisphere and decreased excitability in the other, likely via interhemispheric connections. There is some evidence of interhemispheric interactions between S1 and S2 in humans (Hoechstetter et al., 2001; Stancak et al., 2002; Werhahn et al., 2002; Hlushchuk and Hari, 2006; Ragert et al., 2011). We recently reported that, compared with single-hemisphere tDCS, a single session of dual-hemisphere tDCS over the primary somatosensory area (S1) (Fujimoto et al., 2014a) and secondary somatosensory area (S2) (Fujimoto et al., 2014b) transiently improved tactile discriminative performance in healthy subjects. Given these results, dual-hemisphere tDCS over S1 and/or S2 might improve somatosensory function in stroke patients with sensory deficits.

Abbreviations: FMA, Fugl-Meyer Assessment; GOT, Grating orientation task; SIAS, The stroke impairment assessment set; SWM, Semmes-Weuinstein monofilaments; S1, The primary somatosensory cortex; S2, The secondary somatosensory cortex; tDCS, Transcranial direct current stimulation; VRS, Verbal rating scale.

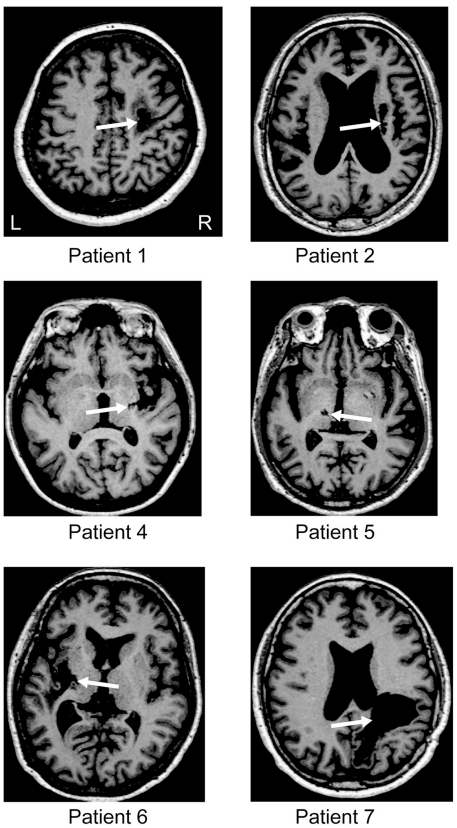


FIGURE 1 | Brain imaging. T1 magnetic resonance imaging (MRI) at the level of the main stroke for each patient. For patients 3 and 8, the MRI data were missing. White arrows indicate the location of the lesion. L and R represent the left and right hemisphere, respectively.

We used a double-blind crossover sham controlled study design to test two hypotheses. The first was that somatosensory performance in stroke patients with sensory dysfunction would be transiently enhanced by a single session of dual-hemisphere tDCS over S1 and S2, compared with sham stimulation. The second was that suppression of excitability in the un-affected hemisphere via cathodal tDCS would further increase excitability in the affected hemisphere, thus enhancing somatosensory performance in stroke patients.

MATERIALS AND METHODS

Patients

Ten patients with chronic stroke participated in this study. However, according to a reviewer's comment, two patients' data (lesions in the brainstem or internal capsule) were excluded from the analysis in order to make the sample more homogenous. Thus, eight patients data (3 males and 5 females; mean age $= 61.6 \pm 9.0$ years) were presented in the present article (**Figure 1** and **Table 1**). It should be noted that even if the two excluded patients' data were added in the analysis, significant effect of tDCS was still observed, supporting our hypothesis. Participants met the following inclusion criteria: they had

TABLE 1 | Patient Information.

Characteristics	Patient No.								Mean ± SD
	1	2	3	4	5	6	7	8	
Age, year	58	74	64	46	66	58	56	71	61.6 ± 9.0
Gender	F	M	M	F	M	F	F	F	
Time after stroke, month	60	47	89	49	10	55	49	105	58.0 ± 28.7
Lesion site	R corona radiata	R corona radiata	R putamen	R putamen	L thalamus	L putamen	R subcortex of parietal lobe	R putamen	
MMSE	26	27	30	27	30	30	30	27	28.4 ± 1.8
Handedness, EDS	R	R	R	R	R	R	R	R	
SIAS MOTOR FUNCITON, U/E									
Knee mouth test	3	3	3	3	3	3	2	1	2.6 ± 0.7
Finger function test	2	3	1a	1a	3	1b	1c	0	2.0 ± 1.4
SIAS SENSORY FUNCTION*, U/E									
Touch	2	2	2	2	1	1	1	1	1.5 ± 0.5
Position	1	2	3	2	2	1	1	2	1.8 ± 0.7
FMA SENSORY FUNCTION, U/E**									
Light touch(palm)	1	1	1	1	1	1	1	1	1.0 (all)
Position(thumb)	1	1	2	2	1	1	1	1	1.3 ± 0.5
SWM***	5	5	5	3	1	1	3	1	3.0 ± 1.9

F, Female; M, Male; R, Right; L, Left; MMSE, Mini Mental State Examination; EDS, Edinburgh Handedness Scale; SIAS, Stroke Impairment Assessment Set; U/E, Upper extremity; FMA, Fugl-Meyer Assessment; SWM, Semmes Weinstein Monofilament.

**The SIAS is a comprehensive instrument that assesses sensory and motor function in stroke patients on a sensory scale of 0–3, where 0 = complete paralysis, 1 = severe paralysis, 2 = moderate paralysis, 3 = no paralysis, and a motor scale of 0–5, where 0 = complete paralysis, 1 = severe paralysis, 2 = moderate to severe paralysis, 3 = light to moderate paralysis, 4 = light to no paralysis, 5 = no paralysis.*

***The FMA is a comprehensive instrument that assesses sensory function in stroke patients on a scale of 0–2, where 0 = anesthesia, 1 = hypoesthesia or dysesthesia, 2 = normal.*

****The Semmes-Weinstein Monofilament Test is an instrument that assesses a light touch function with various narrow monofilaments (2.83, 3.61, 4.31, 4.56, 6.65 Fmg). We counted 2.83, 3.61, 4.31, 4.56, 6.65 Fmg for 5, 4, 3, 2, 1 point.*

1a: Minimal voluntary movement or mass flexion.

1b: Mass extension.

1c: Minimal individual movement.

suffered a supratentorial stroke, they exhibited sensory deficits (excluding complete anesthesia), and they had obtained a Mini Mental Status Examination score of >24 points (Folstein et al., 1975). All participants were right hand dominant according to the Edinburgh Handedness Inventory (Oldfield, 1971), and none had a history of psychiatric or neurological illness. In the present study, participants were defined as having a sensory deficit if they exhibited impaired performance on at least one measure of sensory function via the stroke impairment assessment set (SIAS) (Touch or Position) (Chino et al., 1996), sensory function component of the Fugl-Meyer Assessment (FMA) (touch and position) (Sanford et al., 1993), or the Semmes-Weinstein monofilaments (SWM) exam (Semmes et al., 1960). All participants gave written informed consent before the experiments, which were approved by the local ethics committee at the Tokyo Bay Rehabilitation Hospital (No. 68-3).

Experimental Procedure

We employed a double-blind, crossover, sham-controlled experimental design (Hummel et al., 2005; Gandiga et al., 2006). We measured performance of both index fingers in the grating orientation task (GOT) (Johnson and Phillips, 1981; Van Boven and Johnson, 1994; Nitsche et al., 2003) before, during, and after dual-hemisphere tDCS over S1 or S2, and before, during, and after sham tDCS over S1 or S2. We chose S1 and S2 as target regions because several previous studies have indicated that performance on the GOT task involves both S1 and S2 (Zhang et al., 2005; Fujimoto et al., 2014a,b).

All participants were exposed to 4 conditions (dual-hemisphere S1 tDCS, dual-hemisphere S2 tDCS, and the equivalent sham conditions for both regions), which were conducted in separate sessions at least 3 days apart. In the dual-hemisphere tDCS condition, anodal tDCS was applied over the lesioned hemisphere and cathodal tDCS was applied over the other hemisphere. In the sham condition, tDCS was applied over both hemispheres as in the experimental condition, but for only the first 15 s of the session.

The order of the four conditions was counterbalanced among the participants. Both the participants and the experimenter who measured GOT performance were blind regarding which sessions involved actual vs. sham stimulation. However, the experimenter could discern the S1 from S2 sessions because of the different electrode configurations. Before commencing the first session, the participants were familiarized with the tasks. Each session consisted of 3 task blocks (before, during, and 10 min after the intervention). After each session, we collected verbal rating scale (VRS) scores measuring the attention, fatigue, pain, and discomfort levels of the participants.

Task

We evaluated spatial tactile discrimination performance using the GOT (Van Boven and Johnson, 1994). The GOT is a commonly accepted measure of tactile spatial acuity (Johnson and Phillips, 1981; Van Boven and Johnson, 1994). Additionally, a previous study reported that anodal tDCS over S1 had a facilitative effect on GOT performance (Ragert et al., 2008; Mori et al., 2012). During the task, participants sat on a chair in a comfortable position with their eyes covered by a blindfold. The tactile stimuli were applied using five hemispherical plastic domes with grooves (1.0, 1.2, 1.5, 2, 3, 4, 6, 8 mm in width) cut into their surfaces (Tactile Acuity Grating, Miyuki Giken). Using moderate force, the domes were applied onto the palmar side of the affected and non-affected index fingers for 2 s. The tests were performed separately for each index finger and the test order was counterbalanced among subjects. In each trial, the grooves of the dome were randomly applied in one of two directions: parallel or orthogonal to the axis of the index finger. Immediately after touching the domes, participants were expected to respond verbally, in a two-alternative force-choice paradigm, about whether the orientation of the grating of the presented dome had been parallel or orthogonal. Each dome was presented 20 times in one block (10 trials for the parallel and 10 trials for the orthogonal direction). In each block, the trials started with the largest grating (8.0 mm) and ended with the smallest grating (1.0 mm). To standardize the above procedures, we used a custom-made device that enabled the investigator to control the up-down movements of the domes. To minimize possible performance variance, a single trained investigator tested all of the participants. Using the percentage of correct grating discrimination responses, we calculated the threshold of accurate orientation detection as a primary outcome measurement. The threshold was calculated according to the following formula (Ragert et al., 2008):

$$\text{Threshold} = G_{below} + (0.75 - P_{below}) / (P_{above} - P_{below}) \times (G_{above} - G_{below}) \quad (1)$$

G_{below}: the grating spacing for which the subjects answered correctly in less than 75% of the trials
G_{above}: the grating spacing for which the subjects answered correctly in more than 75% of the trials
P_{below}: the percentage of correct responses for G_{below}
P_{above}: the percentage of correct responses for G_{above}.

Transcranial Direct Current Stimulation

We applied tDCS using a DC Stimulator Plus (NeuroConn, Germany), which delivered direct current through two sponge surface electrodes (each with a surface area of 25 cm^2). The intensity of the stimulation was 2 mA. In the dual-hemisphere tDCS condition, direct current was applied for 15 min (including the initial 15 s during which the current was gradually increased from 0 and the last 15 s during which it was gradually decreased to 0). The current density at the stimulation electrodes was 0.025 mA/cm^2. These parameters are in accordance with previously published safety criteria and are far below the threshold for tissue damage (Nitsche et al., 2003; Poreisz et al., 2007). We used the same procedure in the sham condition, except that we applied current for only 15 s (Gandiga et al., 2006).

To identify the regions over the S1 and S2, we obtained T1-weighted images from all participants using magnetic resonance imaging (Philips, Intera 1.5T, Netherlands) before the tDCS experiment. For each participant, the centers of the stimulation electrodes were placed over the S1 and S2 regions that had been identified in the individual T1-weighted image. These areas were localized using a frameless stereotaxic navigation system (Brainsight2, Rogue Research Inc., Montreal, Canada). Mean Monteal Neurological Institute (MNI) coordinates for the center of the targeted locations across participants are follows: left S1 (x,y,z) = (-31.0 ± 2.1, -34.3 ± 3.9, 59.7 ± 2.7); right S1 = (34.0 ± 3.6, -33.0 ± 4.1, $58.7 \pm 2.$); left S2 = (-42.7 ± 1.6, -32.3 ± 2.0, 14.3 ± 2.3); right S2 (44.0 ± 2.2, -29.7 ± 2.0, 16.3 ± 1.5). Mean coordinates were calculated by means of anatomical normalization based on MNI coordinate system (Friston et al., 1995).

Verbal Rating Scale

To address the possibility that the subjective state of the participants might influence their performance, we asked them to complete questionnaires in which they used a four-point scale to rate their levels of attention (1 = no distraction, 4 = highest level of distraction), fatigue (1 = no fatigue, 4 = highest level of fatigue), pain (1 = no pain, 4 = strongest pain), and discomfort (1 = no discomfort, 4 = strongest discomfort) at the end of each intervention (Poreisz et al., 2007).

Statistical Analysis

First, we separately analyzed the effects of the S1 and S2 stimulation compared with each respective sham condition. For the S1 stimulation condition, we calculated the GOT threshold for each participant in each block, and then subjected the threshold to a three-way repeated measures analysis of variance (ANOVA) with INTERVENTION (dual-hemisphere S1 or sham stimulation), TIME (pre, during, or 10 min after the intervention) and HAND (paretic or non-paretic hands) as within-subject factors. We adopted Bonferroni's test (two-tailed) for multiple-planned comparisons. We then repeated the same procedure for the S2 stimulation condition.

Second, we used a paired *t*-test to directly compare the mean GOT thresholds for the affected index finger during and 10 min after the real S1 and S2 simulations.

Finally, we analyzed the VRS scores using Fisher's exact test. For all statistics in the present study, the level of significance was defined as $p < 0.05$.

RESULTS

Individual data of GOT thresholds was shown in **Table 2**.

Effects of tDCS Over S1

The three-way repeated measures ANOVA revealed significant main effects of INTERVENTION [$F_{(1, 7)} = 18.71$, $p < 0.01$], HAND [$F_{(1, 7)} = 35.32$, $p < 0.01$], and TIME [$F_{(2, 14)} = 14.76$, $p < 0.01$]. Additionally, the three-way interaction among

TABLE 2 | Individual data of grating orientation task (GOT) thresholds (mm).

Case	S1 Sham			S1 Dual			S2 Sham			S2 Dual		
	Pre	During	Post 10 min	Pre	During	Post 10 min	Pre	During	Post 10 min	Pre	During	Post 10 min
AFFECTED INDEX FINGER												
1	2.17	2.29	2.17	2.38	1.15	1.35	2.00	2.17	2.20	2.17	1.14	1.20
2	7.50	8.00	8.00	7.67	5.33	5.00	7.67	8.00	8.00	8.00	5.00	5.33
3	6.00	6.00	6.00	5.50	2.29	2.20	5.00	4.67	5.33	6.00	2.50	3.00
4	8.00	8.00	7.50	7.00	2.25	3.25	7.50	7.67	7.67	7.00	2.83	3.50
5	8.00	8.00	8.00	8.00	7.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
6	7.75	7.43	7.71	8.00	6.50	5.00	8.00	7.67	8.00	8.00	7.11	7.00
7	7.33	8.00	8.00	8.00	6.00	5.20	8.00	8.00	7.67	7.50	5.00	4.80
8	8.00	7.50	8.00	7.50	5.50	7.50	8.00	8.00	7.60	7.67	3.33	6.50
mean	6.84	6.90	6.92	6.76	4.50	4.69	6.77	6.77	6.81	6.79	4.37	4.92
SD	2.00	1.98	2.04	1.96	2.25	2.35	2.18	2.18	2.06	1.99	2.36	2.27
NON-AFFECTED INDEX FINGER												
1	1.20	1.20	1.10	1.28	1.20	1.10	1.20	1.17	1.20	1.13	1.20	1.13
2	2.00	2.20	2.25	2.20	2.00	2.25	2.00	2.20	2.00	2.00	2.00	2.00
3	4.00	3.50	4.00	3.67	3.20	2.25	4.00	3.50	3.80	3.80	3.63	3.83
4	1.35	1.40	1.35	1.38	1.35	1.25	1.37	1.25	1.30	1.45	1.50	1.45
5	4.50	4.00	3.86	4.00	3.25	3.33	3.43	3.75	3.63	3.63	3.63	3.60
6	2.25	2.33	2.40	2.25	2.17	1.25	2.50	2.67	2.50	3.50	3.00	2.50
7	1.43	1.50	1.44	1.50	1.33	1.26	1.50	1.46	1.50	1.43	1.50	1.50
8	2.50	2.33	2.50	2.50	2.50	2.67	8.00	7.00	7.67	2.71	2.67	2.83
mean	2.40	2.31	2.36	2.35	2.12	1.92	3.00	2.87	2.95	2.46	2.39	2.36
SD	1.23	1.00	1.10	1.02	0.82	0.83	2.25	1.94	2.15	1.09	0.97	1.01

INTERVENTION, HAND, and TIME was significant [$F_{(2, 14)} = 14.53$, $p < 0.01$]. This suggests that the real S1 and sham interventions had different effects on the GOT threshold between the paretic and non-paretic hand. To further explore this interaction, we performed a two-way repeated measures ANOVA for each hand.

In the paretic hand, the two-way repeated measures ANOVA revealed significant main effects of INTERVENTION [$F_{(1, 7)} = 15.89$, $p < 0.01$], TIME [$F_{(2, 14)} = 13.17$, $p < 0.01$], and their interaction [$F_{(2, 14)} = 14.90$, $p < 0.01$; **Figure 2A**]. A *post-hoc* analysis revealed that the GOT threshold during tDCS over S1 was significantly lower than that in the sham condition ($p < 0.01$). Additionally, 10 min after tDCS over S1, the GOT threshold was still significantly lower than that after the sham stimulation ($p < 0.01$).

On the non-affected index finger, the main effects of INTERVENTION [$F_{(1, 7)} = 3.69$], TIME [$F_{(2, 14)} = 3.36$], their interaction [$F_{(2, 14)} = 2.53$] were not significant (**Figure 2B**).

Effects of tDCS Over S2

The three-way repeated measures ANOVA revealed significant main effects of INTERVENTION [$F_{(1, 7)} = 6.22$, $p < 0.05$], HAND [$F_{(1, 7)} = 22.41$, $p < 0.01$], and TIME [$F_{(2, 14)} = 12.60$, $p < 0.01$]. Additionally, the three-way interaction among INTERVENTION, HAND, and TIME was significant [$F_{(2, 14)} = 9.32$, $p < 0.01$]. This suggests that the real S2 and sham interventions had different effects on the GOT threshold between the paretic and non-paretic hand. To further explore this interaction, we conducted a two-way repeated measures ANOVA for each hand.

In the paretic hand, the two-way repeated ANOVA revealed significant main effects of INTERVENTION [$F_{(1, 7)} = 15.01$, $p < 0.01$], TIME [$F_{(2, 14)} = 15.40$, $p < 0.01$], and their interaction [$F_{(2,14)} = 12.57$, $p < 0.01$; **Figure 2C**]. A *post-hoc* analysis revealed that the GOT threshold during tDCS over S2 was significantly lower than in the sham condition ($p < 0.01$). Additionally, the GOT threshold 10 min after tDCS over S1 was still significantly lower than that after the sham stimulation ($p < 0.01$).

On the non-affected index finger, the main effects of INTERVENTION [$F_{(1, 7)} = 0.78$], TIME [$F_{(2, 14)} = 0.88$] and their interaction [$F_{(2, 14)} = 0.32$] were not significant (**Figure 2D**). These data indicate that there were no significant differences in GOT thresholds between the real S2 and sham stimulation on the non-affected index finger.

Got Thresholds in S1 and S2 Stimulation Conditions

We compared the mean GOT thresholds for the affected index finger during and 10 min after the S1 and S2 tDCS stimulation sessions. We found no significant differences in the mean GOT thresholds between the S1 and S2 stimulations during [mean threshold of S1 stimulation = 4.50 ± 2.25; mean threshold of S2 stimulation = 4.37 ± 2.36; $t_{(7)} = 0.38$, $p = 0.72$] or 10 min

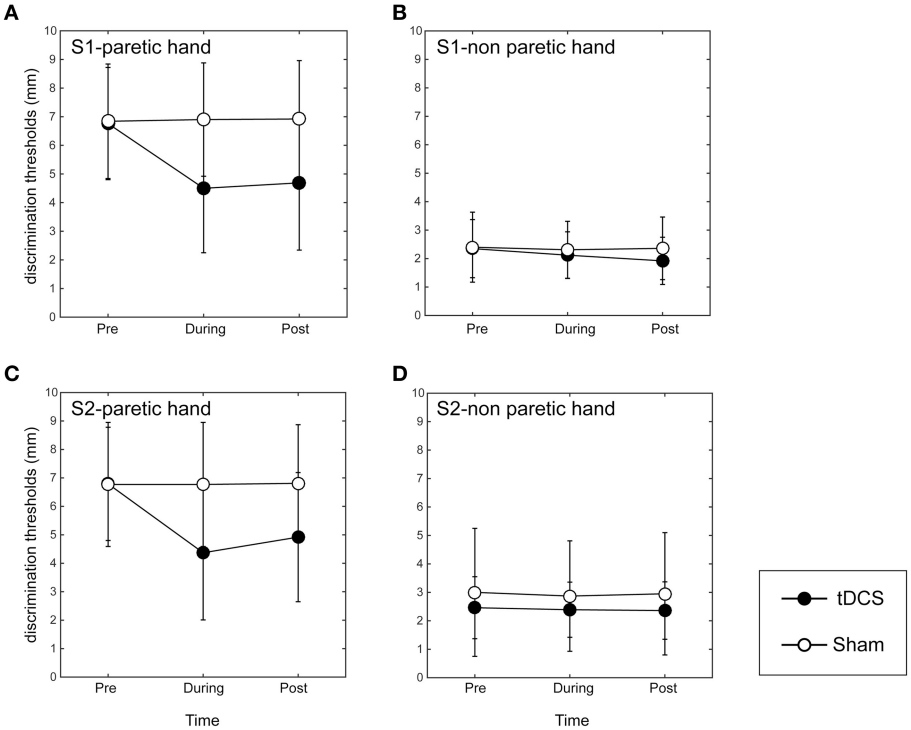


FIGURE 2 | Results of grating orientation task in dual-hemisphere S1 and S2 tDCS. The mean threshold is plotted as a time course relative to the intervention, with bars indicating standard deviation (SD). **(A)** Indicates the effect of the stimulation on the affected index finger when adopting tDCS over S1. **(B)** Indicates the effect of the stimulation on the non-affected index finger when adopting tDCS over S1. **(C)** Indicates the effect of the stimulation over the affected index finger when adopting tDCS over S2. **(D)** Indicates the effect of the stimulation on the non-affected index finger when adopting tDCS over S2. Compared with sham tDCS (white circle, $p < 0.05$), dual-hemisphere tDCS (black circle) significantly improved the grating orientation threshold for the affected index finger during and 10 min after the stimulation over both S1 and S2. However, we found no significant effects of tDCS on the non-affected index finger, regardless of stimulation site.

after the intervention [mean threshold of S1 stimulation = 4.69 ± 2.35; mean threshold of S2 stimulation = 4.92 ± 2.27; $t_{(7)} = 0.73$, $p = 0.49$]. These results suggest that the GOT thresholds were not statistically different between the S1 and S2 stimulation sites.

Psychological Data

None of the participants reported side effects. The VRS scores recorded after each intervention revealed that the tDCS did not significantly influence participant levels of attention, fatigue, pain, or discomfort (**Table 3**). Thus, we expect that the confounding effects of these factors are minimal in this study.

DISCUSSION

Our results indicate that both dual-hemisphere S1 and S2 tDCS transiently improved tactile spatial discrimination task performance compared with sham stimulation in stroke patients with sensory deficits. This effect was specific to the affected index finger. The effecter-specificity of the modulation indicated that general effects, such as changes in attention, fatigue, or pain/discomfort, did not cause the results. The VRS scores supported this notion.

Previous studies with stroke patients have reported that tDCS can enhance motor (Hummel et al., 2005; Hummel and Cohen, 2006; Tanaka et al., 2011), language, and cognitive functions (Shah et al., 2013; Flöel, 2014). Regarding sensory deficits, previous studies have reported on the therapeutic effect of tDCS in patients with multiple sclerosis, peripheral nerve neuropathic pain, and tinnitus (Mori et al., 2012; Nizard et al., 2012; Song et al., 2012). However, to the best of our knowledge, the present study is the first to show that tDCS can improve somatosensory function in stroke patients with sensory deficits.

Our finding that dual-hemisphere tDCS over S1 and S2 improved the GOT threshold in stroke patients is consistent with the findings of a previous study that showed that dual-hemisphere tDCS over the bilateral S1 and S2 enhanced GOT performance in healthy subjects (Fujimoto et al., 2014a,b). In our dual-hemisphere tDCS protocol, the anodal tDCS might have increased the excitability of the affected hemisphere, thus affecting tactile spatial discrimination in the affected index finger. Concurrently, decreased excitability in un-affected hemisphere induced by cathodal tDCS might have further increased excitability in the affected hemisphere through a reduction in interhemispheric inhibition (Werhahn et al., 2002; Hlushchuk and Hari, 2006; Ragert et al., 2011). We speculate that the combined effect of increased excitability in the affected

TABLE 3 | Questionnaire scores after each intervention.

	Dual S1	Sham S1	Dual S2	Sham S2	Statistics (Fisher's exact test)
Attention	1.13 ± 0.35	1.00	1.00	1.00	Non-significant
Fatigue	1.00	1.00	1.00	1.00	Non-significant
Pain	1.13 ± 0.35	1.00	1.13 ± 0.35	1.00	Non-significant
Discomfort	1.38 ± 0.52	1.00	1.25 ± 0.46	1.13 ± 0.35	Non-significant

※ *1.00:All of participants points 1. Data represent the group mean ± SD. Attention was scored on a scale of 1–4 (1 = no distraction; 4 = highest level of distraction). Fatigue was scored on a scale of 1–4 (1 = no fatigue; 4 = highest level of fatigue). Pain was scored on a scale of 1–4 (1 = no pain; 4 = strongest pain). Discomfort was scored on a scale of 1–4 (1 = no discomfort; 4 = strongest discomfort).*

hemisphere via anodal tDCS and decreased excitability in the un-affected hemisphere via cathodal tDCS might have led to the observed behavioral gain.

In the present study, we exclusively used dual-hemisphere tDCS. Thus, we cannot rule out the possibility that single-hemisphere tDCS might have been sufficient to improve tactile discrimination performance in our sample of stroke patients. However, in previous experiments with healthy subjects, dual-hemisphere tDCS elicited a more robust improvement in performance compared with single-hemisphere tDCS (Fujimoto et al., 2014a,b). Therefore, it is reasonable to expect that dual-hemisphere tDCS represents a more powerful strategy for improving tactile spatial discrimination performance in stroke patients compared with single-hemisphere tDCS (Vines et al., 2008; Kasahara et al., 2013; Fujimoto et al., 2014a; Sakai et al., 2014; Koyama et al., 2015). Future studies could clarify this issue by investigating the effects of single-hemisphere stimulation on behavior.

In the present study, we found the degree of improvement in GOT performance elicited by S1 and S2 stimulation to be comparable. Therefore, we cannot make a judgment about which somatosensory cortex is a more suitable target for sensory improvement in stroke patients. This result is consistent with previous neuroimaging findings that both S1 and S2 are important for the performance of tactile spatial discrimination tasks (Zhang et al., 2005).

One limitation in the present study was that the patients were relatively heterogeneous in terms of stroke localization (corona radiate, putamen, thalamus, and subcortical region). Therefore, it is difficult to conclude whether the observed reduction in tactile threshold is due to a potentiation or improvement of an impaired sensory tract by tDCS. Stroke patients with more homogeneus pathology should be investigated in future. On the other hand, the heterogeneity in the present study could be strength when we consider the wider therapeutic impact of tDCS in real life.

To conclude, our study appears to be the first double-blind, cross-over, sham-controlled experiment to demonstrate that dual-hemisphere tDCS over S1 and S2 can enhance tactile spatial discrimination in chronic stroke patients with sensory deficits. Our results provide evidence for the efficacy of tDCS in improving somatosensory function after chronic stroke. Although our small number of subjects may limit the strength of our conclusion, our findings raise the possibility that repeated applications of tDCS, combined with rehabilitation training, might have a long-term beneficial effect on somatosensory performance in stoke patients, as shown with upper limb motor training (Reis et al., 2009; Lefebvre et al., 2012). Testing this hypothesis will be relevant to the clinical application of non-invasive cortical stimulation.

AUTHOR CONTRIBUTIONS

ST conceived and supervised the study. SF, PR, ST designed the experiments. SF, NK, YO, TY, KK carried out the experiments. SF, NK, ST analyzed the data. SF, TN, PR, ST wrote the manuscript. All authors approved the final version of the submitted manuscript.

ACKNOWLEDGMENT

This study was supported by grants from the Grants-in-Aid for Scientific Research (KAKENHI 24680061) to ST. This study was conducted at the Tokyo Bay Rehabilitation Hospital, Chiba, Japan.

REFERENCES

Boggio, P. S., Zaghi, S., Lopes, M., and Fregni, F. (2008). Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. *Eur. J. Neurol.* 15, 1124–1130. doi: 10.1111/j.1468-1331.2008.02270.x

Chino, N., Sonoda, S., Domen, K., Saitoh, E., and Kimura, A. (1996). "Stroke impairment assessment set (SIAS)," in *Functional Evaluation of Stroke Patients*, eds N. Chino and J. L. Melvin (Tokyo: Springer-Verlag), 19–31.

Costa, T. L., Lapenta, O. M., Boggio, P. S., and Ventura, D. F. (2015). Transcranial direct current stimulation as a tool in the study of sensory-perceptual processing. *Atten. Percept. Psychophys.* 77, 1813–1840. doi: 10.3758/s13414-015-0932-3

Dieckhöfer, A., Waberski, T. D., Nitsche, M., Paulus, W., Buchner, H., and Gobbelé R. (2006). Transcranial direct current stimulation applied over the somatosensory cortex - differential effect on low and high frequency SEPs. *Clin. Neurophysiol.* 117, 2221–2227. doi: 10.1016/j.clinph.2006.07.136

Elsner, B., Kugler, J., Pohl, M., and Mehrholz, J. (2013). Transcranial direct current stimulation (tDCS) for improving function and activities of daily living in patients after stroke. *Cochrane Database Syst. Rev.* 11:CD009645. doi: 10.1002/14651858.cd009645.pub2

Elsner, B., Kugler, J., Pohl, M., and Mehrholz, J. (2015). Transcranial direct current stimulation (tDCS) for improving aphasia in patients with aphasia after stroke. *Cochrane Database Syst. Rev*. 5:CD009760. doi: 10.1002/14651858.CD009760.pub3

Flöel, A. (2014). tDCS-enhanced motor and cognitive function in neurological diseases. *Neuroimage* 15, 934–947. doi: 10.1016/j.neuroimage.2013.05.098

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for theclinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Friston, K. J., Ashburner, J., Frith, C. D., Poline, J. B., Heather, J. D., and Frackowiak, R. S. J. (1995). Spatial registration and normalization of images. *Hum. Brain. Mapp*. 2, 165–189. doi: 10.1002/hbm.460030303

Fujimoto, S., Yamaguchi, T., Kon, N., Osu, R., Otaka, Y., Kondo, K., et al. (2014b). Dual-hemisphere direct current stimulation over the secondary somatosensory cortex improves spatial tactile acuity in human. *Neuroscience*. Available online at: http://www.jnss.org/abstract/neuro2014/meeting_planner/sessiondetail.php?id=2014010985&u=1458713243

Fujimoto, S., Yamaguchi, T., Otaka, Y., Kondo, K., and Tanaka, S. (2014a). Dual-hemisphere transcranial direct current stimulation improves performance in a tactile spatial discrimination task. *Clin. Neurophysiol.* 125, 1669–1674. doi: 10.1016/j.clinph.2013.12.100

Furubayashi, T., Terao, Y., Arai, N., Okabe, S., Mochizuki, H., Hanajima, R., et al. (2008). Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. *Exp. Brain Res.* 185, 279–286. doi: 10.1007/s00221-007-1149-z

Gandiga, P. C., Hummel, F. C., and Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin. Neurophysiol.* 117, 845–850. doi: 10.1016/j.clinph.2005.12.003

Hlushchuk, Y., and Hari, R. (2006). Transient suppression of ipsilateral primary somatosensory cortex during tactile finger stimulation. *J. Neurosci.* 26, 5819–5824. doi: 10.1523/JNEUROSCI.5536-05.2006

Hoechstetter, K., Rupp, A., Stancák, A., Meinck, H. M., Stippich, C., Berg, P., et al. (2001). Interaction of tactile input in the human primary and secondary somatosensory cortex–a magnetoencephalographic study. *Neuroimage* 14, 759–767. doi: 10.1006/nimg.2001.0855

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C., et al. (2005). Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain* 128, 490–499. doi: 10.1093/brain/awh369

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

Johnson, K. O., and Phillips, J. R. (1981). Tactile spatial resolution. I. Two-point discrimination, gap detection, grating resolution, and letter recognition. *J. Neurophysiol.* 46, 1177–1192.

Kasahara, K., Tanaka, S., Hanakawa, T., Senoo, A., and Honda, M. (2013). Lateralization of activity in the parietal cortex predicts the effectiveness of bilateral transcranial direct current stimulation on performance of a mental calculation task. *Neurosci. Lett.* 545, 86–90. doi: 10.1016/j.neulet.2013.04.022

Kang, N., Summers, J. J., and Cauraugh, J. H. (2015). Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 87, 345–355. doi: 10.1136/jnnp-2015-311242

Koyama, S., Tanaka, S., Tanabe, S., and Sadato, N. (2015). Dual-hemisphere transcranial direct current stimulation over primary motor cortex enhances consolidation of a ballistic thumb movement. *Neurosci. Lett.* 588, 49–53. doi: 10.1016/j.neulet.2014.11.043

Lefebvre, S., Laloux, P., Peeters, A., Desfontaines, P., Jamart, J., and Vandermeeren, Y. (2012). Dual-tDCS enhances online motor skill learning and long-term retention in chronic stroke patients. *Front. Hum. Neurosci.* 6:343. doi: 10.3389/fnhum.2012.00343

Lüdemann-Podubecká, J., Bösl, K., Rothhardt, S., Verheyden, G., and Nowak, D. A. (2014). Transcranial direct current stimulation for motor recovery of upper limb function after stroke. *Neurosci. Biobehav. Rev.* 47, 245–259. doi: 10.1016/j.neubiorev.2014.07.022

Matsunaga, K., Nitsche, M. A., Tsuji, S., and Rothwell, J. C. (2004). Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans. *Clin. Neurophysiol.* 115, 456–460. doi: 10.1016/S1388-2457(03)00362-6

Mori, F., Nicoletti, C. G., Kusayanagi, H., Foti, C., Restivo, D. A., Marciani, M. G., et al. (2012). Transcranial direct current stimulation ameliorates tactile sensory deficit in multiple sclerosis. *Brain. Stimul.* 6, 654–659. doi: 10.1016/j.brs.2012.10.003

Nakagawa, K., Mochizuki, H., Koyama, S., Tanaka, S., Sadato, N., and Kakigi, R. (2016). A transcranial direct current stimulation over the sensorimotor cortex modulates the itch sensation induced by histamine. *Clin. Neurophysiol.* 127, 827–832. doi: 10.1016/j.clinph.2015.07.003

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

Nitsche, M. A., 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

Nizard, J., Lefaucheur, J. P., Helbert, M., de Chauvigny, E., and Nguyen, J. P. (2012). Non-invasive stimulation therapies for the treatment of refractory pain. *Discov. Med.* 14, 21–31.

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

Otal, B., Olma, M. C., Flöel, A., and Wellwood, I. (2015). Inhibitory non-invasive brainstimulationto homologous language regions as an adjunct to speech and language therapy in post-stroke aphasia: a meta-analysis. *Front. Hum. Neurosci.* 9:236. doi: 10.3389/fnhum.2015.00236

Pollock, A., Farmer, S. E., Brady, M. C., Langhorne, P., Mead, G. E., Mehrholz, J., et al. (2014). Interventions for improving upper limb function after stroke. *Cochrane Database Syst. Rev*. 11:CD010820. doi: 10.1002/14651858.CD010820.pub2

Poreisz, C., Boros, K., Antal, A., and Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res. Bull.* 72, 208–214. doi: 10.1016/j.brainresbull.2007.01.004

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. doi: 10.1097/00001756-199807130-00020

Ragert, P., Nierhaus, T., Cohen, L. G., and Villringer, A. (2011). Interhemispheric interactions between the human primary somatosensory cortices. *PLoS ONE* 6:e16150. doi: 10.1371/journal.pone.0016150

Ragert, P., Vandermeeren, Y., Camus, M., and Cohen, L. G. (2008). Improvement of spatial tactile acuity by transcranial direct current stimulation. *Clin. Neurophysiol.* 119, 805–811. doi: 10.1016/j.clinph.2007.12.001

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

Rogalewski, A., Breitenstein, C., Nitsche, M. A., Paulus, W., and Knecht, S. (2004). Transcranial direct current stimulation disrupts tactile perception. *Eur. J. Neurosci.* 20, 313–316. doi: 10.1111/j.0953-816X.2004.03450.x

Sakai, H., Uchiyama, Y., Tanaka, S., Sugawara, S. K., and Sadato, N. (2014). Prefrontal transcranial direct current stimulation improves fundamental vehicle control abilities. *Behav. Brain Res.* 273, 57–62. doi: 10.1016/j.bbr.2014.07.036

Shah, P. P., Szaflarski, J. P., Allendorfer, J., and Hamilton, R. H. (2013). Induction of neuroplasticity and recovery in post-stroke aphasia by non-invasive brain stimulation. *Front. Hum. Neurosci.* 7:888. doi: 10.3389/fnhum.2013.00888

Sanford, J., Moreland, J., Swanson, L. R., Stratford, P. W., and Gowland, C. (1993). Reliability of the Fugl-Meyer assessment for testing motor performance in patients following stroke. *Phys. Ther.* 73, 447–454.

Semmes, J., Weinstein, S., Ghent, L., and Teueber, H. (1960). *Somatosensory Changes After Penetrating Brain Wounds in Man*. Cambridge: Harvard University Press.

Stancak, A., Hoechstetter, K., Tintera, J., Vrana, J., Rachmanova, R., Kralik, J., et al. (2002). Source activity in the human secondary somatosensory cortex depends on the size of corpus callosum. *Brain Res.* 936, 47–57. doi: 10.1016/S0006-8993(02)02502-7

Sugawara, K., Onishi, H., Yamashiro, K., Kojima, S., Miyaguchi, S., Kirimoto, H., et al. (2015). The effect of anodal transcranial direct current stimulation over the primary motor or somatosensory cortices on somatosensory evoked magnetic fields. *Clin. Neurophysiol.* 126, 60–67. doi: 10.1016/j.clinph.2014.04.014

Song, J. J., Vanneste, S., Van de Heyning, P., and De Ridder, D. (2012). Transcranial direct current stimulation in tinnitus patients: a systemic review and meta-analysis. *Sci. World. J.* 2012:427941. doi: 10.1100/2012/427941

Song, S., Sandrini, M., and Cohen, L. G. (2011). Modifying somatosensory processing with noninvasive brain stimulation. *Restor. Neurol. Neurosci.* 29, 427–437. doi: 10.3233/RNN-2011-0614.

Tanaka, S., Takeda, K., Otaka, Y., Kita, K., Osu, R., Honda, M., et al. (2011). Single session of transcranial direct current stimulation transiently increases knee extensor force in patients with hemiparetic stroke. *Neurorehabil. Neural. Repair.* 25, 565–569. doi: 10.1177/1545968311402091

Tatemoto, T., Yamaguchi, T., Otaka, Y., Kondo, K., and Tanaka, S. (2013). Anodal transcranial direct current stimulation over the lower limb motor cortex increases the cortical excitability with extracephalic reference electrodes. *Conv. Clin. Eng. Res. Neurorehabil. Biosyst. Biorobot.* 1, 831–835. doi: 10.1007/978-3-642-34546-3_135

Van Boven, R. W., and Johnson, K. O. (1994). The limit of tactile spatial resolution in humans: grating orientation discrimination at the lip, tongue, and finger. *Neurology* 44, 2361–2366. doi: 10.1212/WNL.44.12.2361

Vines, B. W., Cerruti, C., and Schlaug, G. (2008). Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. *BMC Neurosci.* 9:103. doi: 10.1186/1471-2202-9-103

Wang, Y., Hao, Y., Zhou, J., Fried, P. J., Wang, X., Zhang, J., et al. (2015). Direct current stimulation over the human sensorimotor cortex modulates the brain's hemodynamic response to tactile stimulation. *Eur. J. Neurosci.* 42, 1933–1940. doi: 10.1111/ejn.12953

Werhahn, K. J., Mortensen, J., Van Boven, R. W., Zeuner, K. E., and Cohen, L. G. (2002). Enhanced tactile spatial acuity and cortical processing during acute hand deafferentation. *Nat. Neurosci.* 5, 936–938. doi: 10.1038/nn917

Yang, S. N., Pyun, S. B., Kim, H. J., Ahn, H. S., and Rhyu, B. J. (2015). Effectiveness of non-invasive brain stimulationin dysphagia subsequent to stroke: a systemic review and meta-analysis. *Dysphagia* 30, 383–391. doi: 10.1007/s00455-015-9619-0

Zhang, M., Mariola, E., Stilla, R., Stoesz, M., Mao, H., Hu, X., et al. (2005). Tactile discrimination of grating orientation: fMRI activation patterns. *Hum. Brain. Mapp.* 25, 370–377. doi: 10.1002/hbm.20107

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 Neuroscience

REVIEW
published: 11 January 2016
doi: 10.3389/fnins.2015.00500

Turning On Lights to Stop Neurodegeneration: The Potential of Near Infrared Light Therapy in Alzheimer's and Parkinson's Disease

Daniel M. Johnstone[1], *Cécile Moro*[2], *Jonathan Stone*[1], *Alim-Louis Benabid*[2] and *John Mitrofanis*[2]*

[1] Department of Physiology, University of Sydney, Sydney, NSW, Australia, [2] University Grenoble Alpes, CEA, LETI, CLINATEC, MINATEC Campus, Grenoble, France, [3] Department of Anatomy, University of Sydney, Sydney, NSW, Australia

OPEN ACCESS

Edited by:
Ignacio Obeso, Hospitales de Madrid and CEU-San Pablo University, Spain

Reviewed by:
Arcady A. Putilov, The Research Institute for Molecular Biology and Biophysics, Russia
Jeri-Anne Lyons, University of Wisconsin-Milwaukee, USA

***Correspondence:**
John Mitrofanis
john.mitrofanis@sydney.edu.au

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

Received: *28 September 2015*
Accepted: *15 December 2015*
Published: *11 January 2016*

Citation:
Johnstone DM, Moro C, Stone J, Benabid A-L and Mitrofanis J (2016) Turning On Lights to Stop Neurodegeneration: The Potential of Near Infrared Light Therapy in Alzheimer's and Parkinson's Disease. Front. Neurosci. 9:500. doi: 10.3389/fnins.2015.00500

Alzheimer's and Parkinson's disease are the two most common neurodegenerative disorders. They develop after a progressive death of many neurons in the brain. Although therapies are available to treat the signs and symptoms of both diseases, the progression of neuronal death remains relentless, and it has proved difficult to slow or stop. Hence, there is a need to develop neuroprotective or disease-modifying treatments that stabilize this degeneration. Red to infrared light therapy (λ =600–1070 nm), and in particular light in the near infrared (NIr) range, is emerging as a safe and effective therapy that is capable of arresting neuronal death. Previous studies have used NIr to treat tissue stressed by hypoxia, toxic insult, genetic mutation and mitochondrial dysfunction with much success. Here we propose NIr therapy as a neuroprotective or disease-modifying treatment for Alzheimer's and Parkinson's patients.

Keywords: disease-modifying, neuroprotection, photobiomodulation, amyloid plaques, tau protein

INTRODUCTION

Several recent studies in animal models of Alzheimer's and Parkinson's disease have reported that low-level near infrared light (NIr) therapy not only mitigates the behavioral deficits associated with these conditions but also has neuroprotective effects, slowing the underlying death of neurons. Current clinical therapies for both diseases do not achieve a comparable slowing of degeneration and neuroprotection, though they do relieve motor signs in Parkinson's disease and, to a lesser extent, the cognitive, and memory deficits in Alzheimer's disease. In this review, we consider the evidence for neuroprotection by NIr in animal models of these diseases, the putative mechanisms by which NIr may work to protect cells against insult, the safety of NIr therapy and finally, the potential effective use of NIr therapy in patients. First, we provide an overview of Alzheimer's and Parkinson's disease and current treatment options for these conditions.

Abbreviations: AchEIs, acetylcholinesterase inhibitors; ATP, adenosine triphosphate; LED, light emitting diode; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NIr, near infrared light; NMDA, N-methyl-d-aspartate receptor; SNc, substantia nigra pars compacta; 6OHDA, 6 hydroxydopamine.

OVERVIEW AND CURRENT TREATMENT OPTIONS FOR ALZHEIMER'S AND PARKINSON'S DISEASE

Neurodegeneration refers to a progressive death of neurons, by either genetic environmental or currently unknown factors. It includes a range of disorders, with the two most common being Alzheimer's and Parkinson's disease. Over time, as more and more neurons die, the signs and symptoms associated with each disorder worsen, making many routine day-day activities increasingly more difficult for patients (Tierney et al., 2013; Schapira et al., 2014; Brettschneider et al., 2015; Coppedè and Migliore, 2015; Goedert, 2015; Herrup, 2015; Nelson and Tabet, 2015). In the sections that follow, the different patterns of neurodegeneration, clinical syndromes and current treatments for each disease will be considered separately.

Alzheimer's Disease

Alzheimer's disease is the name given to an age-related, insidious-onset, progressive dementia. Individuals suffer progressive memory and cognitive decline and an overall loss of executive function (Herrup, 2015; Nelson and Tabet, 2015). There is an insidious death of neurons across large areas of the brain (**Figure 1**); all cortical regions, in particular entorhinal cortex and hippocampus, together with some subcortical regions, including the basal nucleus of Meynert, dorsal raphe, and locus coeruleus, suffer extensive neuronal death (Goedert, 2015; Herrup, 2015). The disease gained its name after the German neurologist Alois Alzheimer (1907, 1911) described three features of the end-stage brain. Two of the three features are proteinopathies (of β-amyloid and hyperphosphorylated tau); the third is now called gliosis or inflammation. Many other abnormalities have since been described in the dementing brain, from small vessel hemorrhage to oxidative stress (see below).

The Alzheimer's brain is characterized by a distinct pathology featuring numerous extracellular β-amyloid plaques and intracellular neurofibrillary tangles. The β-amyloid peptide, forming the bulk of the plaques, results from the cleavage of its precursor, the amyloid precursor protein, while the neurofibrillary tangles are made up of hyperphosphorylated tau protein (Braak and Braak, 1995; Hardy and Selkoe, 2002; Goedert and Spillantini, 2006). Although these pathologies have a similar overall topography across the brain, being found in largely the same regions, they tend to have different patterns of development. The β-amyloid plaques appear first in the cortex and then later across subcortical regions, while hyperphosphorylated tau is first observed in the subcortex (e.g., locus coeruleus) and then later across the cortex (Brettschneider et al., 2015).

Debate concerning the cause of this dementia is robust. In the rare early-onset forms (<65 years), there are strong genetic links, with mutations of amyloid precursor protein or presenilins giving rise to an autosomal dominant inheritance pattern of the disease. The majority of the transgenic animal models of the disease are, in fact, based on mutations of these proteins (e.g., Garcia-Alloza et al., 2006; van Eersel et al., 2010). In the more common late-onset forms (>65 years), genetic associations are not as strong, with the underlying causes and mechanisms being unclear (Coppedè and Migliore, 2015; Goedert, 2015; Herrup, 2015). A number of different hypotheses have been championed, the most popular of which is the amyloid cascade hypothesis, which proposes that the accumulation of β-amyloid in the brain—whether by genetic mutation or other unknown factors—is the primary driver of pathogenesis, namely the formation of tangles and subsequent neuronal death (Hardy and Selkoe, 2002). In more recent times, Alzheimer's pathogenesis has been proposed to be generated by protein assemblies adopting alternative conformations and becoming self-propagating, like prions (Recasens et al., 2014; Brettschneider et al., 2015; Goedert, 2015). An alternative hypothesis suggests that the proteinopathies occur downstream from the prime cause, which is microvascular hemorrhage (Cullen et al., 2005, 2006; Stone, 2008). In this latter view, Alzheimer's disease is a vasculopathy, a form of vascular dementia (De la Torre, 2004). In essence, this hypothesis proposes that the breakdown of cerebral capillaries as a consequence of aging results in microhemorrhages that in turn lead to the formation of plaques, tangles, and subsequent neuronal death (Cullen et al., 2005, 2006; Stone, 2008). We have argued recently that the dementia is best understood as a pulse-induced vascular dementia affecting primarily small cerebral vessels and that the link to age arises from the age-related hardening of the aorta, which intensifies the destructiveness of the pulse; that the pathology and symptoms of the disease are all downstream outcomes of pulse-induced damage to cerebral vessels (Stone et al., 2015). Finally, there is the hypothesis that mitochondrial dysfunction is a major contributor to the neuronal death (Swerdlow and Khan, 2004; Chaturvedi and Beal, 2008; Gonzalez-Lima et al., 2014; Coppedè and Migliore, 2015). As the organelles responsible for fuelling cell function, if mitochondria become damaged or dysfunctional, their efficacy and ATP (adenosine triphosphate) yield would be reduced. This process would lead to an increase in toxic reactive oxygen species, generating oxidative stress and subsequent neuronal death, as observed in Alzheimer's disease (Swerdlow and Khan, 2004; Chaturvedi and Beal, 2008; Gonzalez-Lima et al., 2014; Coppedè and Migliore, 2015). It should be noted that each of these putative pathogenic processes need not be mutually exclusive, and that all probably play some role in the disease process (Stone et al., 2015).

The current treatment options for patients with Alzheimer's disease are limited. These include acetylcholinesterase inhibitors (AChEIs) and N-methyl-d-aspartate receptor (NMDA) antagonists. AChEIs work to slow the rate of cognitive decline by inhibiting the degradation of acetylcholine, the major neurotransmitter associated with attention and memory, while NMDA antagonists work to prevent neurotoxicity in the brain, in particular in regions that are important for memory formation and learning. Unfortunately, these drugs are not efficacious in most patients, may have some toxic side effects and at best provide only minor palliative symptomatic relief (Nelson and Tabet, 2015).

Parkinson's Disease

The clinical syndrome and neuropathology of Parkinson's disease are very different to Alzheimer's disease. Parkinson's patients

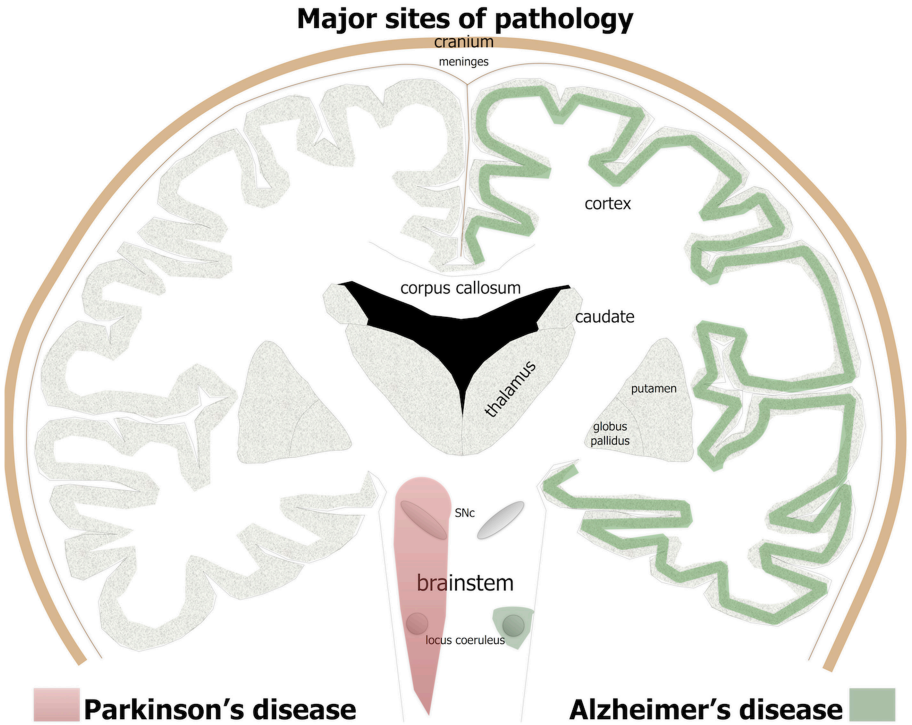


FIGURE 1 | The major brain sites of pathology in Alzheimer's and Parkinson's patients. For Alzheimer's disease, green shade indicates major regions of cell loss and β-amyloid plaques and tau pathology, while in Parkinson's disease, red shade indicates sites of major cell loss and α-synuclein pathology.

have predominately motor signs, including resting tremor, lead-pipe rigidity, akinesia, and/or bradykinesia (Bergman and Deuschl, 2002; Jankovic and Poewe, 2012). There may also be some cognitive impairment but this generally develops very late in the disease process (Cosgrove et al., 2015). Unlike Alzheimer's patients, there are no plaques or tangles and the zones of neurodegeneration are more limited, at least initially. In Parkinson's patients, there is a progressive death of many neurons in the brainstem, in particular the dopaminergic cells in the substantia nigra pars compacta (SNc) of the midbrain (**Figure 1**; Rinne, 1993; Blandini et al., 2000; Bergman and Deuschl, 2002). The loss of these cells leads subsequently to a reduction in the levels of dopamine in the striatum that, in turn, manifests as the distinct signs of the disease (Blandini et al., 2000; Bergman and Deuschl, 2002). In addition to this primary loss of brainstem dopaminergic cells, there are also localized regions of pathology in the olfactory bulb, dorsal motor nucleus of the vagus nerve and locus coeruleus (**Figure 1**) and in much later stages of the disease, across the cortex (Del Tredici and Braak, 2013; Brettschneider et al., 2015).

As with Alzheimer's disease, the factors that cause Parkinson's disease and mechanisms of neuronal death are not clear. In the rarer, early-onset forms of Parkinson's disease (10–15%), strong genetic links have been established, with several gene mutations having been identified (e.g., parkin, PINK1; Corti and Brice, 2013). There are many transgenic animal models of the disease, the most relevant involving mutations of the presynaptic protein, α-synuclein (Blesa et al., 2012; Bezard et al., 2013). In the more common late-onset of forms of the disease, the genetic links are much weaker and the causes remain unknown. As with Alzheimer's disease, several hypotheses have been championed. First, the abnormal accumulation α-synuclein in cells (synucleinopathy)—whether by genetic mutation or other unknown factors—has been suggested to be the primary factor driving the neuronal death (Gitler et al., 2009). The abnormal accumulation of this protein in cells (i.e., Lewy bodies) is thought to have prion-like propagation (Brettschneider et al., 2015; Goedert, 2015). Second, there is evidence that Parkinson's disease arises after exposure to a neurotoxin, for example paraquat, rotenone, 6OHDA (6 hydroxydopamine) or MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Indeed, many of the animal models of the disease are based on exposure to these toxins (Blesa et al., 2012). Third, there are reports proposing a role for vascular dysfunction in Parkinson's pathogenesis. In particular, it has been suggested that the process of neuronal death begins after endothelial cell damage and impairment of blood-brain barrier function (Farkas et al., 2000; Kortekaas et al., 2005; Carvey et al., 2009; Grammas et al., 2011). Further, the toxins that induce parkinsonism in animal models, namely 6OHDA and MPTP, have been shown to generate substantial disruption of the blood-brain barrier (Carvey et al., 2009). Finally, mitochondrial dysfunction—caused by either toxic insult, genetic mutation, vascular damage, or unknown factors—is considered central in the pathogenesis of Parkinson's disease (Fukae et al., 2007;

Exner et al., 2012). This dysfunction leads to a reduction of key cellular functions and subsequent neuronal death (see above). Many of these proposed mechanisms of neuronal death—from mitochondrial dysfunction to vascular compromise and from abnormal protein assemblies to prion-like propagation—are similar to those described above for Alzheimer's disease and are likely to all contribute to the pathological process, not being mutually exclusive.

For Parkinson's patients, there are more treatment options available than for Alzheimer's patients. Most Parkinson's patients are treated initially with dopamine replacement drug therapy, which aims to replace the dopamine lost from the system. This therapy is highly efficacious at reducing motor signs initially, but with prolonged use, its efficacy tapers and side-effects develop (e.g., dyskinesias; Bergman and Deuschl, 2002; Jankovic and Poewe, 2012). At these stages, patients are usually recommended for surgery with high frequency deep brain stimulation, most commonly targeting the subthalamic nucleus (Benabid et al., 2009). This surgery aims to correct the abnormal function of the basal ganglia circuitry caused by the loss of dopamine and, as with the drug therapy, is very effective in treating the signs of the disease. However, for both dopamine drug therapy and surgery, there is little, if any, evidence for neuroprotection in Parkinson's patients (Olanow et al., 2008; Jankovic and Poewe, 2012; Bezard et al., 2013; Schapira et al., 2014).

In summary, the neuropathology and patterns of neurodegeneration across the brain in Alzheimer's and Parkinson's disease are very different, hence resulting in very different signs and symptoms. However, there are similarities in the proposed mechanisms of neuronal death in each disease. The current treatments for patients of both diseases offer at best symptomatic relief (particularly in Parkinson's disease) but do not provide neuroprotection or are not disease-modifying, at least in humans.

FROM THE BENCH TO THE CLINIC: THE EVIDENCE FOR NEUROPROTECTION BY NEAR INFRARED LIGHT (NIr) TREATMENT IN ALZHEIMER'S AND PARKINSON'S DISEASE

Low-level laser or LED (light emitting diode) therapy using red to infrared light ($\lambda = 600$–1070 nm), conflated here to the term "near infrared light" (NIr), is an emerging, putative neuroprotective treatment that is showing promise in several pre-clinical models of disease. For example, NIr has been reported beneficial in animal models of retinal disease (Eells et al., 2004; Natoli et al., 2010, 2013; Albarracin et al., 2013; Begum et al., 2013; Gkotsi et al., 2014), traumatic brain (Ando et al., 2011; Oron et al., 2012; Quirk et al., 2012a; Xuan et al., 2013, 2014, 2015) and optic nerve (Fitzgerald et al., 2010) injury, experimentally-induced stroke (Lapchak et al., 2004; DeTaboada et al., 2006; Oron et al., 2006), familial amyotrophic lateral sclerosis (Moges et al., 2009), multiple sclerosis (Muili et al., 2012), Parkinson's disease (Liang et al., 2008; Whelan et al., 2008; Ying et al., 2008; Shaw et al., 2010; Peoples et al., 2012; Moro et al., 2013, 2014; Purushothuman et al., 2013; Vos et al., 2013; Johnstone et al., 2014a,b; Darlot et al., 2015; El Massri et al., 2015; Reinhart et al., 2015a,b) and Alzheimer's disease (Michalikova et al., 2008; DeTaboada et al., 2011; Grillo et al., 2013; Purushothuman et al., 2014, 2015). In humans, NIr therapy has been reported to improve executive, cognitive, and emotional functions (Barrett and Gonzalez-Lima, 2013; Blanco et al., 2015), together with performance in a range of clinical tests after ischaemic stroke (Lampl et al., 2007; Lapchak, 2010), brain trauma (Naeser et al., 2011, 2014), depression (Schiffer et al., 2009) and in age-related macular degeneration (Merry et al., 2012). The fact that NIr therapy has been reported to be effective in so many different models of disease and in a range of neural systems suggests that it is not a targeted therapy, but instead, acts to mitigate ubiquitous processes relating to cell damage and death. Recent work indicates that NIr is effective in reducing neuronal death induced by apoptosis, but not necrosis (Quirk et al., 2012a). The pathway to apoptosis is likely to involve a critical decline in cellular energy production (Galluzzi et al., 2012), that NIr may help to restore (Hamblin and Demidova, 2006; Liang et al., 2008; Ying et al., 2008; Desmet et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012; Begum et al., 2013; Gkotsi et al., 2014). This mechanism is presumably common to all the above mentioned conditions and is perhaps why NIr therapy has such broad potential applications. In the context of Alzheimer's and Parkinson's disease, although they have distinct initiating causes, both diseases converge on common pathways of inflammation and oxidative stress, mitochondrial dysfunction and neuronal death, indicating that NIr may be beneficial to both through the same protective mechanisms.

NIr for Alzheimer's Disease

The majority of the studies reporting beneficial effects of NIr treatment in Alzheimer's disease or dementia have been in transgenic animal models, in particular those displaying β-amyloid (APP/PS1: DeTaboada et al., 2011; Purushothuman et al., 2014, 2015; TASTPM; Grillo et al., 2013; CD1: Michalikova et al., 2008), or tau (K369I: Purushothuman et al., 2014, 2015) pathologies (**Table 1**). In general, with either acute (weeks; Michalikova et al., 2008) or more chronic (months; DeTaboada et al., 2011; Grillo et al., 2013; Purushothuman et al., 2014, 2015) NIr treatment, these studies have reported reductions in β-amyloid plaques, neurofibrillary tangles of hyperphosphorylated tau protein, inflammation and oxidative stress, together with increased ATP levels and improved overall mitochondrial function. In addition, NIr reduced the characteristic cognitive deficits associated with the CD1 (Michalikova et al., 2008) and APP/PS1 (DeTaboada et al., 2011) transgenic mouse models. One *in vitro* study reported that, after internalization of β-amyloid into human neuroblastoma cells, NIr treatment increased ATP levels and overall cell number, while reducing β-amyloid aggregates (Sommer et al., 2012).

To the best of our knowledge, there have been no major publications—at least in peer-reviewed journals—on the efficacy of NIr in Alzheimer's patients. There are some web pages referring to either an Alzheimer extracranial "helmet," housing many LEDs of wavelengths ranging from 660 to 1070 nm (e.g.,

TABLE 1 | Studies reporting on NIr treatment in Alzheimer's disease.

Findings with NIr application	Study	Model	Species
↑ Cell survival ↑ ATP content ↓ β-amyloid aggregates	Sommer et al., 2012	*In vitro* (neuroblastoma cells internalized with β-amyloid)	Human cells
↓ β-amyloid plaques ↓ Oxidative stress ↓ hyperphosphorylated tau	Purushothuman et al., 2014, 2015	APP/PS1, K3691 transgenics (chronic)	Mouse
↓ β-amyloid plaques ↓ Inflammation ↑ ATP content ↑ Mitochondrial function	DeTaboada et al., 2011	APP transgenic (chronic)	Mouse
↓ β-amyloid plaques ↓ Oxidative stress ↓ Hyperphosphorylated tau ↑ Heat shock proteins	Grillo et al., 2013	TASTPM transgenic (chronic)	Mouse
↑ Cognitive behavioral deficits	Michalikova et al., 2008 DeTaboada et al., 2011	CD1 transgenic (acute) APP transgenic (chronic)	Mouse

http://www.emersonww.com/InfraredHelmet.htm; http://www.science20.com/news_releases/can_this_infra_red_helmet_cure_alzheimers_in_10_minutes_a_day; http://www.instructables.com/id/LED-helmet-for-dementia-alzheimers-parkinsons), or an intranasal device delivering NIr to the brain (http://www.mediclights.com/wp-content/uploads/2013/11/Alzheimer-with-intranasal-light-08-22-13-1.pdf). However, there are no reports, either published, or in progress, of clinical trials on Alzheimer's patients. Two clinical studies by Naeser et al. (2011, 2014) have reported improvements in executive function, learning and memory after NIr treatment—delivered via an extracranial helmet-like device using two LEDs—in a small number of patients suffering chronic traumatic brain injury. Further, there are two human studies in healthy individuals reporting that NIr therapy improves attention and short-term memory (Barrett and Gonzalez-Lima, 2013) and executive functions (Blanco et al., 2015). Although these studies are promising in the sense that NIr therapy resulted in cognitive improvements, the subjects were not Alzheimer's patients.

NIr for Parkinson's Disease

Mainly due to the existence of effective toxin-based *in vitro* and *in vivo* models, there have been considerably more reports on the beneficial effects of NIr for Parkinson's disease (**Table 2**). The first studies to report neuroprotection by NIr after parkinsonian insult demonstrated that NIr treatment reduced cell death, increased ATP content and decreased levels of oxidative stress in rat striatal and cortical cells exposed to the parkinsonian toxins rotenone and MPP^+ (1-methyl-4-phenylpyridium) *in vitro* (Liang et al., 2008; Ying et al., 2008). In cultures of human neuroblastoma cells engineered to overexpress α-synuclein, NIr increased mitochondrial function and reduced oxidative stress after MPP^+ (1-methyl-4-phenylpyridinium) exposure (Trimmer et al., 2009; Quirk et al., 2012b). Further, in hybrid cells bearing mitochondrial DNA from Parkinson's patients, mitochondrial movement along axons improved substantially after NIr treatment, with movement restored to near control levels (Trimmer et al., 2009).

There have also been many *in vivo* studies of NIr-induced neuroprotection in various animal models of Parkinson's disease (**Table 2**). In MPTP-treated mice (Shaw et al., 2010; Peoples et al., 2012; Moro et al., 2013, 2014; Johnstone et al., 2014b; El Massri et al., 2015; Reinhart et al., 2015b) and 6OHDA-lesioned rats (Reinhart et al., 2015a), NIr treatment saved many dopaminergic cells from death. Further, results were similar whether the therapy was applied before, at the same time or well after the insult, indicating that NIr both conditions healthy neurons to resist a subsequent insult and rescues damaged neurons following an insult (Peoples et al., 2012). The rescue of neurons is particularly relevant to the clinical reality of the parkinsonian condition, in which individuals have, at presentation, already suffered significant degeneration, so that treatment follows neuronal loss. In the K369I transgenic mouse model of frontotemporal dementia, which also shows parkinsonian signs and a chronic and progressive degeneration of dopaminergic cells in the SNc, NIr treatment decreased oxidative stress and hyperphosphorylated tau and increased dopaminergic cell survival in the SNc (Purushothuman et al., 2013). Recently, NIr therapy has been used in a non-human primate MPTP model of Parkinson's disease with very promising results. All of the NIr-treated MPTP monkeys had a greater number of surviving dopaminergic nigral cells and striatal terminations compared to those that were not treated (Darlot et al., 2015).

Together with preserving dopaminergic cell survival, NIr has been shown to correct abnormal neuronal activity generated by the parkinsonian condition (Shaw et al., 2012). Using Fos immunohistochemistry (a well-established measure of cell activity), the overactivity of neuronal firing in the subthalamic region, characteristic of parkinsonian cases, was reduced substantially after NIr therapy. This reduction did not quite reach control levels, indicating that the restoration was partial, and was attributed to the functional repair of damaged dopaminergic cells in the SNc, allowing these cells to resume producing and releasing dopamine at their terminals in the striatum (Shaw et al., 2012). This functional restoration may well-underlie the improved motor behavior observed after NIr treatment (see below).

A number of previous studies have reported clear improvements in motor behavior in animal models of Parkinson's disease following NIr treatment. In MPTP-treated mice, NIr therapy improved various parameters of locomotion, for example mobility, and velocity (Whelan et al., 2008; Moro et al., 2013; Reinhart et al., 2015b). NIr treatment also delayed disease

TABLE 2 | Studies reporting on NIr treatment in Parkinson's disease.

Findings with NIr application	Study	Model	Species
↑ Cell survival (striatal and cortical cells) ↑ ATP content ↓ Oxidative stress	Liang et al., 2008; Ying et al., 2008	*In vitro* (rotenone, MPTP)	Rat cells
↑ Mitochondrial function ↓ Oxidative stress	Quirk et al., 2012b	*In vitro* (neuroblastoma cells overexpressing <-synuclein)	Human cells
↑ mitochondrial movement	Trimmer et al., 2009	*In vitro* (hybrid cells with mitochondrial DNA from Parkinson's disease patients)	Human cells
↑ Cell survival (TH^+ cells)	Shaw et al., 2010	MPTP (acute)	Mouse
↑ Cell survival (TH^+ cells)	Peoples et al., 2012	MPTP (chronic)	
↑ Cell survival (TH^+ cells)	Purushothuman et al., 2013	K369I transgenic (chronic)	
↑ Cell survival (TH^+ cells)	Moro et al., 2013, 2014; Johnstone et al., 2014b	MPTP (acute)	
↑ Cell survival (TH^+ cells)	El Massri et al., 2015	MPTP (acute, sub-chronic)	
↑ Cell survival (TH^+ cells)	Reinhart et al., 2015b	MPTP (acute)	
↑ Cell survival (TH^+ cells)	Reinhart et al., 2015a	6OHDA hemi-parkinsonian	Rat
↑ Cell survival (TH^+ and Nissl-stained cells)	Darlot et al., 2015	MPTP (sub-acute)	Monkey
↓ Oxidative stress ↓ Hyperphosphorylated tau	Purushothuman et al., 2013	K369I transgenic (chronic)	Mouse
↑ Flight ↑ Complex IV-dependent respiration ↓ Mutant mitochondria defects	Vos et al., 2013	pink1 mutant	Flies
↓ Abnormal basal ganglia activity (Fos immunoreactivity)	Shaw et al., 2012	MPTP (acute)	Mouse
↑ Locomotive behavior	Whelan et al., 2008	MPTP (acute)	Mouse
	Desmet et al., 2009	MPTP (acute)	
	Quirk et al., 2012b	A53T(<-synuclein transgenic)	
	Moro et al., 2013; Reinhart et al., 2015b	MPTP (acute)	
↓ Apomorphine-induced rotations	Reinhart et al., 2015a	6OHDA hemi-parkinsonian	Rat
↑ Locomotive behavior, clinical signs	Darlot et al., 2015	MPTP (sub-acute)	Monkey
↓ Clinical signs	Zhao et al., 2003; Maloney et al., 2010; Burchman, 2011	Parkinson's patients	Human
	Quietmind Foundation trial (http://www.youtube.com/watch?v=9X-hjgay7pg)		

progression and reduced the severity of the disease phenotype in transgenic mice expressing the A53T human α-synuclein mutation (Quirk et al., 2012b). Further, NIr treatment reduced apomorphine-induced rotational behavior in a 6OHDA-lesioned hemiparkinsonian rat model (Reinhart et al., 2015a). There is also evidence that NIr treatment rescues flight and mutant mitochondria defects, together with promoting complex IV-dependent respiration, in pink1 mutant flies (Vos et al., 2013). Perhaps the strongest evidence for improved behavioral outcomes after NIr treatment has been in the MPTP-treated monkey model of the disease. The NIr-treated MPTP monkeys all had reduced clinical signs compared to untreated MPTP monkeys; these reductions in clinical signs were still evident well after the period of NIr treatment, in fact up to 3 weeks after in many of the cases. This indicates that the therapeutic effects of NIr are long-lasting and not confined to periods when NIr is being applied (Darlot et al., 2015).

As with Alzheimer's disease, there have been few reports to date on the efficacy of NIr treatment in Parkinson's disease patients (**Table 2**). From the Quietmind Foundation trial, there is a linked YouTube video (http://www.youtube.com/watch?v=9X-hjgay7pg) of a Parkinson's patient displaying improved movement and reduced tremor after extracranial application of NIr, but few details are provided. There is a recent non-controlled and non-randomized clinical report indicating improved speech, cognition, freezing episodes and gait after extracranial NIr therapy in parkinsonian patients (Maloney et al., 2010); there are also some clinical reports suggesting improvements in parkinsonian signs in the majority of patients after NIr application through an intranasal device (Zhao et al., 2003). Finally, there is a serendipitous finding in one Parkinson's patient that was treated with NIr for a dental problem. This patient was reported to display a reduction in his parkinsonian signs following NIr treatment to the posterior regions of the cranium/upper neck (Burchman, 2011).

In summary, a number of experimental studies have demonstrated that NIr therapy improves motor behavior and provides neuroprotection in various rodent models of both Alzheimer's and Parkinson's disease; for Parkinson's disease, these benefits have been reported in a non-human primate model as well. However, the evidence for therapeutic benefit at the clinical level is far sparser, prompting the need for systematic, large-scale clinical trials of NIr therapy in Alzheimer's and Parkinson's patients.

HOW DOES NIr WORK TO NEUROPROTECT?

The mechanisms that underpin NIr-induced neuroprotection are not entirely clear, although they appear to operate in at least two different biological levels. First, NIr acts at a cellular level, activating intracellular cascades that ultimately contribute to the survival of the target, and possibly neighboring, cells and/or stimulating neurogenesis. Second, NIr appears capable of triggering systemic protective mechanisms; this presumably involves as yet unidentified circulating cellular or humoral factors that can transduce protective effects to the brain (**Figure 2**).

Direct Stimulation of Cells

There is a large body of work reporting that a number of molecular and cellular systems are influenced by NIr. At a cellular level, NIr displays a biphasic dose-response curve, suggesting that NIr is a low-level stressor of cells and that the activation of endogenous cellular stress response systems is likely to be central to its efficacy (Hamblin and Demidova, 2006; Desmet et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012). The main direct target of NIr appears to be cytochrome c oxidase, a key enzyme of the mitochondrial respiratory chain (**Figure 2A**). This enzyme is a photoacceptor of light in the NIr range; NIr exposure produces a redox change in cytochrome c oxidase which causes a transient change in mitochondrial membrane potential, leading to increase ATP production and a burst in low levels of reactive oxygen species (Hamblin and Demidova, 2006; Desmet et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012). This, in turns, triggers a cascade of secondary downstream signaling pathways that collectively stimulate endogenous cell protection and repair mechanisms (Hamblin and Demidova, 2006; Desmet et al., 2009; Chung et al., 2012; Rojas and Gonzalez-Lima, 2011). This modulation of multiple molecular systems appears capable of both conditioning neurons to resist future damage and accelerating repair of neurons damaged by a previous or continuing insult (e.g., Liang et al., 2008; Ying et al., 2008).

In addition to protecting and repairing damaged or dysfunctional neurons, there is emerging evidence from mouse models of traumatic brain injury that NIr also stimulates neurogenesis and synaptogenesis (**Figure 2A**). In a series of studies using a mouse model of traumatic brain injury, Xuan and colleagues found that a NIr treatment regime that improved neurological performance (Xuan et al., 2013), also increased markers of neuroprogenitor proliferation in the hippocampal region (i.e., dentate gyrus) and subventricular zone (Xuan et al., 2014), brain regions known to harbor neural stems cells. Other early responses in these regions included up-regulation of brain-derived neurotrophic factor, which was associated with subsequent up-regulation of synaptogenesis markers in the lesion site (Xuan et al., 2013). Similar observations of NIr-induced increases in neuroprogenitor cell proliferation in the subventricular zone have been made in a rat model of stroke (Oron et al., 2006).

It should be noted that these studies have focussed on the effect of NIr on neurons; similar NIr-induced cellular mechanisms may also be at play within brain capillary endothelial cells (**Figure 2A**). Mitochondrial dysfunction of these cells has been related to various vascular conditions, including atherosclerosis and hypertension (Tang et al., 2014). In the context of neurodegeneration, both Alzheimer's and Parkinson's disease have been implicated as vascular disorders, with suggestions that the neurodegenerative process begins with the breakdown of the integrity of small cerebral vessels and the blood-brain barrier (see above). This "breakdown" may begin with mitochondrial dysfunction (Grammas et al., 2011). Following, we propose that NIr-induced neuroprotection in Alzheimer's and Parkinson's

disease might involve repair of the damaged mitochondria in local endothelial cells, leading subsequently to a restoration of the integrity of the endothelial network and blood-brain barrier in the region, resulting ultimately in improved neuronal survival (**Figure 2A**).

Indirect Stimulation of Systemic Factors

In addition to direct beneficial actions on damaged cells, there is increasing evidence that NIr treatment might also activate a more global, systemic response (**Figure 2B**). This evidence arises from the observation that local application of NIr to a particular body part can induce beneficial effects in distant body tissues (Braverman et al., 1989; Stone et al., 2013; Johnstone et al., 2014a,b, 2015). For example, neuroprotection of the mouse brain against MPTP insult has been demonstrated following the "remote" application of NIr to the dorsum of the animal, with no direct application to the head (Stone et al., 2013; Johnstone et al., 2014a,b, 2015). While the mechanism remains unknown, it presumably involves the stimulation of one or more circulating molecules or cell types. One possibility is the stimulation of immune cells, for example mast cells and macrophages, that could help neuroprotect cells in the brain (Byrnes et al., 2005; Chung et al., 2012; Muili et al., 2012). There may also be effects on inflammatory mediators, as NIr is associated with down-regulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines (Muili et al., 2012). In addition, bone marrow-derived stem cells may also be involved; a series of studies has demonstrated that NIr exposure increases proliferation of c-kit-positive cells in the bone marrow and that, following myocardial infarction in rats, these cells are mobilized and recruited specifically to the site of damage where they are associated with a reduction in myocardial infarct size and ventricular dilatation (Tuby et al., 2011). These cells, together with immune cells, may release trophic factors (e.g., nerve growth factor, brain-derived neurotrophic factor) that improve the function of dying cells and help their survival (Hou et al., 2008).

Another possibility is for a signaling system between mitochondria in different body tissues. Mitochondria in distress in one body tissue have been suggested to produce an unidentified extracellular signal (mitokine) that is then transmitted to cells in remote body tissues and as a consequence induces a mitochondrial stress response (Durieux et al., 2011; Taylor et al., 2014). In relation to NIr and Alzheimer's and Parkinson's disease, NIr applied to remote tissue may prompt a signal system between mitochondria of peripheral tissues and brain, inducing repair mechanisms in the damaged cells in the brain (Johnstone et al., 2014a,b, 2015). Taken all together, the systemic mechanisms underlying remote NIr-induced neuroprotection may share similarities with other remote tissue protection phenomena—these include remote ischaemic conditioning, where induction of brief ischaemic episodes in one organ provides protection of other distant organs (Hausenloy and Yellon, 2008; Yetgin et al., 2012), and the so-called "abscopal" effect sometimes observed in radiation treatment of metastatic cancer, where treatment targeted at a tumor leads to not only a shrinking of the local tumor but also a shrinking of tumors far from the treated area (Postow et al., 2012).

More research is required to understand the interplay between direct cellular and indirect systemic mechanisms of NIr-induced protection. Both appear capable of acting independently—the findings of numerous *in vitro* cell culture studies reporting that NIr is neuroprotective, indicate clearly that the indirect systemic effect is not necessary for NIr-induced neuroprotection and repair of damaged neurons (Hamblin and Demidova, 2006; Desmet et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012), while accumulating evidence from mouse models suggest remote NIr application provides neuroprotection in the absence of direct NIr stimulation (Johnstone et al., 2014b, 2015; Farfara et al., 2015). The phenomenon of indirect NIr-induced neuroprotection is likely to involve the same mechanisms, at a cellular level, as those that provide neuroprotection to damaged cells with direct NIr stimulation (i.e., stimulation of mitochondrial function; **Figure 2A**). Although the concept of indirect, remote NIr therapy holds promise for future applications, it is not yet as fully understood and developed as direct NIr therapy, thus our subsequent discussion will focus primarily on direct NIr stimulation. Further, some early results in an animal model of Parkinson's disease suggest that, although remote NIr provides neuroprotection, this protection was not as robust as when NIr was applied directly to the head (Stone et al., 2013; Johnstone et al., 2014b; presumably stimulating local neurons and/or endothelial cells). In other words, neuroprotection was achieved with both local and remote NIr treatment, but the local treatment was the more effective. As a working hypothesis, we suggest that direct stimulation of the mitochondria and reparative mechanisms, either in the neurons themselves or in the local endothelial cells (and/or stimulation of neurogenesis), forms the primary mechanism of NIr-induced neuroprotection. A more systemic (indirect) stimulation of immune and/or stem cells may form a secondary and complementary mechanism. We suggest that stimulation of both direct and indirect mechanisms would generate maximum NIr-induced neuroprotection.

IS NIr THERAPY SAFE?

To date, there are no reports of major safety issues nor side-effects after NIr treatment. The commercial LED panels for NIr therapy have already received non-significant risk status by the Food and Drug Administration and previous studies have indicated no adverse impact on brain tissue structure and function after NIr treatment (power range from ∼1 to 700 mW/cm^2; Desmet et al., 2006; Hamblin and Demidova, 2006; Ilic et al., 2006; Zivin et al., 2009; McCarthy et al., 2010; Naeser et al., 2011, 2014; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012; Tata and Waynant, 2012; Quirk et al., 2012a,b; Moro et al., 2014). There is one sole account of some neuronal damage and negative behavioral outcomes in mice, but this was evident after an exceptionally high power intensity (750 mW/cm^2; Ilic et al., 2006), approximately one hundred times higher than the dose required to elicit a therapeutic response (e.g., <10 mW/cm^2). Hence, when taken together, these data indicate that when NIr was applied at

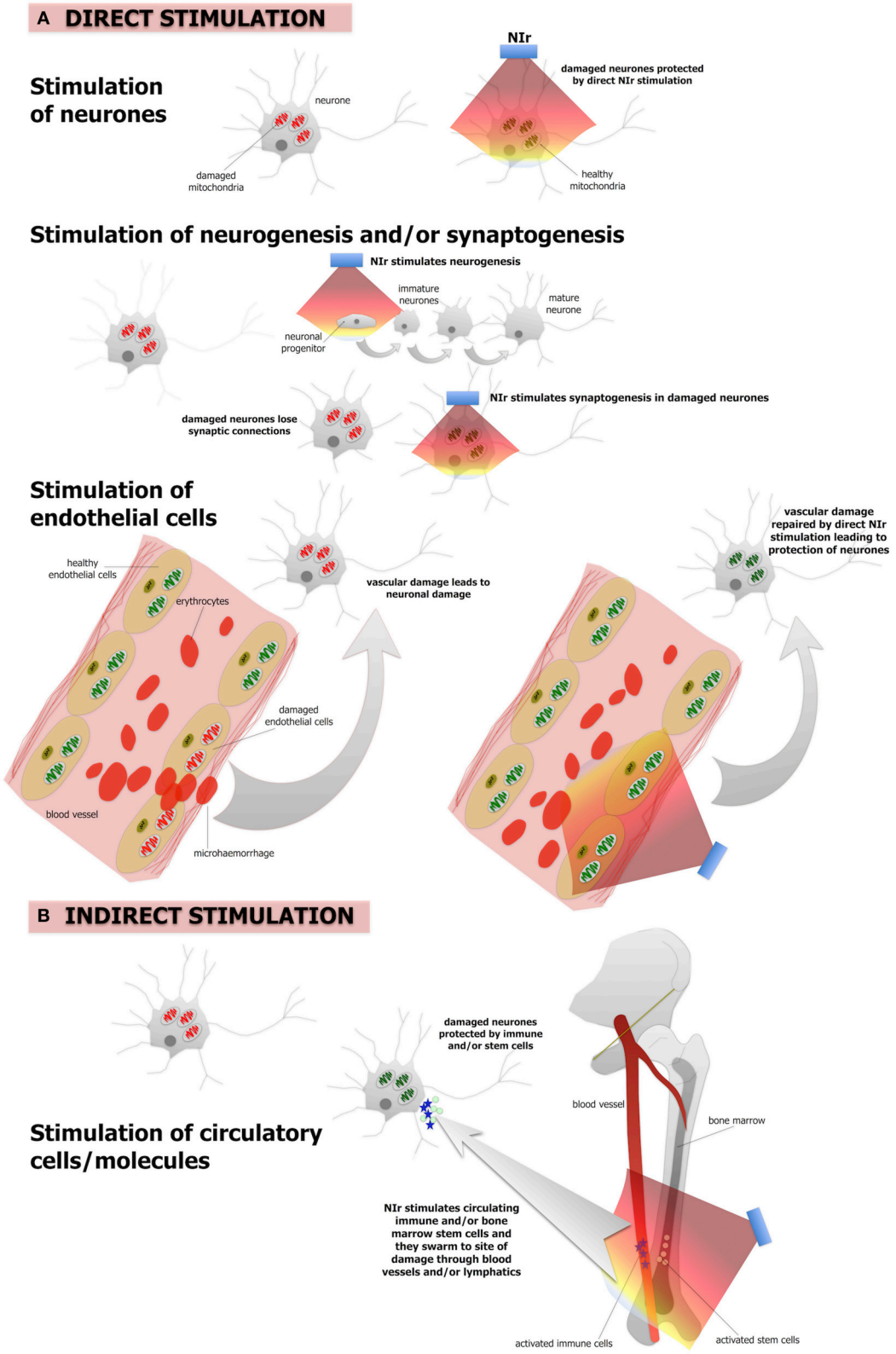


FIGURE 2 | The putative NIr protective mechanisms in the brain. (A) Direct NIr stimulation of the mitochondria of the damaged neurons or endothelial cells. This stimulation would repair the damage leading to neuronal protection. NIr may also stimulate neurogenesis in the hippocampus and/or synaptogenesis in the damaged neurons **(B)** indirect (remote) stimulation via circulating immune cells and/or bone marrow stem cells leading to neuronal protection. The latter is similar to the so-called "abscopal" effect in the treatment of cancer metastasis. We suggest that the primary mechanism is the direct effect, of neurons and/or of endothelial cells, while the systemic indirect effect forms a secondary supportive mechanism.

therapeutic doses (and even well above these doses), its impact on body tissue was overwhelmingly positive, and had a very large safety margin of application (Desmet et al., 2006; Hamblin and Demidova, 2006; Ilic et al., 2006; Zivin et al., 2009; McCarthy et al., 2010; Naeser et al., 2011, 2014; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012; Tata and Waynant, 2012; Quirk et al., 2012a,b; Moro et al., 2014). Further, there appears to be no longer-term side effects associated with NIr application; in a long-term study in rats, no adverse effects were noted after daily treatment for 12 months (McCarthy et al., 2010).

NIr THERAPY IN ALZHEIMER'S AND PARKINSON'S DISEASE PATIENTS: CAN IT WORK?

The key question that still remains is whether NIr therapy can be neuroprotective in humans. In order for maximum effect, the primary goal would be for sufficient NIr signal to reach the main zones of pathology, to elicit a protective, or reparative effect within damaged cells (and perhaps also neurogenesis); a secondary goal would be for the NIr signal to also trigger systemic neuroprotective factors, for example circulating cells or molecules (see above).

The issue of NIr reaching the zones of pathology is of most concern in humans. There are no such concerns when there are few or no tissue barriers, as in the culture dish (Eells et al., 2004; Wong-Riley et al., 2005; Liang et al., 2008; Ying et al., 2008), the retina (Natoli et al., 2010, 2013; Albarracin et al., 2013; Begum et al., 2013) or in the mouse brain (Shaw et al., 2010; Peoples et al., 2012; Moro et al., 2013; Purushothuman et al., 2013, 2014, 2015; Johnstone et al., 2014b; El Massri et al., 2015; Reinhart et al., 2015b). But can NIr be effective when there are many intervening body tissues, namely skin, thick cranium, and meninges, and brain parenchyma, as in humans?

Previous studies have estimated that NIr can be measured—through body tissues—at a distance of 20–30 mm from the transmission source (Lapchak et al., 2004; Byrnes et al., 2005; Zivin et al., 2009), albeit with a considerable dissipation of signal (DeTaboada et al., 2006; Zivin et al., 2009; Shaw et al., 2010; Abdo et al., 2013; Moro et al., 2014). For example, Moro et al. (2014) have noted that at a distance of 10 mm through brain parenchyma, the NIr signal is <1% of that emitted from the source. They estimated a 65% reduction of signal across each millimeter of brain tissue.

For Alzheimer's patients, the NIr signal—when applied from an extracranial source—should be able to reach the main zones of pathology located in the cortex, 8–10 mm below the cranium, and have therapeutic effects (**Figure 3**). Indeed, there have been several human studies reporting that NIr therapy is beneficial when the target region is in the cortex, for example in patients suffering trauma (Naeser et al., 2011, 2014), stroke (Lampl et al., 2007; Lapchak, 2010) or depression (Schiffer et al., 2009). Further, NIr therapy has been shown to improve higher-order cortical functions in healthy individuals, such as sustained attention and short-term memory (Barrett and Gonzalez-Lima, 2013), together with executive functions (Blanco et al., 2015). Hence, in Alzheimer's disease, NIr-induced neuroprotection appears feasible because the main zones of pathology are in superficial structures seemingly within reach from an extracranial source.

For Parkinson's patients, the distance from cranium to the main zone of pathology in the brainstem is much greater, being 80–100 mm (Johnstone et al., 2014a). Hence, it is unlikely that NIr signal from an extracranial source would reach the target cells (**Figure 3**); at these distances, the signal would be at best extremely weak and probably undetectable. This presents a clear limitation in the use of extracranially-applied NIr as a neuroprotective treatment in Parkinson's patients. For these reasons, we have developed a novel method of delivering effective NIr signal to deeper brainstem structures, using an intracranial optical fiber device. This device, when implanted within the brain parenchyma near the region of pathology, delivers NIr in effective doses for neuroprotection, for improved behavioral outcomes and with no toxicity to surrounding tissues in both rodents (Moro et al., 2014; Reinhart et al., 2015a) and non-human primates (Darlot et al., 2015).

We should note that in Parkinson's patients, although extracranially-delivered NIr may not reach the zones of pathology in the brainstem and hence, we argue, have limited neuroprotection, it may nevertheless provide some purely symptomatic effects. In Parkinson's disease, there is much abnormal activity in the cortex (Samuel et al., 1997; Sabatini et al., 2000; Haslinger et al., 2001), a structure that is within range of NIr signal when applied from an extracranial source (see above). NIr may help normalize this neural activity, leading to improvements in movement (Johnstone et al., 2014a). Here, the NIr therapy would impact on the abnormal neural circuitry that has resulted from the loss of dopaminergic cells, rather than on the diseased dopaminergic cells themselves. This form of NIr treatment would be purely symptomatic, rather than neuroprotective. We propose that such symptomatic treatment by NIr, namely clinical improvements without any underlying changes to the pathology, would be short-term; for long-lasting clinical improvements, we suggest that a reduction in the pathology through neuroprotection would be required. Hence, for neuroprotective and maximum therapeutic effects in Parkinson's disease, NIr would need to be applied via the intracranial optical fiber device (**Figure 3**).

In summary, there are clear indications that NIr can be an effective neuroprotective treatment for both neurodegenerative diseases, although the modes of delivery would be different; while extracranial NIr therapy would suffice for Alzheimer's disease, intracranial NIr therapy would be required for Parkinson's disease (**Figure 3**).

WHAT WOULD BE THE ADVANTAGES OF USING NIr THERAPY?

There would be several key advantages for the use of NIr therapy over current treatments for both Alzheimer's and Parkinson's disease. First and foremost, NIr has the potential to be neuroprotective. A growing body of pre-clinical evidence indicates that NIr therapy slows or stops disease pathology (Liang et al., 2008; Ying et al., 2008; Shaw et al., 2010; Peoples et al.,

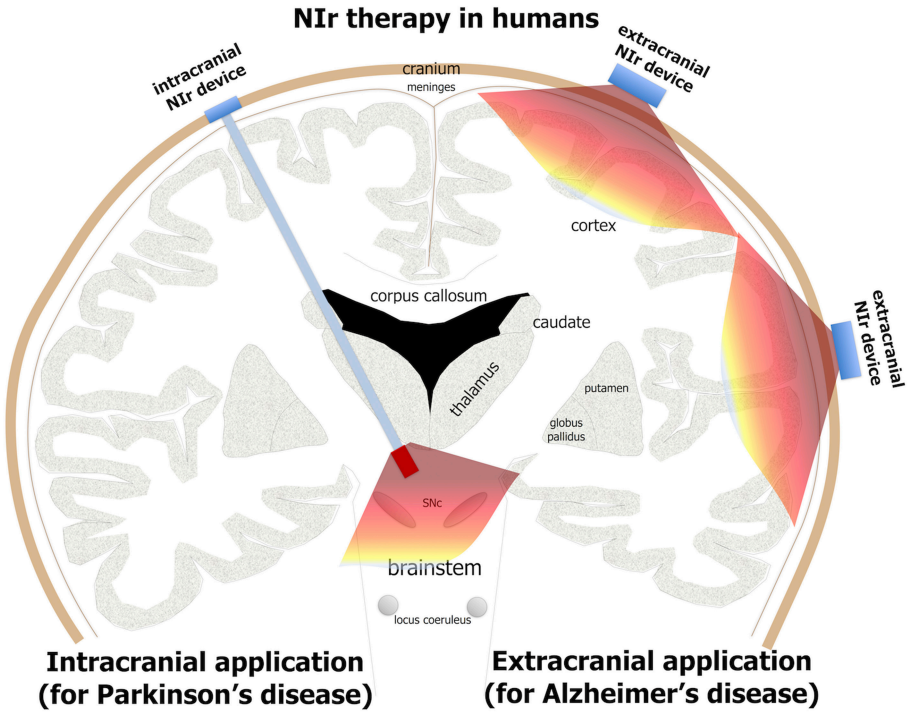


FIGURE 3 | Potential NIr applications in Alzheimer's and Parkinson's patients. For effective neuroprotection, NIr could be applied extracranially in Alzheimer's disease (e.g., in the form of a helmet) and intracranially in Parkinson's disease (e.g., in the form of an optical fiber linked to a LED or laser source). NIr would be delivered very close to the diseased cells in the neocortex (for Alzheimer's) and brainstem SNc (for Parkinson's). In Parkinson's patients selected for deep brain stimulation, the NIr optical fiber could be implanted surgically at the same time, for neuroprotection of remaining dopaminergic cells (see text for details).

2012; Moro et al., 2013; Purushothuman et al., 2013, 2014, 2015; Johnstone et al., 2014b; El Massri et al., 2015; Reinhart et al., 2015a,b). This is something that the current mainstay of treatments for both diseases—drug therapy—does not do. Second, it is safe, with no reported side effects (see above). Third, treatment would be simple. For potential neuroprotection in Alzheimer's disease, patients would apply the NIr extracranially, perhaps in the form of a helmet or a hand held device, over the entire cranium; in Parkinson's disease, patients would require a minimally invasive surgical stereotactic procedure for the insertion of a NIr optical device within the brain; in some cases, this procedure might be undertaken at the same time as stereotactic surgery for deep brain stimulation (see below). This device would be linked to a battery source and pacemaker device (as with patients receiving deep brain stimulation; Benabid et al., 2009) applying the NIr to the brainstem when required. The procedural risks would be comparable to those of single electrode deep brain stimulation.

CONCLUSIONS AND IMPLICATIONS OF FUTURE THERAPY

Although in its infancy, with the bulk of results still at the pre-clinical "proof of concept" stage, NIr therapy has the potential to develop into a safe and effective neuroprotective treatment for patients with Alzheimer's and Parkinson's disease (and presumably other neurodegenerative diseases such multiple sclerosis and amyotrophic lateral sclerosis). If NIr was applied at early stages of the disease process, for example at first diagnosis, it could potentially slow further progression by protecting neurons from death. Consequently, over time, the greater neuronal survival would lessen the clinical signs and symptoms. Further, NIr therapy—because of its lack of side-effects and neuroprotective potential—is amenable to use in conjunction with other treatments. For example, patients may have NIr therapy with a reduced dosage of drugs as a first line treatment; the potential neuroprotective effect of NIr could prolong the efficacy of the drug therapy. Further, in Parkinson's patients selected for deep brain stimulation, they may also have an NIr optical fiber implanted surgically at the same time, thereby potentially offering neuroprotection of the remaining dopaminergic cells. There is much to do in further developing this treatment, but the therapeutic possibilities are many and the potential outcomes very exciting. We await the outcomes of major clinical trials using NIr therapy on these patients with much anticipation.

AUTHOR CONTRIBUTIONS

DJ, CM, JS, AB, and JM are members of staff at their respective institutions. All authors contributed to the design and writing of the manuscript.

ACKNOWLEDGMENTS

We are forever grateful to Tenix corp, Salteri family, Fondation Philanthropique Edmond J. Safra, France Parkinson, Michael J. Fox Foundation, and the French National Research Agency (ANR Carnot Institute) for funding our work. DJ is an Early Career Fellow of the NHMRC Australia. JS was supported by the Foote Foundation and Sir Zelman Cowen Universities Fund; he is Director of CSCM Pty Ltd.

REFERENCES

Abdo, A., Ersen, A., and Sahin, M. (2013). Near-infrared light penetration profile in the rodent brain. *J. Biomed. Opt.* 18:075001. doi: 10.1117/1.JBO.18.7.075001

Albarracin, R., Natoli, R., Rutar, M., Valter, K., and Provis, J. (2013). 670 nm light mitigates oxygen-induced degeneration in C57BL/6 J mouse retina. *BMC Neurosci.* 14:125. doi: 10.1186/1471-2202-14-125

Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnr- inde. *Allgemeine Zeitschrift Psychiatr. Psych.* 146–148.

Alzheimer, A. (1911). Über eigenartige Krankheitsfälle des später- en Alters, Zbl. ges. *Neurol Psychiat.* 4, 356–385. doi: 10.1007/BF02866241

Ando, T., Xuan, W., Xu, T., Dai, T., Sharma, S. K., Kharkwal, G. B., et al. (2011). Comparison of therapeutic effects between pulsed and continuous wave 810-nm wavelength laser irradiation for traumatic brain injury in mice. *PLoS ONE* 6:e26212. doi: 10.1371/journal.pone.0026212

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

Begum, R., Powner, M. B., Hudson, N., Hogg, C., and Jeffery, G. (2013). Treatment with 670 nm light up regulates cytochrome C oxidase expression and reduces inflammation in an age-related macular degeneration model. *PLoS ONE* 8:e57828. doi: 10.1371/journal.pone.0057828

Benabid, A. L., Chabardes, S., Mitrofanis, J., and Pollak, P. (2009). Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol.* 8, 67–81. doi: 10.1016/S1474-4422(08)70291-6

Bergman, H., and Deuschl, G. (2002). Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. *Mov. Disord.* 17(Suppl. 3), S28–S40. doi: 10.1002/mds.10140

Bezard, E., Yue, Z., Kirik, D., and Spillantini, M. G. (2013). Animal models of Parkinson's disease: limits and relevance to neuroprotection studies. *Mov. Disord.* 28, 61–70. doi: 10.1002/mds.25108

Blanco, N. J., Maddox, W. T., and Gonzalez-Lima, F. (2015). Improving executive function using transcranial infrared laser stimulation. *J. Neuropsychol.* doi: 10.1111/jnp.12074. [Epub ahead of print].

Blandini, F., Nappi, G., Tassorelli, C., and Martignoni, E. (2000). Functional changes of the basal ganglia circuitry in Parkinson's disease. *Prog. Neurobiol.* 62, 63–88. doi: 10.1016/S0301-0082(99)00067-2

Blesa, J., Phani, S., Jackson-Lewis, V., and Przedborski, S. (2012). Classic and new animal models of Parkinson's disease. *J. Biomed. Biotechnol.* 2012:845618. doi: 10.1155/2012/845618

Braak, H., and Braak, E. (1995). Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* 16, 271–278. discussion: 278–284. doi: 10.1016/0197-4580(95)00021-6

Braverman, B., McCarthy, R. J., Ivankovich, A. D., Forde, D. E., Overfield, M., and Bapna, M. S. (1989). Effect of helium-neon and infrared laser irradiation on wound healing in rabbits. *Lasers Surg. Med.* 9, 50–58. doi: 10.1002/lsm.1900090111

Brettschneider, J., Tredici, K. D., Lee, V. M.-Y., and Trojanowski, J. Q. (2015). Spreading of pathology in neurodegenerative diseases: a focus on human studies. *Nat. Rev. Neurosci.* 16, 109–120. doi: 10.1038/nrn3887

Burchman, M. (2011). Using photobiomodulation on a severe Parkinson's patient to enable extractions, root canal treatment, and partial denture fabrication. *J. Laser Dent.* 19, 297–300.

Byrnes, K. R., Waynant, R. W., Ilev, I. K., Wu, X., Barna, L., Smith, K., et al. (2005). Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. *Lasers Surg. Med.* 36, 171–185. doi: 10.1002/lsm.20143

Carvey, P. M., Hendey, B., and Monahan, A. J. (2009). The blood-brain barrier in neurodegenerative disease: a rhetorical perspective. *J. Neurochem.* 111, 291–314. doi: 10.1111/j.1471-4159.2009.06319.x

Chaturvedi, R. K., and Beal, M. F. (2008). Mitochondrial approaches for neuroprotection. *Ann. N.Y. Acad. Sci.* 1147, 395–412. doi: 10.1196/annals.1427.027

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

Coppedè, F., and Migliore, L. (2015). DNA damage in neurodegenerative diseases. *Mutat. Res.* 776, 84–97. doi: 10.1016/j.mrfmmm.2014.11.010

Corti, O., and Brice, A. (2013). Mitochondrial quality control turns out to be the principal suspect in parkin and PINK1-related autosomal recessive Parkinson's disease. *Curr. Opin. Neurobiol.* 23, 100–108. doi: 10.1016/j.conb.2012.11.002

Cosgrove, J., Alty, J. E., and Jamieson, S. (2015). Cognitive impairment in Parkinson's disease. *Postgrad. Med. J.* 91, 212–220. doi: 10.1136/postgradmedj-2015-133247

Cullen, K. M., Kócsi, Z., and Stone, J. (2005). Pericapillary haem-rich deposits: evidence for microhaemorrhages in aging human cerebral cortex. *J. Cereb. Blood Flow Metab.* 25, 1656–1667. doi: 10.1038/sj.jcbfm.9600155

Cullen, K. M., Kócsi, Z., and Stone, J. (2006). Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages. *Neurobiol. Aging* 27, 1786–1796. doi: 10.1016/j.neurobiolaging.2005.10.016

Darlot, F., Moro, C., El Massri, N., Chabrol, C., Johnstone, D. M., Reinhart, F., et al. (2015). Near-infrared light is neuroprotective in a monkey model of Parkinson's disease. *Ann. Neurol.* doi: 10.1002/ana.24542. [Epub ahead of print].

De la Torre, J. C. (2004). Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *Lancet Neurol.* 3, 184–190. doi: 10.1016/S1474-4422(04)00683-0

Del Tredici, K., and Braak, H. (2013). Dysfunction of the locus coeruleus-norepinephrine system and related circuitry in Parkinson's disease-related dementia. *J. Neurol. Neurosurg. Psychiatr.* 84, 774–783. doi: 10.1136/jnnp-2011-301817

Desmet, K., Buchmann, E., Henry, M., Wong-Riley, M., Eells, J., VerHoeve, J., et al. (2009). Near-infrared light as a possible treatment option for Parkinson's disease and laser eye injury. *Proc. of SPIE* 7165, 716503-1–716503-10. doi: 10.1117/12.803964

Desmet, K. D., Paz, D. A., Corry, J. J., Eells, J. T., Wong-Riley, M. T. T., Henry, M. M., et al. (2006). Clinical and experimental applications of NIR-LED photobiomodulation. *Photomed. Laser Surg.* 24, 121–128. doi: 10.1089/pho.2006.24.121

DeTaboada, L., Ilic, S., Leichliter-Martha, S., Oron, U., Oron, A., and Streeter, J. (2006). Transcranial application of low-energy laser irradiation improves neurological deficits in rats following acute stroke. *Lasers Surg. Med.* 38, 70–73. doi: 10.1002/lsm.20256

DeTaboada, L., Yu, J., El-Amouri, S., Gattoni-Celli, S., Richieri, S., McCarthy, T., et al. (2011). Transcranial laser therapy attenuates amyloid-β peptide neuropathology in amyloid-β protein precursor transgenic mice. *J. Alzheimers Dis.* 23, 521–535. doi: 10.3233/JAD-2010-100894

Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144, 79–91. doi: 10.1016/j.cell.2010.12.016

Eells, J. T., Wong-Riley, M. T. T., VerHoeve, J., Henry, M., Buchman, E. V., Kane, M. P., et al. (2004). Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy. *Mitochondrion* 4, 559–567. doi: 10.1016/j.mito.2004.07.033

El Massri, N., Johnstone, D. M., Peoples, C. L., Moro, C., Reinhart, F., Torres, N., et al. (2015). The effect of different doses of near infrared light on

dopaminergic cell survival and gliosis in MPTP-treated mice. *Int. J. Neurosci.* doi: 10.3109/00207454.2014.994063

Exner, N., Lutz, A. K., Haass, C., and Winklhofer, K. F. (2012). Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. *EMBO J.* 31, 3038–3062. doi: 10.1038/emboj.2012.170

Farfara, D., Tuby, H., Trudler, D., Doron-Mandel, E., Maltz, L., Vassar, R. J., et al. (2015). Low-level laser therapy ameliorates disease progression in a mouse model of Alzheimer's disease. *J. Mol. Neurosci.* 55, 430–436. doi: 10.1007/s12031-014-0354-z

Farkas, E., De Jong, G. I., de Vos, R. A., Jansen Steur, E. N., and Luiten, P. G. (2000). Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. *Acta Neuropathol.* 100, 395–402. doi: 10.1007/s004010000195

Fitzgerald, M., Bartlett, C. A., Payne, S. C., Hart, N. S., Rodger, J., Harvey, A. R., et al. (2010). Near infrared light reduces oxidative stress and preserves function in CNS tissue vulnerable to secondary degeneration following partial transection of the optic nerve. *J. Neurotrauma* 27, 2107–2119. doi: 10.1089/neu.2010.1426

Fukae, J., Mizuno, Y., and Hattori, N. (2007). Mitochondrial dysfunction in Parkinson's disease. *Mitochondrion* 7, 58–62. doi: 10.1016/j.mito.2006.12.002

Galluzzi, L., Kepp, O., Trojel-Hansen, C., and Kroemer, G. (2012). Mitochondrial control of cellular life, stress, and death. *Circ. Res.* 111, 1198–1207. doi: 10.1161/CIRCRESAHA.112.268946

Garcia-Alloza, M., Robbins, E. M., Zhang-Nunes, S. X., Purcell, S. M., Betensky, R. A., Raju, S., et al. (2006). Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. *Neurobiol. Dis.* 24, 516–524. doi: 10.1016/j.nbd.2006.08.017

Gitler, A. D., Chesi, A., Geddie, M. L., Strathearn, K. E., Hamamichi, S., Hill, K. J., et al. (2009). Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat. Genet.* 41, 308–315. doi: 10.1038/ng.300

Gkotsi, D., Begum, R., Salt, T., Lascaratos, G., Hogg, C., Chau, K.-Y., et al. (2014). Recharging mitochondrial batteries in old eyes. Near infra-red increases ATP. *Exp. Eye Res.* 122, 50–53. doi: 10.1016/j.exer.2014.02.023

Goedert, M. (2015). Alzheimer's and Parkinson's diseases: the prion concept in relation to assembled Aβ, tau, and α-synuclein. *Science* 349:1255555. doi: 10.1126/science.1255555

Goedert, M., and Spillantini, M. G. (2006). A century of Alzheimer's disease. *Science* 314, 777–781. doi: 10.1126/science.1132814

Gonzalez-Lima, F., Barksdale, B. R., and Rojas, J. C. (2014). Mitochondrial respiration as a target for neuroprotection and cognitive enhancement. *Biochem. Pharmacol.* 88, 584–593. doi: 10.1016/j.bcp.2013.11.010

Grammas, P., Martinez, J., and Miller, B. (2011). Cerebral microvascular endothelium and the pathogenesis of neurodegenerative diseases. *Expert Rev. Mol. Med.* 13:e19. doi: 10.1017/S1462399411001918

Grillo, S. L., Duggett, N. A., Ennaceur, A., and Chazot, P. L. (2013). Non-invasive infra-red therapy (1072 nm) reduces β-amyloid protein levels in the brain of an Alzheimer's disease mouse model, TASTPM. *J. Photochem. Photobiol. B Biol.* 123, 13–22. doi: 10.1016/j.jphotobiol.2013.02.015

Hamblin, M. R., and Demidova, T. N. (2006). "Mechanisms of low level light therapy," in *Proceedings of SPIE-The International Society for Optical Engineering*.

Hardy, J., and Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356. doi: 10.1126/science.1072994

Haslinger, B., Erhard, P., Kämpfe, N., Boecker, H., Rummeny, E., Schwaiger, M., et al. (2001). Event-related functional magnetic resonance imaging in Parkinson's disease before and after levodopa. *Brain* 124, 558–570. doi: 10.1093/brain/124.3.558

Hausenloy, D. J., and Yellon, D. M. (2008). Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc. Res.* 79, 377–386. doi: 10.1093/cvr/cvn114

Herrup, K. (2015). The case for rejecting the amyloid cascade hypothesis. *Nat. Neurosci.* 794–799. doi: 10.1038/nn.4017

Hou, S. T., Jiang, S. X., and Smith, R. A. (2008). Permissive and repulsive cues and signalling pathways of axonal outgrowth and regeneration. *Int. Rev. Cell Mol. Biol.* 267, 125–181. doi: 10.1016/S1937-6448(08)00603-5

Ilic, S., Leichliter, S., Streeter, J., Oron, A., DeTaboada, L., and Oron, U. (2006). Effects of power densities, continuous and pulse frequencies, and number of sessions of low-level laser therapy on intact rat brain. *Photomed. Laser Surg.* 24, 458–466. doi: 10.1089/pho.2006.24.458

Jankovic, J., and Poewe, W. (2012). Therapies in Parkinson's disease. *Curr. Opin. Neurol.* 25, 433–447. doi: 10.1097/WCO.0b013e3283542fc2

Johnstone, D., Coleman, K., Moro, C., Torres, N., Eells, J., Baker, G. E., et al. (2014a). The potential of light therapy in Parkinson's disease. *ChronoPhysiology Ther.*4, 1–14. doi: 10.2147/CPT.S57180

Johnstone, D. M., El Massri, N., Moro, C., Spana, S., Wang, X. S., Torres, N., et al. (2014b). Indirect application of near infrared light induces neuroprotection in a mouse model of parkinsonism—an abscopal neuroprotective effect. *Neuroscience* 274, 93–101. doi: 10.1016/j.neuroscience.2014.05.023

Johnstone, D. M., Mitrofanis, J., and Stone, J. (2015). Targeting the body to protect the brain: inducing neuroprotection with remotely-applied near infrared light. *Neural Regen. Res.* 10, 349–351. doi: 10.4103/1673-5374.153673

Kortekaas, R., Leenders, K. L., van Oostrom, J. C. H., Vaalburg, W., Bart, J., Willemsen, A. T. M., et al. (2005). Blood-brain barrier dysfunction in parkinsonian midbrain *in vivo*. *Ann. Neurol.* 57, 176–179. doi: 10.1002/ana.20369

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. (2010). Taking a light approach to treating acute ischemic stroke patients: transcranial near-infrared laser therapy translational science. *Ann. Med.* 42, 576–586. doi: 10.3109/07853890.2010.532811

Lapchak, P. A., Wei, J., and Zivin, J. A. (2004). Transcranial infrared laser therapy improves clinical rating scores after embolic strokes in rabbits. *Stroke* 35, 1985–1988. doi: 10.1161/01.STR.0000131808.69640.b7

Liang, H. L., Whelan, H. T., Eells, J. T., and Wong-Riley, M. T. T. (2008). Near-infrared light via light-emitting diode treatment is therapeutic against rotenone- and 1-methyl-4-phenylpyridinium ion-induced neurotoxicity. *Neuroscience* 153, 963–974. doi: 10.1016/j.neuroscience.2008.03.042

Maloney, R., Shanks, S., and Maloney, J. (2010). The application of low-level laser therapy for the symptomatic care of late stage Parkinson's disease: a non-controlled, non-randomized study [Abstract]. *Am. Soc. Laser Med. Surg.* 185.

McCarthy, T. J., De Taboada, L., Hildebrandt, P. K., Ziemer, E. L., Richieri, S. P., and Streeter, J. (2010). Long-term safety of single and multiple infrared transcranial laser treatments in Sprague-Dawley rats. *Photomed. Laser Surg.* 28, 663–667. doi: 10.1089/pho.2009.2581

Merry, G., Dotson, R., Devenyi, R., Markowitz, S., and Reyes, S. (2012). Photobiomodulation as a new treatment for dry age related macular degeneration. results from the toronto and Oak ridge photobimodulation study in AMD (TORPA). *Invest. Ophthalmol. Vis. Sci.* 53, 2049–2049.

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

Moges, H., Vasconcelos, O. M., Campbell, W. W., Borke, R. C., McCoy, J. A., Kaczmarczyk, L., et al. (2009). Light therapy and supplementary Riboflavin in the SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis (FALS). *Lasers Surg. Med.* 41, 52–59. doi: 10.1002/lsm.20732

Moro, C., El Massri, N., Torres, N., Ratel, D., De Jaeger, X., Chabrol, C., et al. (2014). Photobiomodulation inside the brain: a novel method of applying near-infrared light intracranially and its impact on dopaminergic cell survival in MPTP-treated mice. *J. Neurosurg.* 120, 670–683. doi: 10.3171/2013.9.JNS13423

Moro, C., Torres, N., El Massri, N., Ratel, D., Johnstone, D. M., Stone, J., et al. (2013). Photobiomodulation preserves behaviour and midbrain dopaminergic cells from MPTP toxicity: evidence from two mouse strains. *BMC Neurosci.* 14:40. doi: 10.1186/1471-2202-14-40

Muili, K. A., Gopalakrishnan, S., Meyer, S. L., Eells, J. T., and Lyons, J.-A. (2012). Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by photobiomodulation induced by 670 nm light. *PLoS ONE* 7:e30655. doi: 10.1371/journal.pone.0030655

Naeser, M. A., Saltmarche, A., Krengel, M. H., Hamblin, M. R., and Knight, J. A. (2011). Improved cognitive function after transcranial, light-emitting diode

treatments in chronic, traumatic brain injury: two case reports. *Photomed. Laser Surg.* 29, 351–358. doi: 10.1089/pho.2010.2814

Naeser, M. A., Zafonte, R., Krengel, M. H., Martin, P. I., Frazier, J., Hamblin, M. R., et al. (2014). Significant improvements in cognitive performance post-transcranial, red/near-infrared light-emitting diode treatments in chronic, mild traumatic brain injury: open-protocol study. *J. Neurotrauma* 31, 1008–1017. doi: 10.1089/neu.2013.3244

Natoli, R., Valter, K., Barbosa, M., Dahlstrom, J., Rutar, M., Kent, A., et al. (2013). 670 nm photobiomodulation as a novel protection against retinopathy of prematurity: evidence from oxygen induced retinopathy models. *PLoS ONE* 8:e72135. doi: 10.1371/journal.pone.0072135

Natoli, R., Zhu, Y., Valter, K., Bisti, S., Eells, J., and Stone, J. (2010). Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina. *Mol. Vis.* 16, 1801–1822.

Nelson, L., and Tabet, N. (2015). Slowing the progression of Alzheimer's disease; what works? *Ageing Res. Rev.* 23(Pt B), 193–209. doi: 10.1016/j.arr.2015.07.002

Olanow, C. W., Kieburtz, K., and Schapira, A. H. V. (2008). Why have we failed to achieve neuroprotection in Parkinson's disease? *Ann. Neurol.* 64(Suppl. 2), S101–S110. doi: 10.1002/ana.21461

Oron, A., Oron, U., Chen, J., Eilam, A., Zhang, C., Sadeh, M., et al. (2006). Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits. *Stroke* 37, 2620–2624. doi: 10.1161/01.STR.0000242775.14642.b8

Oron, A., Oron, U., Streeter, J., De Taboada, L., Alexandrovich, A., Trembovler, V., et al. (2012). Near infrared transcranial laser therapy applied at various modes to mice following traumatic brain injury significantly reduces long-term neurological deficits. *J. Neurotrauma* 29, 401–407. doi: 10.1089/neu.2011.2062

Peoples, C., Spana, S., Ashkan, K., Benabid, A.-L., Stone, J., Baker, G. E., et al. (2012). Photobiomodulation enhances nigral dopaminergic cell survival in a chronic MPTP mouse model of Parkinson's disease. *Parkinsonism Relat. Disord.* 18, 469–476. doi: 10.1016/j.parkreldis.2012.01.005

Postow, M. A., Callahan, M. K., Barker, C. A., Yamada, Y., Yuan, J., Kitano, S., et al. (2012). Immunologic correlates of the abscopal effect in a patient with melanoma. *N.Engl. J. Med.* 366, 925–931. doi: 10.1056/NEJMoa1112824

Purushothuman, S., Johnstone, D. M., Nandasena, C., Mitrofanis, J., and Stone, J. (2014). Photobiomodulation with near infrared light mitigates Alzheimer's disease-related pathology in cerebral cortex—evidence from two transgenic mouse models. *Alzheimers. Res. Ther.* 6, 2. doi: 10.1186/alzrt232

Purushothuman, S., Johnstone, D. M., Nandasena, C., van Eersel, J., Ittner, L. M., Mitrofanis, J., et al. (2015). Near infrared light mitigates cerebellar pathology in transgenic mouse models of dementia. *Neurosci. Lett.* 591, 155–159. doi: 10.1016/j.neulet.2015.02.037

Purushothuman, S., Nandasena, C., Johnstone, D. M., Stone, J., and Mitrofanis, J. (2013). The impact of near-infrared light on dopaminergic cell survival in a transgenic mouse model of parkinsonism. *Brain Res.* 1535, 61–70. doi: 10.1016/j.brainres.2013.08.047

Quirk, B. J., Desmet, K. D., Henry, M., Buchmann, E., Wong-Riley, M., Eells, J. T., et al. (2012b). Therapeutic effect of near infrared (NIR) light on Parkinson's disease models. *Front. Biosci. (Elite. Ed).* 4, 818–823.

Quirk, B. J., Torbey, M., Buchmann, E., Verma, S., and Whelan, H. T. (2012a). Near-infrared photobiomodulation in an animal model of traumatic brain injury: improvements at the behavioral and biochemical levels. *Photomed. Laser Surg.* 30, 523–529. doi: 10.1089/pho.2012.3261

Recasens, A., Dehay, B., Bové, J., Carballo-Carbajal, I., Dovero, S., Pérez-Villalba, A., et al. (2014). Lewy body extracts from Parkinson disease brains trigger α-synuclein pathology and neurodegeneration in mice and monkeys: LB-induced pathology. *Ann. Neurol.* 75, 351–362. doi: 10.1002/ana.24066

Reinhart, F., El Massri, N., Darlot, F., Moro, C., Costecalde, T., Peoples, C. L., et al. (2015a). Evidence for improved behaviour and neuroprotection after intracranial application of near infrared light in a hemi-parkinsonian rat model. *J. Neurosurg.* doi: 10.3171/2015.5.JNS15735. [Epub ahead of print].

Reinhart, F., El Massri, N., Darlot, F., Torres, N., Johnstone, D. M., Chabrol, C., et al. (2015b). 810nm near-infrared light offers neuroprotection and improves locomotor activity in MPTP-treated mice. *Neurosci. Res.* 92, 86–90. doi: 10.1016/j.neures.2014.11.005

Rinne, J. O. (1993). Nigral degeneration in Parkinson's disease. *Mov. Disord.* 8(Suppl. 1), S31–S35. doi: 10.1002/mds.870080507

Rojas, J., and Gonzalez-Lima, F. (2011). Low-level light therapy of the eye and brain. *Eye Brain* 3, 49–67. doi: 10.2147/EB.S21391

Sabatini, U., Boulanouar, K., Fabre, N., Martin, F., Carel, C., Colonnese, C., et al. (2000). Cortical motor reorganization in akinetic patients with Parkinson's disease: a functional MRI study. *Brain* 123(Pt 2), 394–403. doi: 10.1093/brain/123.2.394

Samuel, M., Ceballos-Baumann, A. O., Blin, J., Uema, T., Boecker, H., Passingham, R. E., et al. (1997). Evidence for lateral premotor and parietal overactivity in Parkinson's disease during sequential and bimanual movements. A PET study. *Brain* 120(Pt 6), 963–976. doi: 10.1093/brain/120.6.963

Schapira, A. H. V., Olanow, C. W., Greenamyre, J. T., and Bezard, E. (2014). Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. *Lancet* 384, 545–555. doi: 10.1016/S0140-6736(14)61010-2

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

Shaw, V. E., Keay, K. A., Ashkan, K., Benabid, A.-L., and Mitrofanis, J. (2010). Dopaminergic cells in the periaqueductal grey matter of MPTP-treated monkeys and mice; patterns of survival and effect of deep brain stimulation and lesion of the subthalamic nucleus. *Parkinsonism Relat. Disord.* 16, 338–344. doi: 10.1016/j.parkreldis.2010.02.008

Shaw, V. E., Peoples, C., Spana, S., Ashkan, K., Benabid, A.-L., Stone, J., et al. (2012). Patterns of cell activity in the subthalamic region associated with the neuroprotective action of near-infrared light treatment in MPTP-Treated Mice. *Parkinsons. Dis.* 2012:296875. doi: 10.1155/2012/296875

Sommer, A. P., Bieschke, J., Friedrich, R. P., Zhu, D., Wanker, E. E., Fecht, H. J., et al. (2012). 670 nm laser light and EGCG complementarily reduce amyloid-β aggregates in human neuroblastoma cells: basis for treatment of Alzheimer's disease? *Photomed. Laser Surg.* 30, 54–60. doi: 10.1089/pho.2011.3073

Stone, J. (2008). What initiates the formation of senile plaques? The origin of Alzheimer-like dementias in capillary haemorrhages. *Med. Hypotheses* 71, 347–359. doi: 10.1016/j.mehy.2008.04.007

Stone, J., Johnstone, D. M., and Mitrofanis, J. (2013). "The helmet experiment in Parkinson's disease: an observation of the mechanism of neuroprotection by near infra-red light," in *9th WALT Congress* (Gold Coast, QLD).

Stone, J., Johnstone, D. M., Mitrofanis, J., and O'Rourke, M. (2015). The mechanical cause of age-related dementia (Alzheimer's disease): the brain is destroyed by the pulse. *J. Alzheimers Dis.* 44, 355–373. doi: 10.3233/JAD-141884

Swerdlow, R. H., and Khan, S. M. (2004). A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med. Hypotheses* 63, 8–20. doi: 10.1016/j.mehy.2003.12.045

Tang, X., Luo, Y.-X., Chen, H.-Z., and Liu, D.-P. (2014). Mitochondria, endothelial cell function, and vascular diseases. *Front. Physiol.* 5:175. doi: 10.3389/fphys.2014.00175

Tata, D., and Waynant, R. (2012). Laser therapy: a review of its mechanism of action and potential medical applications. *Laser Photonics. Rev.* 1, 1–12. doi: 10.1002/lpor.200900032

Taylor, R. C., Berendzen, K. M., and Dillin, A. (2014). Systemic stress signalling: understanding the cell non-autonomous control of proteostasis. *Nat. Rev. Mol. Cell Biol.* 15, 211–217. doi: 10.1038/nrm3752

Tierney, T. S., Vasudeva, V. S., Weir, S., and Hayes, M. T. (2013). Neuromodulation for neurodegenerative conditions. *Front. Biosci. (Elite. Ed).* 5, 490–499. doi: 10.2741/E630

Trimmer, P. A., Schwartz, K. M., Borland, M. K., De Taboada, L., Streeter, J., and Oron, U. (2009). Reduced axonal transport in Parkinson's disease cybrid neurites is restored by light therapy. *Mol. Neurodegener.* 4:26. doi: 10.1186/1750-1326-4-26

Tuby, H., Maltz, L., and Oron, U. (2011). Induction of autologous mesenchymal stem cells in the bone marrow by low-level laser therapy has profound beneficial effects on the infarcted rat heart. *Lasers Surg. Med.* 43, 401–409. doi: 10.1002/lsm.21063

van Eersel, J., Ke, Y. D., Liu, X., Delerue, F., Kril, J. J., Götz, J., et al. (2010). Sodium selenate mitigates tau pathology, neurodegeneration, and functional deficits in Alzheimer's disease models. *Proc. Natl. Acad. Sci. U.S.A.* 107, 13888–13893. doi: 10.1073/pnas.1009038107

Vos, M., Lovisa, B., Geens, A., Morais, V. A., Wagnières, G., van den Bergh, H., et al. (2013). Near-infrared 808 nm light boosts complex IV-dependent respiration and rescues a Parkinson-related pink1 Model. *PLoS ONE* 8:e78562. doi: 10.1371/journal.pone.0078562

Whelan, H. T., DeSmet, K. D., Buchmann, E. V., Henry, M. M., Wong-Riley, M., Eells, J. T., et al. (2008). Harnessing the cell's own ability to repair and prevent neurodegenerative disease. *SPIE Newsroom* 24, 1–3. doi: 10.1117/2.1200802.1014

Wong-Riley, M. T. 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

Xuan, W., Agrawal, T., Huang, L., Gupta, G. K., and Hamblin, M. R. (2015). Low-level laser therapy for traumatic brain injury in mice increases brain derived neurotrophic factor (BDNF) and synaptogenesis. *J. Biophoton.* 8, 502–511. doi: 10.1002/jbio.201400069

Xuan, W., Vatansever, F., Huang, L., and Hamblin, M. R. (2014). Transcranial low-level laser therapy enhances learning, memory, and neuroprogenitor cells after traumatic brain injury in mice. *J. Biomed. Opt.* 19:108003. doi: 10.1117/1.JBO.19.10.108003

Xuan, W., Vatansever, F., Huang, L., Wu, Q., Xuan, Y., Dai, T., et al. (2013). Transcranial low-level laser therapy improves neurological performance in traumatic brain injury in mice: effect of treatment repetition regimen. *PLoS ONE* 8:e53454. doi: 10.1371/journal.pone.0053454

Yetgin, T., Manintveld, O. C., Groen, F., Tas, B., Kappetein, A. P., van Geuns, R.-J., et al. (2012). The emerging application of remote ischemic conditioning in the clinical arena. *Cardiol. Rev.* 20, 279–287. doi: 10.1097/CRD.0b013e31826c15aa

Ying, R., Liang, H. L., Whelan, H. T., Eells, J. T., and Wong-Riley, M. T. T. (2008). Pretreatment with near-infrared light via light-emitting diode provides added benefit against rotenone- and MPP+-induced neurotoxicity. *Brain Res.* 1243, 167–173. doi: 10.1016/j.brainres.2008.09.057

Zhao, G., Guo, K., and Dan, J. (2003). 36 case analysis of Parkinson's disease treated by endonasal low energy He-Ne laser. *Acta Acad. Med. Qingdao Univ. (Chinese)* 39, 398.

Zivin, J. A., Albers, G. W., Bornstein, N., Chippendale, T., Dahlof, B., Devlin, T., et al. (2009). Effectiveness and safety of transcranial laser therapy for acute ischemic stroke. *Stroke* 40, 1359–1364. doi: 10.1161/STROKEAHA.109.547547

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 Neuroscience

REVIEW
published: 18 April 2016
doi: 10.3389/fnins.2016.00157

Value and Efficacy of Transcranial Direct Current Stimulation in the Cognitive Rehabilitation: A Critical Review Since 2000

Davide Cappon[1]*, *Marjan Jahanshahi*[2]* and *Patrizia Bisiacchi*[1]*

[1] Department of General Psychology, Center for Cognitive Neuroscience, University of Padova, Padua, Italy, [2] Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, London, UK

Non-invasive brain stimulation techniques, including transcranial direct current stimulation (t-DCS) have been used in the rehabilitation of cognitive function in a spectrum of neurological disorders. The present review outlines methodological communalities and differences of t-DCS procedures in neurocognitive rehabilitation. We consider the efficacy of tDCS for the management of specific cognitive deficits in four main neurological disorders by providing a critical analysis of recent studies that have used t-DCS to improve cognition in patients with Parkinson's Disease, Alzheimer's Disease, Hemi-spatial Neglect, and Aphasia. The evidence from this innovative approach to cognitive rehabilitation suggests that tDCS can influence cognition. However, the results show a high variability between studies both in terms of the methodological approach adopted and the cognitive functions targeted. The review also focuses both on methodological issues such as technical aspects of the stimulation (electrode position and dimension; current intensity; duration of protocol) and on the inclusion of appropriate assessment tools for cognition. A further aspect considered is the optimal timing for administration of tDCS: before, during or after cognitive rehabilitation. We conclude that more studies using common methodology are needed to gain a better understanding of the efficacy of tDCS as a new tool for rehabilitation of cognitive disorders in a range of neurological disorders.

Keywords: transcranial direct current stimulation (tDCS), cognitive rehabilitation, neurocognitive disorders, unilateral neglect, Aphasia, Parkinson's disease (PD), Alzheimer's disease (AD)

OPEN ACCESS

Edited by:
Raymond Scott Turner, Georgetown University, USA

Reviewed by:
Robert Paul, University of Missouri, USA
Yixuan Ku, University of California, San Francisco, USA

***Correspondence:**
Davide Cappon
davide.balos.cappon@gmail.com;
Marjan Jahanshahi
m.jahanshahi@ucl.ac.uk;
Patrizia Bisiacchi
patrizia.bisiacchi@unipd.it

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

Received: *11 November 2015*
Accepted: *27 March 2016*
Published: *18 April 2016*

Citation:
Cappon D, Jahanshahi M and Bisiacchi P (2016) Value and Efficacy of Transcranial Direct Current Stimulation in the Cognitive Rehabilitation: A Critical Review Since 2000. Front. Neurosci. 10:157. doi: 10.3389/fnins.2016.00157

INTRODUCTION

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) presented a structure for the diagnosis of neurocognitive disorders. It differentiated "mild" and "major" neurocognitive disorders which may be due to diverse etiologies (Sachdev et al., 2014). Neurocognitive disorders (NCD) are described by decline from a premorbidly reached level of cognitive functioning. The NCD category includes distinct clinical characteristics in which the primary clinical deficit is acquired and is in cognitive function. The prevalence of NCD increases exponentially with age and at the present moment there are no effective pharmacological treatments for these cognitive deficits. Thus, in the context of rapid population aging worldwide, it becomes important to find new strategies to deal with NCD. Specifically, Parkinson's Disease, Alzheimer's

Disease, Vascular Disease are particularly debilitating conditions with cognitive sequelae which have increased in prevalence over the years and are a burden for society.

In the last decades non-invasive brain stimulation (NIBS) techniques have rapidly become an important approach as potential therapeutic tools to improve the outcome of cognitive rehabilitation in patients affected by stroke, neurodegenerative disorders, or psychiatric diseases (Rossini et al., 2015). The two most commonly used techniques for non-invasive brain stimulation (NIBS) are transcranial magnetic stimulation (TMS) (including single pulse TMS, repetitive(rTMS) and theta burst TMS) and transcranial electrical stimulation (tES) (including transcranial direct current stimulation (tDCS), high-definition tDCS, transcranial alternating current stimulation (tACS), transcranial random noise stimulation (tRNS; Peterchev et al., 2012). NIBS apply different electromagnetic principles to non-invasively influence neural activity: TMS involves neurostimulation and neuromodulation of neural tissue, including cerebral cortex, spinal roots, and cranial and peripheral nerves, whereas tES is a purely neuromodulatory intervention (Rothwell, 1997). In other words, tDCS using weak current, unlike TMS is not able to discharge resting axons to produce action potentials, although it can be used to modulate cortical excitability. In tDCS surface electrodes (anode and cathode) inject low amplitude direct current (0.5–2 mA) through the scalp and brain. In early studies tDCS was combined with TMS to investigate modification of primary motor cortex cortical excitability by recording motor evoked potentials (MEPs) (Priori et al., 1998; Nitsche and Paulus, 2000). The mechanisms are not yet clear but presumably the current induces changes in the resting membrane potential of neurons. These changes appear to be polarity specific with anodal depolarization and cathodal hyperpolarization of resting membrane potential (Nitsche and Paulus, 2000; Nitsche et al., 2003). Some studies have been performed in order to understand the physiological mechanisms and it seems that neuroplastic after-effects are N-methyl-D-aspartate (NMDA) receptor dependent (Liebetanz et al., 2002; Nitsche et al., 2004). In fact, it has been shown that the effects can be modified, prolonged or even reversed by drugs acting on the central nervous system (Stagg and Nitsche, 2011). It is noteworthy that NMDA receptors have been reported to have a critical role in synaptic plasticity and long term potentiation (LTP) affecting learning and memory. However, these studies are in the motor domain and it is still not clear to what extent these findings are transferable to other areas of the brain.

Nonetheless, during the last decade a growing body of experimental work have extensively explored the effects of tDCS on brain areas other than the primary motor cortex with encouraging results. These studies have demonstrated significant effects of tDCS on cognitive processes as assessed by a variety of cognitive tasks not only in healthy participants but also in clinical populations. As a consequence, there has been growing interest to use tDCS as a safe and relatively low-cost technique for neurological and neuropsychological rehabilitation as demonstrated by recent reviews of this topic for various cognitive deficits (Fasotti and van Kessel, 2013; Elder and Taylor, 2014; Flöel, 2014; de Aguiar et al., 2015).

The present paper intends to review recent evidence of tDCS for neurocognitive rehabilitation. Our first aim is to discuss the key issues that have emerged from the studies that have demonstrated potential therapeutic applications of t-DCS in neurocognitive disorders. Four clinical conditions will be considered namely Parkinson's Disease, Alzheimer's Disease, Unilateral Hemispatial Neglect and Aphasia. The second aim is give the reader an illustration of the methodological communalities and differences of the studies published so far. Finally, we propose a framework of factors that should be taken into account for an increased understanding of the functional role of tDCS in improving symptoms in patients suffering from neurocognitive disorders.

METHODS

Searches were conducted using the online database Pubmed and manual searches of references in relevant papers. The review period was from 2000 to 2015. Articles were identified by carrying out a comprehensive review of published research papers that have used tDCS to improve cognition in patients with Parkinson's Disease, Alzheimer's Disease, Unilateral Hemispatial Neglect and Aphasia. Search terms were ((Parkinson's Disease[Title/Abstract]) AND tDCS [Title/Abstract]) AND (cognitive OR memory OR executive functions OR semantic fluency); ((Alzheimer's Disease or Alzheimer [Title/Abstract]) AND tDCS [Title/Abstract]) AND ((rehabilitation OR cognitive OR memory OR working memory OR attention OR cognition)); ((Neglect[Title/Abstract]) AND tDCS OR transcranial direct current[Title/Abstract]) AND (rehabilitation OR visuospatial attention); ((Aphasia[Title/Abstract]) AND tDCS OR transcranial direct current [Title/Abstract]) AND (rehabilitation OR language OR anomia). The initial search identified 122 titles and abstracts. The abstracts and full paper were reviewed to eliminate articles according to the following exclusion criteria: (1) review articles (2) papers that did not include patients with a diagnosis of Parkinson's Disease, Alzheimer's Disease, Hemi-spatial Neglect or Aphasia (3) studies that did not focus on cognitive abilities (4) the investigation of other non-motor symptoms or other neuropsychiatric symptoms that were not specified in this review. In total 34 articles met our inclusion criteria (**Figure 1**).

APPLICATION OF t-DCS FOR COGNITIVE REHABILITATION

In this section we will review evidence on the use of tDCS for cognitive rehabilitation in patients with Parkinson's Disease, Alzheimer's Disease, Hemispatial Neglect or Aphasia. For each disorder we start with a concise description of the main features of cognitive deficit, followed by a detailed review of the studies. The methodological details of parameters of stimulation used in these studies are presented in **Table 1**. Patient characteristics, experimental design, cognitive domains targeted, tasks used

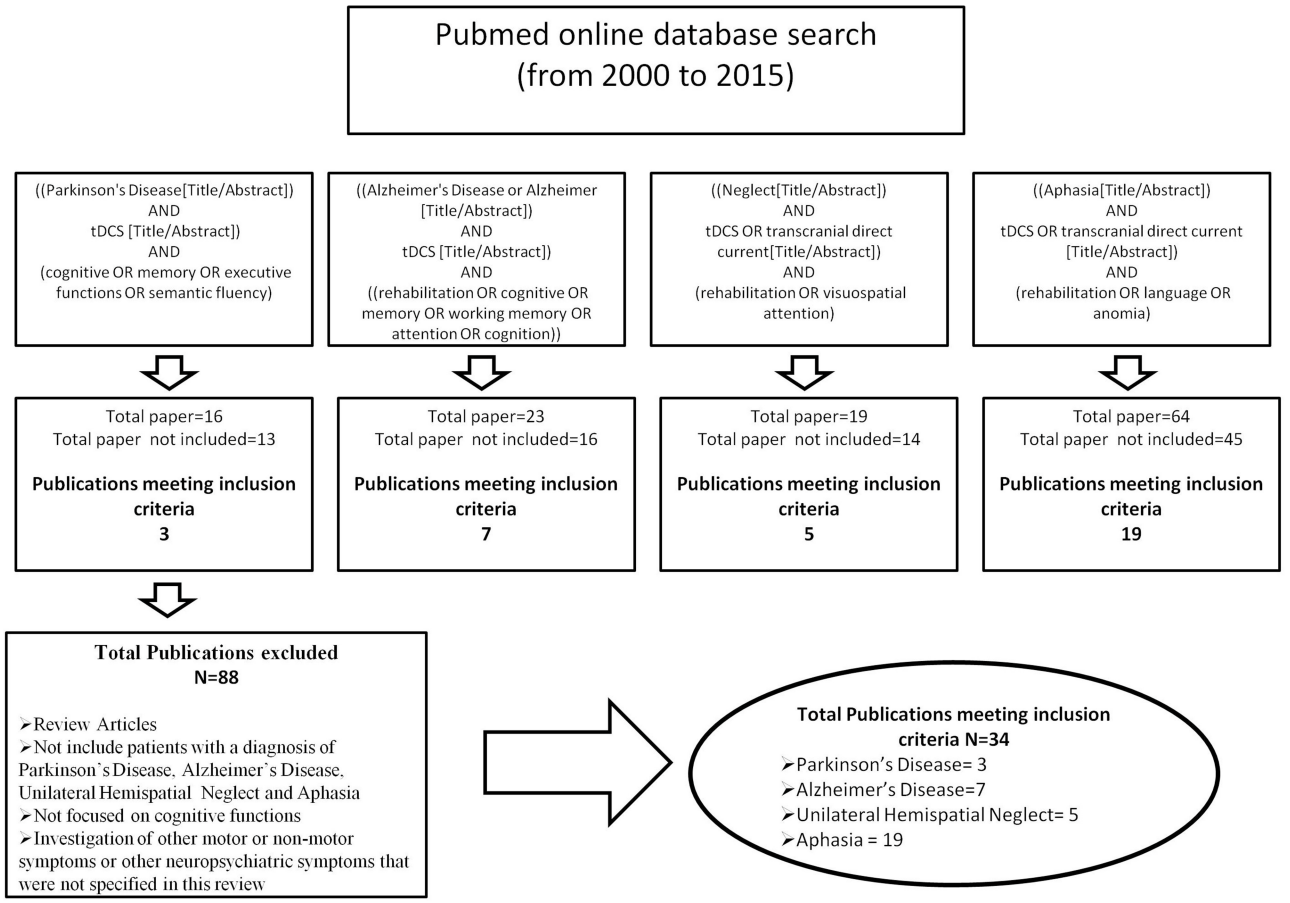


FIGURE 1 | Database key words search strategy.

as outcome measures and main results are summarized in **Table 2**. In **Figures 2A–D** is a visual representation of the electrode montage which could be useful to compare the studies.

Parkinson's Disease (PD)

PD is a chronic and progressive neurodegenerative disorder. PD affects one out of 100 people who are aged older than 60 years in industrialized countries. PD primarily affects dopamine producing neurons in an area of the brain called the substantia nigra pars compacta. The loss of these specific neurons causes motor symptoms characterized by resting tremor, rigidity, bradykinesia and postural instability. These symptoms are the basis for a diagnosis of PD. Mild Neurocognitive disorders (mNCDs) are also common in PD even in the earliest stages of the disease and significantly impair the quality of life (QoL) of patients (Schrag et al., 2000) and caregivers (Schrag et al., 2006). mNCDs in PD include fronto-striatal syndrome due to dopaminergic shortage and include deficits of executive functions, such as planning, mental flexibility and working memory (Kehagia et al., 2010; Dirnberger and Jahanshahi, 2013). As the disease progresses, cognitive deficits spread into other cognitive domains and may deteriorate into major Neurocognitive Disorders interfering with independence in everyday activities (Litvan et al., 2011).

To date, in patients with idiopathic PD three studies have evaluated the efficacy of tDCS on executive functions. Boggio et al. (2006) investigated tDCS effects on 18 patients (mean AGE = 61 45–71; mean MMSE = 24.4) diagnosed idiopathic PD using a three-back working memory task. Patients performed the task during anodal tDCS (A-tDCS) on left dorsolateral prefrontal cortex (L-DLPFC), A-tDCS on motor cortex (M1) and sham. In addition, the authors tested whether the effects depended on the intensity of stimulation; performing a control experiment with different intensities a constant current of 1 mA or 2 mA that was applied for 20 min. The authors found that after a single session of 2 mA A-tDCS over the L-DLPFC patients improved in the accuracy of the 3-back memory task. The other stimulation conditions (sham, 1 mA A-tDCS on L-DLPFC or A-tDCS on M1) were not effective. Their results were recently reinforced by a controlled cross-over, tDCS combined fMRI single session study of Pereira and colleagues. In this study (Pereira et al., 2013) 16 patients (mean

TABLE 1 | Parameters of stimulation in studies of t-DCS for cognitive rehabilitation in Parkinson's disease, Alzheimer's disease, unilateral spatial neglect or aphasia.

Author and year	Electrodes position		Electrodes dimension (cm^2)		Current			Duration (min)	Sessions	Time of stimulation
	"Active"	"Reference"	"Active"	"Reference"	Intensity (mA)	Density (mA/cm^2)				
						"Active"	"Reference"			
PARKINSON'S DISEASE										
Boggio et al. (2006)	(1)Anode L-DLPFC (2)AnodeM1	Cathode C.F. Cathode C.F.	35	35	1 2	0.029 0.057	0.029 0.057	20	1	Online partially during WM task
Pereira et al. (2013)	(1)Anode L-DLPFC (2)Anode L-TPC	Cathode C.F. Cathode C.F.	35	35	2	0.057	0.057	20	1	Rest
Doruk et al. (2014)	(1)Anode L-DLPFC (2)Anode R-DLPFC	Cathode C.F. Cathode C.F.	35	35	2	0.057	0.057	20	10 (2 weeks)	Rest
ALZHEIMER'S DISEASE										
Ferrucci et al. (2008)	(1)Anode L-TPC R-TPC bilaterally (2)Cathode L-TPC R-TPC bilaterally	R-Deltoide	25 25	25	1.5	0.060	0.060	15	1	Rest
Boggio et al. (2008)	(1)Anode L-DLPFC (2)Anode L-TC (T7)	Cathode C.F. Cathode C.F.	35	35	2	0.057	0.057	30	1	Online
Boggio et al. (2012)	Anode L-TPC R-TPC bilaterally (T3-T4)	R-Deltoide	35 35	64	2	0.057 0.057	0.031	30	5	Rest
Cotelli et al. (2014)	Anode L-DLPFC	R-Deltoide	25	60	2	0.080	0.033	25	10 (2 weeks)	Online
Khedr et al. (2014)	(1)Anode L-DLPFC (2)Cathode L-DLPFC	Cathode C.F. Anode C.F.	24	100	2	0.083	0.010	25	10	Rest
Suemoto et al. (2014)	Anode L-DLPFC	Cathode C.F.	35	35	2	0.057	0.057	20	6 (2weeks)	Rest
Penolazzi et al. (2014)	Anode L-DLPFC	Cathode C.F.	35	100	2	0.057	0.010	20	10 (2 weeks)	Rest
UNILATERAL HEMISPATIAL NEGLECT										
Ko et al. (2008)	Anode R-PPC (P4)	Cathode C.F.	25	25	2	0.080	0.080	20	1	Rest
Sparing et al. (2009)	(1)Anode R-PPC (P4) (2) Cathode L- PPC (P3)	Cathode Cz	25	35	1	0.040	0.029	10	1	Rest
Sunwoo et al. (2013)	(1)Dual-mode, Anode R- PPC Cathode L- PPC; (2)Single-mode, R Anode R- PPC	(1)Cathode C.F.; Anode C.F. (2)Cathode C.F.	25	25	1	0.040	0.040	20	1	Rest
Brem et al. (2014)	Anode R-PPC	Cathode L-PPC	35	35	1	0.029	0.029	20	5	Online
Smit et al. (2015)	Anode R-PPC	Cathode L-PPC	n.a	n.a	2	n.a	n.a	20	5	Rest
APHASIA										
Monti et al. (2008)	(1)Anode Broca's area (between T3-Fz and F7-Cz) Cathode Broca's area (between T3-Fz and F7-Cz) (2) Cathode occipital areas (2 cm over the inion)	Cathode R Deltoide Anode R Deltoide Anode R Deltoide	35	35	2	0.057	0.057	10	1	Rest
Baker et al. (2010)	Anode LFC Individually determined (fMRI task)	Cathode R-Deltoide	25	25	1	0.040	0.040	20	5	Online computerized anomia training
Fiori et al. (2011)	Anode L Wernicke's area	Cathode C.F.	35	35	1	0.029	0.029	20	5	Online picture-naming task

(Continued)

TABLE 1 | Continued

Author and year	Electrodes position		Electrodes dimension (cm^2)		Current			Duration (min)	Sessions	Time of stimulation
	"Active"	"Reference"	"Active"	"Reference"	Intensity (mA)	Density (mA/cm^2)				
						"Active"	"Reference"			
Flöel et al. (2011)	(1)Anode R-TPC (Talairach) (2)Cathode R-TPC (Talairach)	(1)Cathode C.F. (2)Anode C.F.	35	100	1	0.029	0.010	20		Online during the first 20 min anomia training
Fridriksson et al. (2011)	Anode LPC Individually determined	Cathode C.F.	25	25	1	0.040	0.040	20	5	Online computerized anomia treatment
Jung et al. (2011)	Cathode R BA 45(between T4-Fz and F8-Cz)	Anode C.F.	35	35	1	0.029	0.029	20	10	Online speech therapy
Kang et al. (2011)	Cathode R- Broca's area	Anode C.F.	25	25	2	0.080	0.080	20	5	Online word-retrieval training
Vines et al. (2011)	Anode R IFG, (2.5 cm posterior to F8)	Cathode C.F.	16	30	1.2	0.075	0.040	20	3	Online Melodic intonation therapy
You et al. (2011)	(1)Anode L sTG (CP5) (2)Cathode R sTG (CP6)	(1)Cathode C.F. (2)Anode C.F.	35	35	2	0.057	0.057	30	10 (2 weeks)	Online speech and language therapy
Lee et al. (2013)	(1)single, Anode L IFG (F7) (2) dual, Anode L IFG (F8) Cathode R IFG	(1)Cathode L buccinator muscle (2)Cathode L buccinator muscle Anode R buccinator muscle	25	25	2	0.080	0.080	30	1	Online speech therapy during the last 15 min
Polanowska et al. (2013)	Anode L-Broca's area (T3-Fz and F7-Cz)	Cathode C.F.	35	35	1	0.029	0.029	10	15	Rest (followed by 45 min language training)
Rosso et al. (2014)	Cathode R Broca's area (Individually determined,neuronavigator)	Anode C.F.	35	35	1	0.029	0.029	15	1	Rest
Santos et al. (2013)	Cathode M1of unaffected side (C3/C4)	Anode C.F.	35	35	2	0.057	0.057	20	10	Rest
Volpato et al. (2013)	Anode L-Broca's Area (between T3-Fz and F7-Cz)	Cathode C.F.	35	35	2	0.057	0.057	20	10 (2 weeks)	Rest
Marangolo et al. (2014)	(1)Anode L Wernicke's area (2)Anode L Broca's area	Cathode C.F.	35	35	1	0.029	0.029	20	5	Offline training for action naming
Vestito et al. (2014)	LF perilesional site (between T3-Fz and F7-Cz)	Cathode C.F.	25	25	1.5	0.060	0.060	20	10 (2weeks)	Online naming training
Manenti et al. (2015)	Anode L-DLPFC (F3)	Cathode R-DLPFC (F4)	35	35	2	0.057	0.057	25	20	Online verb anomia training
Shah-Basak et al. (2015)	Individualized on the individual response (1)Anode L-IFG(F3) (2)Cathode L-IFG (F3) (3)Anode R-IFG (F4) (4)Cathode R-IFG (F4)	Controlateral Mastoide	25	25	2	0.080	0.080	20	10 (2weeks)	Online picture-naming task
Wu et al. (2015)	Anode L Wernicke's area (between T3-P3 and C3-T5)	Cathode unaffected shoulder	25	25	1.2	0.048	0.048	20	20	Rest

BA, Broadmann area; C.F., controlateral forehead; L, left; R, right; LF, left-frontal; DLPFC, dorso lateral prefrontal cortex; IFG, inferior frontal gyrus; mA, milliAmpere; min, minutes; M1, primary motor cotex; TC, temporal cortex; TPC, temporo-parietal cortex; PPC, posterior parietal cortex; sTG, superior temporal gyrus.

TABLE 2 | Patient characteristics, experimental design, cognitive domains, tasks used as outcome measures and main results of studies which used tDCS for cognitive rehabilitation in Parkinson's disease, Alzheimer' disease, unilateral neglect, or aphasia.

Author and year	Sample	Experimental design	Target cognitive domain	Neuropsychological measures	Main results
PARKINSON'S DISEASE					
Boggio et al. (2006)	Idiopathic Parkinson $N = 18$	Randomized controlled cross over	Working Memory	Computerized 3 n-back task	A-tDCS (2mA) of left DLPFC improved accuracy as compared with the other conditions
Pereira et al. (2013)	Idiopathic Parkinson $N = 16$	Randomized controlled cross over	Executive Functions	Computerized verbal fluency task (phonemic fluency, semantic fluency)	A-tDCS L-DLPFC improved performance on the phonemic fluency task as compared L-TPC A-tDCS
Doruk et al. (2014)	Idiopathic Parkinson $N = 18$	Randomized controlled between subject	Abstract Reasoning Executive Functions Selective Attention Visuo-spatial abilities Working Memory	TMT A-B, WCST, DIGSP-BW- FW, HPVOT,CPM, Stroop Test	Both left and right DLPFC A-tDCS groups improved at the 1-month follow-up in TMT-B as compared with sham; no changes in WSCT, PCL, WM, CPM, HVOT,STROOP, and Digit Span
ALZHEIMER'S DISEASE					
Ferrucci et al. (2008)	AD $N = 10$ (criteria MMSE≥20)	Randomized controlled cross over	Episodic Memory Attention	word recognition task visual attention task	Improvement of accuracy of word recognition memory after A-tDCS; no changes in visual attention
Boggio et al. (2008)	AD $N = 10$ (criteria 12<MMSE<25)	Randomized controlled cross over	Executive Functions Selective Attention Working Memory	visual recognition, DIGSP-BW- FW, Stroop	Improvement of visual recognition memory after both temporal and prefrontal A-tDCS;no changes in stroop and digit span
Boggio et al. (2012)	AD $N = 15$ (MMSE>15)	Randomized controlled cross over	Executive Functions Selective Attention Working Memory Global Functioning	Computerized recognition memory task, visual attention task, ADAS-cog, MMSE	Improvement of visual recognition memory after A-tDCS persist for 4 weeks; no changes in other measures
Cotelli et al. (2014)	AD $N = 36$ (Mild to moderate AD)	Randomized controlled between subject	Attention Episodic Memory Executive Functions Functional status Language Praxia Semantic Memory	Computerized Face-name association task, MMSE, ADL, IADL, Picture naming task, BADA, RBMT, RAVLT, ROCFC, TMT A-B	Both sham and real tDCS led to improvement in FNAT performance; persist 12 weeks only for the placebo group. no changes in other measures
Khedr et al. (2014)	AD $N = 34$ (criteria 12<MMSE<23)	Randomized controlled between subject	Global Functioning Intelligence	MMSE,WAIS-III	both A-tDCS and C-tDCS improved MMSE in contrast to sham; only C-tDCS improved performance in the subscales of WAIS-III
Suemoto et al. (2014)	AD $N = 40$ (criteria 10<MMSE<20)	Randomized controlled cross over	Global Functioning	MMSE,ADAS-COG	No effects of repetitive A-tDCS L-DLPFC on cognitive measure tested
Penolazzi et al. (2014)	AD $N = 1$ (MMSE=23)	Single-case controlled cross over	Episodic Memory Executive Functions Working Memory Selective Attention Praxia Visuo-spatial abilities	Computerized word and visual recognition, verbal fluency, CPT, ENB-2	A-tDCS+CT condition had few effects on the cognitive measures; A-tDCS+CT induced a stability of the patient's global cognitive functioning lasting 3 months as compare to sham+CT condition
UNILATERAL HEMISPATIAL NEGLECT					
Ko et al. (2008)	Subacute stroke Neglect $N = 15$	Randomized controlled Cross-over	Neglect Visuo-spatial search Attention	Line bisection, letter and figure cancelation	A-tDCS compare to sham improved both neglect tests performance.
Sparing et al. (2009)	Subacute and chronic stroke Neglect $N = 10$	Randomized controlled Cross-over	Neglect Visuo-spatial search Attention	Computerized line bisection and visual detection tasks	C-tDCS over the unlesioned hemisphere and A-tDCS over lesioned hemisphere reduced symptoms of visuospatial neglect
Sunwoo et al. (2013)	Chronic stroke $N = 10$	Randomized controlled cross over	Neglect Visuo-spatial search Attention	Line Bisection test, Star cancelation test	Both dual- and the single-mode tDCS improved performance in the line bisection test as compare to sham. No changes in the star cancelation test

(Continued)

TABLE 2 | Continued

Author and year	Sample	Experimental design	Target cognitive domain	Neuropsychological measures	Main results
Brem et al. (2014)	Subacute stroke Neglect $N = 1$	Single-case controlled double-blind	Neglect Visuo-spatial search Attention	TAP,NET,ADL	Biparietal tDCS stimulation, improved covert attention allocation toward left-sided invalid stimuli, line bisection and copying as compared to sham stimulation
Smit et al. (2015)	Chronic stroke $N = 5$	Double-blind randomized controlled cross-over	Neglect Visuo-spatial search Attention	BIT	No A-tDCS effects were observed for the BIT subtests
APHASIA					
Monti et al. (2008)	Chronic stroke Non-fluent aphasia $N = 8$ (Broca's $N = 4$; Global $N = 4$)	Randomized controlled Cross-over	Language (naming abilities)	Computerized overt picture naming task	C-tDCS improved accuracy in picture naming as compare to sham and A-tDCS
Baker et al. (2010)	Chronic stroke $N = 10$ (Anomic aphasia $N = 6$; Broca's aphasia $N = 4$)	Randomized controlled Cross-over	Language (naming abilities)	Computerized picture-word matching task	A-tDCS improved naming accuracy as compared to sham; improvement persist after 1 week
Fiori et al. (2011)	Chronic stroke $N = 3$ Non-fluent aphasia	Double-blind randomized controlled cross-over	Language (naming abilities)	Object naming	A-tDCS improved naming accuracy and RTs as compared to sham; improvement persist after 3 weeks in two patients
Flöel et al. (2011)	Chronic stroke Aphasia (type n.a.) $N = 12$	Randomized controlled Cross-over	Language (naming abilities)	Computerized naming task	Both A-tDCS and C-tDCS improved naming accuracy; effects of A-tDCS persist after 2 weeks
Fridriksson et al. (2011)	Chronic stroke Fluent aphasia $N = 8$	Randomized controlled Cross-over	Language (naming abilities)	Verbal word-picture matching task	A-tDCS improved naming RTs as compared to sham; improvement persist after 3 weeks
Jung et al. (2011)	Acute, subacute, chronic stroke Aphasia $N = 37$	Pretest-Posttest Design (no sham control group)	Language	Aphasia quotient and Korean Western Aphasia Battery	C-tDCS improved aphasia symptoms
Kang et al. (2011)	Chronic stroke Aphasia $N = 10$ Global ($n = 3$), Broca's ($n = 4$), anomic (=2), tanscortical motor ($n = 1$)	Double-Blind Randomized controlled Cross-over	LF (naming abilities)	Naming, picture-word Matching task	C-tDCS improved naming accuracy as compared to sham
Vines et al. (2011)	Chronic stroke Moderate to severe Non-fluent aphasia $N = 6$	Randomized controlled Cross-over	Language (naming abilities) (verbal fluency)	Verbal fluency tasks, picture description and picture naming.	A-tDCS improved speech fluency as compared to sham
You et al. (2011)	Subacute stroke Global Aphasia $N = 21$	Randomized controlled between subject	Language (Auditory Verbal Comprehension)	Auditory Verbal Comprehension	C-tDCS improved auditory verbal comprehension as compared to A-tDCS and sham
Lee et al. (2013)	Chronic stroke Aphasia $N = 11$ (Broca's $N = 4$; Anomic $N = 5$; Transcortical Motor $N = 2$)	Randomized controlled Cross-over	Language (naming abilities)	Picture naming test and picture description	Both single and dual tDCS condition improved naming accuracy and RTs as compared to sham
Polanowska et al. (2013)	Subacute stroke Aphasia (moderate to severe) $N = 37$	Randomized, double-blind, controlled	Language	Boston Diagnostic Aphasia Examination	No differences between A-tDCS and sham group (both improved)
Rosso et al. (2014)	Chronic stroke two groups with ($N = 11$) or without ($N = 14$) infarction in the L-Broca's area. Non-fluent aphasia	Randomized controlled cross-over	Language (naming abilities)	Computerized picture-naming task	C-tDCS improved picture naming accuracy in the group with lesion in the L- Broca's area as compared to the other group

(Continued)

TABLE 2 | Continued

Author and year	Sample	Experimental design	Target cognitive domain	Neuropsychological measures	Main results
Santos et al. (2013)	Chronic stroke Aphasia $N = 19$ (Broca's $N = 8$;Anomic $N = 7$; Mixed $N = 4$)	Pretest-Posttest Design (no sham control group)	Language (oral comprehension, writing, naming and verbal fluency)	Oral language comprehension, copying, dictation, reading, writing, naming and verbal fluency	A-tDCS improved comprehension, naming and verbal fluency for animals name; no changes in other outcomes
Volpato et al. (2013)	Chronic stroke $N = 8$ aphasia (Wernike's $N = 2$; Broca's $N = 1$;Anomic $N = 2$; Transcortical sensory $= 1$; Transcortical Motor $N = 1$; Conduction $N = 1$)	Randomized controlled Cross-over	Language (naming abilities)	Computerized picture naming task	No differences between A-tDCS and sham for object and action naming task
Marangolo et al. (2014)	Chronic stroke $N = 7$ Non-fluent aphasia	Randomized controlled Cross-over	Language (naming abilities)	Computerized action naming task	A-tDCS on Broca's area improved naming accuracy as compared with sham; the effects persist at follow-up 1 week and 4weeks
Vestito et al. (2014)	Chronic stroke Aphasia $N = 3$	controlled Cross-over	Language (naming abilities)	Computerized picture naming task	A-tDCS improved naming accuracy as compared to sham; improvement persist after 16 weeks
Manenti et al. (2015)	Chronic stroke non-fluent aphasia $N = 1$	Pre test-Post test Design (no control group)	Language (naming abilities)	Word verb naming	Bi-hemispheric DLPFC tDCS improve verb-naming performances
Shah-Basak et al. (2015)	Chronic stroke non-fluent aphasia (mild to severe) $N = 12$	Randomized controlled Cross-over	Language (naming abilities)	Computerized picture naming task	C-tDCS improved naming as compared to sham
Wu et al. (2015)	Subacute stroke $N = 12$	Randomized controlled Cross-over	Language (naming abilities) (comprehension)	Computerized picture naming auditory word-picture identification	A-tDSC improved picture naming and auditory identification as compared with sham

Randomized Controlled Cross Over, over time, each participant receives an intervention in a random sequence.
Randomized controlled between subject, the various experimental treatments are given to different groups of subjects.
tDCS, transcranial direct current stimulation; A-tDCS, anodal electrode tDCS; C-tDCS, cathodal electrode tDCS; sham, placebo tDCS; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; ADL, activities of daily living; BADA, Batteria per l'Analisi dei Deficit Afasici; BIT, behavioral inattention test; CPM, colored progressive matrices; DIGSP-BW- FW, digit span backwards-forwards; ENB-2, Esame Neuropsicologico Breve-2; FNAT, face-naming association task; HPVOT, Hooper Visual Organization Test; IADL, Indice di dipendenza nelle attività strumentali della vita quotidiana; MMSE, Mini Mental State Examination; NET, Neglect-Test; RAVLT, Rey auditory verbal learning test; RBMT, River mead behavioral memory test; ROCFC, Rey osterrieth Complex figure copy; TAP, Test for Attentional Performance; TMT A-B, Trail making test A-B;WCST, Wisconsin card sorting test; WAIS-III, Wechsler Adult Intelligence Scale-Third edition.

AGE $= 61.5 \pm 0.9$; mean MMSE $= 27.7$) diagnosed as idiopathic PD were randomized to receive A-tDCS on L-DLPFC (F3) or A-tDCS on L-TPC (P3-T5) and immediately after performed a verbal fluency task inside the scanner. The authors found an improvement on the phonemic fluency task after a single session A-tDCS over the L-DLPFC. Furthermore, fMRI analysis of connectivity demonstrated that A-tDCS applied over the L-DLPFC produced a greater activation of the specific functional networks engaged by the task compared to A-tDCS over temporo parietal cortex TPC. While these two studies demonstrated that tDCS may improve specific components of executive function, the effects were short-lasting and did not generalize to everyday functioning. A subsequent multicenter study then investigated the efficacy of a multiple sessions protocol in idiopathic PD patients on multiple cognitive domains including executive function, attention, perceptual-motor abilities, learning and memory. Here, 10 consecutive sessions (over 2 weeks) of A-tDCS over L-DLPFC or A-tDCS over R-DLPFC or sham, were administered by a randomized between subject design on 18 patients (6 in each group). Cognitive functions were evaluated before, at the end of stimulation sessions and at 1 month follow-up. It was found A-tDCS over both the L and R-DLPFC compared to sham improved performance only on Trial Making Test B at the 1-month follow-up but not on the other outcome measures.

Overall, these studies demonstrate that A-tDCS over the prefrontal cortex may be effective for improving executive functions, but it must be emphasized that these studies lack sufficient numbers of patients, statistical power and more importantly transfer of benefits into everyday functioning. Across the studies, there is a general agreement on the parameters of stimulation. While the positions of active electrode A-L-DLPFC(F3) and reference contralateral supraorbital and also the intensity (2 mA) and the duration (20 min) of stimulation are the same or similar in all studies, it is not clear what the criteria are for selection of the outcome criteria, such as reliability or validity. Furthermore, it is unclear what the most sensitive test to measure tDCS efficacy on cognitive domains may be. In sum, this evidence encourages and warrants further investigation. In future studies shared methodology are necessary to allow comparison

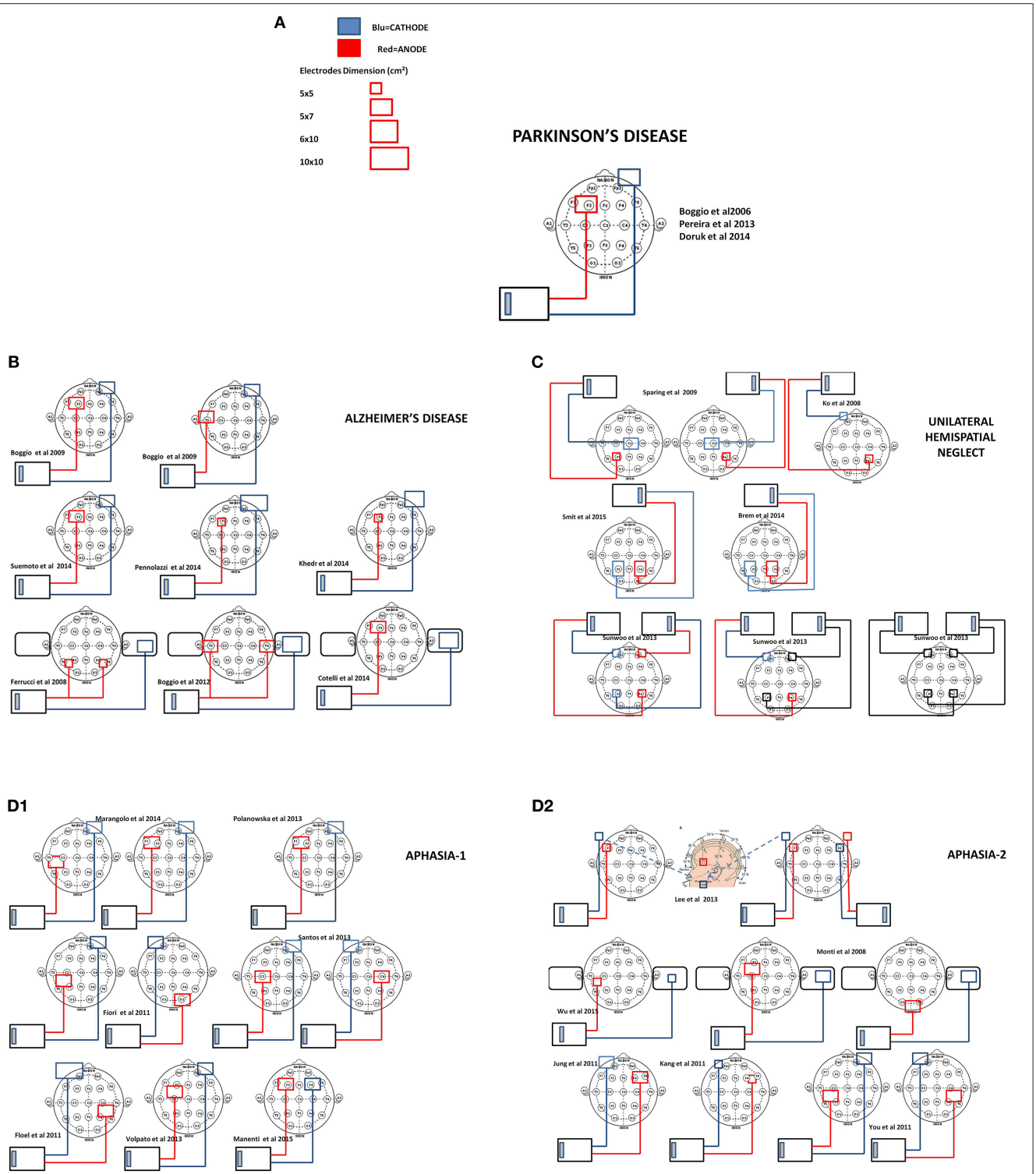


FIGURE 2 | Scale representation of tDCS electrode montage of the reviewed studies with reference to the EEG international 10–20 system. In **(A)** legend of electrodes size and polarity and electrode montage in Parkinson's disease studies (Boggio et al., 2006; Pereira et al., 2013; Doruk et al., 2014), **(B)** Alzheimer's disease (Boggio et al., 2008, 2012; Ferrucci et al., 2008; Cotelli et al., 2014; Khedr et al., 2014; Penolazzi et al., 2014; Suemoto et al., 2014), **(C)** unilateral Neglect (Ko et al., 2008; Sparing et al., 2009; Sunwoo et al., 2013; Brem et al., 2014; Smit et al., 2015), and **(D1,D2)** Aphasia (Monti et al., 2008; Flöel et al., 2011; Fiori et al., 2011; Jung et al., 2011; Kang et al., 2011; You et al., 2011; Lee et al., 2013; Polanowska et al., 2013; Santos et al., 2013; Volpato et al., 2013; Marangolo et al., 2014; Manenti et al., 2015; Wu et al., 2015).

across studies and to assert the usefulness of tDCS for cognitive rehabilitation in PD.

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a progressive disease that arises on a neuropathological background of amyloid plaques (APs) and neurofibrillary tangles (NFTs). AD is the most common form of major NCD, where symptoms gradually progress over a number of years with memory loss and decline of intellectual abilities serious enough to interfere with daily life. This disturbance is related to the degree of brain atrophy in medial temporal lobe involving entorhinal cortex and hippocampus, and also prefrontal areas. Memory disturbances appear early, at first affecting the ability to learn and retrieve information, and later causing impairments in recognition memory and attention. Ferrucci et al. (2008) in a randomized cross-over study tested 10 AD patients (mean AGE = 75.2 years; MMSE = 22.7 overlapping 1.8) on recognition memory and visual attention. Patients underwent a single session protocol of A-tDCS or C-tDCS or sham over bilateral temporo-parietal areas (two electrodes on the scalp and one reference on deltoid). Before and 30 min after stimulation patients performed a word recognition test and a visual attention test. It was found that A-tDCS increased accuracy in word recognition memory, and conversely C-tDCS decreased accuracy. Performance on visual attention did not change. A successive randomized cross over single session study of Boggio et al. (2008) assessed the efficacy of A-tDCS on recognition memory, working memory and attention in 10 AD patients (MMSE between 12 and 25). Patients participated in three separate sessions to receive A-tDCS over left temporal cortex (L-TC) or A-tDCS over the L-DLPFC or sham. For all conditions, the reference, cathode electrode (35 cm^2) was placed over the right supraorbital area. Stimulation was delivered during a Visual Recognition Memory task, Stroop, or Digit Span task, with the order randomized across participants. Tasks started 10 min after stimulation onset and lasted until the end of stimulation. Each condition was separated by at least 48 h. It was found that both A-tDCS over temporal or prefrontal cortex improved Visual Recognition Memory performance compared to sham. Attentional performance measured by the Stroop was unchanged. Albeit these two studies showed that A-tDCS may positively modulate aspects of memory, the effects were small and without any follow-up measures. To overcome these limitations, 3 years later, Boggio and colleagues performed a multicenter, cross-over multiple sessions follow-up study. Here, fifteen AD patients underwent five consecutive A-tDCS over L-TPC and R-TPC bilaterally or sham. Visual Recognition Memory, visual attention and general cognition (MMSE) were assessed before, immediately after the end of stimulation sessions and at 4 weeks follow-up. They found that A-tDCS patients improved on Visual Recognition Memory compared to sham. Moreover, these effect persisted 4 weeks after the end of stimulation. There were no changes in visual attention or general cognition.

To date, two studies have assessed the combined use of tDCS and cognitive training. Cotelli et al. (2014) evaluated for the first time the impact of tDCS combined with individualized associative memory training (iMT-FNAT) on specific associative memory test and learning and memory, attention, language and perceptual-motor domains. Here, 10 consecutive sessions (over 2 weeks) of A-tDCS over the L-DLPFC during iMT or A-tDCS over the L-DLPFC during motor training or sham tDCS during iMT; were administered in a randomized between subject design in 36 patients (12 in each group). Neuropsychological assessment and Face-Name Association memory Task (FNAT) were completed at 4 time points (before, 2 weeks after, 3 and 6 months after). An improvement only in selectively trained stimuli induced by iMT irrespective of site by both A-tDCS and sham tDCS group was found. In other words A-tDCS over the L-DLPFC did not have an additive effect on the FNAT computerized training. Moreover, the improvement was task-stimuli specific and did not generalize to other domains. In a subsequent single case study Pennolazzi and colleagues examined the effectiveness of tDCS combined with Individualized Computerized Task (iCT) performance (Penolazzi et al., 2014). An AD patient of 60 years (MMSE 23) underwent 10 sessions A-tDCS over the L-DLPFC followed by iCT. iCT (based on the patient's impairment) included verbal working memory task, phonemic fluency task and continuous performance task. Effects on cognitive performance were evaluated by the iCT and by extensive neuropsychological assessment of global cognitive functioning. The authors found iCT combined with anodal stimulation to be better than iCT combined with the sham. Thus, combined 10 daily sessions of A-tDCS over the left prefrontal cortex and iCT slowed down the cognitive decline of the patient more than iCT alone.

The differences in the latter two studies (Cotelli et al., 2014; Penolazzi et al., 2014) may emerge from the key methodological variations between them such as training during stimulation (Cotelli) or training follow stimulation (Penolazzi). Moreover, the authors utilized diverse cognitive training together with different outcome measures to assess stimulation effects. In addition Cotelli et al. used an extra-cephalic reference and Penolazzi et al. a cephalic reference which will have resulted in a different current flow.

Recently there have been two studies with a larger number of patients than previous studies. Suemoto et al. (2014) examined the efficacy of A-tDCS in 40 moderately cognitively impaired AD patients (MMSE 10–20) for apathy and global cognitive functioning. Here, six sessions of A-tDCS on L-DLPFC, vs. sham, were administered in a randomized cross-over design. Patients were evaluated at baseline, after the first and the second week of stimulation, and after 1 week without intervention. The authors found that A-tDCS had no effect on apathy or on global cognitive performance, or the ADAS-Cog sub-items. This study shows that repeated A-tDCS over the left prefrontal cortex in patients with a state of relatively advanced deterioration is not able to improve their cognitive deficits or apathy. In a multiple session, 2 months follow up study of Khedr et al. (2014) 34 patients (mean AGE = 69.7 years; mean MMSE = 18.1 range 12–23) were tested. Here, ten sessions of A-tDCS or C-tDCS over the L-DLPFC, vs. sham, were administered in a randomized between subjects study design. Global cognitive functioning (MMSE) and Intelligence (WAIS-III) were assessed at four time points (baseline; end of the 10 sessions; 1 and 2 months after the end). Furthermore, motor cortical excitability and the P300 event-related potential

were assessed at baseline and after the last tDCS session. The authors found that 10 sessions of both A-tDCS or C-tDCS over the L-DLPFC improved MMSE compared to sham with a further increase at 1 and 2 months follow-up. Only C-tDCS seemed to have a minor positive effect on a subscale of the WAIS-III.

To sum up, there is some evidence from randomized controlled clinical studies showing a beneficial effect of A-tDCS on some specific components of memory. However, it is evident that there is a great deal of methodological heterogeneity across these studies. First, there are diverse stimulation protocols adopted, only two studies used the same location and size of the electrodes (Boggio et al., 2008; Suemoto et al., 2014). Additionally, some studies preferred an extra-cephalic reference to avoid unwelcome interference effects from brain areas underlying the reference electrode. In general, a better definition of stimulation protocols needs to be provided. Second, most of these studies do not consider the fact that cognitively impaired patients can be highly variable in the manifestation of their cognitive problems and in some cases group variability between patients and within a patient from 1 day to the next can mask the effectiveness of a treatment. Third, by and large most studies did not measure whether the improvement in a specific task has generalized to everyday life. Indeed it is imperative to discriminate between increase in performance on a specific cognitive task and recovery in more general daily life activities demanding that cognitive function. Further studies should consider the individual characteristics of each patient, better define stimulation parameters and outcome measures and look at translation into everyday cognitive functioning.

Unilateral Spatial Neglect

Unilateral spatial neglect is a neurological syndrome that develops following damage to one hemisphere of the brain. It is characterized by a deficit in attention to and awareness of one side of space. It is defined by the inability of a person to process and perceive stimuli on one side of the body or environment, where that inability is not due to a lack of sensation. Unilateral spatial neglect results most commonly from brain injury to the right cerebral hemisphere, causing visual neglect of the left-hand side of space.

Overall, the rational for the studies using tCDS in patients with unilateral neglect is based on Kinsbourne's interhemispheric conflict model. According to this model parietal lobes may exercise interhemispheric inhibition through the connections of the corpus callosum balancing allocation of visuospatial attention toward both hemifields. Brain lesions, as a result of stroke, damage this balance. For this reason A-tDCS is applied to the lesioned hemisphere to increase cortical excitability and the C-tDCS to inhibit the over-activated unlesioned hemisphere.

In a double-blind, crossover, controlled experiment Ko et al. (2008) enrolled 15 right-handed subacute stroke patients (mean Age = 62.1 ± 8.8 years; mean time post-onset = 29–99 days) with left visuospatial neglect due to right-sided cortical and/or subcortical vascular lesions. Patients participated in a single session protocol of A-tDCS over the right parietal cortex (R-PC) (damaged hemisphere). Before and after "treatment" patients performed a line bisection test and a cancelation test. The authors found an improvement of performance in both tests, indicating a recovery of neglect symptoms, compared to sham. Sparing and colleagues in a randomized cross-over study (Sparing et al., 2009) tested 10 right-handed patients (mean age = 57.3 years; mean time post-onset 2.9–3.5 months) with left visuospatial neglect due to right-sided vascular lesions. Here, a single session of A-tDCS over the right posterior parietal cortex (R-PPC; damaged hemisphere) or C-tDCS over the left posterior parietal cortex (L-PPC) were conducted. A visual search task and a computerized Line Bisection task were administered before and after tDCS. The authors found that both C-tDCS over the undamaged PPC A-tDCS over the damaged PPC reduced symptoms of visuospatial neglect. More recently, a rather unconventional protocol was pursued by Sunwoo et al. (2013) [14], who used two stimulators and four electrodes on the scalp. A double-blind randomized cross-over study was performed to assess the impact of dual-mode montage with A-tDCS over the R-PPC(P4) and C-tDCS over the L-PPC(P3) concurrently, and to compare single-mode A-tDCS over the R-PPC alone and sham on 10 patients with chronic stroke induced neglect (mean age = 62.6 years ± 13.3 mean time post-onset 27.8 ± 60.4 months). Before and after "treatment" patients performed a line bisection test and cancelation test. It was found that both dual-mode and single-mode tDCS were safe and beneficial for neglect symptoms.

Two studies assessed the impact of multiple sessions of tDCS on Neglect patients. A combined approach was followed by Brem et al. (2014), who combined tDCS and cognitive training. Here, five consecutive sessions of ordinary neglect therapy combined with biparietal A-tDCS over the R-PPC and C-tDCS over the L-PPC, vs. sham, were administered in a double-blind, single case cross-over design in a 72-year-old, ambidextrous male patient with stroke of the right posterior cerebral artery. Neuropsychological assessment before and after treatment were evaluated by Test for Attentional Performance (TAP) (which includes covert attention, alertness, visual field) and the Neglect-Test (NET) (line bisection, cancelation, copying). Furthermore, generalization on activities of daily living (ADL) was also evaluated. It was found that with bilaterally active PPC tDCS improvement was significantly higher than during standard neglect therapy alone or sham. The authors highlighted for the first time the additive effects of tDCS and standard neglect therapy on functional improvement. Importantly the beneficial effects of tDCS was maintained over a follow-up period of 1 week and 3 months. A subsequent study by Smit et al. (2015) evaluated the immediate and long-term effects of multiple sessions of tDCS on five severe chronic hemispatial neglect patients. Here, five consecutive sessions of bilateral A-tDCS over the R-PPC and C-tDCS over the L-PPC, vs. sham, were conducted in a randomized double-blind cross-over design. Neuropsychological assessment before and after treatment by Behavioral Attention Test (BIT) indicated no symptomatic improvement after bilaterally PPC tDCS stimulation. While these two studies examined the effects of multiple sessions of tDCS, Brem and colleagues tested a single stroke patient in the subacute phase, while Smit and colleagues tested five stroke patients in the chronic phase.

In summary, these results are encouraging, but further clinical trials with larger number of patients and follow up are needed. Moreover, translation of symptoms amelioration into everyday activities need to be measured.

Aphasia

Aphasia is an impairment of language, affecting the production or comprehension of speech and the ability to read or write. Aphasia is always due to injury to the brain most commonly from a stroke, particularly in older individuals. Aphasia can be so severe as to make communication with the patient almost impossible, or it can be very mild. It may affect mainly a single aspect of language use, such as the ability to retrieve the names of objects, or the ability to put words together into sentences, or the ability to read. Generally multiple aspects of communication are impaired. In this form of aphasia, speech output is severely reduced and is limited mainly to short utterances of less than four words. Vocabulary access is limited and the formation of sounds by individuals with Broca's aphasia is often laborious and clumsy. The person may understand speech relatively well and be able to read, but be limited in writing. Broca's aphasia is often referred to as a 'non fluent aphasia' because of the halting and effortful quality of speech.

In patients who suffer from non-fluent aphasia the studies so far evaluated the immediate effect of tDCS on naming abilities.

The first study was conducted by Monti et al. (2008), who included eight right-handed chronic non-fluent aphasic patients in a randomized controlled cross-over study. They tested the effect of A-tDCS or C-tDCS over the left Broca's area (damaged hemisphere; crossing point between T3-Fz and F7- Cz) and sham on picture naming task accuracy. An improvement in accuracy after C-tDCS compared to A-tDCS and sham was found. It is worth noting that this study is not in line with the Neglect studies cited above in which A-tDCS was applied over the damaged hemisphere and C-tDCS over the intact hemisphere. Even so these study are difficult to compare because of the differences in the parameters adopted.

Subsequent studies evaluated the effect of A-tDCS over the left damaged hemisphere during naming training in post-stroke non-fluent aphasia patients on naming task accuracy with mixed evidence.

Fiori et al. (2011) tested three aphasic patients with anomic difficulties using a picture-naming task. In a randomized double-blind cross-over study, they administered five consecutive sessions of A-tDCS over the Wernicke's area (CP5), vs. sham applied during intensive anomia training. The authors found a significant improvement in the picture-naming task accuracy.

More recently, in eight stroke patients with distinct types of aphasia, Volpato et al. (2013) examined the effect of A-tDCS on naming abilities. Here, ten consecutive sessions over 2 weeks of A-tDCS over the L-Broca's area, vs. sham were administered in a randomized cross-over design. The authors found no significant differences between A-tDCS and sham on naming abilities. Similarly, in a randomized between subjects study, Polanowska et al. (2013) conducted 15 sessions of A-tDCS over L-Broca's area followed by language training. Patients were assessed by Boston Diagnostic Aphasia Examination before, immediately after treatment and at 3 months follow up. Again, the authors found no significant differences between A-tDCS and sham groups. In another small sample study, three patients with chronic stroke, in a cross-over design, received naming training during A-tDCS over the left frontal perilesional areas vs. sham. Vestito et al. (2014) found that naming abilities, as assessed by a computerized naming task, improved in the A-tDCS group compared to the sham group. A rather unconventional protocol was followed by Lee et al. who simultaneously used two stimulators. A randomized cross-over study were performed to assess the impact of a dual-mode montage with A-tDCS over the L-IFG(F7) and C-tDCS over the R-IFG(F8) concurrently, compared to single-mode A-tDCS over the L-IFG alone and sham on 11 patients with chronic stroke-induced aphasia. During the last 15 min of tDCS, speech therapy was provided. Before and after treatment, patients performed a picture naming test and a picture description test. It was found that both dual-mode and single-mode tDCS improved naming accuracy and reaction times compared to sham. More recently, Wu et al. (2015) examined 12 sub-acute stroke patients with aphasia using a picture naming task and an auditory picture identification task. Moreover, they measured cortical excitability by electroencephalography (EEG) nonlinear dynamics analysis. In a randomized controlled cross-over study they administered A-tDCS over the L-posterior perisylvian region vs. sham and patients received 20 sessions of speech therapy. The authors found an improvement in picture naming and auditory comprehension after A-tDCS compared with sham. Furthermore, EEG analysis indicated that naming improvement correlated with higher activation in the brain language network.

Two other studies used an innovative approach to position the electrodes. Baker et al. (2010) in a randomized controlled cross-over study tested 10 patients in the chronic phase with mild to moderate post stroke non-fluent aphasia. They administered five consecutive sessions of A-tDCS over the left frontal cortex vs. sham during computerized anomia training. Each patient performed a naming task inside the scanner. Then fMRI results for each individual was used to place the electrodes. A significant improvement in naming accuracy after A-tDCS compared to sham was reported. The improvement was maintained 1 week after treatment. In a subsequent study Fridriksson et al. (2011) tested eight patients with stroke-induced fluent aphasia utilizing the same picture naming task and electrodes placement procedure. Here, five consecutive sessions of A-tDCS vs. sham were administered in a randomized controlled cross-over design. Reduced RTs during naming were also found after A-tDCS which was maintained after 3 weeks.

Some studies assessed the long-term therapeutic benefits of tDCS on naming. In the chronic stage, Marangolo et al. (2014) included seven patients with stroke-induced non-fluent aphasia in a randomized controlled cross-over study. They administered five consecutive sessions of A-tDCS over the L-Wernicke's area or L-Broca's area vs. sham during training for action naming. Training consisted of three groups of video clips representing actions that patients had to name. Naming accuracy was assessed before treatment, immediately after and at 1 and 2 weeks follow-up. The authors found significantly improved accuracy after

A-tDCS over the Broca's area compared to Wernicke's area and sham. The effect persisted at 4 weeks follow-up. This result highlights the functional importance of Broca's area in verb processing. Manenti et al. (2015) included one chronic stroke patient with non-fluent aphasia in a pretest posttest design study without sham control. Here, twenty consecutive sessions of bi-hemispheric A-tDCS over the L-DLPFC and C-tDCS on R-DLPFC were followed by individualized verb anomia training. An extensive language evaluation was completed before, after treatment and at 12, 24, and 48 weeks after. The authors found an improvement in verb naming and a decrease in self-perceived difficulties in social situations and improved linguistic abilities suggesting an impact of the treatment on the daily life of the patient. Importantly, the authors asserted that this effect persisted 48 weeks after stimulation.

Two studies attempted to improve aphasia symptoms by stimulating the right hemispheric homolog areas. In the chronic stage, Flöel et al. (2011) included 12 patients with moderate to severe aphasia in a randomized controlled cross-over study. Here, A-tDCS or C-tDCS over the R-temporo parietal cortex (R-TPC) vs. sham combined with anomia training were conducted. The authors found that A-tDCS significantly enhanced the overall training effects compared to sham and the effect persisted after 2 weeks. Similarly, in a randomized controlled cross-over study, Vines et al. (2011) enrolled six patients with moderate to severe aphasia. They used A-tDCS over the right inferior frontal gyrus (R-IFG) during melodic intonation therapy (MIT) for three consecutive days. They reported that combining A-tDCS with MIT significantly improved verbal fluency compared to sham with MIT.

Other studies attempted to restore language abilities by suppressing the right homolog language areas with C-tDCS. In the sub-acute stage, You et al. (2011) included 21 patients with comprehension impairment in a randomized controlled between subjects design. Here, ten sessions of conventional speech therapy were combined with A-tDCS over the left superior temporal gyrus or C-tDCS over the right superior temporal gyrus or sham. It was found that auditory verbal comprehension improved after C-tDCS over the right hemisphere compared to A-tDCS and sham. Similarly, in a double-blind randomized controlled study, Kang et al. found that five consecutive sessions of C-tDCS over the R-Broca's area combined with word-retrieval training improved performance in picture-word matching task.

Three studies concentrated on factors associated with response to C-tDCS protocol. Jung et al. (2011) included 37 stroke patients from acute to chronic in a pretest posttest design study without sham control group. Here, ten consecutive sessions of C-tDCS over the R-inferio frontal gyrus were administered. The authors assessed the effect of tDCS by the Korean version of Western aphasia Battery. Using regression statistical models it was found recovery after C-tDCS was more in patients with less severe aphasia who had started "treatment" within the first months after stroke. In a more recent, randomized controlled cross-over study, Rosso et al. (2014) adopted an innovative fMRI combined tDCS approach looking for inter-individual variability. They found C-tDCS over the R-Brocas's area improved performance on a computerized picture naming task. More importantly the authors found that improvements in naming after C-tDCS of the R-Broca's area relies on several structural and functional factors.

One study assessed the efficacy of an individualized tDCS treatment in stroke-induced non fluent aphasia in chronic patients. Shah-Basak et al. (2015) ingeniously took into account the individual variability in response to tDCS. In the first phase of the study the authors individualized the protocol based on individual responses to the A-tDCS or C-tDCS over the L-IFG or R-IFG. Then in a randomized cross-over study, 10 sessions of active tDCS or sham were administered during a picture naming task. Language abilities were assessed before, after treatment, 2 weeks and 2 months after. Aphasia symptoms improved after the active tDCS treatment compared to sham and the improvement remained 2 month after the end of treatment. This study suggests that an individualized protocol may be effective in improving stroke-induced chronic aphasia symptoms overcoming the high variability between patients.

An unusual approach was followed by Santos et al. (2013). They included nine teenaged chronic stroke sufferers from non–fluent aphasia in a pretest posttest design study without sham control group. Here, ten consecutive sessions of A-tDCS over the primary motor cortex (M1) of the healthy hemisphere were administered. Language level was assessed before and immediately after the treatment. They found a significantly improved performance in sentence comprehension, naming and specific animal name category verbal fluency.

In sum, there are some randomized controlled evidence that indicated a favorable effect of tDCS in improving language symptoms related to aphasia. Again, there is a great deal of methodological heterogeneity across these studies. Various approaches have been undertaken including the application of A-tDCS over the left damaged hemisphere concomitant to a naming training or to restore naming abilities by suppressing the activation of the right homolog language areas with C-tDCS. In a rather original fashion, one study took individual differences in response of tDCS into account.

METHODOLOGICAL ISSUES

Clinical and Demographic Characteristics of Samples

It is important to remember that neurodegeneration or insult or injury to the brain does not affect two people identically. Such individual differences also lead to differences in the evolution of the disease. Even though patients have been diagnosed with the same disorder there are substantial differences between them. In the case of progressive degenerative diseases such as PD and AD, the evolution and progression of the disease is unique in each case and each person responds differently to treatment.

Furthermore, numerous studies have argued that there are some important factors that can affect the evolution of NCD. Cognitive Reserve (CR), for instance, is a factor that would be reasonable to consider in the case of neurodegenerative disorders (Stern, 2002). CR is a term describing the resilience of the brain following the brain damage. CR is defined as the

ability to optimize or maximize performance through differential recruitment of brain networks (Scarmeas et al., 2003). It depends on factors such as education, profession, lifestyle and leisure activities which play an important role in determining how many alternative resources are available to be used to compensate for the cognitive deficits.

With regards to medical conditions that occur after a brain injury such as unilateral spatial neglect and aphasia there are many points to consider. First, it is almost impossible to find two patients with damage that affects exactly the same part of the brain because of anatomical differences between individuals. Cerebral infarction and hemorrhage may be more or less circumscribed involving diverse brain areas. Second, even if we find two patients with exactly the same injury the two individuals could have a different ability to recover or to compensate. Third, in patients who have suffered a stroke an important aspect to consider is whether patients are treated in the subacute phase (within 6 months) or in the chronic phase. It has been suggested that the brain is more sensitive to reorganization during the months immediately after the stroke. Fourth, it would be important to consider the pre-morbid cognitive state of the participants.

Selection of patients for inclusion in the experimental group is an important and sometimes difficult process in this areas of research. Group variability can affect the outcome of a study. It is extremely important to minimize the heterogeneity of patients in order to gain a better understanding of tDCS as a therapeutic technique. Bearing this in mind, there are remarkable differences in the demographic and clinical characteristics of patients undergoing tDCS treatment in the studies examined (see **Table 2**). For example, regarding AD in the study of Boggio et al. (2008) there is a huge intragroup variability. A patient with an MMSE score of 12 (moderate cognitive impairment) is in the same group as a patient with an MMSE score of 25 (mild cognitive impairment). These patients were comparable for age, respectively 85 and 89, but different for years of education, respectively 4 and 11 years. Suemoto and colleagues recruited patients and divided them into two groups with mean ages of 79.4 and 81.6 years; 5 and 4.5 years of education and a MMSE score of 15 and 15.4 (Suemoto et al., 2014); while in the single case study of Penolazzi et al the patient's age was 60 years, with 18 years of education and an MMSE of 23 (Penolazzi et al., 2014). In the study of Khedr et al. (2014) the average age of the three groups of patients recruited was 68.5, 70.7, 67.3 years and MMSE scores of 18.4, 18.8, and 16.9; and years of education was not reported.. Regarding PD, Boggio et al. (2006) recruited patients with a score of 36.8 for Experiment 1 and 43 for Experiment 2 on the UPDRS while in the study of Pereira et al. (2013) patients were recruited with a mean score of 13.3 on the UPDRS. Furthermore, in the study of Boggio et al the average years of education of the patients was 4.7 years for Experiment 1 and 5.3 years for Experiment 2; while in the study of Pereira et al the patients' average schooling was 12.3 years. With regards to unilateral spatial neglect, there are remarkable intragroup differences in the site of damage of the patients. In the studies reviewed in the same experimental group there are patients with damage limited to the basal ganglia, patients with more extensive lesions covering frontal, temporal and parietal lobes or frontal parietal occipital lobes. Another factor on which the patients differed is the duration of illness post onset. Most of the studies recruited patients in the subacute phase within 6 months after stroke (Ko et al., 2008; Sparing et al., 2009; Brem et al., 2014). Only two studies enrolled patients in the chronic phase (Sunwoo et al., 2013; Smit et al., 2015).

In the existing studies, it is often neglected that clinical features of patients may affect the outcome of tDCS. To date, little importance has been given to patient characteristics which could in part explain the variability in the response to the tDCS. Future studies should try to control as much as possible factors that may influence the outcome of therapeutic application of tDCS in cognitive rehabilitation.

tDCS Parameters, Electric Fields and Neuroanatomy

tDCS scalp surface anodal and cathodal electrodes inject low amplitude direct currents (0.5–2 mA) through the head and these currents are applied from few seconds to several minutes. This results in an electric field and a current density generated in the scalp and brain. In the studies which first measured the impact of this electric field on the human brain, tDCS was combined with TMS to investigate modification of cortical excitability. The first study to explore cortical excitability investigated the effects of up to 0.5 mA currents applied using an M1-chin montage on the size of the motor evoked potential (MEP) (Priori et al., 1998). However, the first "modern" study to use the standard current and electrode parameters was published 2 years later (Nitsche and Paulus, 2000).

Generally if the anode is placed above the motor cortex, after DC stimulation, single pulse TMS will result in a larger MEP (Day et al., 1987; Rothwell, 1997). If the cathode is placed over the motor cortex, the MEP size will be reduced. Thus, long-lasting and polarity-dependent changes in neural excitability of the human cortex are elicited. This effect is conceivably due to depolarization of somatic membrane potentials by anodal currents and hyperpolarization of soma by cathodal currents, as observed in animal studies (Bindman et al., 1964).

Several studies have been performed in humans in order to understand the physiological mechanisms of tDCS. It has been shown that the effects on the MEP can be modified, prolonged or even reversed by drugs acting on the central nervous system (Stagg and Nitsche, 2011). Importantly, it seems that neuroplastic after-effects of tDCS are NMDA-receptor dependent (Liebetanz et al., 2002). Moreover, anodal after-effects can be selectively suppressed by both the sodium channel blocker carbamazepine and the calcium channel blocker flunarizine (Nitsche et al., 2003). These studies demonstrated that is possible to measure in humans the effects of direct current application by TMS at the motor cortex.

Based upon what is known about the process of MEP production a growing interest for examining the anodal and cathodal tDCS effects on other brain areas has emerged. It is worth noting that it is absolutely unclear whether it is possible to generalize these processes in the modification of MEPs to other more complex cognitive processes. In spite of this during the last

decade a considerable amount of literature has been published on the capacity of tDCS to alter human brain functions over numerous brain areas and in the treatment of a wide range of diseases. This interest has been facilitated by the fact that from a neuroscience point of view, the causal and interventional nature of tDCS is particularly exciting. This exponential growth of published works is somewhat surprising if we consider that the understanding of the basic principles of tDCS have not yet been achieved. Perceiving, remembering, reasoning and language are more complex processes than MEPs. Moreover, many studies are based on the theoretical assumption that placing the anode electrode over the area of interest would enhance precisely the activity of the target region and conversely placing the cathode would decrease the activity, which raises a number of problematic points. One problem with this approach is the low spatial resolution of tDCS. The rationale that putting an electrode on the scalp over a region of the brain results in a precise stimulation of that region, and only the target region is unlikely to be accurate. Indeed the major drawback is that the amount and distribution of current flow fluctuates extensively as a function of individual physiology and anatomy. So investigators who use tDCS are not in a position to make accurate inferences about the operation of a specific brain area. It is not sufficient to only examine the behavioral outcome to ascertain the specific involvement of a brain area and rule out the possible role of another area.

It therefore follows that an urgent question that needs to be asked is how the current is distributed in the brain during tDCS. To answer this question recently modern mathematical models that integrate structural resonance magnetic images (MRI), have been developed to understand the distribution of the electric field in the brain (Bikson et al., 2012; Datta et al., 2012). These modeling approaches showed that the effects of administering a current in the brain using a particular configuration of the electrodes are the result of many factors such as the spatial distribution of the electric field induced in the gray matter (GM) and white matter (WM), the orientation of the electric field relative to the neurons and many other factors (Miranda et al., 2013).

In light of this complexity, the application of tDCS to neurocognitive disorders should consider the brain morphological heterogeneity of patients. Along these lines it is difficult to conceive that the same stimulation protocols with the same parameters of stimulation may be optimal in different patients. For instance, in the case of degenerative disorders characterized by marked atrophy such as AD it is difficult to conceive that the same dose of tDCS is optimal in two different patients as suggested by Mahdavi et al. (2014). An interesting parallel in this regard is with deep brain stimulation (DBS). DBS is a neurosurgical procedure in which an electrode is implanted in the brain and is controlled by a neurostimulator. In DBS the patient's behavioral state is used as an indicator of how to change the parameters. That is to say that the frequency, pulse width and voltage of stimulation are adjusted based on the positive response of the symptoms of each patient and simultaneous avoidance of side-effects (Kringelbach et al., 2007).

It is evident that tDCS of both the normal and the diseased brain depends on a number of factors such as the stimulation parameters including the electrode localization, duration and intensity (see **Table 3** and **Figure 3**) of stimulation and also the patient characteristics such as age, disease stage, years of education and premorbid level of functioning which influence cognitive reserve. The studies we reviewed above show remarkable differences regarding the criteria for selecting the patients, the placement of the electrodes, the duration and intensity of stimulation (see **Tables 1**, **2**) and this makes it very difficult to compare the results across studies. More research into the complex dynamics of the current flow it is essential before obtaining a definitive optimization of stimulation protocols (see de Berker et al., 2013).

Further research in this area may include an integration of data coming from other techniques such as functional magnetic resonance imaging (fMRI) and magnetoenchephalografy (MEG). In the coming years, it is important to work toward optimizing tDCS protocols for cognitive rehabilitation based on the initial response of each patient to this therapeutic application.

State of the Brain during Stimulation

An important and fundamental question that remains to be addressed is "Why does depolarizing cells by administering

TABLE 3 | Current Density (mA/cm^2) of different electrode dimensions.

	Max Current Intensity (mA)			
Electrode size (cm^2)	**1**	**1,2**	**1,5**	**2**
16	0.063	0.075	0.094	0.130
24	0.042	0.050	0.062	0.083
25	0.040	0.048	0.060	0.080
30	0.033	0.040	0.050	0.067
35	0.029	0.034	0.043	0.057
60	0.017	0.020	0.025	0.033
64	0.016	0.019	0.023	0.031
100	0.010	0.012	0.015	0.020

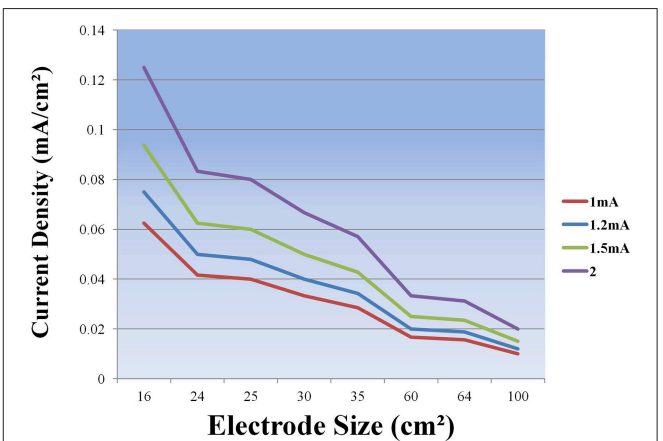


FIGURE 3 | Current Density (mA/cm^2) as a function of electrode size.

a very weak current in the brain modify elaborate cognitive processes?" The theoretical model that may be relevant to answering this complex question is stochastic resonance (SR). SR has been observed throughout nature and it has been reported in physiological neural populations and networks (McDonnell and Ward, 2011). "*SR is observed when noise added to a system changes the system's behavior. Stochastic resonance (SR) is a phenomenon in which a signal that is normally too weak to be detected by a sensor, can be enhanced by adding white noise to the signal, which contains a spectrum of frequencies. A proportioned amount of added noise results in the maximum enhancement a disproportionate noise intensity degrade detectability or information*" (Moss, 2004).

Along similar lines, conceptualizing the administration of tDCS as adding noise to the brain system, one can argue that when a proportionate amount of noise enters the system it would maximize behavioral performance, and conversely if disproportionate noise enters the system it would not produce any effect or worse behavioral performance. This model seems appropriate to explain the high variability in the reported effects of tDCS (Jacobson et al., 2011; Horvath et al., 2015). The implication of the SR model is that the activity status of the system is important. In this case the system is the brain. It follows that the activity of the brain during tDCS is extremely important in determining the overall effect of the stimulation as previously suggested by Silvanto et al. (2008) and more recently by Miniussi et al. (2013). First, a critical factor which is necessary to consider is whether stimulation should be applied during behavioral / cognitive treatment or whether stimulation should be applied offline. Second, following the SR model, it is necessary to consider how many sessions are needed to change the behavior of the "brain system." Third, not only the timing of stimulation and the number of sessions but also the difficulty of the task or training must be considered. Depending on the level of difficulty of the task that the patients have to engage in, more or less cognitive resources would be required, which is also an important variable. Fourth, it is extremely important to determine whether any improvement generalized on untrained cognitive tasks. Evidences indicates that cognitive enhancement can occur at the expense of other cognitive functions (Iuculano and Cohen Kadosh, 2013). To our knowledge very few publications in the literature have also measured other cognitive domains (different from that central for the study) to control for possible cognitive side effects. Future studies should consider all these factors for a more effective therapeutic protocol.

GENERAL CONCLUSIONS

The present review considered the application of tDCS for the cognitive rehabilitation of four neurocognitive disorders: Parkinson's Disease, Alzheimer's Disease, Unilateral Hemispatial Neglect and Aphasia. While in PD there is a general agreement on the parameters of stimulation, what might constitute the most sensitive test to measure t-DCS efficacy on cognitive domains remains unclear. By contrast, for AD, unilateral neglect and aphasia, the variability across studies in the stimulation parameters used, the target site of tDCS stimulation and on the intensity of the stimulation, makes drawing firm conclusions about efficacy more difficult.

Nevertheless, most of the studies reviewed reported a positive effect of tDCS in all these neurocognitive disorders. However, in cognitive rehabilitation it is critical to move beyond statistical significance and consider clinical significance of effects. Such positive evidence of tDCS-induced cognitive benefit cannot be considered as fully reliable due to methodological limits of the studies, particularly the lack of long-term follow-up to establish the durability and longevity of the observed beneficial effects and specific testing to establish whether the beneficial effects of tDCS observed in the laboratory/clinic generalized to everyday cognitive functioning and activities of daily living. Production of long-lasting and generalizable cognitive improvement by tDCS is essential to ensure clinical significance and meaningfulness of the benefits.

The field may benefit from drawing up some guidelines for application of tDCS as a therapeutic approach for NCDs.

AUTHOR CONTRIBUTIONS

Study conception and design: DC, MJ, PB. Acquisition of data: DC. Analysis and interpretation of data: DC, PB. Drafting of manuscript: DC, PB, and MJ.

FUNDING

PB is funded by the Bial Foundation (GA 84/2012).

REFERENCES

Baker, J. M., Rorden, C., and Fridriksson, J. (2010). Using transcranial direct-current stimulation to treat stroke patients with aphasia. *Stroke* 41, 1229–1236. doi: 10.1161/STROKEAHA.109.576785

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

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, 369–82.

Boggio, P. S., Ferrucci, R., Mameli, F., Martins, D., Martins, O., Vergari, M., et al. (2012). Prolonged visual memory enhancement after direct current stimulation in Alzheimer's disease. *Brain Stimul.* 5, 223–230. doi: 10.1016/j.brs.2011.06.006

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. S., Martins, O. E. M. S., de Macedo, E. C., and Fregni, F. (2008). Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease. *J. Neurol. Neurosurg. Psychiatry* 80, 444–447. doi: 10.1136/jnnp.2007.141853

Brem, A.-K., Unterburger, E., Speight, I., and Jäncke, L. (2014). Treatment of visuospatial neglect with biparietal tDCS and cognitive training: a single-case study. *Front. Syst. Neurosci.* 8:180. doi: 10.3389/fnsys.2014.00180

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

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

Day, B. L., Rothwell, J. C., Thompson, P. D., Dick, J. P., Cowan, J. M., Berardelli, A., et al. (1987). Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain* 110 (Pt 5), 1191–209.

de Aguiar, V., Paolazzi, C. L., and Miceli, G. (2015). tDCS in post-stroke aphasia: the role of stimulation parameters, behavioral treatment and patient characteristics. *Cortex* 63, 296–316. doi: 10.1016/j.cortex.2014.08.015

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

Dirnberger, G., and Jahanshahi, M. (2013). Executive dysfunction in Parkinson's disease: a review. *J. Neuropsychol.* 7, 193–224. doi: 10.1111/jnp.12028

Doruk, D., Gray, Z., Bravo, G. L., Pascual-Leone, A., and Fregni, F. (2014). Effects of tDCS on executive function in Parkinson's disease. *Neurosci. Lett.* 582, 27–31. doi: 10.1016/j.neulet.2014.08.043

Elder, G. J., and Taylor, J.-P. (2014). Transcranial magnetic stimulation and transcranial direct current stimulation: treatments for cognitive and neuropsychiatric symptoms in the neurodegenerative dementias? *Alzheimer's Res. Ther.* 6:74. doi: 10.1186/s13195-014-0074-1

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

Ferrucci, R., Mameli, F., Guidi, I., Mrakic-Sposta, S., Vergari, M., Marceglia, S., et al. (2008). Transcranial direct current stimulation improves recognition memory in Alzheimer disease. *Neurology* 71, 493–498. doi: 10.1212/01.wnl.0000317060.43722.a3

Fiori, V., Coccia, M., Marinelli, C. V., Vecchi, V., Bonifazi, S., Ceravolo, M. G., et al. (2011). Transcranial direct current stimulation improves word retrieval in healthy and nonfluent aphasic subjects. *J. Cogn. Neurosci.* 23, 2309–2323. doi: 10.1162/jocn.2010.21579

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

Flöel, A., Meinzer, M., Kirstein, R., Nijhof, S., Deppe, M., Knecht, S., et al. (2011). Short-term anomia training and electrical brain stimulation. *Stroke* 42, 2065–2067. doi: 10.1161/STROKEAHA.110.609032

Fridriksson, J., Richardson, J. D., Baker, J. M., and Rorden, C. (2011). Transcranial direct current stimulation improves naming reaction time in fluent aphasia: a double-blind, sham-controlled study. *Stroke* 42, 819–821. doi: 10.1161/STROKEAHA.110.600288

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

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

Jacobson, L., Koslowsky, M., and Lavidor, M. (2011). 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

Jung, I.-Y., Lim, J. Y., Kang, E. K., Sohn, H. M., and Paik, N.-J. (2011). The factors associated with good responses to speech therapy combined with transcranial direct current stimulation in post-stroke aphasic patients. *Ann. Rehabil. Med.* 35, 460–469. doi: 10.5535/arm.2011.35.4.460

Kang, E. K., Kim, Y. K., Sohn, H. M., Cohen, L. G., and Paik, N.-J. (2011). Improved picture naming in aphasia patients treated with cathodal tDCS to inhibit the right Broca's homologue area. *Restor. Neurol. Neurosci.* 29, 141–152. doi: 10.3233/RNN-2011-0587

Kehagia, A. A., Barker, R. A., and Robbins, T. W. (2010). Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. *Lancet Neurol.* 9, 1200–1213. doi: 10.1016/S1474-4422(10)70212-X

Khedr, E. M., Gamal, N. F., El, El-Fetoh, N. A., Khalifa, H., Ahmed, E. M., Ali, A. M., et al. (2014). A double-blind randomized clinical trial on the efficacy of cortical direct current stimulation for the treatment of Alzheimerâ€^{TM}s disease. *Front. Aging Neurosci.* 6:275. doi: 10.3389/fnagi.2014.00275

Ko, M.-H., Han, S.-H., Park, S.-H., Seo, J.-H., and Kim, Y.-H. (2008). Improvement of visual scanning after DC brain polarization of parietal cortex in stroke patients with spatial neglect. *Neurosci. Lett.* 448, 171–174. doi: 10.1016/j.neulet.2008.10.050

Kringelbach, M. L., Jenkinson, N., Owen, S. L. F., and Aziz, T. Z. (2007). Translational principles of deep brain stimulation. *Nat. Rev. Neurosci.* 8, 623–635. doi: 10.1038/nrn2196

Lee, S. Y., Cheon, H.-J., Yoon, K. J., Chang, W. H., and Kim, Y.-H. (2013). Effects of dual transcranial direct current stimulation for aphasia in chronic stroke patients. *Ann. Rehabil. Med.* 37, 603–610. doi: 10.5535/arm.2013.37.5.603

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(Pt 10), 2238–2247.

Litvan, I., Aarsland, D., Adler, C. H., Goldman, J. G., Kulisevsky, J., Mollenhauer, B., et al. (2011). MDS Task Force on mild cognitive impairment in Parkinson's disease: critical review of PD-MCI. *Movem. Disord.* 26, 1814–1824. doi: 10.1002/mds.23823

Mahdavi, S., Yavari, F., Gharibzadeh, S., and Towhidkhah, F. (2014). Modeling studies for designing transcranial direct current stimulation protocol in Alzheimer's disease. *Front. Comp. Neurosci.* 8:72. doi: 10.3389/fncom.2014.00072

Manenti, R., Petesi, M., Brambilla, M., Rosini, S., Miozzo, A., Padovani, A., et al. (2015). Efficacy of semantic-phonological treatment combined with tDCS for verb retrieval in a patient with aphasia. *Neurocase* 21, 109–119. doi: 10.1080/13554794.2013.873062

Marangolo, P., Fiori, V., Campana, S., Calpagnano, M. A., Razzano, C., Caltagirone, C., et al. (2014). Something to talk about: enhancement of linguistic cohesion through tdCS in chronic non fluent aphasia. *Neuropsychologia* 53, 246–256. doi: 10.1016/j.neuropsychologia.2013.12.003

McDonnell, M. D., and Ward, L. M. (2011). The benefits of noise in neural systems: bridging theory and experiment. *Nat. Rev. Neurosci.* 12, 415–426. doi: 10.1038/nrn3061

Miniussi, C., Harris, J. A., and Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neurosci. Biobehav. Rev.* 37, 1702–1712. doi: 10.1016/j.neubiorev.2013.06.014

Miranda, P. C., Mekonnen, A., Salvador, R., and Ruffini, G. (2013). The electric field in the cortex during transcranial current stimulation. *Neuroimage* 70, 48–58. doi: 10.1016/j.neuroimage.2012.12.034

Monti, A., Cogiamanian, F., Marceglia, S., Ferrucci, R., Mameli, F., Mrakic-Sposta, S., et al. (2008). Improved naming after transcranial direct current stimulation in aphasia. *J. Neurol. Neurosurg. Psychiatr.* 79, 451–453. doi: 10.1136/jnnp.2007.135277

Moss, F. (2004). Stochastic resonance and sensory information processing: a tutorial and review of application. *Clin. Neurophysiol.* 115, 267–281. doi: 10.1016/j.clinph.2003.09.014

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., and Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology* 29, 1573–1578. doi: 10.1038/sj.npp.1300517

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., 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(Pt 1), 293–301. doi: 10.1113/jphysiol.2003.049916

Penolazzi, B., Bergamaschi, S., Pastore, M., Villani, D., Sartori, G., and Mondini, S. (2014). Transcranial direct current stimulation and cognitive training in the rehabilitation of Alzheimer disease: a case study. *Neuropsychol. Rehabil.* 25, 799–817. doi: 10.1080/09602011.2014.977301

Pereira, J. B., Junqué, C., Bartrés-Faz, D., Martí, 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

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

Polanowska, K. E., Lesniak, M. M., Seniów, J. B., Czepiel, W., and Członkowska, A. (2013). Anodal transcranial direct current stimulation in early rehabilitation of patients with post-stroke non-fluent aphasia: a randomized, double-blind, sham-controlled pilot study. *Restor. Neurol. Neurosci.* 31, 761–771. doi: 10.3233/RNN-130333

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.

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin. Neurophysiol.* 126, 1071–1107. doi: 10.1016/j.clinph.2015.02.001

Rosso, C., Perlbarg, V., Valabregue, R., Arbizu, C., Ferrieux, S., Alshawan, B., et al. (2014). Broca's area damage is necessary but not sufficient to induce after-effects of cathodal tDCS on the unaffected hemisphere in post-stroke aphasia. *Brain Stimul.* 7, 627–635. doi: 10.1016/j.brs.2014.06.004

Rothwell, J. C. (1997). Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *J. Neurosci. Methods* 74, 113–22.

Sachdev, P. S., Blacker, D., Blazer, D. G., Ganguli, M., Jeste, D. V., Paulsen, J. S., et al. (2014). Classifying neurocognitive disorders: the DSM-5 approach. *Nat. Rev. Neurol.* 10, 634–642. doi: 10.1038/nrneurol.2014.181

Santos, M. D., Gagliardi, R. J., Mac-Kay, A. P. M. G., Boggio, P. S., Lianza, R., and Fregni, F. (2013). Transcranial direct-current stimulation induced in stroke patients with aphasia: a prospective experimental cohort study. *Sao Paulo Med. J.* 131, 422–426. doi: 10.1590/1516-3180.2013.1316595

Scarmeas, N., Zarahn, E., Anderson, K. E., Hilton, J., Flynn, J., Van Heertum, R. L., et al. (2003). Cognitive reserve modulates functional brain responses during memory tasks: a PET study in healthy young and elderly subjects. *Neuroimage* 19, 1215–1227. doi: 10.1016/S1053-8119(03)00074-0

Schrag, A., Hovris, A., Morley, D., Quinn, N., and Jahanshahi, M. (2006). Caregiver-burden in parkinson's disease is closely associated with psychiatric symptoms, falls, and disability. *Parkinsonism Relat. Disord.* 12, 35–41. doi: 10.1016/j.parkreldis.2005.06.011

Schrag, A., Jahanshahi, M., and Quinn, N. (2000). What contributes to quality of life in patients with Parkinson' s disease ? 69, 308–312. doi: 10.1136/jnnp.69.3.308

Shah-Basak, P. P., Norise, C., Garcia, G., Torres, J., Faseyitan, O., and Hamilton, R. H. (2015). Individualized treatment with transcranial direct current stimulation in patients with chronic non-fluent aphasia due to stroke. *Front. Hum. Neurosci.* 9:201. doi: 10.3389/fnhum.2015.00201

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

Smit, M., Schutter, D. J. L. G., Nijboer, T. C. W., Visser-Meily, J. M. A., Kappelle, L. J., Kant, N., et al. (2015). Transcranial direct current stimulation to the parietal cortex in hemispatial neglect: a feasibility study. *Neuropsychologia* 74, 152–161. doi: 10.1016/j.neuropsychologia.2015.04.014

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(Pt 11), 3011–3020. doi: 10.1093/brain/awp154

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

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *J. Intern. Neuropsychol. Soc.* 8, 448–60. doi: 10.1017/S1355617702813248

Suemoto, C. K., Apolinario, D., Nakamura-Palacios, E. M., Lopes, L., Paraizo Leite, R. E., Sales, M. C., et al. (2014). Effects of a non-focal plasticity protocol on apathy in moderate Alzheimer's Disease: a randomized, double-blind, sham-controlled trial. *Brain Stimul.* 7, 308–313. doi: 10.1016/j.brs.2013.10.003

Sunwoo, H., Kim, Y.-H., Chang, W. H., Noh, S., Kim, E.-J., and Ko, M.-H. (2013). Effects of dual transcranial direct current stimulation on post-stroke unilateral visuospatial neglect. *Neurosci. Lett.* 554, 94–98. doi: 10.1016/j.neulet.2013.08.064

Vestito, L., Rosellini, S., Mantero, M., and Bandini, F. (2014). Long-term effects of transcranial direct-current stimulation in chronic post-stroke aphasia: a pilot study. *Front. Hum. Neurosci.* 8:785. doi: 10.3389/fnhum.2014.00785

Vines, B. W., Norton, A. C., and Schlaug, G. (2011). Non-invasive brain stimulation enhances the effects of melodic intonation therapy. *Front. Psychol.* 2:230. doi: 10.3389/fpsyg.2011.00230

Volpato, C., Cavinato, M., Piccione, F., Garzon, M., Meneghello, F., and Birbaumer, N. (2013). Transcranial direct current stimulation (tDCS) of Broca's area in chronic aphasia: a controlled outcome study. *Behav. Brain Res.* 247, 211–216. doi: 10.1016/j.bbr.2013.03.029

Wu, D., Wang, J., and Yuan, Y. (2015). Effects of transcranial direct current stimulation on naming and cortical excitability in stroke patients with aphasia. *Neurosci. Lett.* 589C, 115–120. doi: 10.1016/j.neulet.2015.01.045

You, D. S., Kim, D.-Y., Chun, M. H., Jung, S. E., and Park, S. J. (2011). Cathodal transcranial direct current stimulation of the right Wernicke's area improves comprehension in subacute stroke patients. *Brain Lang.* 119, 1–5. doi: 10.1016/j.bandl.2011.05.002

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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on