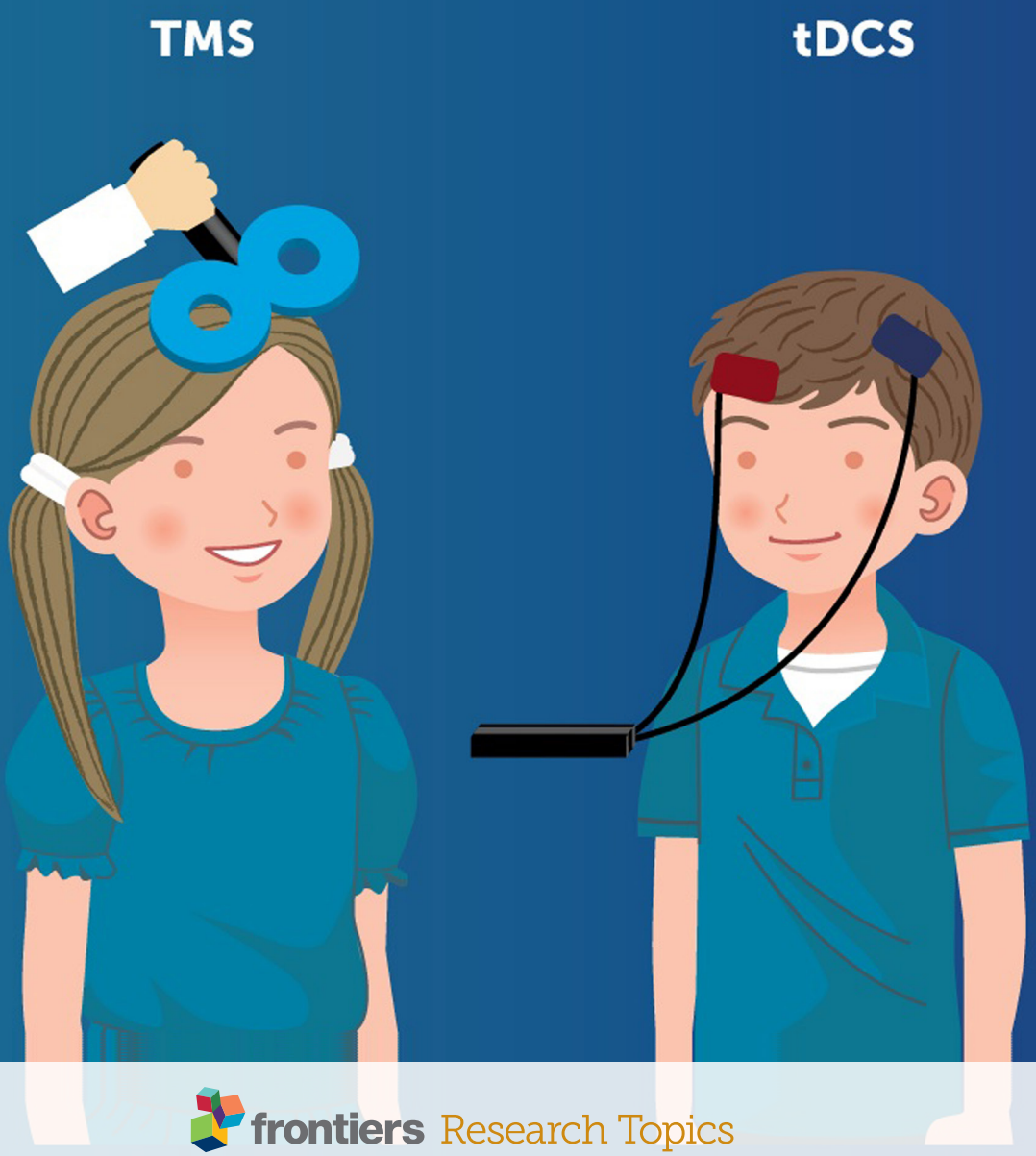
THE SAFETY AND EFFICACY OF NONINVASIVE BRAIN STIMULATION IN DEVELOPMENT AND NEURODEVELOPMENTAL DISORDERS

EDITED BY: Lindsay M. Oberman and Peter G. Enticott
PUBLISHED IN: Frontiers in Human Neuroscience



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88919-699-9
DOI 10.3389/978-2-88919-699-9

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**

THE SAFETY AND EFFICACY OF NONINVASIVE BRAIN STIMULATION IN DEVELOPMENT AND NEURODEVELOPMENTAL DISORDERS

Topic Editors:
Lindsay M. Oberman, E. P. Bradley Hospital and Warren Alpert Medical School, Brown University, USA
Peter G. Enticott, Deakin University, Australia

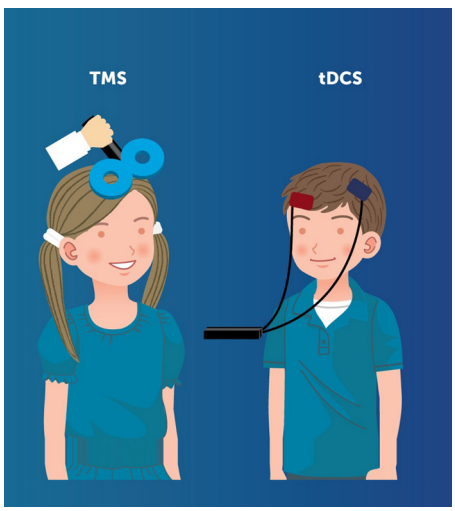


Image provided by Boston Children's Hospital.

Noninvasive brain stimulation (including Transcranial Magnetic Stimulation (TMS) and Transcranial Current Brain Stimulation (TCS)) can be used both experimentally and therapeutically. In the experimental domain TMS can be applied in single pulses to depolarize a small population of neurons in a targeted brain region. This protocol can be used, for example, to map cortical motor outputs, study central motor conduction time, or evaluate the cortical silent period (a measure of intracortical inhibition) all of which are relevant to neurodevelopment. TMS can also be applied in pairs of pulses (paired pulse stimulation, ppTMS) where two pulses are presented in rapid succession to study intracortical inhibition and facilitation. Trains of repeated TMS (rTMS) pulses can be applied at various stimulation frequencies and patterns to modulate local cortical excitability beyond the duration of the stimulation itself. Depending on the parameters of stimulation the excitability can be either facilitated or suppressed. TCS (including Transcranial Direct Current Stimulation (tDCS), alternating current (tACS), and random noise current stimulation (tRNS) also have the potential to modulate cortical excitability and have also been used to study and modulate cortical activity in healthy and patient populations. The after-effects of rTMS and TCS are thought to be related to changes in efficacy (in either the positive or negative direction) of synaptic connections of the neurons being stimulated, thus these techniques have been used to study and modulate cortical plasticity mechanisms in a number of populations. Recently, researchers have begun to apply these techniques to the study of neurodevelopmental mechanisms as well as the pathophysiology and development of novel treatments for neurodevelopmental disorders. Though there is much promise, caution is warranted given the vulnerability of pediatric and clinical populations and the potential that these techniques have to modify circuit development in a cortex that is in a very dynamic state.

This Research Topic hopes to provide an opportunity to share ideas across areas (human and animal researchers, clinicians and basic scientists). We are particularly interested in papers that address issues of choosing a protocol (intensity, frequency, location, coil geometry etc.), populations where noninvasive brain stimulation may have direct impact on diagnostics and treatment, as well as the safety and ethics of applying these techniques in pediatric populations. As many may not be aware of the potential and limitations of noninvasive brain stimulation and its use for research and treatment in this area, this Research Topic promises to have broad appeal. Submissions for all Frontiers article types are encouraged.

Citation: Oberman, L. M., Enticott, P. G., eds. (2015). The Safety and Efficacy of Noninvasive Brain Stimulation in Development and Neurodevelopmental Disorders. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-699-9

Table of Contents

frontiers in Human Neuroscience

EDITORIAL
published: 02 October 2015
doi: 10.3389/fnhum.2015.00544

Editorial: The safety and efficacy of noninvasive brain stimulation in development and neurodevelopmental disorders

Lindsay M. Oberman[1]* *and Peter G. Enticott*[2]

[1] *Neuroplasticity and Autism Spectrum Disorder Program, Department of Psychiatry and Human Behavior, E.P. Bradley Hospital and Warren Alpert Medical School, Brown University, Providence, RI, USA,* [2] *Cognitive Neuroscience Unit, School of Psychology, Deakin University, Burwood, VIC, Australia*

Keywords: noninvasive brain stimulation, pediatric, transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), safety

Noninvasive brain stimulation (NIBS) techniques including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are emerging as neuroscientific techniques that can be used as *in vivo* probes of brain function as well as therapeutic tools in a number of psychiatric and neurological disorders. Though much of the research and applications with these techniques have been applied to adult psychiatry and neurology, recent years have seen a number of researchers applying these tools to study brain development in typically developing children as well as those with neurodevelopmental and child psychiatric and neurological disorders. Clinical trials and case series designs have also been used to develop novel therapeutic interventions using these NIBS techniques in pediatric clinical populations and researchers are forming working groups dedicated to the application of NIBS to specific neurodevelopmental disorders (e.g., Autism Spectrum Disorder, Oberman et al., 2014a).

The papers in this research topic highlight the excitement in the field and the promise of these techniques both for the understanding of neurodevelopment (Pedapati et al., 2015) and neuropathology of neurodevelopmental disorders (Croarkin et al., 2014; Oberman et al., 2014b) as well as novel treatment development for neurodevelopmental disorders (Casanova et al., 2014; Gillick et al., 2014). This excitement and promise, however, is appropriately tempered by other papers in this research topic that highlight the unknown risks and potential ethical concerns related to applying these techniques in pediatric populations (Davis, 2014; Maslen et al., 2014).

A recent metaanalysis (Rajapakse and Kirton, 2013) reviewed the studies to date involving all rTMS protocols in children (approximately 1000 children have been studied across all rTMS protocols to date) and concluded "Its minimal risk, excellent tolerability and increasingly sophisticated ability to interrogate neurophysiology and plasticity make it an enviable technology for use in pediatric research with future extension into therapeutic trials." This was supported by a paper in this topic highlighting the safety and tolerability of a specific paradigm, Theta Burst stimulation (Hong et al., 2015).

The most serious possible TMS-related adverse event is induction of a seizure. To date, 16 cases of TMS-induced seizures have been reported out of tens of thousands of examined subjects over the past 25 years. Overall the risk of seizure is considered to be less than 0.01% across all patients and all paradigms (Rossi et al., 2009). The risk of overall adverse event burden from TMS, however, may be underestimated due to the lack of systematic identification, tracking, and reporting of adverse events in study publications. Thus, the safety, tolerability, and efficacy have not been characterized sufficiently to justify off-label clinical use of NIBS, especially in pediatric populations. At this point, use of these technologies either for investigational or clinical use should be under the context of an

OPEN ACCESS

Edited and reviewed by:
Srikantan S. Nagarajan, University of California, San Francisco, USA

***Correspondence:**
Lindsay M. Oberman
loberman@lifespan.org

Received: *27 May 2015*
Accepted: *17 September 2015*
Published: *02 October 2015*

Citation:
Oberman LM and Enticott PG (2015) Editorial: The safety and efficacy of noninvasive brain stimulation in development and neurodevelopmental disorders. Front. Hum. Neurosci. 9:544. doi: 10.3389/fnhum.2015.00544

investigational device exemption (IDE) or IRB approved research trial. Unfortunately, there have been instances of "do-it-yourself" brain stimulation devices entering the marketplace, raising the possibility that these techniques will be applied to individuals with neurodevelopmental disorders without an evidence-base, regulatory oversight, or appropriate expertise.

Despite the therapeutic promise of repetitive TMS for neurodevelopmental disorders, translation to "treatment-based" protocols poses a number of important challenges and complexities. For instance, there are various considerations in selecting pulse sequences (e.g., frequency, intensity), regions of stimulation, and coil type, each combination of which is likely to have different efficacy and side-effect profiles. While TMS has been the primary technique employed in neurodevelopment thus far, electrical stimulation techniques (e.g., tDCS, transcranial alternating current stimulation [tACS]) have very different mechanisms of action and risk profiles (e.g., seizure induction is not generally indicated in tDCS/tACS). Brain stimulation protocols can also have differing effects across participants, and these effects might be exacerbated when considering the heterogeneity of neurodevelopmental disorders such as autism spectrum disorder (ASD).

Another important factor to consider in trialing therapeutic interventions is the optimal age of intervention. It might be argued that the greatest effects will be seen if NIBS is applied early in development, when the brain is considered more plastic. As noted, however, there are important ethical and feasibility concerns around NIBS in children. At present, a relatively small number of typically developing children and children with neurodevelopmental disorder have undergone NIBS. Single pulse TMS has been applied to study development of corticospinal projections in neonates within hours of birth (Eyre et al., 2001), however, repetitive (rTMS) has been limited to older children and adolescents. Thus, any interaction between repetitive brain stimulation and neurodevelopment is currently unknown. This is particularly important in the context of developmental disorders where in most cases the developmental neuropathology has yet to be fully elucidated.

In conclusion, there is an obvious need for further research in this area. Specifically, studies focusing on developmental trajectories and how the effects of NIBS change across childhood would be extremely useful. The use of NIBS in children is a burgeoning field whose full potential has yet to be realized. The papers in this research topic speak to both the promise and the challenges that researchers and clinicians face when applying NIBS techniques to study typical development, developmental pathophysiology, and as potential nonpharmacological, brain-based treatments for neurodevelopmental disorders.

Author Contributions

LO and PE co-wrote the manuscript.

Acknowledgments

PE is supported by a Career Development Fellowship from the National Health and Medical Research Council (NHMRC) of Australia. LO is supported by the Nancy Lurie Marks Family Foundation.

References

Casanova, M. F., Hensley, M. K., Sokhadze, E. M., El-Baz, A. S., Wang, Y., Li, X., et al. (2014). Effects of weekly low-frequency rTMS on autonomic measures in children with autism spectrum disorder. *Front. Hum. Neurosci.* 8:851. doi: 10.3389/fnhum.2014.00851

Croarkin, P. E., Nakonezny, P. A., Lewis, C. P., Zaccariello, M. J., Huxsahl, J. E., Husain, M. M., et al. (2014). Developmental aspects of cortical excitability and inhibition in depressed and healthy youth: an exploratory study. *Front. Hum. Neurosci.* 8:669. doi: 10.3389/fnhum.2014.00669

Davis, N. J. (2014). Transcranial stimulation of the developing brain: a plea for extreme caution. *Front. Hum. Neurosci.* 8:600. doi: 10.3389/fnhum.2014.00600

Eyre, J. A., Taylor, J. P., Villagra, F., Smith, M., and Miller, S. (2001). Evidence of activity-dependent withdrawal of corticospinal projections during human development. *Neurology* 57, 1543–1554. doi: 10.1212/WNL.57.9.1543

Gillick, B. T., Kirton, A., Carmel, J. B., Minhas, P., and Bikson, M. (2014). Pediatric stroke and transcranial direct current stimulation: methods for rational individualized dose optimization. *Front. Hum. Neurosci.* 8:739. doi: 10.3389/fnhum.2014.00739

Hong, Y. H., Wu, S. W., Pedapati, E. V., Horn, P. S., Huddleston, D. A., Laue, C. S., et al. (2015). Safety and tolerability of theta burst stimulation vs. single and paired pulse transcranial magnetic stimulation: a comparative study of 165 pediatric subjects. *Front. Hum. Neurosci.* 9:29. doi: 10.3389/fnhum.2015.00029

Maslen, H., Earp, B. D., Cohen Kadosh, R., and Savulescu, J. (2014). Brain stimulation for treatment and enhancement in children: an ethical analysis. *Front. Hum. Neurosci.* 8:953. doi: 10.3389/fnhum.2014.00953

Oberman, L. M., Enticott, P. G., Casanova, M. F., Rotenberg, A., Pascual-Leone, A., and McCracken, J. T. (2014a). Transcranial magnetic stimulation (TMS) therapy for autism: an international consensus conference held in conjunction with the international meeting for autism research on May 13th and 14th, 2014. *Front. Hum. Neurosci.* 8:1034. doi: 10.3389/fnhum.2014.01034

Oberman, L. M., Pascual-Leone, A., and Rotenberg, A. (2014b). Modulation of corticospinal excitability by transcranial magnetic stimulation in children and adolescents with autism spectrum disorder. *Front. Hum. Neurosci.* 8:627. doi: 10.3389/fnhum.2014.00627

Pedapati, E. V., Gilbert, D. L., Horn, P. S., Huddleston, D. A., Laue, C. S., Shahana, N., et al. (2015). Effect of 30 Hz theta burst transcranial magnetic stimulation on the primary motor cortex in children and adolescents. *Front. Hum. Neurosci.* 9:91. doi: 10.3389/fnhum.2015.00091

Rajapakse, T., and Kirton, A. (2013). Non-invasive brain stimulation in children: applications and future directions. *Transl. Neurosci.* 4, 217–223. doi: 10.2478/s13380-013-0116-3

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

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
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 04 February 2015
doi: 10.3389/fnhum.2015.00029

Safety and tolerability of theta burst stimulation vs. single and paired pulse transcranial magnetic stimulation: a comparative study of 165 pediatric subjects

Yaejee H. Hong[1], Steve W. Wu[2]*, Ernest V. Pedapati[2,3], Paul S. Horn[2], David A. Huddleston[2], Cameron S. Laue[3] and Donald L. Gilbert[2]

[1] College of Medicine, University of Cincinnati, Cincinnati, OH, USA
[2] Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[3] Division of Psychiatry, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Edited by:
Lindsay M. Oberman, Brown University, USA

Reviewed by:
Richard Eugene Frye, Children's Hospital Boston/Harvard University, USA
Paul Croarkin, Mayo Clinic, USA

****Correspondence:***
Steve W. Wu, Division of Neurology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave. MLC 2015, Cincinnati, OH 45229, USA
e-mail: steve.wu@cchmc.org

Background: Although single- and paired-pulse (sp/pp) transcranial magnetic stimulation (TMS) studies are considered minimal risk in adults and children, the safety profile for theta-burst TMS (TBS) is unknown.

Objective: In this comparative analysis, we explored the rate, severity, and specific symptoms of TMS-related adverse effects (AEs) between sp/ppTMS and TBS in subjects between ages 6 and 18 years.

Method: Data from 165 participants from 2009 to 2014 were analyzed. Assessment of AEs was performed based on baseline and post-TMS administration of a symptom-based questionnaire that rated AEs on a 5-level ordinal scale (minimal, mild, moderate, marked, severe). AE rates and severity were compared using Chi Square or Fisher's Exact Test depending on data characteristics.

Result: Overall, no seizures or severe-rated AEs were reported by 165 pediatric participants. The rate of AE in all TBS sessions was 10.5% (n = 76, 95% CI: 4.7–19.7%), whereas the rate of AE in all sp/ppTMS sessions was 12.4% (n = 89, 95% CI: 6.3–21.0%). There was no statistical difference in AE rates between TBS and sp/ppTMS (p = 0.71). In all sp/ppTMS and TBS sessions, 20 subjects reported a total of 35 AEs, among these 31 (∼88.6%) were rated as "minimal" or "mild". There was no difference in the severity of AE between TBS and sp/ppTMS (p = 1.0). Only one of 76 TBS participants reported an AE rated as more than minimal/mild.

Conclusion: Our comparative analysis showed that TBS appears to be as safe as sp/ppTMS in terms of AE rate and severity. This report supports further investigation of TBS in children.

Keywords: children, youth, transcranial magnetic stimulation, repetitive transcranial magnetic stimulation, theta burst stimulation, safety

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive form of brain stimulation that has been increasingly used to develop physiological biomarkers and in therapeutic applications of neurological and psychiatric conditions across a wide range of subjects. Currently, consensus guidelines have suggested that single- and paired-pulse TMS (sp/ppTMS) may be considered as minimal risk in children (Gilbert et al., 2004; Rossi et al., 2009). In contrast, repetitive TMS (rTMS) which may include rapid trains of TMS pulses do not have clear guidelines for use in the pediatric population and carry a potential risk of epileptogenesis (Oberman and Pascual-Leone, 2009). Theta Burst Stimulation (TBS), a type of rTMS, can induce effects on cortical excitability that outlast the stimulation period (Huang et al., 2005). Although TBS and conventional rTMS have been shown to elicit comparable cortical neurophysiologic changes (Zafar et al., 2008; Di Lazzaro et al., 2011), the TBS procedure has two advantages: (1) shorter stimulation duration; and (2) lower stimulation intensity. These features decrease the likelihood of discomfort from TMS pulses, thus making TBS a potentially ideal rTMS protocol to use in pediatric studies.

Several systematic reviews have suggested that TBS is relatively well-tolerated in the adult population including two recent studies that have estimated an approximately 5% rate of adverse events of adults undergoing TBS, which are primarily mild (Oberman et al., 2011; Maizey et al., 2013). To our knowledge, only one serious adverse event (AE), seizure, was reported in

a healthy adult male during continuous TBS performed at 100% of resting motor threshold (RMT; Oberman and Pascual-Leone, 2009). In children, we recently reported a total AE rate of 11.6% in 40 children undergoing TBS (Wu et al., 2012).

Future applications of TBS in children as a biomarker or as a therapeutic modality are contingent on a clearer estimate of potential risks of adverse events including sharing sensitive safety data between laboratories (Rossi et al., 2009). In the present report, we compare AE rates between TBS and sp/ppTMS in a cohort of youth over a five year period in a TMS lab within a large stand-alone children's hospital. We additionally explored the incidence of adverse events across protocol parameters and examined predictors of adverse events.

MATERIALS AND METHODS

PARTICIPANTS

Data from 165 unique participants (69 females, 96 males) between ages 6–18 years were analyzed from Institutional Review Board (IRB)-approved protocols which were active in our TMS Lab between 2009 and 2014. Subjects with epilepsy, hearing problems, serious medical condition(s), or implanted medical device(s) were excluded from participation. Recruitment occurred through sub-specialty clinics, hospital wide emails, and from the community. Safety data for TBS was drawn from two studies: (1) TBS technique optimization and biomarker studies which involved healthy and Tourette Syndrome (TS) youths; and (2) a sham-controlled continuous TBS study in TS (Wu et al., 2014). Sp/ppTMS safety data was summarized from studies involving youth with attention deficit hyperactivity disorder (ADHD) and typically developed controls. For the sp/ppTMS studies, ADHD subjects on non-stimulant medications (e.g., atomoxetine) were excluded and those on stimulants were instructed to hold the medication for at least 24 h prior to participation. None of the typically developed controls were on any neuropsychiatric medications at the time of participation. All parent(s)/guardian(s) gave written informed consent for the studies.

TRANSCRANIAL MAGNETIC STIMULATION

Sp/ppTMS was performed with a Magstim 200/Bistim stimulator and a 70 mm figure-8 coil (Magstim Co., Wales, UK). Surface electromyography (EMG) leads were placed over the dominant first dorsal interosseous (FDI) muscle. The coil was placed over the dominant primary motor cortex at the optimal site for obtaining a motor-evoked potential (MEP). RMT and active motor thresholds, cortical silent period, and single and paired pulse amplitudes and ratios were quantified using standard methods, requiring approximately 200 TMS pulses (Rossini et al., 1994; Mills and Nithi, 1997). In paired pulse TMS studies, the intensities of the conditioning and test pulses were predetermined and set at 60% and 120% of RMT respectively. TBS was performed with Magstim SuperRapid2 (Magstim Co., Wales, UK). TBS stimulation intensities ranged from 60–90% RMT. Three pulses were administered at 30 to 50 Hz pulse frequency, 5 Hz burst frequency, with a total number of pulses of either 300 or 600 (Huang et al., 2005). TBS was preceded and followed by spTMS used for post-TBS MEP measurement. This required approximately 200 spTMS pulses. Nine participants received both intermittent and continuous TBS (iTBS, cTBS). TBS administration for the sham controlled trial (Wu et al., 2014) did not involve post TBS assessment of MEP amplitudes.

ASSESSMENT OF ADVERSE EVENTS

A sixteen-question review of systems (ROS) questionnaire was administered to rate the subjective symptom (headache, scalp pain, arm/hand pain, other pain(s), numbness/tingling, other sensation(s), weakness, loss of dexterity, vision/hearing change(s), ear ringing, nausea/vomiting, appetite loss, rash, skin change(s) or any other symptom(s)) on a scale of 0 to 5 (none, minimal, mild, moderate, marked, severe) prior to any TMS application. At the end of the study after the entire TMS session, this ROS was repeated to detect any AE. The presence of an AE was defined as a positive increase in any of the ROS criteria compared to pre-TMS. The rate of AE was defined as the ratio of sessions with adverse events divided by total sessions. In the Tourette Syndrome study (Wu et al., 2014), patients received two consecutive days of real or sham TBS. Only day 1 AEs were used for data analysis.

STATISTICAL ANALYSIS

T-test or Wilcoxon Mann Whitney test was used to compare demographics depending on data distribution. Comparison of AE rates were analyzed using either Chi Square or Fisher's Exact Test depending on whether any cell of the 2×2 table has a count <5. All types of TBS were combined into one group. TS, ADHD, and other motor disorder were considered "affected". Logistic regression analyses were used to estimate effects of additional predictors. Analyses including power calculations were performed using SAS v9.3 (Cary, NC).

RESULTS

DEMOGRAPHICS

Demographics and clinical data are shown in **Table 1**. Among 76 children receiving TBS, 68% were typically developing healthy controls, 25% had a diagnosis of TS, or 7% had other motor disorders. Among 89 participants receiving sp/ppTMS, 21% participants were healthy controls, and 79% participants were affected with ADHD. Of the 24 TBS participants with either TS or other motor disorders, 15 of them were taking neuropsychiatric medications at the time of the TMS session. Collectively, these medications included amitriptyline, atomoxetine, baclofen, citalopram, clonidine, dexmethylphenidate, escitalopram, guanfacine, melatonin, methylphenidate, pimozide, quetiapine, risperidone and sertraline.

ADVERSE EVENT RATES

AE rates are shown in **Table 2**. All participants completed sp/ppTMS or TBS sessions without seizures and there were no serious adverse events. There was no statistical difference in AE rates between sp/ppTMS and TBS sessions ($p = 0.71$). There was no difference in frequency of AE between sham and real TBS in

Table 1 | All participant characteristics and adverse events.

Participant characteristics	TBS protocols (n = 76)	Sp/ppTMS protocols (n = 89)	p-value
Age, mean (SD)	12.3 (2.9)	10.3 (2.5)	<0.0001
Sex (% male)	57.9%	58.4%	0.63
Diagnosis (% control)	68.4%	21.3%	<0.0001
% Sessions with adverse events (95% CI)	10.5% (4.7–19.7%)	12.4% (6.3–21.0%)	0.71

CI = confidence interval, SD = standard deviation, TBS = Theta Burst Stimulation, Sp/ppTMS = single and paired pulse transcranial magnetic stimulation.

the clinical trial. The AE rates were not statistically significant (p = 1.0) between cTBS-only (12.5%, n = 8) and iTBS-only sessions (11.9%; n = 59).

SEVERITY AND SPECIFIC SYMPTOMS

In TBS sessions, no "marked" or "severe" symptoms were reported. Of thirteen post-TBS AEs, twelve (92.3%) were rated "minimal' or "mild" with one described as "moderate". There were twenty-two post-sp/ppTMS AEs: twenty (90.9%) were rated "minimal" or "mild" and two were "moderate" or "marked". Proportions of minimal/mild AEs did not differ between TBS and sp/ppTMS (p = 1.0). Specific symptoms were comparable (**Table 2**).

ADVERSE EVENT RATES: HEALTHY VS. AFFECTED CHILDREN

Healthy control participants reported AEs in 11.5% of TBS vs. 5.3% of sp/ppTMS sessions (p = 0.67) (**Table 3**). Children with neurological diagnoses reported AEs in 8.3% of TBS vs. 14.3% of sp/ppTMS sessions (p = 0.72).

ADVERSE EVENT RATES: SHAM VS. ACTIVE TBS

In a small sample of participants who received either active or sham cTBS (Wu et al., 2014), no difference was detected between sham vs. active TBS (p = 1.0).

Table 2 | Percentages of participants experiencing specific adverse events.

Symptom	TBS (n = 76)	Sp/ppTMS (n = 89)
Headache	6.6%	6.7%
Scalp pain	–	4.5%
Arm/hand/other pain	2.6%*	2.2%
Numbness/tingling	2.6%	5.6%
Other sensations	2.6%	1.1%**
Weakness	1.3%	–
Ringing in ears	–	1.1%*
Nausea/vomiting	–	1.1%
Other	1.3%	1.1%

*All adverse events were rated minimal to mild except: *one subject each rated "moderate"; **one subject rated "marked". "Other sensations**" referred to chest pain that was present at baseline due to one subject having an upper respiratory infection at the time of the visit. The "ringing in ears*" and "other sensations**" ratings were from the same individual within one sp/ppTMS session. TBS = Theta Burst Stimulation, Sp/ppTMS = single and paired pulse transcranial magnetic stimulation.*

PREDICTORS OF AEs

No predictors of the odds of an AE were identified after data relating to all participants (Age, Sex, Diagnosis, RMT, mode of TMS) were entered into a backward logistic regression analysis.

DISCUSSION

In this brief report, we found no greater rate of adverse events of TBS compared to sp/ppTMS in a large cohort of pediatric subjects. The majority of AEs reported were classified minimal or mild with no severe or serious AE such as seizure. Headache was the most commonly reported specific AE in both groups. These findings represent the largest published sample analyzed to address an important gap in the safety literature regarding youth who have underwent TBS and contribute to other safety studies of TBS (Oberman et al., 2011; Wu et al., 2012; Maizey et al., 2013). The findings of this study are reassuring with regards of continued judicious use of similar TBS methods in youth.

The purpose of comparing TBS-induced AE rates to that of sp/ppTMS is because single and paired-pulse stimulations have been suggested to be "minimal risk" in children (Gilbert et al., 2004). The most recent international TMS Consensus Group "cautiously conclude that single-pulse and paired-pulse TMS in pediatrics is safe for children two years and older (Rossi et al., 2009)". However, not all local IRB or ethics boards may agree with this statement. In the Code of Federal Regulations Section 46.102 of the United States of America, Minimal Risk means that the "probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examination or tests". According to this definition, sp/ppTMS may be considered as Minimal Risk for the following reasons. First, children have rated the sp/ppTMS experience more enjoyable than several common life events (long car ride, throwing up, go to dentist, shot at the doctor's) (Garvey et al., 2001). Second, like other medical tests commonly used in pediatric patients (e.g., brain MRI, computer tomography, nerve conduction study and EMG), TMS delivers "energy" into the human body. Contrarily, one can certainly argue that healthy children would not otherwise receive any of these medical tests and therefore TMS participation might constitute greater than minimal risk. Ultimately when working with the IRB/ethics board in developing any pediatric TMS (sp/pp or rTMS) study, it is critical to concisely present the TMS technology, scientific background/rationale, screening procedure (Rossi et al., 2011) and safety monitoring (Rossi et al., 2009; Krishnan et al., 2015)

Table 3 | Healthy control participant characteristics and adverse events.

Participant characteristics	TBS protocols ($n = 52$)	Sp/ppTMS protocols ($n = 19$)	p-value
Age, mean (SD)	12.6 (2.8)	12.1 (3.2)	0.44
Sex (% male)	51.9%	47.4%	0.79
% Sessions with adverse events (95% CI)	11.5% (4.4–23.4%)	5.3% (0.13–26.0%)	0.67

CI = confidence interval, SD = standard deviation, TBS = Theta Burst Stimulation, Sp/ppTMS = single and paired pulse transcranial magnetic stimulation.

for the proposed study. As more pediatric TMS safety data emerges, it may become easier for investigators and IRB/ethical boards to objectively decide whether the proposed study is safe to proceed.

The two primary limitations of the study are sample size, and the timing of AE assessments after both sp/ppTMS and TBS in TBS protocols. Verifying small differences in rates of AEs may not be feasible without much larger samples. Using our data, we estimate approximately 4,400 children would be needed per a group to have an 80% power to detect this ∼2% difference using an alpha of 0.05. AE assessments at the end of the session captured added spTMS plus TBS effects as participants also received spTMS for TBS sessions. Our finding of comparable AE rates thus suggests that the majority of AEs in TBS sessions occurred may be due to spTMS. This is further supported by findings in the sham-controlled study (Wu et al., 2014) which, consistent with the adult data (Maizey et al., 2013), were equivalent in the true and sham TBS arms. While the AE rates may also have been influenced by differences in age and case mix, the negative regression analysis suggests these effects were, at most, small. Another significant limitation is that we pooled the safety data from several studies over a five year period. Therefore, this analysis represents a heterogeneous group, including typically developing youth as well as affected pediatric population across different TMS protocols. Only one of our studies in this time period employed a sham TBS stimulation, therefore, we analyzed the data separately. Although we did not detect a difference in the sham vs. active TBS AE rates, this analysis is limited by the small sample size ($n = 10$) (Wu et al., 2014). Due to the same issue of small sample size, another concern that cannot be fully addressed in this report is the effect(s) of concurrent neuropsychiatric medication(s) on TBS safety. At the time of TBS sessions, fifteen participants were actively taking neuropsychiatric medications, some of which are known to lower seizure threshold (Pisani et al., 2002) and change cortical excitability (Ziemann et al., 2014). Given that some children were on medications that can potentially lower seizure threshold, it is encouraging that no one developed any TBS-induced seizures. Finally, the epilepsy exclusionary criterion is a limiting factor. Although available data suggests that seizure induction during rTMS for epilepsy patients is relatively low, most of the data are from adult populations (Bae et al., 2007). As TMS is increasingly used in epilepsy research, the risk of seizure provocation by various forms of rTMS, including TBS, in children with epilepsy is a knowledge gap that needs to be addressed.

TBS and other forms of rTMS hold promise for future pediatric neurophysiologic studies (Oberman et al., 2010, 2014; Damji et al., 2013) and clinical trials (Kirton et al., 2008; Wu et al., 2014). With comprehensive ongoing safety monitoring, published frequencies, and subthreshold intensities, further investigation of TBS within previously reported parameters in children appears to confer no greater risk than single and paired-pulse TMS.

ACKNOWLEDGMENTS

These studies were supported by Cincinnati Children's Hospital Medical Center Division of Neurology, the USA Tourette Syndrome Association and National Institute of Health (R01MH081854, R01MH078160).

REFERENCES

Bae, E. H., Schrader, L. M., Machii, K., Alonso-Alonso, M., Riviello, J. J. Jr., Pascual-Leone, A., et al. (2007). Safety and tolerability of repetitive transcranial magnetic stimulation in patients with epilepsy: a review of the literature. *Epilepsy Behav.* 10, 521–528. doi: 10.1016/j.yebeh.2007.03.004

Damji, O., Roe, J., Shinde, S., Kotsovsky, O., and Kirton, A. (2013). P 174. Effects of paired associative stimulation on developmental motor plasticity in children. *Clin. Neurophysiol.* 124, e147–e148. doi: 10.1016/j.clinph.2013.04.251

Di Lazzaro, V., Dileone, M., Pilato, F., Capone, F., Musumeci, G., Ranieri, F., et al. (2011). Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *J. Neurophysiol.* 105, 2150–2156. doi: 10.1152/jn.00781.2010

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

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

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

Kirton, A., Chen, R., Friefeld, S., Gunraj, C., Pontigon, A. M., and Deveber, G. (2008). Contralesional repetitive transcranial magnetic stimulation for chronic hemiparesis in subcortical paediatric stroke: a randomised trial. *Lancet Neurol.* 7, 507–513. doi: 10.1016/s1474-4422(08)70096-6

Krishnan, C., Santos, L., Peterson, M. D., and Ehinger, M. (2015). Safety of noninvasive brain stimulation in children and adolescents. *Brain Stimul.* 8, 76–87. doi: 10.1016/j.brs.2014.10.012

Maizey, L., Allen, C. P., Dervinis, M., Verbruggen, F., Varnava, A., Kozlov, M., et al. (2013). Comparative incidence rates of mild adverse effects to transcranial magnetic stimulation. *Clin. Neurophysiol.* 124, 536–544. doi: 10.1016/j.clinph.2012.07.024

Mills, K. R., and Nithi, K. A. (1997). Corticomotor threshold to magnetic stimulation: normal values and repeatability. *Muscle Nerve* 20, 570–576. doi: 10.1002/(sici)1097-4598(199705)20:5<570::aid-mus5>3.3.co;2-f

Oberman, L., Edwards, D., Eldaief, M., and Pascual-Leone, A. (2011). Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. *J. Clin. Neurophysiol.* 28, 67–74. doi: 10.1097/WNP.0b013e318205135f

Oberman, L., Ifert-Miller, F., Najib, U., Bashir, S., Woollacott, I., Gonzalez-Heydrich, J., et al. (2010). Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile x syndrome and autism spectrum disorder. *Front. Synaptic Neurosci.* 2:26. doi: 10.3389/fnsyn.2010.00026

Oberman, L. M., and Pascual-Leone, A. (2009). Report of seizure induced by continuous theta burst stimulation. *Brain Stimul.* 2, 246–247. doi: 10.1016/j.brs.2009.03.003

Oberman, L. M., Pascual-Leone, A., and Rotenberg, A. (2014). Modulation of corticospinal excitability by transcranial magnetic stimulation in children and adolescents with autism spectrum disorder. *Front. Hum. Neurosci.* 8:627. doi: 10.3389/fnhum.2014.00627

Pisani, F., Oteri, G., Costa, C., Di Raimondo, G., and Di Perri, R. (2002). Effects of psychotropic drugs on seizure threshold. *Drug Saf.* 25, 91–110. doi: 10.2165/00002018-200225020-00004

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clin. Neurophysiol.* 122:1686. doi: 10.1016/j.clinph.2010.12.037

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., 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

Wu, S. W., Maloney, T., Gilbert, D. L., Dixon, S. G., Horn, P. S., Huddleston, D. A., et al. (2014). Functional MRI-navigated repetitive transcranial magnetic stimulation over supplementary motor area in chronic tic disorders. *Brain Stimul.* 7, 212–218. doi: 10.1016/j.brs.2013.10.005

Wu, S. W., Shahana, N., Huddleston, D. A., Lewis, A. N., and Gilbert, D. L. (2012). Safety and tolerability of theta-burst transcranial magnetic stimulation in children. *Dev. Med. Child Neurol.* 54, 636–639. doi: 10.1111/j.1469-8749.2012.04300.x

Zafar, N., Paulus, W., and Sommer, M. (2008). Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. *Clin. Neurophysiol.* 119, 1393–1399. doi: 10.1016/j.clinph.2008.02.006

Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., et al. (2014). TMS and drugs revisited 2014. *Clin. Neurophysiol.* doi: 10.1016/j.clinph.2014.08.028. [Epub ahead of print].

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

Received: 23 December 2014; paper pending published: 29 December 2014; accepted: 12 January 2015; published online: 04 February 2015.

Citation: Hong YH, Wu SW, Pedapati EV, Horn PS, Huddleston DA, Laue CS and Gilbert DL (2015) Safety and tolerability of theta burst stimulation vs. single and paired pulse transcranial magnetic stimulation: a comparative study of 165 pediatric subjects. Front. Hum. Neurosci. ***9****:29. doi: 10.3389/fnhum.2015.00029*

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

frontiers in
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 25 February 2015
doi: 10.3389/fnhum.2015.00091

Effect of 30 Hz theta burst transcranial magnetic stimulation on the primary motor cortex in children and adolescents

Ernest V. Pedapati[1,2]*, Donald L. Gilbert[1], Paul S. Horn[1,3], David A. Huddleston[1], Cameron S. Laue[2], Nasrin Shahana[1] and Steve W. Wu[1]

[1] *Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
[2] *Division of Child and Adolescent Psychiatry, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
[3] *Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Edited by:
Lindsay M. Oberman, University of California-San Diego, USA

Reviewed by:
Marco Iacoboni, University of California Los Angeles, USA
Michael A. Nitsche, Georg-August-University, Germany
Nick J. Davis, Swansea University, UK

****Correspondence:***
Ernest V. Pedapati, Division of Child and Adolescent Psychiatry, Division of Neurology, E-2 Psychiatry, 3333 Burnett Avenue, Cincinnati, OH 45229, USA
e-mail: ernest.pedapati@cchmc.org

Fourteen healthy children (13.8 ± 2.2 years, range 10–16; M:F = 5:9) received 30 Hz intermittent theta burst transcranial magnetic stimulation (iTBS) with a stimulation intensity of 70% of resting motor threshold (RMT) with a total of 300 (iTBS300) pulses. All volunteers were free of neurologic, psychiatric and serious medical illnesses, not taking any neuropsychiatric medications, and did not have any contraindications to transcranial magnetic stimulation. Changes in the mean amplitudes of motor-evoked potentials from baseline following iTBS were expressed as a ratio and assessed from 1 to 10 min (BLOCK1) and 1–30 min (BLOCK2) using repeated-measures analysis of variance. All 14 subjects completed iTBS300 over the dominant primary motor cortex (M1) without any clinically reported adverse events. ITBS300 produced significant M1 facilitation [$F_{(5,\ 65)} = 3.165$, $p = 0.01$] at BLOCK1 and trend level M1 facilitation at BLOCK2 [$F_{(10,\ 129)} = 1.69$, $p = 0.089$]. Although iTBS300 (stimulation duration of 92 s at 70% RMT) delivered over M1 in typically developed children was well-tolerated and produced on average significant facilitatory changes in cortical excitability, the post-iTBS300 neurophysiologic response was variable in our small sample. ITBS300-induced changes may represent a potential neuroplastic biomarker in healthy children and those with neuro-genetic or neuro-psychiatric disorders. However, a larger sample size is needed to address safety and concerns of response variability.

Keywords: repetitive transcranial magnetic stimulation, theta burst stimulation, long-term potentiation, pediatric, neuroplasticity

INTRODUCTION

Neuroplasticity broadly describes the ability of the nervous system to reorganize in response to intrinsic or environmental demands and underlies the conceptual framework of learning, memory and development (Lamprecht and LeDoux, 2004; Pascual-Leone et al., 2005). Though genetic and early environmental factors dictate the potential scope of brain development, neuroplastic processes play a critical role following birth to configure and optimize neural circuits, including the maturation of complex sensory, cognitive and regulatory functions throughout life (Tau and Peterson, 2009). Moreover, there is evidence that, for a broad group of neurodevelopmental disorders, abnormalities in the mechanisms of neuroplasticity, including maladaptive plasticity (Johnston, 2004), may best explain the fundamental pathophysiology of these disorders, including Fragile X Syndrome (Huber et al., 2002), Neurofibramatosis-1 (Costa et al., 2002), Gilles de la Tourette's syndrome (Wu and Gilbert, 2012), and autism spectrum disorders (Markram and Markram, 2010).

Despite relevance of aberrant neuroplasticity in animal models of multiple neurodevelopmental disorders, little is known of the role of long-term potentiation (LTP) and the relationship with behavioral plasticity in the typical developing human cortex (Martin et al., 2000). LTP describes the long-lasting modification of neuronal connections, including changes in synaptic efficacy, which is commonly cited as the cellular basis of learning and memory (Brown et al., 1988). LTP been studied extensively in mammalian hippocampus including hippocampal slices from humans undergoing temporal lobe surgery (Brown et al., 1988; Beck et al., 2000). Though investigation of cellular LTP in children have obviously been limited, electrophysiological studies of neonate and juvenile animals have shed light on the purpose and mechanisms of LTP during development. Developmental age in rodents has been associated with varying susceptibility and efficacy of induced-LTP in hippocampal slices (Harris et al., 1992; Swartzwelder et al., 1995; Leinekugel et al., 2002; Cao and Harris, 2012). In young rats, periods of susceptibility to LTP in the visual cortex coincides with developmental critical periods which can be prolonged by rearing animals in darkness (Kirkwood et al., 1995).

Transcranial magnetic stimulation (TMS) under certain stimulation parameters can lead to changes in corticospinal and corticocortical excitability that outlast the stimulation period, thus representing a surrogate marker of cellular LTP and LTD from the intact human cortex (Pascual-Leone et al., 1994). These phenomena share a remarkable similarity to cellular measurements

of LTP or LTD, including the loss of TMS-induced LTP- and LTD-like effects after N-methyl-D-aspartate receptor blockade (Stefan et al., 2002; Wolters et al., 2003; Huang et al., 2007) and methods of physiological induction, either through tetanic stimulation, such as theta burst stimulation (TBS) (Pascual-Leone et al., 1994; Huang and Rothwell, 2004; Huang et al., 2005) or through associative methods, such as paired associative stimulation (PAS) (Stefan et al., 2000). Though details regarding individual synaptic connections are at best speculative, TMS techniques can grossly quantify the final output of a specific region of the neocortex and test hypotheses regarding the configuration of established neural networks.

Here we report the effect of a modified 30 Hz intermittent TBS (iTBS) protocol, previously reported to generate primary motor (M1) cortical facilitation in adults (Wu et al., 2012a) and now optimized for the pediatric population, on M1 excitability of typically-developing children/adolescents. Despite several pediatric repetitive TMS (rTMS) studies (Kirton et al., 2008; Oberman et al., 2010, 2014; Wu and Gilbert, 2012; Gillick et al., 2014; Wu et al., 2014), there is limited data on the effect of rTMS and TBS on the developing cortex. In addition, the reported inter-individual variability to iTBS potentially limits the use of the technique as a diagnostic or prognostic tool (Hamada et al., 2013; Lopez-Alonso et al., 2014). The overall goal of this work was to establish a safe biomarker of pediatric neuronal plasticity using iTBS. Such a marker would provide an additional tool to explore the cortical physiology of suspected neuroplastic abnormalities across a host of pediatric illnesses of the central nervous system. In addition, we systematically discuss the rationale for the modification of TBS parameters based on safety concerns and feasibility for use in children. The present study, to our knowledge, represents the first published cohort of healthy children who have undergone iTBS. We hypothesized that 30 Hz iTBS to M1 in healthy children would elicit a brief physiological facilitation of motor-evoked potential (MEP) amplitudes following stimulation.

MATERIALS AND METHODS

Parents of pediatric patients gave written informed consent and child participants gave written informed assent for the study, which were approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Participants were reimbursed for time and travel.

PARTICIPANTS

Healthy children ages 8–17 were recruited through advertising flyers and email through the local institution and community. All volunteers were free of neurologic, psychiatric and serious medical illnesses, were not taking any neuropsychiatric medications, and did not have any contraindications to TMS (Rossi et al., 2011). Handedness was either determined through Physical And Neurological Examination for Soft Signs (Denckla, 1985) or the Edinburgh Handedness Inventory (Oldfield, 1971).

SINGLE PULSE TRANSCRANIAL MAGNETIC STIMULATION (spTMS)

A monophasic Magstim 200 stimulator connected to a figure-8, 70 mm coil (Magstim Ltd., Whitland, UK) was used to determine resting motor threshold (RMT) and obtain MEPs measured by surface electromyography (EMG) in the first dorsal interosseous (FDI) muscle of the dominant hand. A second set of EMG leads was placed on dominant extensor carpi radialis for monitoring during iTBS. Participants were seated comfortably with both arms fully supported on a pillow. Full muscle relaxation was monitored visually and by EMG. The figure-8 coil (handle pointing posteriorly at 45°) was placed tangentially to the scalp over the dominant M1 at the optimal site for obtaining maximal peak-to-peak amplitude of MEPs from the dominant FDI using standard methods (Mills and Nithi, 1997). This "hot spot" was marked with a wax pencil for consistent placement of the figure-8 coils during application of spTMS and rTMS. We opted not to employ neuronavigation as (1) TBS of the motor cortex has been routinely performed with a non-technical approach (Huang et al., 2005) and (2) to maximize the potential feasibility of the protocol for widespread biomarker use. TMS pulses separated by 6 s (±5%; generated by Signal software version 2.15; Cambridge Electronic Design Limited, Cambridge, UK) were administered at intensities of 1.2*baseline RMT to obtain MEP amplitudes at 11 time points: 20 pulses (114 s) at baseline (T0), and 10 pulses (54 s) at 1 (T1), 3 (T2), 5 (T3), 7 (T4), 10 (T5), 12.5 (T6), 15 (T7), 17.5 (T8), 20 (T9), and 30 (T10) min following iTBS. Surface EMG signals were amplified and filtered (100/1000 Hz; Coulbourn Instruments, Allentown, PA) before being digitized at 2 kHz and stored for analysis, using Signal software and a Micro1401 interface (Cambridge Electronic Design, Cambridge, UK). Each surface EMG tracing was reviewed offline and tagged for removal if it contained muscle movements prior to the TMS pulse (∼1% of all tracings). Due to technical difficulties, there was missing data for the T8 time point for one subject.

MEASUREMENT OF RESTING MOTOR THRESHOLD

RMT was defined for each Magstim stimulator separately as the minimal intensity of stimulation to the dominant M1 to induce MEPs in at least 3 out of 6 consecutive trials following determination of the optimal site (Conforto et al., 2004). Stimulation began well above threshold intensity, usually 75% of maximal stimulator output) and decreased until RMT was identified within a 1% increment. Due to the influence of phasic and tonic finger movements on TBS outcome (Gentner et al., 2008; Huang et al., 2008; Iezzi et al., 2008), we chose not to measure active motor threshold and instead used RMT as a reference for stimulation intensity.

INTERMITTENT THETA BURST STIMULATION

ITBS was performed using a biphasic 115V version of Magstim SuperRapid^{2}Plus^1 (Magstim Ltd., Whitland, UK) connected to a figure-8, 70 mm coil applied to the M1 "hot spot" as designated above. We did not use additional hearing protection as in laboratory measurement of mean (less than 57.9 dB) and peak decibel levels (less than 69.1 dB) of single pulse TMS and iTBS fell within well-established hearing safety standards and consistent with previous reports (Dhamne et al., 2014). The use of Magstim 200 in addition to the SuperRapid^{2}Plus^1 allowed for the measurement of RMT in children who generally have higher thresholds (Garvey et al., 2003). All iTBS sessions were performed in the afternoon. Subjects received iTBS300 (**Figure 1**), which consisted

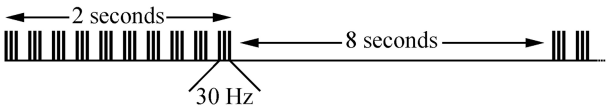


FIGURE 1 | Pulse pattern for iTBS300. Three TMS pulses per burst were given at 30 Hz. A burst was delivered every 200 ms so that 10 bursts were given in a 2-s train. Ten trains were given every 10 s for a total of 300 magnetic pulses per iTBS300.

of bursts of 3 magnetic pulses at 30 Hz repeating every 200 ms for 2 s (one train) with trains repeated every 10 s apart for total of 300 pulses (92 s) at a stimulation intensity of 70%*RMT. Full muscle relaxation and generation of evoked potentials by iTBS was monitored visually and by continuous EMG throughout the iTBS stimulation period.

Thirty Hz iTBS was used, rather than the more typical 50 Hz, as this allows for higher stimulation intensities (i.e., 30 Hz TBS can be delivered at up to 89% power vs. only 57% for 50 Hz TBS with Magstim SuperRapid^{2}Plus^1). Moreover, 30 Hz TBS has been shown to produce the expected LTP- and LTD-like changes in M1 (Goldsworthy et al., 2012; Wu and Gilbert, 2012). Higher stimulation intensity is often necessary for TBS research in children as they have higher motor thresholds (Garvey et al., 2003). The 70%*RMT intensity was chosen to balance safety (the only case of TBS-induced seizure occurred at 100%*RMT) (Oberman and Pascual-Leone, 2009) and efficacy (i.e., we expected higher facilitatory changes in M1 excitability with higher stimulation intensity). Before and immediately after the 30-min time point, a structured diagnostic interview with detailed 16-question review of systems [headache, scalp pain, arm/hand pain, other pain(s), numbness/tingling, other sensation(s), weakness, loss of dexterity, vision/hearing change(s), ear ringing, nausea/vomiting, appetite loss, rash, skin change(s) or any other symptom(s)] was conducted to rate any potential adverse events on an ordinal scale (none, minimal, mild, moderate, marked, or severe) (Wu et al., 2012b).

STATISTICAL ANALYSIS

Descriptive statistics were applied to demographic and baseline physiological measures. Mean MEP fold change was normalized to be expressed as a ratio of average post-TBS/pre-TBS MEP peak-to-peak amplitudes for each time point. Since the iTBS300 protocol has half the total pulses compared to the original description, we anticipated a shorter duration of iTBS effect on cortical excitability (Huang et al., 2005). Therefore, two repeated measures analysis of variance (RM-ANOVA) were performed, analyzing MEP-fold change by a within-subject factor for 10 min—BLOCK1 (6 levels: T0; T1; T2; T3; T4; T5)—and for 30 min—BLOCK2 (11 levels: T0; T1; T2; T3; T4; T5; T6; T7; T8; T9; T10). We tested the hypotheses that the modified iTBS protocol would produce facilitation of mean MEP fold-change across BLOCK1 and BLOCK2. All analyses were performed in SAS (SAS Institute Inc., Cary, NC, USA) with a two-tailed $p < 0.05$ considered significant. To determine whether age had an effect on the post-iTBS300 change, it was included as a covariate in the RM-ANOVA.

In addition to RM-ANOVA, which has been used in most prior published TBS studies, we performed a secondary linear mixed model (LMM) analysis which has several potential advantages. This analysis incorporates intrasubject correlations, accounts well for missing observations, and, by using raw MEPs, accounts for inter-individual variability in the baseline MEP amplitudes (Huang et al., 2005; Wu et al., 2012a; Dhamne et al., 2014). This is a special case of a linear mixed model (LMM) with the added component of a within subject covariance structure to account for the repeated measures over time. We used an unstructured covariance model in which the correlation between any two values within subject is estimated from the data (West et al., 2006). Our a priori hypothesis expected the adjusted mean amplitudes at each post-TBS time point differed from baseline. For each comparison, the resultant p-value were corrected for a False Discovery Rate (FDR) to account for the multiple testing (Benjamini and Hochberg, 1995); with 5 and 10 contrasts, respectively, for the two blocks.

RESULTS

DEMOGRAPHICS AND SAFETY

Fourteen healthy children (13.8 ± 2.2 years, range 10–16; M:F = 5:9) completed the study (**Table 1**). Thirteen subjects were right-handed. No adverse events were reported or identified by structured diagnostic interviews and no seizure occurred.

iTBS 300

Average RMT was 50.7 ± 9.7% of Magstim200 maximal output and 63.7 ± 13.6% of SuperRapid^{2}Plus^1 maximal output. The "hot-spot" scalp location was identical for both machines. Mean iTBS stimulation intensity was 44.6 ± 9.5% (range: 31–62%) of SuperRapid^{2}Plus^1 maximal output.

The mean MEP fold changes for each time point and RM-ANOVA and LMM results following iTBS are summarized in **Table 1**. One-Way RM-ANOVA revealed a significant facilitation during BLOCK1 (1–10 min) and trend level facilitation during BLOCK2 (1–30 min) (**Figure 2**). For LMM, the main effect Time was statistically significant for both blocks. For BLOCK1, after adjusting for multiple comparisons, the MEP-amplitudes at 3 min were significantly larger than baseline (FDR adjusted $p = 0.021$). For BLOCK2, after adjusting for multiple comparisons, the MEP amplitudes at 3 min were significantly larger than baseline (FDR adjusted $p = 0.042$). Adding age as a covariate did not have a significant effect in either analysis (not shown).

DISCUSSION

In the present study, we demonstrated that a 300 pulse, intermittent theta burst stimulation (iTBS) protocol delivered at sub-motor threshold intensity resulted in facilitation of M1 cortical excitability in healthy children. Nearly all prior studies have been performed in adults (Oberman et al., 2011; Wu et al., 2012a). In place of the originally described 50 Hz bursts (Huang et al., 2005), we used 30 Hz TBS to create a frequency/intensity paradigm that was compatible with the mechanical parameters of the SuperRapid^{2}Plus^1 device for use in pediatric research (Wu et al., 2012a). Using the commonly employed method of analysis (repeated-measures ANOVA) in rTMS studies, the iTBS300

Table 1 | Effect of modified iTBS300 on M1 as measured by MEP-fold change from baseline over time in healthy children ($n = 14$, 13.8 ± 2.2 years).

Minutes after iTBS300	1	3	5	7	10	12.5	15	17.5	20	30
Mean MEP fold change	1.40	1.45	1.30	1.20	1.13	1.23	1.32	1.51	1.55	1.25
SD	0.36	0.45	0.51	0.47	0.47	0.67	0.59	1.08	0.98	0.56
SEM	0.10	0.12	0.14	0.12	0.12	0.18	0.16	0.30	0.26	0.15
N	14	14	14	14	14	14	14	13	14	14
RM-ANOVA	BLOCK1, $F_{(5, 65)} = 3.17$, $p = 0.01$*									
	BLOCK2, $F_{(10, 129)} = 1.69$, $p = 0.089$									
LMM	BLOCK1, $F_{(5, 13)} = 4.72$, $p = 0.01$*									
	BLOCK2, $F_{(10, 13)} = 6.28$, $p = 0.002$*									

*BLOCK1, analysis of 1–10 min; BLOCK2, analysis of 1–30 min. SD, standard deviation; SEM, standard error of the mean; n, number of observations; MEP, Motor-Evoked Potentials; iTBS, intermittent Theta Burst Stimulation; M1, primary motor cortex; RM-ANOVA, repeated measures analysis of variance; LMM, Linear Mixed Model; *indicates statistical significance.*

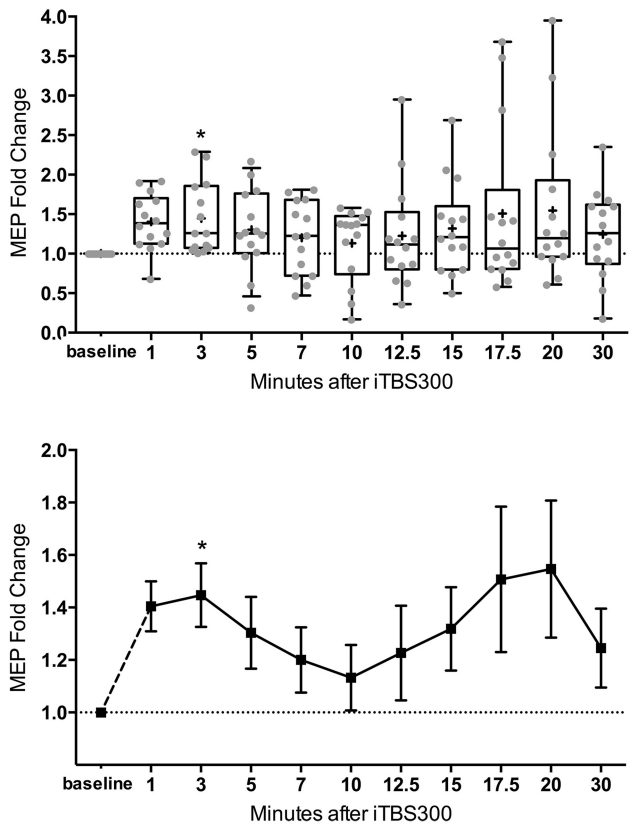


FIGURE 2 | M1 excitability changes after 30 Hz iTBS (300 pulses, 70% of RMT) in healthy children ($n = 14$, mean age = 13.8 ± 2.2 years old) from 1 to 30 min. (Top) Box (25th–75th) and Whiskers (5th–95th) percentiles with line at median and cross at mean. Mean MEP-fold change of each subject is overlaid as gray circles. **(Bottom)** Mean line plot of MEP-fold changes with error bars representing standard error of means. MEP, Motor-Evoked Potentials; RMT, resting motor threshold; iTBS, intermittent Theta Burst Stimulation; M1, primary motor cortex; * denotes statistically significant increase compared to baseline in *post-hoc* analysis after correcting for multiple comparison.

protocol demonstrated a statistically significant M1 facilitation from 1 to 10 min. However, there was significant variability in M1 response. *Post-hoc* analysis using an alternative analytical technique (LMM) and correcting for multiple comparisons showed that this facilitatory effect on M1 was primarily seen at the 3-min time point following iTBS300. Even with these limitations, the main conclusion of this study is that we were able to safely deliver iTBS to typically developing children with statistically significant facilitatory changes in M1 excitability thus lending support for further judicious use of iTBS to understand neuroplasticity in the developing cortex in children with developmental disorders.

iTBS-INDUCED FACILITATION IN M1 EXCITABILITY

To our knowledge, this is the first report of iTBS induced M1 neurophysiologic changes in healthy children. These results demonstrate similar magnitude of facilitation as we found in adults using 30 Hz iTBS with 600 pulses at 90% of RMT (Wu et al., 2012a). A brief, non-invasive method of inducing LTP- and LTD-like changes in cortical excitability holds tremendous potential to advance the study of neurodevelopmental processes (Morris et al., 2014). The optimization and validation of these techniques can also provide insight into the neural mechanisms of learning and rehabilitation (Johnston, 2009) and bridge decades of electrophysiological research from *in vivo* and *in vitro* models of central nervous system disease with clinically recognized motor, cognitive, or emotional impairments in humans (Freitas et al., 2011; Castren et al., 2012). To date, the most commonly used TMS techniques to induce cortical excitability changes are paired associative stimulation (PAS), rTMS, or TBS (Stefan et al., 2000; Di Lazzaro et al., 2011). In sensitive populations, rTMS and PAS may be limited by the discomfort of prolonged periods of stimulation above motor threshold.

Virtually all other TBS studies of M1 plasticity in adults have been performed using 50 Hz stimulation with relatively lower stimulation intensities (Huang et al., 2005; Cardenas-Morales et al., 2010; Hoogendam et al., 2010). Although there are several similarities in our results, including the time course and

maximal changes in MEP amplitudes (Huang et al., 2005; Wu et al., 2012a; Cardenas-Morales et al., 2014), comparing effects in adults vs. children and 50 Hz vs. 30 Hz iTBS will require further study.

Of interest and relevance to future studies, we also confirmed the statistical significance of the facilitation effect over 10 min (BLOCK1) using a linear mixed model (LMM) analysis. Using LMM, the BLOCK2 time effect was also statistically significant, whereas for the more commonly used RM-ANOVA found significance at the trend level. A repeated-measures LMM has several advantages over a traditional multivariate approach where there is no ability to model the correlation between observations on the same subject (Krueger and Tian, 2004). An LMM allows the incorporation of intrasubject correlations and since each observation is considered individually (including continuous or categorical covariates at a particular time) this model can also account for missing observations without list-wise deletion. We created an LMM model that used an unstructured covariance for the raw MEP amplitudes that closely fit our data. By using the raw MEPs, we were able to maintain the variability of individual subjects baseline, which is lost when looking at a MEP-fold change and transforming the baseline to 1. This may account for the discrepancy between the multivariate approach and the LMM results for BLOCK2. *Post-hoc* testing against this baseline in both time blocks, including a stringent correction for multiple comparisons, found a significant contrast at 3 min. Thus, both performed analyses support a statistically significant facilitating effect on cortical excitability within the first 10 min.

VARIABILITY IN iTBS-INDUCED FACILITATION IN M1 EXCITABILITY

One significant concern about TBS induced plasticity is the variability of the magnitude and direction of the MEP response (Player et al., 2012; Hamada et al., 2013; Hinder et al., 2014). In adults, several factors have been identified to contribute to this variability including age, gender, time of day, genetic background, and attention (Cardenas-Morales et al., 2010; Hoogendam et al., 2010; Ridding and Ziemann, 2010). Furthermore, intrinsic mechanisms such as inter-individual differences in the recruitment of interneuron networks by TMS may play a larger role than previously realized (Hamada et al., 2013). Although covariate analysis of our data did not find an effect of age, this and other factors should be analyzed in future, larger studies. Over a 30 min time course BLOCK2, as has also been reported after conventional TBS (Huang et al., 2005), we observed a second "peak" (see **Table 1** and **Figure 2**). In cellular models, LTP has come to be recognized as a phenomenon that represents a series of phases, including early and late, that can be more precisely categorized based on molecular mechanisms and order of persistence (Raymond, 2007).

METAPLASTICITY

The concept of the previous brain activity affecting synaptic response is termed metaplasticity. Several studies have shown that tonic or phasic finger movements before TBS can change the expected outcome of the tetanic stimulation (Gentner et al., 2008; Huang et al., 2008; Iezzi et al., 2008). Prior brain stimulation may also "prime" TBS response (Todd and Ridding, 2010), such as prolonging the duration of the stimulation (i.e., more pulses). In addition, extending the number of pulses seems to influence the results. One study found that facilitation and inhibition could be reversed simply by doubling the TBS pulses delivered from 600 to 1200 (iTBS1200) (Gamboa et al., 2010), while another reported that 1800 pulses of iTBS resulted in significantly higher facilitation of MEP-amplitudes than iTBS600 or iTBS1200 (Nettekoven et al., 2014). Given concerns for feasibility in pediatric populations, we were interested to study iTBS with fewer pulses. The iTBS300 protocol presented in this study produced an increase in M1 excitability in most pediatric subjects. So far, there has been one iTBS150 study that showed no significant M1 changes in adults (Huang et al., 2008). Future studies in children could evaluate iTBS150 to determine if this is sufficient to modulate cortical excitability.

It is possible that the 0.17 Hz test pulses used prior to (baseline) and after iTBS may themselves induce metaplastic effects as was suggested by a recent study of PAS-induced LTP and LTD (Delvendahl et al., 2010). However, the 0.1 Hz rTMS precondition in this study abolished PAS-induced neuroplastic effects whereas we observed statistically significant increase in M1 excitation in our study. Furthermore, very low frequency rTMS (0.1 and 0.2 Hz) have not been shown to exert direct effect on MEP amplitudes (Chen et al., 1997; Delvendahl et al., 2010; Furukawa et al., 2010). This could create a trade-off between using more frequent TMS to capture the temporal characteristics of induced cortical excitability vs. less frequent TMS to avoid inducing metaplastic effects. Future sham controlled TBS studies (Davis et al., 2013), or perhaps studies outside of motor cortex with different outputs, may clarify this.

SAFETY

A key finding of this study is that iTBS was delivered safely and without any reported clinical adverse effects in all 14 children who participated in this study. This is an important finding as there is limited data on the use of TBS in the pediatric population (Oberman et al., 2010, 2014; Wu et al., 2014; Hong et al., 2015). A small number of these participants reported mild adverse events after TBS: fatigue, headache/scalp pain, arm/hand pain, paresthesia, weakness, nausea, tinnitus, abdominal pain and dry eyes. We recently compared the adverse event rates between TBS and single-/paired-pulse TMS sessions in 165 children and found no significant difference (Hong et al., 2015). In the present study, systematic review of systems following iTBS found no significant adverse effects. There are a few possible explanations for this. First, the iTBS protocol contained only 300 pulses rather than the originally described 600 pulses. Thus, this 92-s TBS stimulation duration may have a lower probably of causing adverse effects. Second, based on a systematic review of >1000 adults who received >4500 TBS sessions, a crude risk of 1.1% was identified for mild adverse events (Oberman et al., 2011). Furthermore, another safety report of various forms of TMS/rTMS in 113 adults showed that TBS sessions were associated with less adverse events (Maizey et al., 2013). These adult safety data may also explain why we did not detect any adverse events in our small pediatric sample.

LIMITATIONS

The results of this study are vulnerable to a type II error given the small sample size. Thus, we could not adequately examine factors that might lead to variability in iTBS300 response. Generalization of our findings may be limited by the predominance of females in our cohort, as gender difference may be a determinant of TMS-induced plasticity (Ridding and Ziemann, 2010).

In addition, further work needs to be done to extend these types of assessments to younger children. Although we attempted to recruit younger children for the study, several participants' motor thresholds were too high to proceed with TBS. The youngest subject in our cohort was 9 years old but his RMT was relatively low for his age (54% on Magstim200, 68% on SuperRapid^{2}Plus^1) which allowed us to complete the iTBS protocol. In rodent models, the maturation of the cortex with advancing developmental age influences the conditions necessary to induce LTP-effects (Meredith et al., 2003). Such an analysis lies outside the scope of the present study, however, with a larger sample with younger age groups may allow the quantification of such effects in the future. In addition, repeated sessions could be used to evaluate the extent of intra-individual variability, for example, related to time of day, stress, fatigue, or hormonal fluctuations in females.

CONCLUSION

This is the first report of iTBS- induced M1 neurophysiologic effects in healthy children. All participants safely completed the iTBS300 session which involved just 92 s of bursts of subthreshold TMS pulses without any serious adverse events. We were able to show statistically significant increase in M1 excitability in the first 10 min after iTBS300. Future pediatric TBS studies to acquire normative data are needed. We speculate the demonstrated physiological effects of this protocol to M1 could also be further investigated in non-motor regions for neuromodulation or for repeated applications in clinical trials. This data supports further, judicious use of iTBS as a technique for studying brain development, neuropsychiatric and neuro-developmental disorders.

ACKNOWLEDGMENTS

This study was supported by the Cincinnati Children's Hospital Medical Center Division of Neurology, Cincinnati Children's Hospital Research Foundation, and American Academy of Child and Adolescent Psychiatry Pilot Award.

REFERENCES

Beck, H., Goussakov, I. V., Lie, A., Helmstaedter, C., and Elger, C. E. (2000). Synaptic plasticity in the human dentate gyrus. *J. Neurosci.* 20, 7080–7086.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57, 289–300.

Brown, T. H., Chapman, P. F., Kairiss, E. W., and Keenan, C. L. (1988). Long-term synaptic potentiation. *Science* 242, 724–728. doi: 10.1126/science.2903551

Cao, G., and Harris, K. M. (2012). Developmental regulation of the late phase of long-term potentiation (L-LTP) and metaplasticity in hippocampal area CA1 of the rat. *J. Neurophysiol.* 107, 902–912. doi: 10.1152/jn.00780.2011

Cardenas-Morales, L., Nowak, D. A., Kammer, T., Wolf, R. C., and Schonfeldt-Lecuona, C. (2010). Mechanisms and applications of theta-burst rTMS on the human motor cortex. *Brain Topogr.* 22, 294–306. doi: 10.1007/s10548-009-0084-7

Cardenas-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

Castren, E., Elgersma, Y., Maffei, L., and Hagerman, R. (2012). Treatment of neurodevelopmental disorders in adulthood. *J. Neurosci.* 32, 14074–14079. doi: 10.1523/JNEUROSCI.3287-12.2012

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

Conforto, A. B., Z'Graggen, W. J., Kohl, A. S., Rosler, K. M., and Kaelin-Lang, A. (2004). Impact of coil position and electrophysiological monitoring on determination of motor thresholds to transcranial magnetic stimulation. *Clin. Neurophysiol.* 115, 812–819. doi: 10.1016/j.clinph.2003.11.010

Costa, R. M., Federov, N. B., Kogan, J. H., Murphy, G. G., Stern, J., Ohno, M., et al. (2002). Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature* 415, 526–530. doi: 10.1038/nature711

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

Delvendahl, I., Jung, N. H., Mainberger, F., Kuhnke, N. G., Cronjaeger, M., and Mall, V. (2010). Occlusion of bidirectional plasticity by preceding low-frequency stimulation in the human motor cortex. *Clin. Neurophysiol.* 121, 594–602. doi: 10.1016/j.clinph.2009.09.034

Denckla, M. B. (1985). Revised Neurological Examination for Subtle Signs. *Psychopharmacol. Bull.* 21, 773–800.

Dhamne, S. C., Kothare, R. S., Yu, C., Hsieh, T. H., Anastasio, E. M., Oberman, L., et al. (2014). A measure of acoustic noise generated from transcranial magnetic stimulation coils. *Brain Stimul.* 7, 432–434. doi: 10.1016/j.brs.2014.01.056

Di Lazzaro, V., Dileone, M., Pilato, F., Capone, F., Musumeci, G., Ranieri, F., et al. (2011). Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *J. Neurophysiol.* 105, 2150–2156. doi: 10.1152/jn.00781.2010

Freitas, C., Perez, J., Knobel, M., Tormos, J. M., Oberman, L., Eldaief, M., et al. (2011). Changes in cortical plasticity across the lifespan. *Front. Aging Neurosci.* 3:5. doi: 10.3389/fnagi.2011.00005

Furukawa, T., Toyokura, M., and Masakado, Y. (2010). Suprathreshold 0.2 Hz repetitive transcranial magnetic stimulation (rTMS) over the prefrontal area. *Tokai J. Exp. Clin. Med.* 35, 29–33.

Gamboa, O. L., Antal, A., Moliadze, V., and Paulus, W. (2010). Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. *Exp. Brain Res.* 204, 181–187. doi: 10.1007/s00221-010-2293-4

Garvey, M. A., Ziemann, U., Bartko, J. J., Denckla, M. B., Barker, C. A., and Wassermann, E. M. (2003). Cortical correlates of neuromotor development in healthy children. *Clin. Neurophysiol.* 114, 1662–1670. doi: 10.1016/S1388-2457(03)00130-5

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

Gillick, B. T., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., Thomas, W., et al. (2014). Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: a randomized controlled trial. *Dev. Med. Child Neurol.* 56, 44–52. doi: 10.1111/dmcn.12243

Goldsworthy, M. R., Pitcher, J. B., and Ridding, M. C. (2012). A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. *Clin. Neurophysiol.* 123, 2256–2263. doi: 10.1016/j.clinph.2012.05.001

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

Harris, K. M., Jensen, F. E., and Tsao, B. (1992). Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *J. Neurosci.* 12, 2685–2705.

Hinder, M. R., Goss, E. L., Fujiyama, H., Canty, A. J., Garry, M. I., Rodger, J., et al. (2014). Inter- and Intra-individual variability following intermittent theta

burst stimulation: implications for rehabilitation and recovery. *Brain Stimul.* 7, 365–371. doi: 10.1016/j.brs.2014.01.004

Hong, J. H., Wu, S. W., Pedapati, E. V., Horn, P. S., Huddleston, D. A., Laue, C. S., et al. (2015). Safety and tolerability of theta burst stimulation versus single and paired pulse transcranial magnetic stimulation: a comparative study of 165 pediatric subjects. *Front. Hum. Neurosci.* 9:29. doi: 10.3389/fnhum.2015.00029

Hoogendam, J. M., Ramakers, G. M., and Di Lazzaro, V. (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimul.* 3, 95–118. doi: 10.1016/j.brs.2009.10.005

Huang, Y. Z., and Rothwell, J. C. (2004). The effect of short-duration bursts of high-frequency, low-intensity transcranial magnetic stimulation on the human motor cortex. *Clin. Neurophysiol.* 115, 1069–1075. doi: 10.1016/j.clinph.2003.12.026

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

Huang, Y. Z., Rothwell, J. C., Edwards, M. J., and Chen, R. S. (2008). Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cereb. Cortex* 18, 563–570. doi: 10.1093/cercor/bhm087

Huber, K. M., Gallagher, S. M., Warren, S. T., and Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. U.S.A.* 99, 7746–7750. doi: 10.1073/pnas.122205699

Iezzi, E., Conte, A., Suppa, A., Agostino, R., Dinapoli, L., Scontrini, A., et al. (2008). Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *J. Neurophysiol.* 100, 2070–2076. doi: 10.1152/jn.90521.2008

Johnston, M. V. (2004). Clinical disorders of brain plasticity. *Brain Dev.* 26, 73–80. doi: 10.1016/S0387-7604(03)00102-5

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

Kirkwood, A., Lee, H.-K., and Bear, M. F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature* 375, 328–331. doi: 10.1038/375328a0

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

Krueger, C., and Tian, L. (2004). A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. *Biol. Res. Nurs.* 6, 151–157. doi: 10.1177/1099800404267682

Lamprecht, R., and LeDoux, J. (2004). Structural plasticity and memory. *Nat. Rev. Neurosci.* 5, 45–54. doi: 10.1038/nrn1301

Leinekugel, X., Khazipov, R., Cannon, R., Hirase, H., Ben-Ari, Y., and Buzsaki, G. (2002). Correlated bursts of activity in the neonatal hippocampus *in vivo*. *Science* 296, 2049–2052. doi: 10.1126/science.1071111

Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., and Fernandez-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul.* 7, 372–380. doi: 10.1016/j.brs.2014.02.004

Maizey, L., Allen, C. P., Dervinis, M., Verbruggen, F., Varnava, A., Kozlov, M., et al. (2013). Comparative incidence rates of mild adverse effects to transcranial magnetic stimulation. *Clin. Neurophysiol.* 124, 536–544. doi: 10.1016/j.clinph.2012.07.024

Markram, K., and Markram, H. (2010). The intense world theory - a unifying theory of the neurobiology of autism. *Front. Hum. Neurosci.* 4:224. doi: 10.3389/fnhum.2010.00224

Martin, S., Grimwood, P., and Morris, R. (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu. Rev. Neurosci.* 23, 649–711. doi: 10.1146/annurev.neuro.23.1.649

Meredith, R. M., Floyer-Lea, A. M., and Paulsen, O. (2003). Maturation of long-term potentiation induction rules in rodent hippocampus: role of GABAergic inhibition. *J. Neurosci.* 23, 11142–11146.

Mills, K. R., and Nithi, K. A. (1997). Corticomotor threshold to magnetic stimulation: normal values and repeatability. *Muscle Nerve* 20, 570–576.

Morris, S. E., Rumsey, J. M., and Cuthbert, B. N. (2014). Rethinking mental disorders: the role of learning and brain plasticity. *Restor. Neurol. Neurosci.* 32, 5–23. doi: 10.3233/rnn-139015

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

Oberman, L. M., and Pascual-Leone, A. (2009). Report of seizure induced by continuous theta burst stimulation. *Brain Stimul.* 2, 246–247. doi: 10.1016/j.brs.2009.03.003

Oberman, L. M., Pascual-Leone, A., and Rotenberg, A. (2014). Modulation of corticospinal excitability by transcranial magnetic stimulation in children and adolescents with autism spectrum disorder. *Front. Hum. Neurosci.* 8:627. doi: 10.3389/fnhum.2014.00627

Oberman, L., Edwards, D., Eldaief, M., and Pascual-Leone, A. (2011). Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. *J. Clin. Neurophysiol.* 28, 67–74. doi: 10.1097/WNP.0b013e31820 5135f

Oberman, L., Ifert-Miller, F., Najib, U., Bashir, S., Woollacott, I., Gonzalez-Heydrich, J., et al. (2010). Transcranial magnetic stimulation provides means to assess cortical plasticity and excitability in humans with fragile x syndrome and autism spectrum disorder. *Front. Synaptic Neurosci.* 2:26. doi: 10.3389/fnsyn.2010.00026

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

Pascual-Leone, A., Amedi, A., Fregni, F., and Merabet, L. B. (2005). The plastic human brain cortex. *Annu. Rev. Neurosci.* 28, 377–401. doi: 10.1146/annurev.neuro.27.070203.144216

Pascual-Leone, A., Valls-Sole, 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

Player, M. J., Taylor, J. L., Alonzo, A., and Loo, C. K. (2012). Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. *Clin. Neurophysiol.* 123, 2220–2226. doi: 10.1016/j.clinph.2012.03.081

Raymond, C. R. (2007). LTP forms 1, 2 and 3: different mechanisms for the "long" in long-term potentiation. *Trends Neurosci.* 30, 167–175. doi: 10.1016/j.tins.2007.01.007

Ridding, M. C., and Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J. Physiol.* 588, 2291–2304. doi: 10.1113/jphysiol.2010.190314

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clin. Neurophysiol.* 122, 1686. doi: 10.1016/j.clinph.2010.12.037

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., and Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J. Physiol.* 543(Pt 2), 699–708. doi: 10.1113/jphysiol.2002.023317

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., and Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* 123, 572–584. doi: 10.1093/brain/123.3.572

Swartzwelder, H. S., Wilson, W., and Tayyeb, M. (1995). Age-dependent inhibition of long-term potentiation by ethanol in immature versus mature Hippocampus. *Alcohol. Clin. Exp. Res.* 19, 1480–1485. doi: 10.1111/j.1530-0277.1995.tb0 1011.x

Tau, G. Z., and Peterson, B. S. (2009). Normal development of brain circuits. *Neuropsychopharmacology* 35, 147–168. doi: 10.1038/npp.2009.115

Todd, G., and Ridding, M. C. (2010). The response to repetitive stimulation of human motor cortex is influenced by the history of synaptic activity. *Restor. Neurol. Neurosci.* 28, 459–467. doi: 10.3233/RNN-2010-0565

West, B. T., Welch, K. B., and Galecki, A. T. (2006). *Linear Mixed Models: a Practical Guide Using Statistical Software*. Boca Raton, FL: CRC Press.

Wolters, A., Sandbrink, F., Schlottmann, A., Kunesch, E., Stefan, K., Cohen, L. G., et al. (2003). A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J. Neurophysiol.* 89, 2339–2345. doi: 10.1152/jn.00900.2002

Wu, S. W., and Gilbert, D. L. (2012). Altered neurophysiologic response to intermittent theta burst stimulation in Tourette syndrome. *Brain Stimul.* 5, 315–319. doi: 10.1016/j.brs.2011.04.001

Wu, S. W., Maloney, T., Gilbert, D. L., Dixon, S. G., Horn, P. S., Huddleston, D. A., et al. (2014). Functional MRI-navigated repetitive transcranial magnetic

stimulation over supplementary motor area in chronic tic disorders. *Brain Stimul.* 7, 212–218. doi: 10.1016/j.brs.2013.10.005

Wu, S. W., Shahana, N., Huddleston, D. A., and Gilbert, D. L. (2012a). Effects of 30Hz theta burst transcranial magnetic stimulation on the primary motor cortex. *J. Neurosci. Methods* 208, 161–164. doi: 10.1016/j.jneumeth.2012.05.014

Wu, S. W., Shahana, N., Huddleston, D. A., Lewis, A. N., and Gilbert, D. L. (2012b). Safety and tolerability of theta-burst transcranial magnetic stimulation in children. *Dev. Med. Child Neurol.* 54, 636–639. doi: 10.1111/j.1469-8749.2012.04300.x

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

Received: 30 December 2014; paper pending published: 18 January 2015; accepted: 04 February 2015; published online: 25 February 2015.

Citation: Pedapati EV, Gilbert DL, Horn PS, Huddleston DA, Laue CS, Shahana N and Wu SW (2015) Effect of 30 Hz theta burst transcranial magnetic stimulation on the primary motor cortex in children and adolescents. Front. Hum. Neurosci. ***9****:91. doi: 10.3389/fnhum.2015.00091*

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

frontiers in
HUMAN NEUROSCIENCE

OPINION ARTICLE
published: 06 January 2015
doi: 10.3389/fnhum.2014.01034

Transcranial magnetic stimulation (TMS) therapy for autism: an international consensus conference held in conjunction with the international meeting for autism research on May 13th and 14th, 2014

Lindsay M. Oberman[1]*, Peter G. Enticott[2], Manuel F. Casanova[3], Alexander Rotenberg[4,5], Alvaro Pascual-Leone[5] and James T. McCracken[6]

[1] *Neuroplasticity and Autism Spectrum Disorder Program, Department of Psychiatry and Human Behavior, E.P. Bradley Hospital and Warren Alpert Medical School, Brown University, Providence, RI, USA*
[2] *Cognitive Neuroscience Unit, School of Psychology, Deakin University, Burwood, VIC, Australia*
[3] *Department of Psychiatry and Behavioral Science, University of Louisville, Louisville, KY, USA*
[4] *Neuromodulation Program, Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA*
[5] *Berenson-Allen Center for Noninvasive Brain Stimulation, Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA*
[6] *Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA, USA*
**Correspondence: loberman@lifespan.org*

Edited by:
Rachael D. Seidler, University of Michigan, USA

Reviewed by:
Jorge Leon Morales-Quezada, Laboratory of Neuromodulation, USA

Keywords: Autism Spectrum Disorder, transcranial magnetic stimulation, pathophysiology, therapy, consensus

The Centers for Disease Control and Prevention currently estimate the prevalence of Autism Spectrum Disorder (ASD) in the U.S. at 1:68 children (Baio, 2014). Despite decades of research across multiple levels of analysis, we currently lack a reliable biomarker that may facilitate diagnosis, illuminate pathophysiology, or guide treatment. The development of novel treatment strategies for ASD will require efforts for better clinical characterization, identification of more homogeneous subgroups for studies, and improved understanding of underlying pathophysiology. There is growing support for early intensive interventions in this population (Reichow, 2012). Pharmacological treatments have been shown to be effective in treating some of the common secondary and comorbid features of ASD (Hampson et al., 2012), but there is currently no pharmacotherapy conclusively shown to improve the core symptoms (Oberman, 2012).

Recently a number of investigators have begun to explore the use of transcranial magnetic stimulation (TMS) as a tool to characterize ASD pathophysiology, and to test its therapeutic potential. TMS is a safe and well-tolerated method for non-invasive focal cortical stimulation where small intracranial electrical currents are generated by a rapidly fluctuating extracranial magnetic field. In an effort to share recent progress in the use of TMS in ASD, promote collaboration across laboratories, and establish consensus on parameters that may be useful for the study of pathophysiology and the potential treatment of ASD, leading experts in the field gathered in Atlanta, GA on May 13th and 14th 2014 for the "Transcranial Magnetic Stimulation (TMS) Therapy for Autism Consensus Conference" organized and supported by the Clearly Present Foundation with additional support from Neuronetics, Inc. and Autism Speaks.

Alvaro Pascual-Leone began the conference by discussing the basic mechanisms and safety of TMS in clinical populations. TMS can be applied in single pulses to investigate corticospinal excitability, pairs of pulses to study intracortical inhibition and facilitation, and repeated trains of TMS (rTMS) to both to study and therapeutically modulate excitability and plasticity in a number of neurological and psychiatric conditions (Kobayashi and Pascual-Leone, 2003). The effects of rTMS can be expected to differ considerably by virtue of varying parameters of stimulation and knowledge of underlying symptom pathophysiology. TMS is considered quite safe if applied within current safety guidelines; however, it does pose some risk for adverse side-effects (Rossi et al., 2009). Though relatively few patients with ASD have participated in TMS protocols, the frequency and quality of side-effects shown thus far approximates that seen in the general population (Oberman et al., 2013). As with any other condition, factors including medications and medical history need to be assessed when determining risk for an individual. There are currently no identified ASD-specific risk factors for TMS-induced adverse effects. Even though ASD can be associated with an increased risk for seizures, in TMS studies to date, there is no evidence of increased epileptogenic risk in ASD when safety guidelines and recommendations are followed.

Manuel Casanova then provided a targeted review of the literature on the pathophysiology of ASD. Postmortem studies have shown evidence of abnormalities of neuronal migration in the brains of individuals with ASD (Bailey et al., 1998), which include displaced neurons manifesting as focal cortical dysplasias in a majority of individuals with ASD (Casanova

et al., 2013). Morphometric analysis of cells within the malformed cortex has suggested a reduced number of interneurons (Casanova et al., 2013). This is consistent with previous reports of abnormalities in ASD within the peripheral cortical minicolumn neuropil space, the compartment where most inhibitory cells are located (Casanova et al., 2002). Both EEG and vibrotactile studies corroborate a deficit of cortical lateral inhibition (Keita et al., 2011; Puts et al., 2014). He proposed that this deficit could account for the seizures and sensory abnormalities often reported in ASD.

Lindsay Oberman discussed the use of TMS as an investigative device to study cortical excitability and plasticity in ASD. These studies show that a number of basic mechanisms and circuits are atypical while other measures appear to be normal (see Oberman et al., 2013). Specifically, motor thresholds and baseline motor-cortical excitability measures appear to be normal. There is heterogeneity in the response to paired-pulse paradigms with impaired inhibition in some individuals, typical response in others, and paradoxical facilitation in another subgroup. Studies exploring corticospinal plasticity mechanisms, using two different rTMS protocols [theta burst stimulation (TBS) and paired associative stimulation (PAS)], have shown abnormalities. However, the direction of the abnormality is unclear with TBS studies showing enhanced response (Oberman et al., 2012) and PAS showing reduced response (Jung et al., 2013). There are a number of open questions related to the use of TMS as an investigative device in ASD including developmental effects, effects related to intellectual disability and functioning, and what underlying mechanisms are driving the observed heterogeneity in the population.

Peter Enticott discussed the efficacy of rTMS as a therapeutic intervention in ASD. A number of studies using low-frequency rTMS in an effort to enhance cortical inhibitory tone in dorsolateral prefrontal cortex have resulted in improvements in EEG indices of attention, information processing, and error monitoring as well as behavioral improvements in repetitive behaviors and irritability (Sokhadze et al., 2014). Low-frequency stimulation to left pars triangularis resulted in improved object naming in a single session study (Fecteau et al., 2011). High-frequency stimulation, designed to enhance excitability, has suggested improvements in self-reported social relating and social anxiety following medial prefrontal cortex stimulation (Enticott et al., 2014) and significant improvements in eye-hand coordination following premotor stimulation (Panerai et al., 2013). Although an emerging literature, these studies collectively provide support for the potential efficacy of rTMS in ASD (Oberman et al., 2013). However, the small study samples, lack of blind assessments, and limited use of control or comparison conditions limit the interpretation of these early investigations.

James McCracken concluded the conference by discussing key factors to consider when designing clinical trials for ASD. These factors included identification of valid and reliable endpoints, incorporation of blind assessments, need for credible control conditions, establishment of effective stimulation parameters, need to relate changes in electrophysiologic endpoints to functional change, and identification of biomarkers that can be used to reduce the heterogeneity of the sample and stratify participants to treatment strategies that are best matched to their underlying pathophysiology. To this end, those present discussed the utility of developing functional imaging and TMS indices as potential standardized biomarkers and the need for larger, multisite trials to establish validity of these measures across development and levels of functioning and reliability of these measures across centers.

At the conclusion of the conference, there was enthusiasm for the potential use of TMS in ASD. Further work is necessary to achieve consensus on the key factors discussed by Dr. McCracken, but the expertise and commitment is present in the research and clinical community to work toward the end goal of designing and implementing large-scale, double blind, multisite clinical trials of rTMS for ASD in the near future. Those present committed to collaborate across laboratories to establish mutually agreed upon protocols and to meet again within 1 year.

AUTHOR CONTRIBUTIONS

Lindsay M. Oberman, Peter G. Enticott, Manuel F. Casanova, Alvaro Pascual-Leone, and James T. McCracken contributed to the organization of the conference, were invited speakers to the conference, and lead the discussion during the conference. Alexander Rotenberg contributed to the organization of the conference, and the discussion during the conference. All authors contributed to the writing of the manuscript.

ACKNOWLEDGMENT

The content is solely the responsibility of the authors and does not necessarily represent the official views of Clearly Present Foundation, Autism Speaks, or Neuronetics, Inc.

REFERENCES

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., et al. (1998). A clinicopathological study of autism. *Brain* 121 (Pt 5), 889–905. doi: 10.1093/brain/121.5.889

Baio, J. (2014). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill. Summ.* 63, 1–21.

Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., and Roy, E. (2002). Minicolumnar pathology in autism. *Neurology* 58, 428–432. doi: 10.1212/WNL.58.3.428

Casanova, M. F., El-Baz, A. S., Kamat, S. S., Dombroski, B. A., Khalifa, F., Elnakib, A., et al. (2013). Focal cortical dysplasias in autism spectrum disorders. *Acta Neuropathol. Commun.* 1:67. doi: 10.1186/2051-5960-1-67

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

Fecteau, S., Agosta, S., Oberman, L., and Pascual-Leone, A. (2011). Brain stimulation over Broca's area differentially modulates naming skills in neurotypical adults and individuals with Asperger's syndrome. *Eur. J. Neurosci.* 34, 158–164. doi: 10.1111/j.1460-9568.2011.07726.x

Hampson, D. R., Gholizadeh, S., and Pacey, L. K. (2012). Pathways to drug development for autism spectrum disorders. *Clin. Pharmacol. Ther.* 91, 189–200. doi: 10.1038/clpt.2011.245

Jung, N. H., Janzarik, W. G., Delvendahl, I., Munchau, A., Biscaldi, M., Mainberger, F., et al. (2013). Impaired induction of long-term potentiation-like plasticity in patients with high-functioning autism and Asperger syndrome. *Dev. Med. Child Neurol.* 55, 83–89. doi: 10.1111/dmcn.12012

Keita, L., Mottron, L., Dawson, M., and Bertone, A. (2011). Atypical lateral connectivity: a neural basis for altered visuospatial processing

in autism. *Biol. Psychiatry* 70, 806–811. doi: 10.1016/j.biopsych.2011.07.031

Kobayashi, M., and Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol.* 2, 145–156. doi: 10.1016/S1474-4422(03)00321-1

Oberman, L., Eldaief, M., Fecteau, S., Ifert-Miller, F., Tormos, J. M., and Pascual-Leone, A. (2012). Abnormal modulation of corticospinal excitability in adults with Asperger's syndrome. *Eur. J. Neurosci.* 36, 2782–2788. doi: 10.1111/j.1460-9568.2012.08172.x

Oberman, L. M. (2012). mGluR antagonists and GABA agonists as novel pharmacological agents for the treatment of autism spectrum disorders. *Expert Opin. Investig. Drugs* 21, 1819–1825. doi: 10.1517/13543784.2012.729819

Oberman, L. M., Rotenberg, A., and Pascual-Leone, A. (2013). Use of transcranial magnetic stimulation in autism spectrum disorders. *J. Autism Dev. Disord.* doi: 10.1007/s10803-013-1960-2. [Epub ahead of print].

Panerai, S., Tasca, D., Lanuzza, B., Trubia, G., Ferri, R., Musso, S., et al. (2013). Effects of repetitive transcranial magnetic stimulation in performing eye-hand integration tasks: four preliminary studies with children showing low-functioning autism. *Autism* 18, 638–650. doi: 10.1177/1362361313495717

Puts, N. A., Wodka, E. L., Tommerdahl, M., Mostofsky, S. H., and Edden, R. A. (2014). Impaired tactile processing in children with autism spectrum disorder. *J. Neurophysiol.* 111, 1803–1811. doi: 10.1152/jn.00890.2013

Reichow, B. (2012). Overview of meta-analyses on early intensive behavioral intervention for young children with autism spectrum disorders. *J. Autism Dev. Disord.* 42, 512–520. doi: 10.1007/s10803-011-1218-9

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., and Safety Of, T. M. S. C. G. (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

Sokhadze, E. M., El-Baz, A. S., Sears, L. L., Opris, I., and Casanova, M. F. (2014). rTMS neuromodulation improves electrocortical functional measures of information processing and behavioral responses in autism. *Front. Syst. Neurosci.* 8:134. doi: 10.3389/fnsys.2014.00134

Conflict of Interest Statement: Alvaro Pascual-Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Axilum Robotics, Magstim, Neuroelectrics, and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI). Alexander Rotenberg is listed as an inventor on a patent for apparatus and method of use of TMS in epilepsy. He is a co-founder of Neuromotion Inc. This conference was supported by the Clearly Present Foundation, Autism Speaks, and Neuronetics, Inc. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 November 2014; accepted: 09 December 2014; published online: 06 January 2015.

Citation: Oberman LM, Enticott PG, Casanova MF, Rotenberg A, Pascual-Leone A and McCracken JT (2015) Transcranial magnetic stimulation (TMS) therapy for autism: an international consensus conference held in conjunction with the international meeting for autism research on May 13th and 14th, 2014. Front. Hum. Neurosci. ***8****:1034. doi: 10.3389/fnhum.2014.01034*

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

frontiers in
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 13 August 2014
doi: 10.3389/fnhum.2014.00627

Modulation of corticospinal excitability by transcranial magnetic stimulation in children and adolescents with autism spectrum disorder

Lindsay M. Oberman [1,2,3,4] ****, Alvaro Pascual-Leone*** [1] ***and Alexander Rotenberg*** [1,2] *******

[1] *Department of Neurology, Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center – Harvard Medical School, Boston, MA, USA*
[2] *Neuromodulation Program and Division of Epilepsy and Clinical Neurophysiology, Department of Neurology, Boston Children's Hospital – Harvard Medical School, Boston, MA, USA*
[3] *Neuroplasticity and Autism Spectrum Disorder Program, E. P. Bradley Hospital, East Providence, RI, USA*
[4] *Department of Psychiatry and Human Behavior, Warren Alpert Medical School of Brown University, East Providence, RI, USA*

Edited by:
Peter G. Enticott, Deakin University, Australia

Reviewed by:
Paul Croarkin, Mayo Clinic, USA
Melissa Kirkovski, Monash University, Australia

****Correspondence:***
Lindsay M. Oberman, Neuroplasticity and Autism Spectrum Disorder Program, E. P. Bradley Hospital, 1011 Veterans Memorial Parkway, East Providence, RI 02915, USA
e-mail: loberman@lifespan.org;
Alexander Rotenberg, Neuromodulation Program and Division of Epilepsy and Clinical Neurophysiology, Department of Neurology, Boston Children's Hospital – Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA
e-mail: alexander.rotenberg@childrens.harvard.edu

The developmental pathophysiology of autism spectrum disorders (ASD) is currently not fully understood. However, multiple lines of evidence suggest that the behavioral phenotype may result from dysfunctional inhibitory control over excitatory synaptic plasticity. Consistent with this claim, previous studies indicate that adults with Asperger's Syndrome show an abnormally extended modulation of corticospinal excitability following a train of repetitive transcranial magnetic stimulation (rTMS). As ASD is a developmental disorder, the current study aimed to explore the effect of development on the duration of modulation of corticospinal excitability in children and adolescents with ASD. Additionally, as the application of rTMS to the understanding and treatment of pediatric neurological and psychiatric disorders is an emerging field, this study further sought to provide evidence for the safety and tolerability of rTMS in children and adolescents with ASD. Corticospinal excitability was measured by applying single pulses of TMS to the primary motor cortex both before and following a 40 s train of continuous theta burst stimulation. 19 high-functioning males ages 9–18 with ASD participated in this study. Results from this study reveal a positive linear relationship between age and duration of modulation of rTMS after-effects. Specifically we found that the older participants had a longer lasting response. Furthermore, though the specific protocol employed typically suppresses corticospinal excitability in adults, more than one third of our sample had a paradoxical facilitatory response to the stimulation. Results support the safety and tolerability of rTMS in pediatric clinical populations. Data also support published theories implicating aberrant plasticity and GABAergic dysfunction in this population.

Keywords: autism spectrum disorders, transcranial magnetic stimulation, development, plasticity, GABA, theta burst stimulation

INTRODUCTION

Autism spectrum disorder (ASD) is diagnosed clinically, based on the key symptoms including qualitative impairments in social communication and the presence of restricted and repetitive behaviors (APA, 2013). However, the variability of the clinical phenotype of ASD is quite large and symptoms can manifest over a range of ages in childhood. Thus, ASD diagnosis can be challenging and is often not made until 3–5 years of age. For this reason, a physiologic ASD biomarker is highly desirable.

Several lines of evidence suggest that an impairment of GABAergic transmission may be critical in the pathophysiology of ASD (see Coghlan et al., 2012 for a review). GABA plays a key role in regulating neuronal excitability via feedback and feed-forward inhibition (Sutor and Luhmann, 1995; Petroff, 2002; Madsen et al., 2008; Huang, 2009). While in the mature brain GABA acts as an inhibitory neurotransmitter, during the embryonic and the perinatal period, GABA is excitatory (Cherubini et al., 1991). It is hypothesized that at least some forms of autism result from an imbalance between excitation and inhibition in local circuits involved in sensory, mnemonic, social, and emotional processes (Rubenstein and Merzenich, 2003; Markram and Markram, 2010). Ben-Ari et al. (2012), for instance, suggest that a dysfunction in the shift of GABA from excitation to inhibition may contribute to this imbalance.

Empirical support for the role of aberrant GABA signaling in the pathophysiology of ASD comes from both human and animal model research. A recent study conducted by Tyzio et al. (2014) found that GABA had excitatory action in two animal models of ASD [rats exposed to valproate in utero (VPA) and mice carrying the fragile X mutation (FRX)]. Furthermore, maternal pretreatment with bumetanide, forcing the shift of GABA in the offspring from excitatory to inhibitory, resulted in the restoration of typical electrophysiological and behavioral phenotypes in affected animals (Tyzio et al., 2014).

Human studies have found reduced GABA receptor expression (Fatemi et al., 2009a,b, 2010) as well as a 50% reduction in enzymes that synthesize GABA [*glutamic acid decarboxylase* (GAD) 65 and 67; Fatemi et al., 2002; Yip et al., 2007] in individuals with ASD. Furthermore, a recent study (Gaetz et al., 2014) identified significant reduction in the GABA MRS signal in the motor cortex of patients with ASD, and a marginally significant ($p = 0.054$) positive correlation between the GABA signal and age. Among the many roles of GABAergic circuits during development, one is lateral inhibition across neighboring minicolumns in the cortex. Consistent with an impairment in GABAergic transmission, postmortem studies have found a reduction in the horizontal spacing between minicolumns (Casanova et al., 2002). These abnormalities in the GABA system may directly contribute to altered anatomical and functional connectivity, and suggest a mechanism underlying the neurological and behavioral phenotype of ASD (Blatt, 2011).

In addition to and perhaps as a consequence of excitation/inhibition imbalance, recent studies in both human and animal models implicate synaptic plasticity mechanisms in the pathophysiology of ASD (see Oberman et al., in press). While most synaptic plasticity data in ASD are derived from *in vitro* rodent brain slice models, direct measures of circuit level plasticity in humans can be obtained by transcranial magnetic stimulation (TMS) paradigms (Ziemann, 2004; Huang et al., 2005; Thickbroom, 2007; Huerta and Volpe, 2009). In TMS, the cortex is stimulated focally by small intracranial electrical currents that are generated by a powerful and fluctuating extracranial magnetic field (Barker et al., 1985; Kobayashi and Pascual-Leone, 2003; Hallett, 2007). A number of experimental TMS measures of brain plasticity have been introduced, and provide the only noninvasive capacity to measure human phenomena that closely resemble long-term potentiation (LTP) and long-term depression (LTD). TMS is safe and well-tolerated, even in pediatric populations, if appropriately guidelines and recommendations are followed (Garvey and Gilbert, 2004; Rajapakse and Kirton, 2013).

Single-pulse TMS combined with EMG, EEG, fMRI, or other brain imaging methods can be used to quantify cortical reactivity before and following a given intervention (Pascual-Leone et al., 2011) providing an index of brain plasticity in response to said intervention. Recently, patterned bursting protocols have been developed that mimic paradigms used to assess synaptic plasticity in animal models (Huang et al., 2005, 2008). Specifically, theta burst stimulation (TBS) involves application of three bursts of 50-Hz rTMS repeated every 200 ms either continuously for a total of 40 s or intermittently (every 8 s) for about 3 min. When applied to the motor cortex, continuous TBS (cTBS) and intermittent TBS (iTBS) result in depression and potentiation of cortical reactivity as indexed through suppression and facilitation of motor evoked potentials (MEPs), respectively (Huang et al., 2005). Results from animal and human studies indicate that TBS modulatory effects on cortical reactivity reflect synaptic plasticity mechanisms (Cardenas-Morales et al., 2011). Specifically, and relevant to the present experiment, published data suggest that cTBS leads to enhancement of GABAergic inhibition (Stagg et al., 2009; Benali et al., 2011).

Notably, compared to other rTMS protocols, TBS has the advantage of lower stimulation intensities and shorter durations than conventional protocols making this protocol more suitable for use in clinical and pediatric populations. The safety and tolerability of this protocol has recently been evaluated and shown to be safe in healthy children and in children with Tourette Syndrome (Wu et al., 2012).

In a recent study (Oberman et al., 2012) we used the cTBS paradigm in 20 adults with Asperger's syndrome (high functioning ASD), and found them to show greater and longer-lasting suppression of cortical reactivity in the motor cortex following cTBS as compared to age-, gender-, and IQ-matched controls. The latency to return to baseline following TBS was on average between 80 and 90 min in the ASD group compared to 25–30 min in the controls. This finding was confirmed in a separate cohort of 15 individuals (Oberman et al., 2012). Interestingly, and consistent with other studies, there was no significant group difference in measures of basic excitability as assessed by resting and active motor threshold (Theoret et al., 2005; Oberman et al., 2012; Enticott et al., 2013) or response to single pulse TMS (Oberman et al., 2012). Thus, the excessive modulation of excitability in response to stimulation (a putative measure of plasticity) is not primarily attributable to differences in baseline excitability.

In the current study, we extended our age range to include data from 19 children and adolescents with high-functioning ASD (HF ASD) to explore the effect of development on the response to the cTBS paradigm. As the application of rTMS to the understanding and treatment of pediatric neurological and psychiatric disorders is an emerging field (Frye et al., 2008; Croarkin et al., 2011), this study additionally aimed to provide evidence for the safety and tolerability of TBS in HF children and adolescents with ASD.

MATERIALS AND METHODS

PARTICIPANTS

We studied 19 males with HF ASD, age 9–18 years (See **Table 1** for demographic characteristics of the sample). All participants gave informed consent or assent, which was also obtained from a parent or guardian to participate in the study. The study was reviewed and approved by the institutional review board at Boston Children's Hospital. Participants were recruited through local community advertisement. All participants had IQ > 80 based on the Weschler Abbreviated Scale of Intelligence (WASI). All met DSM-IV-TR criteria for Autism, Asperger's Syndrome or PDD-NOS, and met criteria for ASD on the Autism Diagnostic Observation Schedule, Module 4 (ADOS). Some participants also had comorbid symptoms including inattention, anxiety, irritability, and obsessive-compulsive behaviors (See **Table 1**). All participants were given a comprehensive neurological exam by a board-certified pediatric neurologist (Alexander Rotenberg) to confirm normal gross motor and fine motor function. Lastly, all participants were screened following published recommendations (Rossi et al., 2009) to ensure that they did not have any condition that would put them at greater risk of an adverse event related to TMS (e.g., a personal or immediate family history of epilepsy).

STIMULATION AND RECORDING

To evaluate modulation of corticospinal excitability (a putative index of cortical plasticity mechanisms) and specifically GABAergic inhibition, cTBS was applied to the primary motor cortex. The

Table 1 | Sample characteristics.

Participant number	Age	IQ	ADOS Score	Comorbid symptoms	Neuroactive medications	Response to cTBS
1	11	100	13	Anxiety	Citalopram	Suppression
2	9	94	7	None	None	Facilitation
3	12	86	10	Anxiety, Inattention	Citalopram, atomoxetine	Suppression
4	9	87	9	Anxiety ADHD	None	Suppression
5	14	115	8	Anxiety, ADHD	Citalopram, atomoxetine	Facilitation
6	14	99	9	None	None	Facilitation
7	10	106	12	None	None	Suppression
8	9	88	7	None	None	Facilitation
9	10	89	10	ADHD	Buspirone	Suppression
10	11	93	9	Obsessive-Compulsive Behaviors, Anxiety	Sertraline, citalopram	Facilitation
11	11	115	7	ADHD	Methylphenidate	Suppression
12	13	129	9	Inattention	Atomoxetine	Suppression
13	14	115	8	ADHD	Atomoxetine	Suppression
14	10	102	7	Irritability	Risperidone	Suppression
15	11	103	14	Inattention	Guanfacine, methylphenidate	Facilitation
16	13	102	7	ADHD	Methylphenidate	Facilitation
17	18	121	12	None	None	Suppression
18	18	83	13	None	None	Suppression
19	17	81	13	None	None	Suppression
AVERAGE	**12.26**	**100.42**	**9.58**			

cTBS paradigm used in the current study was identical to that described by Huang et al. (2005) and applied in previous studies in our laboratory (Oberman et al., 2010, 2012). The protocol consisted of three pulses of 50 Hz stimulation repeated at 200-ms intervals for 40 s (for a total of 600 pulses) at an intensity of 80% of active motor threshold (AMT). Corticospinal excitability was assessed prior to and following cTBS by measuring peak-to-peak amplitude of MEPs induced in the contralateral first dorsal interosseus (FDI) muscle in response to single-pulse TMS. These single pulses were applied at a rate of approximately 0.1 Hz (a random jitter of ± 1 s was introduced to avoid any train effects). Three batches of 10 MEPs were recorded prior to cTBS and used as a baseline. Beginning at 5 min following cTBS, batches of 10 MEPs were measured at periodic intervals (5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 105, and 120 min) until the MEPs returned to baseline levels to track changes in MEP amplitude over time. The participant was asked to remain relaxed during the entire study. Muscle activity was monitored throughout the session with EMG surface electrodes. TMS was only applied when the EMG signal indicated that the participant's FDI muscle was in a relaxed state. Any trials where the participant voluntarily contracted the muscle within 1000 ms of the TMS pulse were not included in the analysis.

To measure TMS induced MEPs, EMG surface electrodes were placed in a belly tendon montage over the FDI muscle of participants' right hands. Raw signals were amplified and bandpass-filtered between 20 and 2000 Hz. EMG signals were sampled at a rate of 5000 Hz. All stimulation (single-pulse TMS and TBS) was delivered using a hand-held 70 mm figure-of-eight coil attached to a Magstim Super Rapid stimulator (The MagStim Company Ltd., Whitland, UK). The coil was placed tangentially to the scalp with the handle pointing posteriorly. All stimulation was applied over the hand area of the left motor cortex and individually localized for each participant based on the optimal position for eliciting MEPs in the right FDI. The stimulation intensity for baseline and post-TBS single pulses was set at 120% of each individual's resting motor threshold (RMT) while the TBS itself was delivered at 80% of AMT. RMT and AMT were defined following recommendation from the International Federation of Clinical Neurophysiology. RMT was defined as the minimum single-pulse TMS intensity required to induce an MEP in the contralateral FDI of >50 μV peak-to-peak amplitude on more than five out of ten consecutive trials while the target muscle was at rest. AMT was defined as the minimum single-pulse TMS intensity required to induce an MEP in the contralateral FDI of >200 μV peak-to-peak amplitude on more than five out of ten consecutive trials while the target muscle was held at approximately 20% of the maximal contraction. To precisely target the stimulation site (primary motor cortex) and keep the brain target constant throughout the stimulation session, we used a frameless

stereotactic neuronavigation system (Brainsight, Rogue Research Inc., Montreal, QC, Canada).

DATA ANALYSIS

Data were analyzed using MatLab version 8.1 and SPSS version 22. Data analysis followed the methods described and applied in previous studies in our laboratory (Oberman et al., 2010, 2012). Average MEP amplitude values were calculated at baseline prior to TBS and starting five minutes after TBS and continuing until the average amplitude returned to within the 95% confidence interval of the baseline amplitude and did not return to outside that interval on subsequent time-point measures. MEP amplitudes were standardized, forming a ratio of MEP amplitudes following TBS relative to average baseline MEP amplitude for each individual.

Cubic spline interpolation was used to create smooth curves through the data points. Spline interpolation is a piecewise continuous function defined by third-degree polynomials in the intervals of a limited range of known data points (in this case, the time-points at which MEP data were collected with batches of 10 single TMS pulses). The use of spline interpolation on TMS data has been validated (Borghetti et al., 2008) and used in previous studies to evaluate degree and duration of modulation of MEP amplitudes following cTBS (Freitas et al., 2011). As an index of the duration of the TBS-induced modulation of cortico-spinal excitability, we defined, for each participant, the time-point ("time to baseline") at which post-cTBS MEP amplitude returned to the average MEP amplitude at baseline, i.e., the time-point at which the spline crossed the MEP threshold.

A natural log transformation was applied to the data prior to analysis as tests of normality indicated that the data was significantly different than normal. A Pearson product-moment correlation coefficient was calculated to assess the degree of relationship between age and duration of response to cTBS.

SIDE EFFECT MONITORING

Immediately following the TMS session a side effects questionnaire was completed by the experimenter. Participants were asked to report whether they experienced any of the following side effects: headache, neck pain, scalp pain or irritation, difficulty hearing, thinking or concentrating, change in mood, or any other change or side effect they experienced. The experimenter also noted whether the participant experienced a syncopal event or seizure. The participant also received a call the day after the TMS session and was once again asked to report whether they experienced any of the above side effects or to report any other side effect they experienced after they left the hospital. If the participant reported any side effect either immediately following the stimulation or the following day, its severity and duration were documented.

RESULTS

TMS SAFETY AND TOLERABILITY

All participants tolerated TBS and single-pulse stimulation without serious adverse event. One participant had a mild headache after stimulation that was alleviated with a single acetaminophen dose. Two participants had mild fatigue after the session that resolved the following day. No other adverse events were reported.

AGE-DEPENDENT RESPONSE TO cTBS

A Pearson product-moment correlation coefficient was calculated to test the hypothesis that a linear relationship existed between age and duration of response to cTBS. As described above, response to the cTBS protocol was defined as duration of effect as defined by the number of minutes following cTBS before the participant returned to baseline excitability levels ("time-to-baseline"; $M = 46.3$ min, SD $= 29.3$ min). The results of the analysis indicated that there was a significant positive linear relationship between age and duration of response [$r(17) = 0.660$, $p < 0.01$; **Figure 1**].

In addition to the planned analyses, it was noted that unlike our previous study that included only adult participants, a third of the participants (7 out of 19; ages 9, 11, 13, and 14) had a paradoxical facilitation in response to the cTBS protocol (**Figure 2**). Additional analyses were conducted excluding the seven participants who facilitated and the significant positive correlation between age and duration of response remained [$r(10) = 0.62$, $p < 0.05$] with a Mean duration of 49.2 min (SD $= 33.8$ min). The subgroup of participants who displayed the paradoxical facilitation response was not predicted by comorbid symptoms or medication ($\chi^2 = 0.091$, $p = 0.76$).

DISCUSSION

As the application of rTMS protocols to children increases, it is critical to evaluate the safety and tolerability of this procedure in these vulnerable populations. In the current study, all participants tolerated the stimulation and reported only minor discomforts that resolved quickly following the procedures. These findings add to the literature suggesting that rTMS is safe and well tolerated in children and in individuals with ASD (Garvey and Gilbert, 2004; Frye et al., 2008; Croarkin et al., 2011; Wu et al., 2012; Oberman et al., 2013; Rajapakse and Kirton, 2013). Systematic monitoring and documentation of side effects is

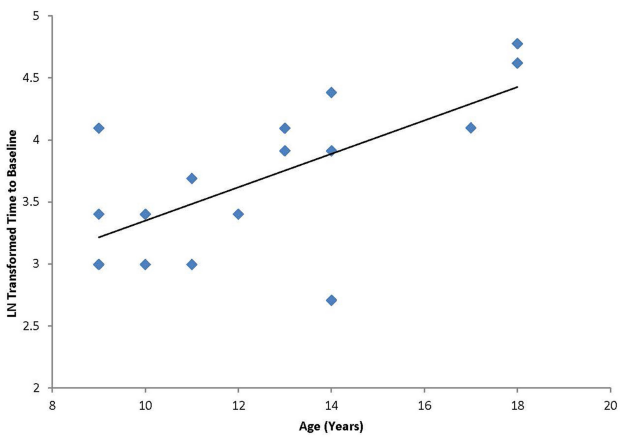


FIGURE 1 | Correlation between age and the natural log transformed "time to baseline" for each individual following cTBS. Graph shows a significant positive relationship between duration of modulation and age.

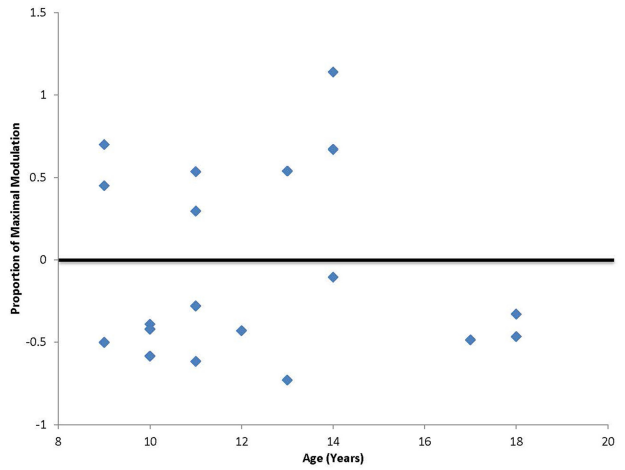


FIGURE 2 | Degree of maximal suppression or facilitation following cTBS. Values falling above zero indicate paradoxical facilitation in response to the typically suppressive cTBS protocol. Seven out of 20 participants in this study, ages 9, 11, 13, and 14 showed this paradoxical facilitation.

critical moving forward to ensure that both participants and investigators have an accurate sense of both the range and frequency of side effects of rTMS in clinical and pediatric populations.

Our findings are also the first step toward the study of the developmental regulation of the cTBS effect and reveal a positive linear relationship between age and duration of modulation of cTBS. Specifically we found that the older participants had a longer lasting response. On the surface this may appear counter intuitive if we consider that response to TBS has been used by our group and others to indicate degree of plasticity. One would imagine that younger children have a greater, not lesser, capacity for plasticity (Huttenlocher, 2002). However, cTBS is thought to model LTD-like plasticity (Huang et al., 2008) and is related to GABAergic inhibitory tone. Thus, perhaps LTD-like plasticity or GABAergic inhibition increases over development, especially during adolescence (Selemon, 2013). As we did not perform iTBS, we cannot speak to the development of LTP-like plasticity in this sample, however, it would be important to evaluate this process as well. Additionally, the current study did not include a sham control condition or any other rTMS protocol, thus it is unclear whether the results are specific to the cTBS paradigm.

Recently, the molecular mechanisms underlying the changes in cortical excitability induced by cTBS have been studied using MRS (Stagg et al., 2009). The findings reveal that the effects of cTBS are mediated by changes in the local activity of inhibitory interneuronal cortical pathways (as measured by changes in cortical GABA concentration in the primary sensorimotor cortex; Stagg et al., 2009). Consistent with the idea that younger children have less inhibitory tone, studies using paired pulse measures of intracortical inhibition have found that children display decreased levels of suppression as compared to adolescents or adults (Walther et al., 2009). This study further claimed that reduced GABA mediated intracortical inhibition may facilitate excitatory (LTP-like) cortical plasticity and motor learning in children. Thus, the current results, although obtained from individuals with ASD, provide further evidence of increasing capacity for LTD-like suppression of cortical excitability across childhood.

Additionally, the finding that over one third of our sample had a paradoxical facilitatory response to cTBS supports the notion of GABAergic dysfunction in ASD. During typical development, GABA currents shift from excitatory to inhibitory through a maturation of chloride transport mechanisms and an age-dependent reduction of intracellular chloride concentration [(Cl^-)$_i$; Ben-Ari et al., 2007)]. However, a recent study finds that two ASD animal models (rodent valproate and fragile X models) show excitatory GABA activity well beyond the age where wild-type animals' GABA activity has shifted to inhibition (Tyzio et al., 2014). In these animals administration of a GABA agonist (isoguvacine) led to an *increase* in spike frequency in neurons recorded from hippocampal slices as compared to a decrease in wild-type animals. Additionally, the in utero administration of bumetanide, a chloride importer antagonist that reduces intracellular chloride accumulation thereby promoting the shift of GABA from excitation to inhibition, resulted in the restoration of typical electrophysiological and behavioral phenotypes in affected offspring (Tyzio et al., 2014). These preclinical data support the hypothesis that a dysfunction in this shift may contribute to the pathophysiology of ASD (Ben-Ari et al., 2012). Ben-Ari and colleagues have proposed that this dysfunction may be a result of increased intracellular ($[Cl^-]_i$) concentrations in individuals with ASD. This is further supported by a study reporting paradoxical *increases* in hyperactivity in six out of seven and aggression in seven out of seven children with ASD who were treated with diazepam (Marrosu et al., 1987). Furthermore, in a recent clinical trial where bumetanide, was given to children with ASD results showed improvement in ASD symptoms as measured by the Childhood Autism Rating Scale (CARS) and the Repetitive and Restricted Behavior Scale (RRB) as well as a reduction in aberrant behavior as measured by the Aberrant Behavior Checklist (ABC; Lemonnier and Ben-Ari, 2010). Our findings suggest that one could do an analogous study in humans to explore whether bumetanide would normalize the cTBS modulation in those with a paradoxical facilitation and if this normalization corresponded to improved behavioral symptoms.

We recently suggested that the neurological and behavioral ASD phenotypes are associated with altered brain plasticity that can be measured noninvasively by TMS (Oberman et al., in press). As our data showing age-dependence of the cTBS response suggest, the timing of plastic brain changes may be important for optimal development of cortical circuitry. As ASD is a developmental disorder it would be critical to evaluate the developmental trajectory of abnormalities in a putative mechanism underlying the phenotype.

As the current study did not include healthy control participants or females, it is not clear whether the observed developmental trajectory shown by these males with ASD is similar to what may be obtained in neurotypical individuals or females with ASD. It is possible that variables such as head size or myelination

could have led to the observed correlation with age. However, as reviewed above, multiple lines of evidence point toward aberrant GABAergic transmission in ASD. Thus, it will be important to evaluate these measures in a healthy developing population and female ASD population to compare the typical developmental trajectory to that shown in males with ASD. Additionally, follow-up translational studies, analogous to what has been done in animal models, directly testing the relationship between GABA receptor expression [measured by [^{11}C] FMZ PET (Maziere et al., 1984)] or concentration [measured by MRS (Mescher et al., 1998)] and measures of cortical reactivity in humans with ASD are needed.

In the current study, we focused on primary motor cortex in the left hemisphere. Thus, it is unclear whether other cortical regions would show similar developmental trajectories or whether there would be a laterality effect in these individuals. The left primary motor cortex was chosen in this study for two reasons. First, MEPs are the standard index used to quantify the effect of TBS protocols. Other indices of cortical excitability outside the motor cortex (e.g., based on electroencephalographic measures) have not yet been well validated for this application. We chose the left hemisphere as it is typically the dominant hemisphere for both right- and left-handed individuals. Second, although motor abnormalities are not considered core symptoms of ASD, many studies have reported motor deficits in individuals with ASD, including alterations in motor milestone development (Teitelbaum et al., 1998), clumsiness, motor incoordination, disturbances in reach-to-grasp movement (Miyahara et al., 1997; Ghaziuddin and Butler, 1998; Mari et al., 2003), deficits in gross and fine motor movement (Noterdaeme et al., 2002), and impaired postural control (Kohen-Raz et al., 1992; Minshew et al., 2004). It has also been suggested that these motor deficits may underlie the core deficits in ASD (Mostofsky and Ewen, 2011).

Our results support the safety and tolerability of TBS in the pediatric ASD populations. As we continue to enhance our understanding of the relationship between the response to cTBS with GABAergic inhibition and GABAergic dysfunction with ASD pathophysiology we suggest that cTBS may be a practical biomarker of GABAergic dysfunction in this population.

AUTHOR CONTRIBUTIONS

Lindsay M. Oberman designed the study, collected the data, analyzed the data, and wrote the manuscript. Alvaro Pascual-Leone contributed to the study design, interpretation of the data, and the writing of the manuscript, Alexander Rotenberg contributed to the study design, interpretation of the data, supervised and contributed to data collection, and contributed to writing of the manuscript.

ACKNOWLEDGMENTS

Work on the project is supported grants the Boston Children's Hospital Translational Research Program (Alexander Rotenberg), National Institutes of Health and National Institute of Mental Health (1R01MH100186), and Harvard Catalyst, The Harvard Clinical and Translational Science Center (NCRR and the NCATS NIH 8KL2TR000168-05). Lindsay M. Oberman is further supported by grants from the Harvard Clinical and Translational Science Center (8UL1TR000170-05), the Epilepsy Research Foundation, the Simons Foundation and the Nancy Lurie Marks Family Foundation. Alvaro Pascual-Leone is further supported by grants from the National Institutes of Health (R01HD069776, R01NS073601, R21 MH099196, R21 NS082870, R21 NS085491, R21 HD07616, UL1 RR025758), Michael J. Fox Foundation and Sidney R. Baer Foundation. Alexander Rotenberg is further supported by the Center for Integration of Medicine and Innovative Technology (CIMIT), Department of Defense PR121509, Autism Speaks Grant #8702, and grants from Eisai Inc. and the Epilepsy Research Foundation and epilepsy therapy project.

The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or any of the listed granting agencies.

REFERENCES

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

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

Benali, A., Trippe, J., Weiler, E., Mix, A., Petrasch-Parwez, E., Girzalsky, W., et al. (2011). Theta-burst transcranial magnetic stimulation alters cortical inhibition. *J. Neurosci.* 31, 1193–1203. doi: 10.1523/JNEUROSCI.1379-10.2011

Ben-Ari, Y., Gaiarsa, J. L., Tyzio, R., and Khazipov, R. (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* 87, 1215–1284. doi: 10.1152/physrev.00017.2006

Ben-Ari, Y., Khalilov, I., Kahle, K. T., and Cherubini, E. (2012). The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *Neuroscientist* 18, 467–486. doi: 10.1177/1073858412438697

Blatt, G. J., and S. H. Fatemi (2011). Alterations in GABAergic biomarkers in the autism brain: research findings and clinical implications. *Anat. Rec. (Hoboken)* 294, 1646–1652. doi: 10.1002/ar.21252

Borghetti, D., Sartucci, F., Petacchi, E., Guzzetta, A., Piras, M. F., Murri, L., et al. (2008). Transcranial magnetic stimulation mapping: a model based on spline interpolation. *Brain Res. Bull.* 77, 143–148. doi: 10.1016/j.brainresbull.2008.06.001

Cardenas-Morales, L., Gron, G., and Kammer, T. (2011). Exploring the after-effects of theta burst magnetic stimulation on the human motor cortex: a functional imaging study. *Hum. Brain Mapp.* 32, 1948–1960. doi: 10.1002/hbm.21160

Casanova, M. F., Buxhoeveden, D. P., and Brown, C. (2002). Clinical and macroscopic correlates of minicolumnar pathology in autism. *J. Child Neurol.* 17, 692–695. doi: 10.1177/088307380201700908

Cherubini, E., Gaiarsa, J. L., and Ben-Ari, Y. (1991). GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.* 14, 515–519. doi: 10.1016/0166-2236(91)90003-D

Coghlan, S., Horder, J., Inkster, B., Mendez, M. A., Murphy, D. G., and Nutt, D. J. (2012). GABA system dysfunction in autism and related disorders: from synapse to symptoms. *Neurosci. Biobehav. Rev.* 36, 2044–2055. doi: 10.1016/j.neubiorev.2012.07.005

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

Enticott, P. G., Kennedy, H. A., Rinehart, N. J., Tonge, B. J., Bradshaw, J. L., and Fitzgerald, P. B. (2013). GABAergic activity in autism spectrum disorders: an investigation of cortical inhibition via transcranial magnetic stimulation. *Neuropharmacology* 68, 202–209. doi: 10.1016/j.neuropharm.2012.06.017

Fatemi, S. H., Folsom, T. D., Reutiman, T. J., and Thuras, P. D. (2009a). Expression of GABA(B) receptors is altered in brains of subjects with autism. *Cerebellum* 8, 64–69. doi: 10.1007/s12311-008-0075-73

Fatemi, S. H., Halt, A. R., Stary, J. M., Kanodia, R., Schulz, S. C., and Realmuto, G. R. (2002). Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced

in autistic parietal and cerebellar cortices. *Biol. Psychiatry* 52, 805–810. doi: 10.1016/S0006-3223(02)01430-0

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., Rooney, R. J., Patel, D. H., and Thuras, P. D. (2010). mRNA and protein levels for GABAAalpha4, α5, β1 and GABABR1 receptors are altered in brains from subjects with autism. *J. Autism Dev. Disord.* 40, 743–750. doi: 10.1007/s10803-009-0924-z

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., and Thuras, P. D. (2009b). GABA(A) receptor downregulation in brains of subjects with autism. *J. Autism Dev. Disord.* 39, 223–230. doi: 10.1007/s10803-008-0646-647

Freitas, C., Perez, J., Knobel, M., Tormos, J. M., Oberman, L., Eldaief, M., et al. (2011). Changes in cortical plasticity across the lifespan. *Front. Aging Neurosci.* 3:5. doi: 10.3389/fnagi.2011.00005

Frye, R. E., Rotenberg, A., Ousley, M., and Pascual-Leone, A. (2008). Transcranial magnetic stimulation in child neurology: current and future directions. *J. Child Neurol.* 23, 79–96. doi: 10.1177/0883073807307972

Gaetz, W., Bloy, L., Wang, D. J., Port, R. G., Blaskey, L., Levy, S. E., et al. (2014). GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. *Neuroimage* 86, 1–9. doi: 10.1016/j.neuroimage.2013.05.068

Garvey, M. A., and Gilbert, D. L. (2004). Transcranial magnetic stimulation in children. *Eur. J. Paediatr. Neurol.* 8, 7–19. doi: 10.1016/j.ejpn.2003.11.002

Ghaziuddin, M., and Butler, E. (1998). Clumsiness in autism and Asperger syndrome: a further report. *J. Intellect. Disabil. Res.* 42, 43–48. doi: 10.1046/j.1365-2788.1998.00065.x

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

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., Edwards, M. J., and Chen, R. S. (2008). Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cereb. Cortex* 18, 563–570. doi: 10.1093/cercor/bhm087

Huang, Z. J. (2009). Activity-dependent development of inhibitory synapses and innervation pattern: role of GABA signalling and beyond. *J. Physiol.* 587, 1881–1888. doi: 10.1113/jphysiol.2008.168211

Huerta, P. T., and Volpe, B. T. (2009). Transcranial magnetic stimulation, synaptic plasticity and network oscillations. *J. Neuroeng. Rehabil.* 6:7. doi: 10.1186/1743-0003-6-7

Huttenlocher, P. R. (2002). *Neural Plasticity.* Cambridge: Harvard University Press.

Kobayashi, M., and Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol.* 2, 145–156. doi: 10.1016/S1474-4422(03)00321-1

Kohen-Raz, R., Volkmar, F. R., and Cohen, D. J. (1992). Postural control in children with autism. *J. Autism Dev. Disord.* 22, 419–432. doi: 10.1007/BF01048244

Lemonnier, E., and Ben-Ari, Y. (2010). The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. *Acta Paediatr.* 99, 1885–1888. doi: 10.1111/j.1651-2227.2010.01933.x

Madsen, K. K., Larsson, O. M., and Schousboe, A. (2008). Regulation of excitation by GABA neurotransmission: focus on metabolism and transport. *Results Probl. Cell Differ.* 44, 201–221. doi: 10.1007/400_2007_036

Mari, M., Castiello, U., Marks, D., Marraffa, C., and Prior, M. (2003). The reach-to-grasp movement in children with autism spectrum disorder. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 358, 393–403. doi: 10.1098/rstb.2002.1205

Markram, K., and Markram, H. (2010). The intense world theory – a unifying theory of the neurobiology of autism. *Front. Hum. Neurosci.* 4:224. doi: 10.3389/fnhum.2010.00224

Marrosu, F., Marrosu, G., Rachel, M. G., and Biggio, G. (1987). Paradoxical reactions elicited by diazepam in children with classic autism. *Funct. Neurol.* 2, 355–361.

Maziere, M., Hantraye, P., Prenant, C., Sastre, J., and Comar, D. (1984). Synthesis of ethyl 8-fluoro-5,6-dihydro-5-[11C]methyl-6-oxo-4H-imidazo [1,5-a][1,4]benzodiazepine-3-carboxylate (RO 15.1788-11C): a specific radioligand for the in vivo study of central benzodiazepine receptors by positron emission tomography. *Int. J. Appl. Radiat. Isot.* 35, 973–976. doi: 10.1016/0020-708X(84)90215-1

Mescher, M., Merkle, H., Kirsch, J., Garwood, M., and Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR Biomed.* 11, 266–272. doi: 10.1002/(SICI)1099-1492(199810)11:6<266::AID-NBM530>3.0.CO;2-J

Minshew, N. J., Sung, K., Jones, B. L., and Furman, J. M. (2004). Underdevelopment of the postural control system in autism. *Neurology* 63, 2056–2061. doi: 10.1212/01.WNL.0000145771.98657.62

Miyahara, M., Tsujii, M., Hori, M., Nakanishi, K., Kageyama, H., and Sugiyama, T. (1997). Brief report: motor incoordination in children with Asperger syndrome and learning disabilities. *J. Autism Dev. Disord.* 27, 595–603. doi: 10.1023/A:1025834211548

Mostofsky, S. H., and Ewen, J. B. (2011). Altered connectivity and action model formation in autism is autism. *Neuroscientist* 17, 437–448. doi: 10.1177/1073858410392381

Noterdaeme, M., Mildenberger, K., Minow, F., and Amorosa, H. (2002). Evaluation of neuromotor deficits in children with autism and children with a specific speech and language disorder. *Eur. Child Adolesc. Psychiatry* 11, 219–225. doi: 10.1007/s00787-002-0285-z

Oberman, L., Eldaief, M., Fecteau, S., Ifert-Miller, F., Tormos, J. M., and Pascual-Leone, A. (2012). Abnormal modulation of corticospinal excitability in adults with Asperger's syndrome. *Eur. J. Neurosci.* 36, 2782–2788. doi: 10.1111/j.1460-9568.2012.08172.x

Oberman, L. M., Horvath, J. C., and Pascual-Leone, A. (2010). TMS: using the theta-burst protocol to explore mechanism of plasticity in individuals with Fragile X syndrome and autism. *J. Vis. Exp.* pii: 2272. doi: 10.3791/2272

Oberman, L. M., Rotenberg, A., and Pascual-Leone, A. (2013). Use of transcranial magnetic stimulation in autism spectrum disorders. *J. Autism Dev. Disord.* doi: 10.1007/s10803-013-1960-1962 [Epub ahead of print].

Oberman, L. M., Rotenberg, A., and Pascual-Leone, A. (in press). "Aberrant brain plasticity in autism spectrum disorders," in *Plasticity of Cognition in Neurologic Disorders*, eds J. Tracy, B. Hampstead, and K. Sathian (New York: Oxford University Press).

Pascual-Leone, A., Freitas, C., Oberman, L., Horvath, J. C., Halko, M., Eldaief, M., et al. (2011). Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI. *Brain Topogr.* 24, 302–315. doi: 10.1007/s10548-011-0196-198

Petroff, O. A. (2002). GABA and glutamate in the human brain. *Neuroscientist* 8, 562–573. doi: 10.1177/1073858402238515

Rajapakse, T., and Kirton, A. (2013). Non-invasive brain stimulation in children: applications and future directions. *Transl. Neurosci.* 4, 217–233. doi: 10.2478/s13380-013-0116-3

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

Rubenstein, J. L., and Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* 2, 255–267. doi: 10.1034/j.1601-183X.2003.00037.x

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Transl. Psychiatry* 3:e238. doi: 10.1038/tp.2013.7

Stagg, C. J., Wylezinska, M., Matthews, P. M., Johansen-Berg, H., Jezzard, P., Rothwell, J. C., et al. (2009). Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *J. Neurophysiol.* 101, 2872–2877. doi: 10.1152/jn.91060.2008

Sutor, B., and Luhmann, H. J. (1995). Development of excitatory and inhibitory postsynaptic potentials in the rat neocortex. *Perspect. Dev. Neurobiol.* 2, 409–419.

Teitelbaum, P., Teitelbaum, O., Nye, J., Fryman, J., and Maurer, R. G. (1998). Movement analysis in infancy may be useful for early diagnosis of autism. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13982–13987. doi: 10.1073/pnas.95.23.13982

Theoret, H., Halligan, E., Kobayashi, M., Fregni, F., Tager-Flusberg, H., and Pascual-Leone, A. (2005). Impaired motor facilitation during action observation in individuals with autism spectrum disorder. *Curr. Biol.* 15, R84–R85. doi: 10.1016/j.cub.2005.01.022

Thickbroom, G. W. (2007). Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. *Exp. Brain Res.* 180, 583–593. doi: 10.1007/s00221-007-0991-993

Tyzio, R., Nardou, R., Ferrari, D. C., Tsintsadze, T., Shahrokhi, A., Eftekhari, S., et al. (2014). Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* 343, 675–679. doi: 10.1126/science.1247190

Walther, M., Berweck, S., Schessl, J., Linder-Lucht, M., Fietzek, U. M., Glocker, F. X., et al. (2009). Maturation of inhibitory and excitatory motor cortex pathways in children. *Brain Dev.* 31, 562–567. doi: 10.1016/j.braindev.2009.02.007

Wu, S. W., Shahana, N., Huddleston, D. A., Lewis, A. N., and Gilbert, D. L. (2012). Safety and tolerability of theta-burst transcranial magnetic stimulation in children. *Dev. Med. Child Neurol.* 54, 636–639. doi: 10.1111/j.1469-8749.2012.04300.x

Yip, J., Soghomonian, J. J., and Blatt, G. J. (2007). Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta Neuropathol.* 113, 559–568. doi: 10.1007/s00401-006-0176-173

Ziemann, U. (2004). TMS induced plasticity in human cortex. *Rev. Neurosci.* 15, 253–266. doi: 10.1515/REVNEURO.2004.15.4.253

Conflict of Interest Statement: Alvaro Pascual-Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Neuroelectrics, and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI). Alexander Rotenberg is listed as an inventor on a patent for apparatus and method of use of TMS in epilepsy. He is a co-founder of Neuromotion Inc.

Received: 16 June 2014; accepted: 28 July 2014; published online: 13 August 2014.
Citation: Oberman LM, Pascual-Leone A and Rotenberg A (2014) Modulation of corticospinal excitability by transcranial magnetic stimulation in children and adolescents with autism spectrum disorder. Front. Hum. Neurosci. ***8****:627. doi: 10.3389/fnhum.2014.00627*
This article was submitted to the journal Frontiers in Human Neuroscience.

frontiers in
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 21 October 2014
doi: 10.3389/fnhum.2014.00851

Effects of weekly low-frequency rTMS on autonomic measures in children with autism spectrum disorder

Manuel Fernando Casanova[1,2]*, Marie K. Hensley[2], Estate M. Sokhadze[1,2], Ayman S. El-Baz[1,2], Yao Wang[1,3], Xiaoli Li[3] and Lonnie Sears[4]

[1] *Department of Psychiatry and Behavioral Sciences, University of Louisville, Louisville, KY, USA*
[2] *Department of Bioengineering, University of Louisville, Louisville, KY, USA*
[3] *College of Brain and Cognitive Neurosciences, Bejing Normal University, Bejing, China*
[4] *Department of Pediatrics, University of Louisville, Louisville, KY, USA*

Edited by:
Lindsay M. Oberman, Brown University, USA

Reviewed by:
Marco Iacoboni, University of California Los Angeles, USA
Paul Croarkin, Mayo Clinic, USA

****Correspondence:***
Manuel Fernando Casanova, Department of Psychiatry and Behavioral Sciences, University of Louisville, 500 South Preston Street, Louisville, 40202 KY, USA
e-mail: m0casa02@louisville.edu

The term autism spectrum disorder (ASD) describes a range of conditions characterized by impairments in social interactions, communication, and by restricted and repetitive behaviors. Autism spectrum disorder may also present with symptoms suggestive of autonomic nervous system (ANS) dysfunction. The objective of this study was to determine the effect of 18 sessions of low frequency (LF) repetitive transcranial magnetic stimulation (rTMS) on autonomic function in children with ASD by recording electrocardiogram (ECG) and electrodermal activity (EDA) pre- post- and during each rTMS session. The autonomic measures of interest in this study were R-R cardiointervals in EKG (R-R), time and frequency domain measures of heart rate variability (HRV) and skin conductance level (SCL). Heart rate variability measures such as R-R intervals, standard deviation of cardiac intervals, pNN50 (percentage of cardiointervals >50 ms different from preceding interval), power of high frequency (HF) and LF components of HRV spectrum, LF/HF ratio, were then derived from the recorded EKG. We expected that the course of 18 weekly inhibitory LF rTMS applied to the dorsolateral prefrontal cortex (DLPFC) would enhance autonomic balance by facilitating frontal inhibition of limbic activity thus resulting in decreased overall heart rate (HR), increased HRV (in a form of increased HF power), decreased LF power (resulting in decreased LF/HF ratio), and decreased SCL. Behavioral evaluations post-18 TMS showed decreased irritability, hyperactivity, stereotype behavior and compulsive behavior ratings while autonomic measures indicated a significant increase in cardiac interval variability and a decrease of tonic SCL. The results suggest that 18 sessions of LF rTMS in ASD results in increased cardiac vagal control and reduced sympathetic arousal.

Keywords: autism spectrum disorder, TMS, autonomic nervous system, electrocardiogram, skin conductance

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by difficulties in social interactions communication, and restricted and repetitive patterns of behaviors. In 2014, it was estimated by the Centers for Disease Control and Prevention (CDC) that ASD affects approximately 1 in 68 children (CDC's Morbidity and Mortality Weekly Report, 2014). In addition to affecting neural development, it is also thought that ASD can manifest itself in abnormalities of autonomic nervous system (ANS) activity. Recent research suggests that some autistic individuals manifest an over-activation of the sympathetic branch of the ANS on a background of parasympathetic activity deficits (Ming et al., 2011). This bias creates an autonomic imbalance evidenced by a faster heart rate (HR) of little variability and increased tonic electrodermal activity (EDA; Zahn et al., 1987).

AUTONOMIC DYSFUNCTIONS IN AUTISM

Heart rate variability

Several types of autonomic dysfunctions have been reported in autism, including increased basal sympathetic tone (Hirstein et al., 2001), as well as reduced baseline parasympathetic activity in association with increased baseline sympathetic tone (Toichi et al., 1999; Julu et al., 2001; Porges, 2001; Toichi and Kamio, 2003; Ming et al., 2004, 2005, 2011). Heart rate variability (HRV) measures are widely used in psychopathology research (Cohen et al., 2000; Thayer and Friedman, 2002) for assessment of phasic and tonic cardiac autonomic control (Berntson et al., 1997). Reduced HRV, specifically the attenuated power of high frequency (HF) component of the HRV (also called "respiratory sinus arrhythmia" [RSA]), is an indicator of limited psychophysiological flexibility (Berntson et al., 1997, 2008; Eckberg, 1997; Friedman and Thayer, 1998; Stein and Kleiger, 1999; Cohen et al., 2000). Several

studies have shown that typical children show more HRV than autistic children (Hutt et al., 1975; Althaus et al., 1999; Jenkins et al., 2002), and that autistic children have unusually small deceleratory HR responses to stimuli (Palkovitz and Wiesenfeld, 1980; Coronoa et al., 1998; Porges, 2001). A recently published paper by Ming et al. (2011) reported evidence of reduced baseline parasympathetic activity and increased sympathetic tone in children with ASD. Another study by Bal et al. (2010) used RSA as a measure of cardiac vagal tone and compared RSA values between children with and without ASD. The study found that children with ASD had significantly lower RSA values and faster HR than those without ASD, which suggests decreased vagal cardiac regulation in autism. The clinical implications of chronic increased sympathetic activity and decreased vagal tone are poor control of HR and a tendency for tachycardia (Berntson et al., 1997, 2008). Therefore, analysis of the HRV, in particular the HF component of HRV along with other measures of heart beat variability (e.g., standard deviation of R-R intervals in electrocardiogram (ECG)) associated with parasympathetic activity, may provide important information regarding autonomic dysfunctions in autism.

Poor control of HR and vulnerability to tachycardia is an important consequence of chronic increased sympathetic activity and decreased vagal tone (Berntson et al., 1997, 2008; Coronoa et al., 1998; Friedman and Thayer, 1998). The baseline sympathetic over-arousal found in autism may reflect a condition of disinhibition, resulting from compromised baseline parasympathetic tone. Reduced fronto-limbic connectivity and poor prefrontal tonic inhibitory control over the limbic system (Loveland et al., 2008) might be one of the reasons for excessive excitation by the sympathetic branch of the ANS in ASD. Application of inhibitory rTMS to frontal cortex aimed at reducing the high cortical excitation/inhibition (E/I) ratio could be an effective technique for restoring normative fronto-limbic tonic inhibition, and for improving sympatho-vagal cardiac balance in autism.

Electrodermal activity

Studies of the ANS in autism have demonstrated several manifestations of abnormal sympathetic functions (Ming et al., 2004, 2005, 2011). Skin conductance response (SCR) studies in autistic children have shown a lack of the normal habituation in the magnitudes of SCR to the same stimulus over time (Udupa et al., 2007). Palkovitz and Wiesenfeld (1980) did not find differences in electrodermal reactivity to auditory stimulation compared to controls, but reported that the autistic group had a higher baseline skin conductance level (SCL). In addition, it has been reported that children with autism have a blunted autonomic arousal as indexed by SCL and SCR to visual or auditory social stimuli (Zahn et al., 1987; Hirstein et al., 2001; Ming et al., 2004, 2005, 2011). Angus (1970) found that children with ASD displayed more fluctuations in SCL compared to controls. Skin conductance response studies in autistic children have shown a lack of the normal SCR habituation to the same stimulus over time (Toichi and Kamio, 2003). Abnormal autonomic activity in during rest and during responses to stimulation in ASD was recently reported also in other studies (Benevides and Lane, 2013; Eilam-Stock et al., 2014). Furthermore, several of our own pilot studies also support excessive but less differentiated SCR to affective sounds, visual, and audio-visual stimuli in various affective stimulation tests (Sokhadze et al., 2012c; Dombroski et al., 2013) and positive changes following several experimental treatment approaches (Hensley et al., 2012, 2013; Sokhadze et al., 2012b; Dombroski et al., 2013). Since SCL is controlled solely by the sympathetic inputs (Williams et al., 2004; Boucsein, 2012), the above-mentioned effects are indicative of high sympathetic tone and low selectivity of sympathetic responses in autism.

NEUROMODULATION APPROACHES IN TREATMENT OF AUTISM

Recently there has been considerable interest on the effects of repetitive transcranial magnetic stimulation (rTMS) on cortical excitability. Biophysical foundations underlying TMS effects are reviewed in Wagner et al. (2009), while results of investigation of connectivity of the cortical structures during TMS using positron emission tomography (PET) was reported by Paus et al. (1997). Transcranial magnetic stimulation operates based on Faraday's law of electromagnetic induction, which describes the process by which a changing magnetic field induces the flow of electric current in a nearby conductor, one preferentially standing at 90% to the magnetic field. Studies have indicated that low-frequency or "slow" rTMS (<1 Hz) increases inhibition of stimulated cortex, whereas high-frequency rTMS (>5 Hz) increases excitability of stimulated cortex. It has been proposed that the effect of slow rTMS arises from increases in the activation of inhibitory circuits (Pascual-Leone et al., 2000). We theorize that contrary to other inhibitory cells (i.e., basket and chandelier), whose projections keep no constant anatomical relation to the surface of the cortex, the geometrically exact orientation of double-bouquet cells and their location at the periphery of the minicolumn (the so-called inhibitory surround) makes them an appropriate candidate for induction by a magnetic field applied tangentially to the cortex. Over a course of treatment, slow rTMS may selectively depotentiate enhanced synaptic weights associated with pathological conditions, and, in the case of ASD, may lower the ratio of cortical excitation to cortical inhibition. Safety of TMS application in children were reviewed in several reports (Quintana, 2005; Garvey and Mall, 2008).

Transcranial magnetic stimulation has already shown to be an effective neuromodulatory tool capable of altering ANS functions. In a paper by Udupa et al. (2007) researchers compared rTMS with antidepressant therapy to address the autonomic imbalance associated with depression. The authors found that rTMS not only produced antidepressant effects, but also "corrected" the autonomic balance. The researchers used HRV measures as evidence that rTMS did in fact reduce the sympathetic-to-parasympathetic ratio thus improving the sympatho-vagal balance. In our previous studies slow rTMS was shown to improve both evoked EEG gamma activity and error processing in individuals with ASD. Baruth et al. (2010a) compared evoked gamma activity in the early stages of visual processing between individuals with ASD and neurotypicals using Kanizsa illusory figures in a visual oddball task. In autistic individuals, evoked gamma activity was not discriminative of stimulus type, whereas control subjects displayed

early gamma-power differences between target and non-target stimuli (for a review of gamma activity see Casanova et al., 2013). Individuals with ASD underwent 12 sessions of rTMS and repeated the Kanizsa test. Results showed improvement in discriminatory gamma activity between target and non-target stimuli, as well as improvement in responses on behavioral questionnaires. In a study by Sokhadze et al. (2012a) TMS was used to improve error processing in children with ASD, as measured by event-related potentials (ERP) associated with response to errors, such as error-related negativity (ERN). Post-TMS results showed significant differences in the response-locked ERPs such as ERN, as well as behavioral response monitoring measures indicative of improved error monitoring and correction function (Sokhadze et al., 2012a). In another pilot study we reported minute-by-minute changes of HR, HRV indices, and SCL during 12 session of rTMS in children with autism (Hensley et al., 2012, 2013). In particular, we noted a decrease in the LF component of HRV and a decrease of SCL during 10 min of rTMS session indicative of decreased sympathetic activity.

The dorsolateral prefrontal cortex (DLPFC) was selected as a target for stimulation in our rTMS studies based on the topographical analysis of minicolumnar morphometry in cortices varying in cytoarchitectural differentiation: paralimbic, high-order (heteromodal) association, modality specific (unimodal) association, and idiotypic areas (Casanova et al., 2006). Neuroanatomical studies indicated that minicolumnar abnormalities in autism occur in a gradient that parallels connectivity; high-order association areas exhibiting salient abnormalities while idiotypic areas apparently being sparred. In addition, several of our recent publications have demonstrated positive behavioral, clinical and electrophysiological functional outcomes of rTMS when stimulating the DLPFC in children with autism (Sokhadze et al., 2009a,b, 2010a,b, 2012a; Baruth et al., 2010a,b, 2011; Casanova et al., 2012).

It is doubtful whether a pervasive neurodevelopmental disorder such as ASD could be explained in terms of pathology within a single brain area, i.e., DLPC. However, "normalizing" an area like the DLPFC whose physiology depends on distributed networks may provide beneficial cascading effects at secondary sites (Walsh and Pascual-Leone, 2003). Due to the anatomical and functional connectivity of the DLPC, we expected the TMS-based intervention not to be limited to the site of magnetic stimulation but rather to generalize to other cortical and subcortical areas. In effect results of our pilot studies (Sokhadze et al., 2009a,b, 2010a, 2012a) have shown changes of ERP and induced electroencephalographic (EEG) gamma oscillations not only in the frontal lobe but also in distal cortical areas (parietal, parieto-occipital, etc.). Effects of rTMS over DLPFC are possibly extended to paralimbic and limbic structures as well and may manifest themselves in ANS activity changes.

We hypothesized that rTMS stimulation applied bilaterally to the DLPFC would improve autonomic measures, more specifically, it was predicted that it would lower sympathetic arousal and normalize autonomic balance. Heart rate variability and SCL measurements were used to track changes in autonomic balance caused by rTMS. We chose to use HRV and SCL as indicators of the effectiveness of rTMS treatment because they are largely controlled by the ANS. The first measure, HRV, allowed us to observe differences in cardiac autonomic control, while the second measure, SCL, is controlled solely by sympathetic inputs and is therefore an excellent indicator of sympathetic nervous system activity. The expected outcomes were an increase in average R-R intervals in ECG, an increase in standard deviation of R-R intervals, an increase in the HF component of HRV, a decrease in the LF component of HRV, a decrease in the LF/HF ratio, increase in pNN50, as well as a decrease of SCL. We also predicted that the proposed intervention would provide for improvements in irritability, hyperactivity and repetitive behavior rating scales on the Aberrant Behavior Checklist (ABC; Aman and Singh, 1994) and Repetitive Behavior Scale (RBS; Bodfish et al., 1999). This is a proof of concept study aimed at defining the putative existence of positive effects as well as the effect size of our TMS intervention in a population of ASD individuals. It is hoped that the study will establish the potential to pursue future trials of adequate sample size using a sham control population. In this regard the present study does not constitute a clinical trial.

METHODS

SUBJECTS

In this study, we investigated the activity of the ANS during rTMS treatment in 18 children with ASD (14 boys and 4 girls, mean age 13.1 years, SD = 2.2). Participants with ASD were recruited through the University of Louisville Weisskopf Child Evaluation Center (WCEC). Diagnosis was made according to the DSM-IV-TR and further ascertained with the Autism Diagnostic Interview-Revised (ADI-R; Le Couteur et al., 2003) by Dr. Sears, who also did pre- and post-TMS clinical evaluations. All participants were high-functioning children with ASD and with full-scale IQs >80 assessed using the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler, 2004). Participating subjects and their parents (or legal guardians) were provided with all information regarding the study, and the consent and assent forms approved by the IRB were reviewed and signed. Sixteen ASD subjects out of 18 enrolled in the study completed all 18 sessions of rTMS. Two subjects (both boys) completed only 14 sessions and dropped out of study due to family circumstances. Therefore, our retention rate in the study was 88.8%. Two subjects (one boy, one girl) were excluded from data analysis because they were active junior track-and-field athletes (long distance runners) and their cardiac activity was affected by the changes in their intense physical exercise regimen.

The study complied with all relevant national regulations and institutional policies and has been approved by the local Institutional Review Board (IRB). Participating subjects and their parents (or legal guardians) were provided with full information about the study including the purpose, requirements, responsibilities, reimbursement, risks, benefits, alternatives, and role of the local IRB. The consent and assent forms approved by the IRB were reviewed and explained to all subjects who expressed interest to participate. All questions were answered before consent signature was requested. If the individual agreed to participate, both she/he and parent/guardian signed and dated the consent or assent form and received a copy countersigned by the investigator who obtained consent.

LOW FREQUENCY REPETITIVE TMS PROCEDURE

A trained electrophysiologist delivered rTMS using a Magstim Rapid 220 system (Magstim Co, Whitland, UK). Patients were seated in a leather chair and fitted with a swimming head cap. Motor threshold (MT) was determined in the following manner: mild supra-threshold stimulations was administered over the left motor cortex to determine the optimal area for stimulation of the *abductor pollicis brevis* (APB) muscle. The output of the machine was increased by 7% each time until the least amount of machine power that induces a 50 μV deflection or a visible twitch is identified in four out of five trials over the cortical area controlling the contralateral APB. Surface electrodes were attached over the APB and *first dorsal interossi (FDI)* areas. Electromyographic (EMG) responses (motor evoked potentials) were recorded using the C2 J&J Engineering Inc. (Poulsbo, WA) physiological data acquisition system interfaced with Magstim TMS device. Similar procedure was applied to determine MT for the right hemisphere. The TMS treatment course was administered once per week for 18 weeks over the DLPFC (six over the left, six over right, and six equally over the both left and right hemispheres). The site for stimulation was placed 5 cm anterior to, and in a parasagital plane to the site of maximal APB stimulation. The figure-eight coil, with a 70-mm wing diameter was kept flat over the scalp. Stimulation was performed at 0.5 Hz and 90% of resting MT, with a total of 160 pulses/per day (session had 8 trains by 20 pulses, with a 20-s interval between the trains, for additional procedure detail see Casanova et al., 2012; Sokhadze et al., 2012a).

AUTONOMIC MONITORING PROCEDURE

Physiological monitoring

For 3–5 min before rTMS, during ∼10–12 min rTMS session, and immediately after the completion of the TMS for another 3–5 min the subjects had their physiological activity monitored and recorded. Therefore, all autonomic measures were recorded during each rTMS session in every participant for several minutes preceding TMS administration, then during TMS procedure, and also for several minutes after TMS session. For data analysis in this particular study were included only data during administration of TMS. We used approximately 10 min long period to calculate HRV variability measures (RR intervals, SDRR, LF and HF of HRV) derived from an artifact free ECG recording and mean SCL. In our other pilot studies (Hensley et al., 2012, 2013) in addition to analysis of mean values of autonomic measures it was analyzed as well minute-by-minute values of HR and SCL.

The monitoring of ANS activity was conducted using C2 J&J Engineering Inc. (Poulsbo, WA) device with specialist USE-3 software application. The procedure of autonomic monitoring includes presentation of HRV measures in a form of cascading HRV spectrum, individual HRV components and SCL (both tonic and phasic changes) with visual and auditory feedback for experimenter. All physiological measures were analyzed both on- and off-line. Schematic presentation of the procedure is depicted at the **Figure 1**.

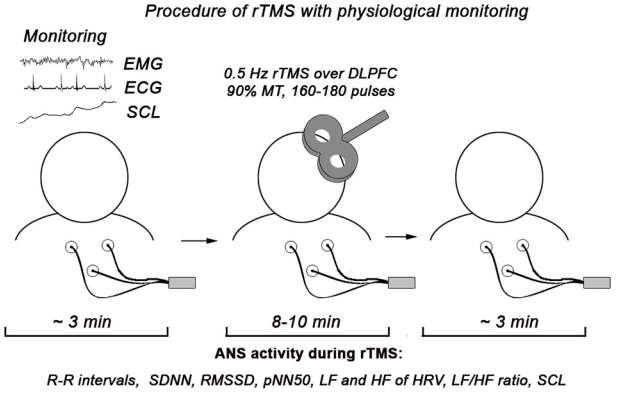


FIGURE 1 | Procedure of physiological monitoring during rTMS session. In the statistical analysis we used only autonomic data recorded during rTMS (approximately 10 min).

Measurement of the ANS dependent variables

Electrocardiogram, electromyogram (EMG), pneumogram (PNG), and EDA were acquired (1024 Hz sampling rate for EMG and ECG, 128 Hz for PNG and EDA) by a C-2 J&J Engineering Inc. physiological monitoring system with USE-3 software (Physiodata, Poulsbo, WA). Three Ag/AgCl electrodes (El-503, Biopac Systems, Inc., CA) were attached for measurement of Lead II ECG, 3 Ag/AgCl electrodes (EL-501 from Biopac) for EMG recording from the right hand, and PNG was recorded with a strain gauge transducer. Electrodermal activity was recorded by Ag/AgCl electrodes (EL-507 by Biopac with Unibase isotonic gel) attached to the distal phalanx of index and middle fingers to measure SCL.

Cardiovascular activity. Average R-R intervals in ECG (R-R), standard deviation of all normal R-R (NN) intervals (SDNN), Square root of the mean of the squares of successive NN interval differences (or the average change in interval between beats)—RMSSD, the percentage of intervals >50 ms different from preceding interval (pNN50); frequency domain HRV measures such as power of HF, LF, very low frequency (VLF) components, and the ratio of the LF over the HF (LF/HF ratio is used as an indirect autonomic balance index) of HRV are calculated as time domain and frequency domain cardiac activity measures (Kleiger et al., 2005). Artifact-corrected at least 5 min long recording epochs were analyzed with Fast Fourier Transformation (FFT) to assess HRV. Integrals of the spectrum in 0.04–0.15 Hz (LF of HRV) and 0.15–0.40 Hz (HF of HRV) bands were measured (in ms^2). All HRV data was analyzed off-line using Kubios HRV software v. 2.0 (University of Kuopio, Finland). Heart rate variability interpretation was following concepts: (1) The HF component of HRV is often referred to as RSA and is assumed to be the non-invasive index of parasympathetic influences on the heart (Berntson et al., 1997; Sohn et al., 2001); (2) the LF component of HRV has been linked to sympathetic nervous system activity and sympathovagal balance by numerous studies (Pagani et al., 1986; Malliani

et al., 1994). Other studies have shown that the LF variability is rather a reflection of both sympathetic and vagal influences related to baroreflex mechanisms (Berntson et al., 1997). It is thought that changes in blood pressure amplitude may cause a vagally-mediated baroreflex responses as well as changes in LF variability.

Respiratory activity. Respiration rate on per minute basis and peak respiration frequency were calculated. These measures were used to control HF peak in HRV related to respiratory frequencies in HRV and were not used as dependent measures.

Electrodermal activity. Skin conductance level (in μS) and amplitude of the SCR, defined as fluctuation with more than 0.02 μS increment (Boucsein, 2012), NS.SCR—number of non-specific SCR (per min) were calculated, but only SCL was used as dependent variable in this study. The main reason of excluding NS.SCR measure from analysis was related to the consideration that some of the SCR might reflect auditory stimulation response to clicks produced by the TMS coil and could be considered as non-specific SCRs.

BEHAVIORAL OUTCOMES

For the evaluation of social and behavioral functioning we utilized caregiver reports and clinician ratings of improvement. Every participant was evaluated before TMS course and within 2 weeks following TMS treatment. Aberrant Behavior Checklist (Aman and Singh, 1994; Aman, 2004) is a clinician administered rating scale to assess Irritability, Lethargy/Social Withdrawal, Stereotypy, Hyperactivity, and Inappropriate Speech based on parent/caregiver report. *Social Responsiveness Scale (SRS).Repetitive Behavior Scale-Revised* (*RBS-R*, Bodfish et al., 1999) is a caregiver completed rating scale assessing stereotyped, self-injurious, compulsive, ritualistic, sameness, and restricted range (Bodfish et al., 1999).

STATISTICAL ANALYSIS

The primary statistical analyses included linear regression estimation of each autonomic dependent variable over 18 sessions of rTMS course, paired sample *t*-test of mean values of dependent ANS variables at the first and last session of the rTMS course, and paired sample *t*-test of pre-TMS and post-TMS behavioral measures. For each dependent autonomic variable analyzed using *t*-test, normality of distribution was analyzed to ensure appropriateness for the test, and 95% confidence intervals (95% CI) were included in outcome.

RESULTS

AUTONOMIC ACTIVITY MEASURES

Time-domain measures of HRV (R-R intervals, SDNN, RMSSD, pNN50)

Cardiointervals in ECG (R-R intervals) showed a statistically significant linear regression over 18 sessions of rTMS ($R = 0.661$, $R^2 = 0.437$, $y = 2.69x + 684.5$ ms, $t = 3.52$, $p = 0.003$, observed power = 0.868 at $\alpha = 0.05$, **Figure 2A**). *T*-test showed that R-R intervals increased statistically from the first to the last rTMS session (from 684.7 ± 90.9 ms to 723.8 ± 96.5 ms, mean increase being 39.08 ± 53.6 ms, 95% CI from 70.04 to 8.13 ms, $t_{(13)} = 2.72$, $p = 0.017$). Standard Deviations of R-R intervals showed statistically significant linear increase over 18 sessions of rTMS ($R = 0.645$, $R^2 = 0.417$, $y = 2.09x + 52.2$ ms, $t = 3.38$, $p = 0.004$, observed power = 0.844 at $\alpha = 0.05$, **Figure 2B**) and *t*-test showed that SDNN increased statistically from the first to the last rTMS session (from 60.6 ± 20.4 ms to 99.7 ± 74.7 ms, mean increase being 39.09 ± 66.7 ms, 95% CI from 77.6 to 0.53 ms, $t_{(13)} = 2.19$, $p = 0.047$). Increase of the RMSSD was only marginally linear ($R = 0.473$, $R^2 = 0.224$, $y = 1.48x + 52.8$, $t = 2.15$, $p = 0.047$, observed power = 0.512 at $\alpha = 0.05$, i.e., below the desired power of 0.800). Changes in pNN50 both across 18 sessions and between the first and last session of rTMS did not reach significance level (both >0.05).

Frequency-domain measures of HRV (LF and HF of HRV, LF/HF ratio index)

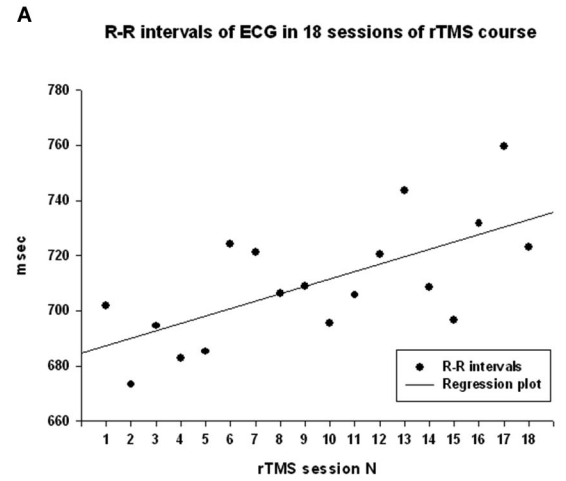
Power of HF component of HRV showed a strong statistically significant linear increase ($R = 0.788$, $R^2 = 0.621$, $y = 68.6x + 671.9$ ms^2, $t_{(18)} = 5.12$, $p < 0.001$, observed power = 0.985 at $\alpha = 0.05$ **Figure 3A**), *t*-test did show statistical increase (by 1249 ± 1556 ms^2, 95% CI from 2147 to 350 ms, $t_{(13)} = 3.00$, $p = 0.01$). The Power of LF component of HRV showed a tendency towards linear regression but was not statistically significant ($R = 0.247$, $y = -15.23x + 1775.4$ ms^2, $t = -1.02$, $p = 0.323$, observed power = 0.163 at $\alpha = 0.05$, not significant, well below the desired power of 0.800. **Figure 4A**), *t*-test also did not show statistical difference ($p > 0.05$). The LF/HF ratio index (linear regression shown at **Figure 3B**) did show statistically significant linear decrease, $R = 0.691$, $R^2 = 0.478$ $y = -0.28x + 1.619$, $t = -3.83$, observed power = 0.913 at $\alpha = 0.05$) and also decreased significantly from the first to last rTMS session (by 0.48 ± 0.81, 95% CI from 7.64 to 2.89, $t_{(13)} = 2.23$, $p = 0.044$).

Skin conductance level

Skin conductance level showed statistically significant liner regression over 18 sessions of rTMS ($R = 0.681$, $R^2 = 0.464$, $y = -17x + 8.65$, $t = -3.71$, $p > 0.002$, observed power = 0.948 at $\alpha = 0.05$, **Figure 4B**), and *t*-test yielded statistically significant decrease from the first to the last rTMS session (from 10.22 ± 4.53 to 5.84 ± 3.41 μS, mean decrease −4.37 ± 5.65 μS, $t_{(13)} = 2.89$, $p = 0.013$).

BEHAVIORAL EVALUATIONS POST- TMS

The ABC and RBS behavioral checklists showed significant improvements in several areas. We found a significant decrease in stereotype repetitive and restricted behavior patterns following 18 sessions of bilateral rTMS as measured by the RBS-R (Bodfish et al., 1999) when analyzed using a paired sample Student's *t*-test. Total RBS-R score decreased from 25.4 ± 14.0 to 19.8 ± 10.9, with the mean decrease being −5.44 ± 6.49, $t_{(13)} = 3.55$, $p = 0.002$. Changes in individual subscale rating scores are shown in **Figure 5**, where Stereotypic Behavior Subscale shows significant decrease (from 5.94 ± 4.30 to 4.76 ± 3.84, mean change −1.17 ± 1.59, $t_{(13)} = 3.05$, $p = 0.008$) and Ritualistic/Sameness Behavior Subscale scores show a significant



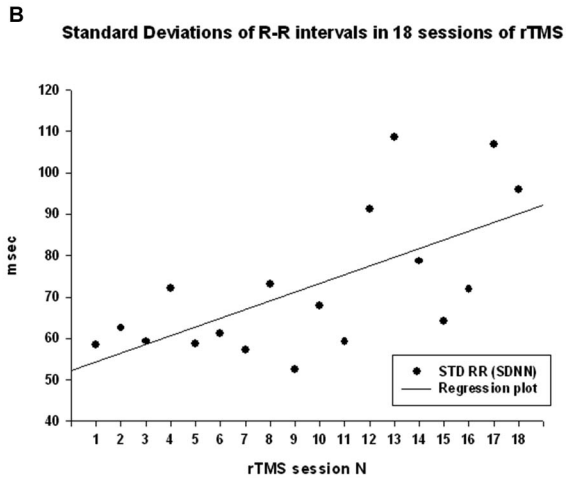
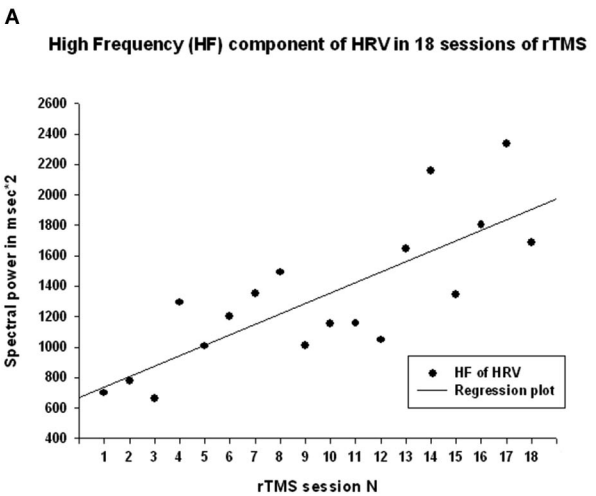


FIGURE 2 | (A) Scattergram of linear regression of R-R intervals in ECG across 18 sessions of rTMS in 14 children with ASD. The mean values of R-R intervals show linear increase over the rTMS course. **(B)** Scattergram of linear regression of standard deviations of R-R intervals (SDNN) across 18 sessions of rTMS course shows linear increase.



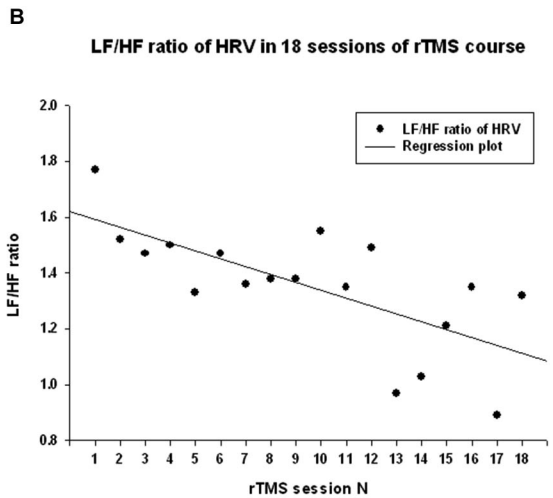


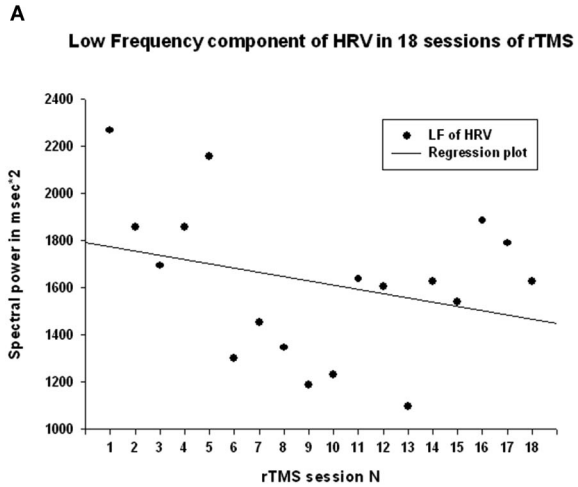
FIGURE 3 | (A) Scattergram of linear regression of HF component of HRV across 18 sessions of rTMS in 14 children with ASD. The mean values of HF component of HRV show linear increase over the rTMS course. **(B)** Scattergram of linear regression of LF/HF ratio (cardiac autonomic balance index) across 18 sessions of rTMS course shows linear decrease.

decrease (-1.35 ± 2.02, $t_{(13)} = 2.52$, $p = 0.022$). We also found a significant reduction in Irritability subscale as measured by the ABC (from 10.53 ± 6.86 to 7.95 ± 5.56, mean change -2.57 ± 5.17, $t_{(13)} = 2.17$, $p = 0.044$). Lethargy subscale of the ABC showed a similar score reduction (-2.55 ± 4.32, $t_{(13)} = 2.50$, $p = 0.023$) while Hyperactivity showed an even greater reduction (from 13.53 ± 10.91 to 10.37 ± 9.36, -3.15 ± 6.08, $t_{(13)} = 2.27$, $p = 0.035$). Changes of individual subscale rating scores are depicted at the **Figure 6**.

OVERVIEW OF RESULTS

Results of the HRV analysis show several measures with significant differences between pre- and post-TMS therapy. **Table 1** below shows the *t*-test results for RR interval, SDNN, HF component of HRV, LF/HF ratio, and SCL. **Table 1** shows that there was a significant increase in the R-R interval from pre- to post-TMS treatment. This result can also be interpreted as a decrease in HR since the R-R interval is the time between successive heartbeats. The *t*-test also reveals significant increases in SDNN and HF power, as well as significant decreases in the LF/HF ratio and SCL.

Regression analysis was completed to observe trends during the entire 18 session TMS course. **Table 2** shows the results of regression analysis. Regression analysis shows that the trend in each measure was significant for all analyzed,



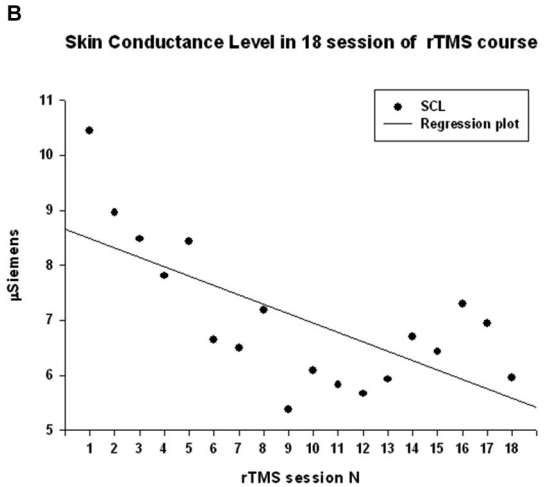


FIGURE 4 | (A) Scattergram of linear regression of LF component of HRV across 18 sessions of rTMS in 14 children with ASD. The mean values of LF show tendency to decrease over the rTMS course but the trend was not reaching significance level. **(B)** Scattergram of linear regression of skin conductance level (SCL) across 18 sessions of rTMS course shows significant linear decrease.

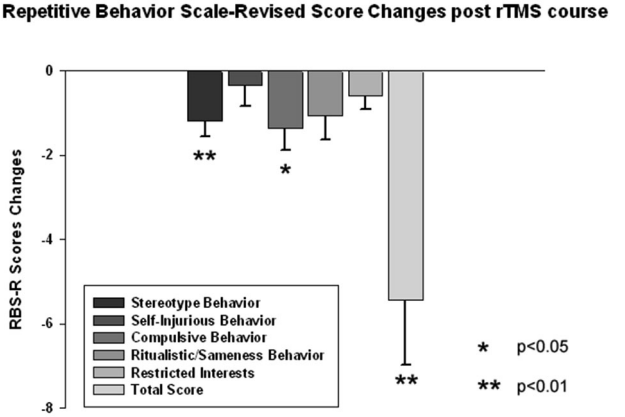


FIGURE 5 | Changes of Repetitive Behavior Scale (RBS-R) scores post-TMS as compared to baseline levels in children with ASD (*N* = 14). Stereotype Behavior, Ritualistic Behavior and Total RBS scores decreased significantly.

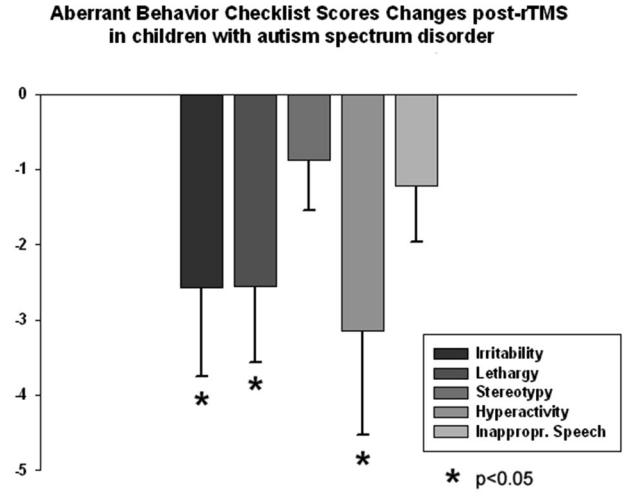


FIGURE 6 | Changes of Aberrant Behavior Checklist (ABC) scores post-TMS as compared to baseline levels in children with ASD (*N* = 14). Irritability, Lethargy, and Hyperactivity rating scores decreased significantly post-TMS.

expect for LF power. Although there was a negative trend for LF power observed over the 18 sessions of TMS, it did not reach significance. NN50 (count of R-R intervals differing by >50 ms from the preceding interval) does show a significant positive trend; however, because the length of each physiological recording session was not uniform, regression analysis results of NN50 are not a reliable reflection of this measure. pNN50, the percent of RR intervals differing by >50 ms from the preceding interval, did not show a significant positive trend. **Figures 2–4** show the regression analysis for individual measures. Behavioral questionnaires also demonstrated significant improvements both for the ABC and RBS rating scales (**Figures 5**, **6**).

DISCUSSION

All novel studies involving neuromodulation techniques in children should proceed with caution. Transcranial magnetic stimulation is a non-invasive intervention, which could be a potential strategy for early intervention for autism; however, the dose, duration, and type of rTMS stimulation for such intervention in children as well as effects on vital functions need to be carefully investigated and documented. This need requires proof of concept studies when testing the feasibility of using rTMS in order to modulate autonomic activity in ASD.

Table 1 | Regression equations and statistics of linear regression of autonomic dependent variables over the 18 session long rTMS course in 14 children with ASD.

Measure	Units	*t*	*p*-value	*R*	R^2	Regression equation	Power at $\alpha = 0.05$
RR	ms	3.52	0.003	0.661	0.437	$y = 2.696x + 684.57$	0.868
SDNN	ms	3.38	0.004	0.645	0.417	$y = 2.098x + 52.28$	0.844
RMSSD	ms^2	2.15	0.047	0.473	0.224	$y = 1.480x + 52.80$	0.512
LF power	ms^2	−1.02	0.323	0.247	0.061	$y = -15.23x + 1775.4$	0.163
HF power	ms^2	5.12	<0.001	0.788	0.621	$y = 68.65x + 671.9$	0.985
LF/HF ratio	N/A	−3.83	0.001	0.691	0.478	$y = -0.028x + 1.619$	0.913
SCL	μS	−3.71	0.002	0.681	0.464	$y = -0.17x + 8.65$	0.948

Table 2 | Changes of dependent variables of autonomic activity from the first to the last session of rTMS treatment course in 14 children with ASD.

Pairs	Paired differences							
	Units	Mean	Std. Dev.	95% CI		*t*	df	*p*-value
				Lower	Upper			
RR post-pre	ms	39.08	53.61	70.04	8.13	2.73	13	0.017
SDRR post-pre	ms	39.09	66.78	77.65	0.54	2.19	13	0.047
HF post-pre	ms^2	1249.3	1556.1	2147.8	350.8	3.00	13	0.010
LF/HF post-pre	N/A	−0.48	0.81	−0.01	−0.95	−2.23	13	0.044
SCL post-pre	μS	−4.37	5.65	−1.11	−7.64	−2.89	13	0.013

Results of our study indicate that HRV and EDA are noninvasive and effective ways of gathering information about ANS functioning during rTMS therapy in autism. Accelerated HR in association with lower HRV indexed by high LF/HF ratio and low SDNN along with high electrodermal activity (SCL) found in children with ASD at the pre-treatment stage are indicators of excessive sympathetic and reduced parasympathetic activation in ASD resulting in limited psychophysiological flexibility and behavioral rigidity. We investigated changes in autonomic activity during 18 rTMS sessions in the same children with ASD. Our hypothesis was that children with ASD would show improved HRV measures (decreased overall HR indexed by longer R-R intervals, increased STDRR, higher pNN50 index, increased HF power, decreased LF power, decreased LF/HF ratio, increased pNN50) and lower SCL measures. Our results showed that, except for a reduction in LF power and pNN50, all dependent HRV variables changed in the predicted way, as indexed by statistically significant liner regression coefficients over TMS sessions and statistically significant pre- *vs.* post-TMS changes (first *vs.* last TMS session). The LF power decrease showed a trend towards decrease but it did not reach significance level.

Time-domain HRV results showed that the most significant changes from TMS treatment were an increase in R-R cardiointerval length and a higher standard deviation of R-R intervals. Frequency-domain HRV results showed increase of HF power in HRV, and decreased LF/HF ratio. Electrodermal activity also showed a decrease in the form of lower tonic SCL. The increased standard deviation in cardiointervals along with higher power of HF of HRV and decreased LF/HF ratio are promising because this suggests more prominent parasympathetic activity and more flexibility in HR overall. Significant change was also observed in mean R-R interval lengths, which means a lower HR. Outcomes within the frequency-domain of HRV showed increased HF component of HRV, which is also of importance as it suggests enhancement of the parasympathetic tone. As we did not observe a statistical change in the LF component, it can be inferred that restoration of autonomic balance was achieved mainly through an increased HF component of HRV, which correlates to parasympathetic (vagus) cardiac neural control. However, while the change in the LF component was not significant, we did observe a decrease in SCL over the 18 sessions. This result suggests a withdrawal of sympathetic tone as SCL is controlled by sympathetic inputs. It should be noted that cardiac sympathetic influences are predominantly mediated through beta-adrenergic drives, while peripheral sympathetic control of sweat glands is exerted through alpha-adrenergic drives.

The question remains as to how does prefrontal rTMS affect autonomic functions? Only a few papers have looked at the effects of rTMS on the autonomic system, despite the fact that many frontal cortical areas are directly implicated in ANS control (Filippi et al., 2000; Czéh et al., 2002). It has been reported (Ben-Shachar et al., 1997) that there might be neurohumoral changes after treatment with rTMS. A hypothesis was also proposed suggesting that anxiolytic effects of rTMS may act through normalization of the hypothalamic-pituitary-adrenocortical (HPA) axis (Holsboer, 2000). Chronic rTMS-induced changes in stress-related corticotropin and corticosterone levels have been found in animal models (Keck et al., 2000; Hedges et al., 2002) providing support for the suggestion that rTMS, directed at the prefrontal lobe, may attenuate the activity of the HPA system.

Low frequency rTMS can influence autonomic balance when using HRV (Yoshida et al., 2001). Udupa et al. (2007) reported that HRV measures indicated that rTMS produced a significant reduction in the cardiac sympathetic/vagal ratio, suggesting improvements in the sympatho-vagal cardiac balance. Lower post-TMS sympathetic activity was reported in one additional study (Jenkins et al., 2002). It is possible that rTMS effects are mediated through fronto-limbic connections. The limbic system is a complex network of structures central to anxiety and mood regulation (Mayberg, 2003; Seminowicz et al., 2004). Originally rTMS was investigated as a potential antidepressant therapeutic device under the assumption that magnetic stimulation of the prefrontal cortex (PFC) would engage the

connected limbic regions involved in mood and anxiety regulation (George et al., 1999). The hypothesis is consistent with the PFC rTMS modulating the function of fronto-limbic circuits.

Another important question is how TMS affects cortical E/I balance. Several studies have outlined a disruption in the ratio between cortical excitation and inhibition in ASD (Casanova et al., 2002; Rubenstein and Merzenich, 2003; Casanova, 2006; Yizhar et al., 2011). One possible explanation for an increase in cortical excitation to inhibition in ASD is the recent finding of abnormalities in cortical minicolumns (Casanova et al., 2002; Casanova, 2005). Double-bouquet cells in the peripheral neuropil space of minicolumns impose a strong vertically directed stream of inhibition (Mountcastle, 2003) surrounding the minicolumnar core. In ASD our preliminary studies indicate that cortical minicolumns are reduced in size and increased in number, especially within the prefrontal cortex (Casanova et al., 2002, 2006; Casanova, 2005, 2006). Disturbances in the ratio of cortical excitation to inhibition may lead to an increase in cortical "noise" that may influence functional cortical connectivity and may hinder the "binding" of associated cortical areas. It has been proposed that the effect of "slow" rTMS arises from increases in the activation of inhibitory circuits. We theorize that contrary to other inhibitory cells (i.e., basket and chandelier), whose projections keep no constant relation to the surface of the cortex, the geometrically exact orientation of double-bouquet cells and their location at the periphery of the minicolumn makes them an appropriate candidate for induction by a magnetic field applied parallel to cortex. Over a course of treatment rTMS may selectively lower the ratio of cortical excitation to cortical inhibition. Low frequency rTMS over DLPFC may therefore lead to improvement in frontal functions, including fronto-limbic function. We have already reported positive effects of rTMS in autism as expressed in improved ERP and evoked and induced gamma frequency oscillations during performance on visual oddball task (Sokhadze et al., 2009a,b, 2010a,b, 2012a; Baruth et al., 2010a,b; Casanova et al., 2012). The main finding was improved target discrimination and attenuated responses to non-target items, indicative of better differentiation of targets vs. non-targets and improved early stage filtering of task-irrelevant stimuli.

By convention, rTMS in 0.3–1 Hz frequency range is referred to as "slow," whereas "fast" rTMS refers to stimulation greater than 5 Hz. Hoffman and Cavus (2002) in their review of slow rTMS studies proposed long-term depression and long-term depotentiation as models for understanding the mechanism of slow rTMS. Neocortical long-term depression and changes in the cortical excitability induced by slow rTMS appear to accumulate in an additive fashion as the number of stimulations is increased over many days. Studies of both slow rTMS and long-term depression suggest additive efficacy when higher numbers of spaced, daily stimulations are administered. The reversal, or depotentiation, of previously enhanced synaptic transmission due to long-term potentiation may be the most relevant model for slow rTMS when used as a therapeutic tool. Our study used relatively high number of slow rTMS sessions (18 sessions on weekly rate). The mechanism of low-frequency TMS involves increasing inhibition of the stimulated cortex. For this study the stimulated region was the DLPFC, which is linked to the tonic inhibitory control of the ANS activity. The findings of our study indicate that TMS applied to the DLPFC was successful in the positive modulation of the autonomic balance in ASD through activation of the parasympathetic tone and withdrawal of sympathetic tone.

Some potential implications of TMS based neuromodulation could be considered in the context of other stimulation approaches and comorbidities proper to ASD. Excessive sympathetic arousal is often associated with anxiety. For children with ASD, especially during adolescence, anxiety is one of the most common presenting problems in clinical settings. Several research groups have reported that over 55% of sampled children with ASD meet criteria for at least one anxiety disorder (de Bruin et al., 2007; McPheeters et al., 2011). Anxiety disorder, as in social phobia, may be related to reduced functional connectivity between the frontal lobes and the limbic system (Hahn et al., 2011). Development of new neuromodulation methods aimed at regulating the effect of the frontal lobe on autonomic functions may thus provide a potential therapeutic intervention in ASD. Some potential implications of TMS based neuromodulation in autism could be considered also in the context of other stimulation approaches, for instance Vagal Nerve Stimulation (VNS) applications in ASD (Levy et al., 2010). Future studies of adequate sample size and sham controls are needed to explore the use of rTMS as a novel treatment for improving autonomic balance in ASD.

ACKNOWLEDGMENTS

The study was partially supported by National Institutes of Health Eureka R01 grant MH86784 to Manuel Fernando Casanova, M.D.

REFERENCES

Althaus, M., Mulder, L. J., Mulder, G., Aarnoudse, C., and Minderaa, R. (1999). Cardiac adaptivity to attention-demanding tasks in children with a pervasive developmental disorder not otherwise specified (PDD-NOS). *Biol. Psychiatry* 46, 799–809. doi: 10.1016/s0006-3223(98)00374-6

Aman, M. G. (2004). Management of hyperactivity and other acting out problems in patients with autism spectrum disorder. *Semin. Pediatr. Neurol.* 11, 225–228. doi: 10.1016/j.spen.2004.07.006

Aman, M. G., and Singh, N. N. (1994). *Aberrant Behavior Checklist—Community. Supplementary Manual.* East Aurora, NY: Slosson Educational Publications.

Angus, Z. (1970). Autonomic and cognitive functions in childhood psychosis. *Bull. Brit. Psychol. Soc.* 23, 228–229.

Bal, E., Harden, E., Lamb, D., Van Hecke, A. V., Denver, J. W., and Porges, S. W. (2010). Emotion recognition in children with autism spectrum disorders: relations to eye gaze and autonomic state. *J. Autism Dev. Disord.* 40, 358–370. doi: 10.1007/s10803-009-0884-3

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

Baruth, J. M., Casanova, M. F., Sears, L., and Sokhadze, E. (2010b). Early-stage visual processing abnormalities in high-functioning autism spectrum disorder (ASD). *Transl. Neurosci.* 1, 177–187. doi: 10.2478/v10134-010-0024-9

Baruth, J., Williams, E., Sokhadze, E., El-Baz, A., Sears, L., and Casanova, M. F. (2011). Repetitive transcranial stimulation (rTMS) improves electroencephalographic and behavioral outcome measures in autism spectrum disorders (ASD). *Autism Sci. Digest* 1, 52–57.

Benevides, T. W., and Lane, S. J. (2013). A review of cardiac autonomic measures: considerations for examination of physiological response in children with autism spectrum disorder. *J. Autism Dev. Disord.* doi: 10.1007/s10803-013-1971-z. [Epub ahead of print].

Ben-Shachar, D., Belmaker, R. H., Grisaru, N., and Klein, E. (1997). Transcranial magnetic stimulation induces in brain monoamines. *J. Neural Transm.* 104, 191–197. doi: 10.1007/BF01273180

Berntson, G. G., Bigger, J. T. Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods and interpretive caveates. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Norman, G. J., Hawkley, L. C., and Cacioppo, J. T. (2008). Cardiac autonomic balance versus cardiac regulatory capacity. *Psychophysiology* 45, 643–652. doi: 10.1111/j.1469-8986.2008.00652.x

Bodfish, J. W., Symons, F. J., and Lewis, J. (1999). *Repetitive Behavior Scale.* Cullowhee, NC: Western Carolina Center Research Reports.

Boucsein, W. (2012). *Electrodermal Activity.* 2nd Edn. New York: Springer.

Casanova, M. F. (2005). "Minicolumnar pathology in autism," in *Recent Developments in Autism Research*, ed M. F. Casanova (New York: Nova Biomedical Books), 133–144.

Casanova, M. F. (2006). Neuropathological and genetic findings in autism: the significance of a putative minicolumnopathy. *Neuroscientist* 12, 435–441. doi: 10.1177/1073858406290375

Casanova, M. F., Baruth, J., El-Baz, A. S., Sokhadze, G. E., Hensley, M., and Sokhadze, E. M. (2013). "Evoked and induced gamma-frequency oscillations in autism," in *Imaging the Brain in Autism*, eds M. F. Casanova, A. S. El-Baz and J. S. Suri (New York: Springer), 87–106.

Casanova, M. F., Baruth, J. M., El-Baz, A., Tasman, A., Sears, L., and Sokhadze, E. (2012). Repetitive transcranial magnetic stimulation (rTMS) modulates event-related potential (ERP) indices of attention in autism. *Transl. Neurosci.* 3, 170–180. doi: 10.2478/s13380-012-0022-0

Casanova, M. F., Buxhoeveden, D. P., and Brown, C. (2002). Clinical and macroscopic correlates of minicolumnar pathology in autism. *J. Child Neurol.* 17, 692–695. doi: 10.1177/088307380201700908

Casanova, M. F., van Kooten, I., Switala, A. E., van England, H., Heinsen, H., Steinbuch, H. W. M., et al. (2006). Abnormalities of cortical minicolumnar organization in the prefrontal lobes of autistic patients. *Clin. Neurosci. Res.* 6, 127–133. doi: 10.1016/j.cnr.2006.06.003

CDC's Morbidity and Mortality Weekly Report (2014). CDC estimates 1 in 68 children has been identified with autism spectrum disorder. Available online at: http://www.cdc.gov/media/releases/2014/p0327-autism-spectrum-disorder.html

Cohen, N., Benjamin, J., Geva, A. B., Matar, M. A., Kaplan, Z., and Kotler, M. (2000). Autonomic dysregulation in panic disorder and in post-traumatic stress disorder: application of power spectrum analysis of heart rate variability at rest and in response to recollection of trauma or panic attack. *Psychiatry Res.* 96, 1–13. doi: 10.1016/s0165-1781(00)00195-5

Coronoa, R., Dissanayake, C., Arbelle, S., Wellington, P., and Sigman, M. (1998). Is affect aversive to young children with autism? Behavioral and cardiac responses to experimenter distress. *Child Dev.* 69, 1494–1502. doi: 10.1111/j.1467-8624.1998.tb06172.x

Czéh, B., Welt, T., Fischer, A. K., Erhardt, A., Schmitt, W., Müller, M. B., et al. (2002). Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. *Biol. Psychiatry* 52, 1057–1065. doi: 10.1016/s0006-3223(02)01457-9

de Bruin, E. L., Ferdinand, R. F., Meester, S., de Nijs, P. F., and Verheij, F. (2007). High rates of psychiatric co-morbidity in PDD-NOS. *J. Autism Dev. Disord.* 37, 877–886. doi: 10.1007/s10803-006-0215-x

Dombroski, B., Kaplan, M., Kotsamanidis, B., Edelson, S. M., Sokhadze, G., Casanova, M. F., et al. (2013). Ambient lenses and visuomotor exercise effects on autonomic reactivity in autism. *Appl. Psychophysiol. Biofeedback* 38, 235.

Eckberg, D. L. (1997). Sympathovagal balance: a critical appraisal. *Circulation* 96, 3224–3232. doi: 10.1161/01.cir.96.9.3224

Eilam-Stock, T., Xu, P., Cao, M., Gu, X., Van Dam, N. T., Anagnostou, E., et al. (2014). Abnormal autonomic and associated brain activities during rest in autism spectrum disorder. *Brain* 137, 153–171. doi: 10.1161/01.cir.96.9.3224

Filippi, M. M., Oliveri, M., Vernieri, F., Pasqualetti, P., and Rossini, P. M. (2000). Are autonomic signals influencing cortico-spinal motor excitability? A study with transcranial magnetic stimulation. *Brain Res.* 881, 159–164. doi: 10.1016/s0006-8993(00)02837-7

Friedman, B. H., and Thayer, J. F. (1998). Anxiety and autonomic flexibility: a cardiovascular approach. *Biol. Psychol.* 49, 303–323.

Garvey, M. A., and Mall, V. (2008). Transcranial magnetic stimulation in children. *Clin. Neurophysiol.* 119, 973–984. doi: 10.1016/j.clinph.2007.11.048

George, M. S., Lisanby, S. H., and Sackeim, H. A. (1999). Transcranial magnetic stimulation: applications in neuropsychiatry. *Arch. Gen. Psychiatry* 56, 300–311. doi: 10.1001/archpsyc.56.4.300

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., et al. (2011). Reuced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *Neuroimage* 56, 881–889. doi: 10.1016/j.neuroimage.2011.02.064

Hedges, D. W., Salyer, D. L., Higginbotham, B. J., Lund, T. D., Hellewell, J. L., Ferguson, D., et al. (2002). Transcranial magnetic stimulation (TMS) effects on testosterone, prolactin and corticosterone in adult male rats. *Biol. Psychiatry* 51, 417–421. doi: 10.1016/s0006-3223(01)01266-5

Hensley, M., El-Baz, A., Casanova, M. F., and Sokhadze, E. (2013). Heart rate variability and cardiac autonomic measures changes during rTMS course in autism. *Appl. Psychophysiol. Biofeedback* 38, 238.

Hensley, M., El-Baz, A., Sokhadze, G., Sears, L., Casanova, M. F., and Sokhadze, E. M. (2012). TMS effects on cardiac autonomic control in children with autism. *Psychophysiology* 49, S40.

Hirstein, W., Iversen, P., and Ramachandran, V. S. (2001). Autonomic responses of autistic children to people and objects. *Proc. Biol. Sci.* 268, 1883–1888. doi: 10.1098/rspb.2001.1724

Hoffman, R. E., and Cavus, I. (2002). Slow transcranial magnetic stimulation, long-term depotentiation and brain hyperexcitability disorders. *Am. J. Psychiatry* 159, 1093–1102. doi: 10.1176/appi.ajp.159.7.1093

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23, 477–501. doi: 10.1016/s0893-133x(00)00159-7

Hutt, C., Forresst, S. J., and Richer, J. (1975). Cardiac arrhythmia and behavior in autistic children. *Acta Psychiatr. Scand.* 51, 361–372. doi: 10.1111/j.1600-0447.1975.tb00014.x

Jenkins, J., Shajahan, P. M., Lappin, J. M., and Ebmeier, K. P. (2002). Right and left prefrontal transcranial magnetic stimulation at I Hz does not affect mood in healthy volunteers. *BMC Psychiatry* 2:1. doi: 10.1186/1471-244X-2-1

Julu, P. O., Kerr, A. M., Apartipoulos, F., Al-Rawas, S., Engerström, I. W., Engerström, L., et al. (2001). Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. *Arch. Dis. Child.* 85, 29–37. doi: 10.1136/adc.85.1.29

Keck, M. E., Engelmann, M., Müller, M. B., Henniger, M. S., Hermann, B., Rupprecht, R., et al. (2000). Repetitive transcranial magneticstimulation induces active coping strategies and attenuates the neuroendocrine stress response in rats. *J. Psychiatr. Res.* 34, 265–276. doi: 10.1016/s0022-3956(00)00028-5

Kleiger, R. E., Stein, P. K., and Bigger, J. T. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474x.2005.10101.x

Le Couteur, A., Lord, C., and Rutter, M. (2003). *The Autism Diagnostic Interview—Revised (ADI-R).* Los Angeles, CA: Western Psychological Services.

Levy, M. L., Levy, K. M., Hoff, D., Amar, A. P., Park, M. S., Conklin, J. M., et al. (2010). Vagus nerve stimulation therapy in patients with autism spectrum disorder and intractable epilepsy: results from the vagus nerve stimulation therapy patient outcome registry. *J. Neurosurg. Pediatr.* 5, 595–602. doi: 10.3171/2010.3.PEDS09153

Loveland, K. A., Bachevalier, J., Pearson, D. A., and Lane, D. M. (2008). Fronto-limbic functioning in children and adolescents with and without autism. *Neuropsychologia* 46, 49–62. doi: 10.1016/j.neuropsychologia.2007.08.017

Malliani, A., Pagani, M., and Lombardi, F. (1994). Physiology and clinical implications of variability of cardiovascular parameters with focus on heart rate and blood pressure. *Am. J. Cardiol.* 73, 3C–9C. doi: 10.1016/0002-9149(94)90617-3

Mayberg, H. S. (2003). Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimized treatment. *Br. Med. Bull.* 65, 193–207. doi: 10.1093/bmb/65.1.193

McPheeters, M. L., Davis, A., Nayarre, J. R., and Scott, T. A. (2011). Family report of ASD concomitant with depression or anxiety among US children. *J. Autism Dev. Disord.* 41, 646–653. doi: 10.1007/s10803-010-1085-9

Ming, X., Bain, J. M., Smith, D., Brimacombe, M., Gold von-Simson, G., and Axelrod, F. B. (2011). Assessing autonomic dysfunction symptoms in children: a pilot study. *J. Child Neurol.* 26, 420–427. doi: 10.1177/0883073810381921

Ming, X., Julu, P. O., Brimacombe, M., Connor, S., and Daniels, M. L. (2005). Reduced cardiac parasympathetic activity in children with autism. *Brain Dev.* 27, 509–516. doi: 10.1016/j.braindev.2005.01.003

Ming, X., Julu, P. O., Wark, J., Apartopoulos, F., and Hansen, S. (2004). Discordant mental and physical efforts in an autistic patient. *Brain Dev.* 26, 519–524. doi: 10.1016/j.braindev.2004.02.005

Mountcastle, V. B. (2003). Introduction. Computation in cortical columns. *Cereb. Cortex* 13, 2–4. doi: 10.1093/cercor/13.1.2

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., et al. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. *Circ. Res.* 59, 178–193. doi: 10.1161/01.res.59.2.178

Palkovitz, R. J., and Wiesenfeld, A. R. (1980). Differential autonomic responses of autistic and normal children. *J. Autism Dev. Disord.* 10, 347–360. doi: 10.1007/bf02408294

Pascual-Leone, A., Walsh, V., and Rothwell, J. (2000). Transcranial magnetic stimulation in cognitive neuroscience—virtual lesion, chronometry and functional connectivity. *Curr. Opin. Neurobiol.* 10, 232–237. doi: 10.1016/s0959-4388(00)00081-7

Paus, T., Jech, R., Thompson, C. J., Comeau, R., Peters, T., and Evans, A. C. (1997). Transcranial magnetic stimulation during positron emission tomography: a new method for studying connectivity of the human cerebral cortex. *J. Neurosci.* 17, 3178–3184.

Porges, S. W. (2001). The polyvagal theory: phylogenetic substrates of a social nervous system. *Int. J. Psychophysiol.* 42, 123–146. doi: 10.1016/s0167-8760(01)00162-3

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

Rubenstein, J. L. R., and Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Gen. Brain Behav.* 2, 255–267. doi: 10.1034/j.1601-183x.2003.00037.x

Seminowicz, D. A., Mayberg, H. S., McIntosh, A. R., Goldapple, K., Kennedy, S., Segal, Z., et al. (2004). Limbic-frontal circuitry in major depression: a path modeling meta-analysis. *Neuroimage* 22, 409–418. doi: 10.1016/j.neuroimage.2004.01.015

Sohn, J.-H., Sokhadze, E., and Watanuki, S. (2001). Electrodermal and cardiovascular manifestations of emotions in children. *J. Physiol. Anthropol. Appl. Human Sci.* 20, 55–64. doi: 10.2114/jpa.20.55

Sokhadze, E., Baruth, J., El-Baz, A., Horrell, T., Sokhadze, G., Carroll, T., et al. (2010a). Impaired error monitoring and correction function in autism. *J. Neurother.* 14, 79–95. doi: 10.1080/10874201003771561

Sokhadze, E. M., Baruth, J. M., Sears, L., Sokhadze, G. E., El-Baz, A. S., and Casanova, M. F. (2012a). Prefrontal neuromodulation using rTMS improves error monitoring and correction functions in autism. *Appl. Psychophysiol. Biofeedback* 37, 91–102. doi: 10.1007/s10484-012-9182-5

Sokhadze, E., Baruth, J., Tasman, A., El-Baz, A., Mansoor, M., Ramaswamy, R., et al. (2010b). Low-frequency repetitive transcranial magnetic stimulation (rTMS) affects event-related potential measures of novelty processing in autism. *Appl. Psychophysiol. Biofeedback* 35, 147–161. doi: 10.1007/s10484-009-9121-2

Sokhadze, E., Baruth, J., Tasman, A., Sears, L., Mathai, G., El-Baz, A., et al. (2009a). Event-related potential study of novelty processing abnormalities in autism. *Appl. Psychophysiol. Biofeedback* 34, 37–51. doi: 10.1007/s10484-009-9074-5

Sokhadze, E., El-Baz, A., Baruth, J., Mathai, G., Sears, L., and Casanova, M. (2009b). Effect of a low-frequency repetitive transcranial magnetic stimulation (rTMS) on induced gamma frequency oscillations and event-related potentials during processing of illusory figures in autism spectrum disorders. *J. Autism Dev. Disord.* 39, 619–634. doi: 10.1007/s10803-008-0662-7

Sokhadze, G., El-Baz, A., Sokhadze, E., Sears, L., and Casanova, M. (2012b). Effects of TMS on autonomic nervous system in children with autism. *Appl. Psychophysiol. Biofeedback* 37, 302.

Sokhadze, G., Kaplan, M., Edelson, S. M., Sokhadze, E., El-Baz, A., Hensley, M., et al. (2012c). Effects of ambient prism lenses on autonomic reactivity to emotional stimuli in autism. *Appl. Psychophysiol. Biofeedback* 37, 303.

Stein, P. K., and Kleiger, R. E. (1999). Insights from the study of heart rate variability. *Annu. Rev. Med.* 50, 249–261. doi: 10.1146/annurev.med.50.1.249

Thayer, J. F., and Friedman, B. H. (2002). Stop that: inhibition, sensitization and their neurovisceral concomitants. *Scand. J. Psychol.* 43, 123–130. doi: 10.1111/1467-9450.00277

Toichi, M., and Kamio, Y. (2003). Paradoxical autonomic response to mental task in autism. *J. Autism Dev. Disord.* 33, 417–426. doi: 10.1023/A:1025062812374

Toichi, M., Kubota, Y., Murai, T., Kamio, Y., Sakihama, M., Toriuchi, T., et al. (1999). The influence of psychotic states on the autonomic nervous system in schizophrenia. *Int. J. Psychophysiol.* 31, 147–154. doi: 10.1016/s0167-8760(98)00047-6

Udupa, K., Sathyaprabha, T. N., Thirthalli, J., Kishore, K. R., Raju, T. R., and andGangadhar, B. N. (2007). Modulation of cardiac autonomic functions in patients with major depression treated with repetitive transcranial magnetic stimulation. *J. Affect. Disord.* 104, 231–236. doi: 10.1016/j.jad.2007.04.002

Wagner, T., Rushmore, J., Eden, U., and Valero-Cabre, A. (2009). Biophysical foundations underlying TMS: Setting the stage for an effective use of neurostimulation in the cognitive neurosciences. *Cortex* 45, 1025–1034. doi: 10.1016/j.cortex.2008.10.002

Walsh, V., and Pascual-Leone, A. (2003). *Transcranial Magnetic Stimulation: A Neurochronometrics of Mind.* Cambridge, Massachusetts: MIT Press.

Wechsler, D. (2004). *Wechsler Intelligence Scale for Children-Fourth Edition Integrated (WISC-IV Integrated).* San Antonio, TX: Harcourt.

Williams, L. M., Brown, K. J., Das, P., Boucsein, W., Sokolov, E. N., Brammer, M. J., et al. (2004). The dynamics of cortico-amygdala and autonomic activity over the experimental time course of fear perception. *Brain Res. Cogn. Brain Res.* 21, 114–123. doi: 10.1016/j.cogbrainres.2004.06.005

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., et al. (2011). Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477, 171–178. doi: 10.1038/nature10360

Yoshida, T., Yoshino, A., Kobayashi, Y., Inoue, M., Kamakura, K., and Nomura, S. (2001). Effects of slow repetitive transcranial magnetic stimulation on heart rate variability according to power spectrum analysis. *J. Neurol. Sci.* 184, 77–80. doi: 10.1016/s0022-510x(00)00505-0

Zahn, T. P., Rumsey, J. M., and Van Kammen, D. P. (1987). Autonomic nervous system activity in autistic, schizophrenic and normal men: effects of stimulus significance. *J. Abnorm. Psychol.* 96, 135–144. doi: 10.1037//0021-843x.96.2.135

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

Received: 31 July 2014; accepted: 03 October 2014; published online: 21 October 2014.
Citation: Casanova MF, Hensley MK, Sokhadze EM, El-Baz AS, Wang Y, Li X and Sears L (2014) Effects of weekly low-frequency rTMS on autonomic measures in children with autism spectrum disorder. Front. Hum. Neurosci. ***8****:851. doi: 10.3389/fnhum.2014.00851*
This article was submitted to the journal Frontiers in Human Neuroscience.

frontiers in
HUMAN NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 02 September 2014
doi: 10.3389/fnhum.2014.00669

Developmental aspects of cortical excitability and inhibition in depressed and healthy youth: an exploratory study

Paul E. Croarkin[1]*, Paul A. Nakonezny[2,3], Charles P. Lewis[1], Michael J. Zaccariello[1], John E. Huxsahl[1], Mustafa M. Husain[4], Betsy D. Kennard[3], Graham J. Emslie[3] and Zafiris J. Daskalakis[5]

[1] *Division of Child and Adolescent Psychiatry, Department of Psychiatry and Psychology, Mayo Clinic, Rochester, MN, USA*
[2] *Division of Biostatistics, Department of Clinical Sciences, UT Southwestern Medical Center, Dallas, TX, USA*
[3] *Department of Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA*
[4] *Department of Psychiatry and Behavioral Sciences, Duke University School of Medicine, Durham, NC, USA*
[5] *Department of Psychiatry, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada*

Edited by:
Lindsay M. Oberman, E.P. Bradley Hospital and Warren Alpert Medical School of Brown University, USA

Reviewed by:
Sudha Kilaru Kessler, University of Pennsylvania School of Medicine, USA
Motoaki Nakamura, Kanagawa Psychiatric Center, Japan

***Correspondence:**
Paul E. Croarkin, Department of Psychiatry and Psychology, Mayo Clinic, 200 First Street SW, Generose, Rochester, MN 55905, USA
e-mail: croarkin.paul@mayo.edu

Objectives: The objective of this *post-hoc* exploratory analysis was to examine the relationship between age and measures of cortical excitability and inhibition.

Methods: Forty-six participants (24 with major depressive disorder and 22 healthy controls) completed MT, SICI, ICF, and CSP testing in a cross-sectional protocol. Of these 46 participants, 33 completed LICI testing. Multiple linear robust regression and Spearman partial correlation coefficient were used to examine the relationship between age and the TMS measures.

Results: In the overall sample of 46 participants, age had a significant negative relationship with motor threshold (MT) in both the right ($r_S = -0.49$, adjusted $p = 0.007$; $\beta = -0.08$, adjusted $p = 0.001$) and left ($r_S = -0.42$, adjusted $p = 0.029$; $\beta = -0.05$, adjusted $p = 0.004$) hemispheres. This significant negative relationship of age with MT was also observed in the sample of depressed youth in both the right ($r_S = -0.70$, adjusted $p = 0.002$; $\beta = -0.09$, adjusted $p = 0.001$) and left ($r_S = -0.54$, adjusted $p = 0.034$; $\beta = -0.05$, adjusted $p = 0.017$) hemispheres, but not in healthy controls. In the sample of the 33 participants who completed LICI testing, age had a significant negative relationship with LICI (200 ms interval) in both the right ($r_S = -0.48$, adjusted $p = 0.05$; $\beta = -0.24$, adjusted $p = 0.007$) and left ($r_S = -0.64$, adjusted $p = 0.002$; $\beta = -0.23$, adjusted $p = 0.001$) hemispheres. This negative relationship between age and LICI (200 ms interval) was also observed in depressed youth in both the right ($r_S = -0.76$, adjusted $p = 0.034$; $\beta = -0.35$, adjusted $p = 0.004$) and left ($r_S = -0.92$, adjusted $p = 0.002$; $\beta = -0.25$, adjusted $p = 0.001$) hemispheres.

Conclusion: These findings suggest that younger children have higher MTs. This is more pronounced in depressed youth than healthy controls. LICI inhibition may also increase with age in youth.

Keywords: adolescents, depression, neurodevelopment, CSP, ICF, SICI, LICI, TMS

INTRODUCTION

Transcranial magnetic stimulation (TMS) measures of cortical excitability and inhibition have shown initial promise as biomarkers and for the study of neurophysiology in youth with attention deficit hyperactivity disorder (Gilbert et al., 2011), neurodevelopmental disorders (Garvey et al., 2001, 2003; Garvey and Gilbert, 2004; Enticott et al., 2013; Oberman et al., 2013) and mood disorders (Croarkin et al., 2013, 2014). These TMS paradigms involve quantifying the effects of brief TMS pulses on the motor cortex through the measurement of a motor evoked potential (MEP) with surface electromyography (EMG) applied to hand muscles such as the abductor pollicis brevis (APB) (Levin et al., 2014). The amplitude of a MEP reflects the excitatory/inhibitory balance of cortical pyramidal cells. Based on prior work, these measures have good reliability and validity (Farzan et al., 2010). Notably, many of these measures are indirect indices gamma-aminobutyric acid (GABA) and glutamate receptor mediated neurotransmission (Radhu et al., 2013).

Two measures of cortical excitability are the motor threshold (MT) which is a single pulse TMS measure and intracortical facilitation (ICF) which is a paired-pulse TMS measure. Prior human pharmacologic studies indicate that the MT is at least partially dependent on voltage-gated sodium channels while ICF is an indirect measure of glutamatergic N-methyl-D-aspartate (NMDA) mediated neurotransmission (Ziemann et al., 1996a,b, 1998). The MT is operationally defined as the stimulus intensity which produces a reliable MEP with a stimulation intensity of at least 50 microvolts in 5 out of 10 trials. During

ICF measures, a subthreshold condition stimulation (set to 80% of resting MT) precedes a suprathreshold test stimulation with interstimulus intervals of 10–20 ms. The conditioned MEP is compared to the MEP produced by a suprathreshold test stimulus to examine the degree of change in amplitude. Higher ratios reflect increased cortical facilitation. Measures of cortical inhibition include short-interval intracortical inhibition (SICI) (Kujirai et al., 1993), long-interval cortical inhibition (LICI) (Daskalakis et al., 2008; Farzan et al., 2010), and the cortical silent period (CSP) (Garvey and Mall, 2008). Prior research suggests that SICI is a measure of $GABA_A$ receptor mediated neurotransmission (Kujirai et al., 1993; Paulus et al., 2008), while LICI and CSP index $GABA_B$ receptor mediated neurotransmission (Connors et al., 1988; Kujirai et al., 1993; McDonnell et al., 2006). For SICI measures, a subthreshold conditioning stimulation (set to 80% of resting MT) precedes a suprathreshold test stimulation with interstimulus intervals of 1–5 ms. The conditioned MEP is compared to the MEP produced by a suprathreshold test stimulus to examine the degree of change in amplitude. Lower SICI ratios reflect increased cortical inhibition. During LICI measurements, suprathreshold conditioning and suprathreshold test stimulations are applied to the motor cortex with 50–200 ms interstimulus intervals (Kujirai et al., 1993; Garvey and Mall, 2008). The conditioned MEP is compared to the MEP produced by a suprathreshold test stimulus to examine the degree of change in amplitude. Lower LICI ratios reflect increased cortical inhibition. During CSP testing the participant contracts the muscle of interest submaximally while a suprathreshold, single-pulse stimulation is administered to the motor cortex. The resultant period of EMG silence corresponds to the amount of cortical inhibition (Farzan et al., 2013). Contralateral silent period measures are typically collected in psychiatric research, although it is possible to collect ipsilateral silent period data as well (Garvey and Gilbert, 2004). Both CSP measures reflect the function of cortical inhibitory interneurons and the corpus callosum (Müller et al., 1997; Garvey et al., 2003).

These neurophysiologic indices have potential for classifying psychiatric illnesses and guiding pharmacologic treatments (Ziemann, 2004; Paulus et al., 2008). Research regarding the developmental courses of cortical excitability and inhibition measures in adulthood is emerging (McGinley et al., 2010; Fling and Seidler, 2012; Heise et al., 2013; Levin et al., 2014; Liguz-Lecznar et al., 2014), but remarkably little is known about the early trajectory of each measure in health and disease (Moll et al., 1999; Mall et al., 2004; Garvey, 2008). Although there is a paucity of research, it is generally accepted that MT measures are higher in children than in adults (Garvey and Gilbert, 2004; Garvey, 2008). At some point in adolescence, MT values fall to adult levels (Garvey and Gilbert, 2004). Some TMS studies suggest that the CSP durations increase with age while other studies others do not (Moll et al., 1999; Garvey et al., 2003). Research regarding early age-related differences in SICI and ICF is also inconclusive (Moll et al., 1999; Garvey et al., 2003; Mall et al., 2004; Gilbert et al., 2011). Further, there are few published reports examining LICI measures in children and adolescents (Croarkin et al., 2014). An enhanced understanding of developmental changes in these neurophysiologic measures would have great utility. This knowledge would advance understanding of neurodevelopment, the ontogeny of GABA and glutamate neurotransmission, and inform neurophysiologic study protocols (Paulus et al., 2008; Radhu et al., 2013). The aim of this exploratory study was to examine the current suppositions regarding developmental changes in motor cortex excitability and inhibition in early life. In particular, we examined the association of age with cortical inhibition (SICI, LICI, and CSP) and excitability (MT, ICF).

METHODS

STUDY DESIGN AND OVERVIEW

This was a cross-sectional study of depressed youth and healthy controls. All participants had a clinical evaluation and TMS testing. Cortical inhibition (SICI, CSP) and excitability (MT and ICF) were collected during a single session. A subgroup of this cohort completed LICI testing during the same testing session. Study design details have been previously published (Croarkin et al., 2013, 2014). All study procedures were approved by the local institutional review board prior to the enrollment of subjects. Below is a brief description of relevant design aspects of this study.

STUDY PARTICIPANTS

This study involved the 46 male and female, children and adolescents, aged 9–17 years from our parent study (Croarkin et al., 2013, 2014). In particular, 24 participants had major depressive disorder (MDD) and 22 participants were healthy controls. Of the 46 youth in our parent study (Croarkin et al., 2013, 2014), 33 of these participants (14 with MDD and 19 healthy controls) completed the LICI testing protocol. This sample was recruited from a pediatric mood disorders clinic and local advertising. After obtaining written assent from youth and informed consent from legal guardians, participants, and families were evaluated by a board-certified child and adolescent psychiatrist (P.E.C.). This included a clinical history, psychiatric history, medical history, mental status exam, neurological exam, physical exam, urine pregnancy test for females who had reached menarche, and a semi-structured interview with Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997). Depression severity was assessed with the Children's Depression Rating Scale, Revised (CDRS-R) (Poznanski et al., 1983). The Oldfield's Edinburgh Handedness Inventory (Oldfield, 1971) was completed to confirm handedness. The TMS Adult Safety Screen (Keel et al., 2001) was used to confirm safety for single- and paired-pulse TMS testing. Depressed patients and healthy controls were not taking psychotropic medications or receiving psychotherapy prior to the TMS testing session.

A personal or family history of seizure disorders was exclusionary. A prior history of neurosurgery was exclusionary. Exclusionary psychiatric disorders in depressed participants included autism spectrum disorders, bipolar disorder, conduct disorder, eating disorders, mental retardation, obsessive-compulsive disorder, posttraumatic stress disorder, schizophrenia, substance use disorders, and tic disorders. Screening for mental retardation involved a detailed review of records and questions regarding academic performance. If

below-average cognitive ability was considered, formal intellectual screening was completed with the Kaufman Brief Intelligence Test-2 (Kaufman and Kaufman, 2004). This encompassed verbal knowledge, matrices, and riddles. Youth with an estimated IQ less than 80 were not enrolled. Healthy control participants were in excellent health and did not meet present or past diagnostic criteria for any psychiatric illness based on the evaluation and the K-SADS-PL interview.

PROCEDURES AND MEASURES

TMS testing

Cortical excitability and inhibition TMS testing was performed as described in prior literature (Kujirai et al., 1993; Ziemann, 2004; Paulus et al., 2008). Participants were seated and wore a swim cap during the procedures. Participants and study team wore earplugs during the testing. Stimulations were delivered with a Magstim 200 magnetic simulator (Magstim Co Ltd.) with a figure-of-eight coil (each loop is 70 mm in diameter). MEP data were collected with surface EMG recordings of the APB muscle. Audio feedback was examined to assess muscle relaxation of the participant. Single and paired-pulse TMS was delivered to the hand area of the contralateral motor cortex with the coil placed tangentially on the scalp at a 45° from the midline. The motor cortex testing site was ascertained after moving the coil in 1-cm increments to locate the site producing maximal MEP. The location was marked for reliable testing throughout the session. The resting MT was established as the stimulation intensity producing a MEP greater than 50 μV in 5 of 10 TMS pulses with a relaxed muscle. The suprathreshold test stimulus was obtained but adjusting stimulation intensity to produce a mean MEP 0.5–1.5 mV peak-to-peak in amplitude in the dominant hand muscle. The conditioning stimulus was set to 80% of the participants resting MT. For SICI and ICF measures, the conditioning and test stimuli were delivered to the test site at interstimulus intervals of 2, 4, 10, 15, 20 ms in a random, counterbalanced fashion. Each interstimulus interval was tested 12 times and results were averaged. The left hemisphere was stimulated first in all subjects. For LICI testing, test stimulus intensity was adjusted to reliably produce MEP 0.5–1.5 mV peak-to-peak in amplitude in the dominant hand muscle. For LICI testing the conditioning and test stimuli were both delivered at this suprathreshold intensity with interstimulus intervals of 100, 150, and 200 ms in a random, counterbalanced fashion. Each interstimulus interval was tested 10 times and results were averaged. The MEP data from these paired-pulse TMS measures was expressed as the percentage of the mean MEP elicited with the test (unconditioned pulse). For CSP testing, the participant submaximally (20%) contracted the APB with contralateral single-pulse stimulations at 140% of the participants resting MT. Ten trials were performed and averaged to determine the durations of the silent period. The TMS testing was performed bilaterally for this study providing data from both hemispheres.

Dependent variables

The outcomes were TMS measures of cortical inhibition (SICI, LICI, CSP) and excitability (MT, ICF). The TMS measures were log transformed to obtain a more normal distribution (because of skewness).

Independent variable and covariates

The primary independent variable was patient age in years. Sex and CDRS-R total score were included as covariates in the models to bolster precision in the evaluation of the relationship between age and each measure of cortical inhibitory or excitatory functioning. Some (Cuypers et al., 2014) but not all (Wasserman, 2002) studies suggest that sex may contribute to variation in TMS MEP measures. Although, it is not well understood, depression severity (CDRS-R) may also impact TMS MEP measures (Croarkin et al., 2013).

STATISTICAL ANALYSIS

This was an exploratory study with the aim of investigating the impact of age on measures of cortical inhibition and excitability in youth. Analyses were performed on the entire sample and then on subgroups of healthy and depressed youth. The rationale for this approach was to maximize limited data and with the realization that the impact of depression or depression severity on these neurophysiological measures is not definitively known. Demographic and clinical characteristics for the overall sample of patients and for depressed youth and healthy controls were described using the sample mean and standard deviation for continuous variables and the frequency and percentage for categorical variables. Multiple linear robust regression (with MM estimation) and the Spearman partial correlation coefficient (r_s) were used to examine the relationship between age and TMS measures of cortical inhibition (SICI, LICI, CSP) and excitability (MT, ICF) in the overall sample, while adjusting for sex and CDRS-R total score, and then separately in depressed youth and healthy controls, while adjusting for sex. The estimated slope from the regression model indicates the mean change in each TMS measure per one-year increase in age, while the sample correlation coefficient indicates not only direction, but also strength of the linear relationship between age and each TMS measure. The Spearman partial correlation coefficient can also be interpreted as the effect size estimator in evaluating the magnitude of the relationship between age and each TMS measure.

Regression coefficients found to have statistical significance for each of the two groups in the abovementioned regression analysis, were then tested in subsequent (*post-hoc*) regression models (similar to that described above) by evaluating the interaction effect of group (Healthy Controls vs. MDD Patients) with age on the TMS measure. Comparing the regression parameters (slopes) between groups was used to assess if the mean change in the TMS measure per one-year increase in age was different between healthy controls and MDD patients.

All statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Inc., Cary, NC, USA). The level of significance for all tests was set at $\alpha = 0.05$ (two-tailed). We implemented the False Discovery Rate procedure (Benjamini and Hochberg, 1995) to control false-positives over the sets of multiple tests associated with the correlation coefficient and regression coefficient for the groups of participants (e.g., overall sample, depressed youth, and healthy controls).

RESULTS

PARTICIPANT CHARACTERISTICS

Twenty-four, medication-naïve participants (14 female) with MDD completed MT, SICI, ICF, and CSP testing. Demographics

of the sample are also characterized our prior publication. In comparing participants with MDD and healthy controls, there were no statistically significant differences in age, sex, or handedness (Croarkin et al., 2013). These participants were 9–17 years of age, with a mean [SD] age of 13.8 [2.1] years. The mean [SD] CDRS-R total score of the MDD youth was 58.9 [8.5] and the mean [SD] episode duration was 10.9 [9.7] months. Eight participants with MDD had comorbid anxiety disorders, four had comorbid attention-deficit/hyperactivity disorder, one had comorbid oppositional defiant disorder, and one had comorbid Type I diabetes mellitus. Two MDD participants were left handed. Twenty-two healthy controls (11 female) completed MT, SICI, ICF, and CSP testing. These participants were 9–17 years of age, with a mean [SD] age of 13.7 [2.2] years. The mean [SD] CDRS-R total score of the healthy controls was 19.6 [1.6]. Two healthy controls were left handed. Of the 46 adolescents in the current study, 23 (50%) were Caucasian, 13 (28.2%) were African American, 4 (8.7%) were Hispanic, and 13 (28.2%) had a family history of mood disorder. Family history of mood disorder occurred in 13 of the 24 depressed adolescents (54.2%) and in 0 of the 22 healthy controls. Fourteen patients with MDD (8 female), with a mean [SD] age of 14.0 [2.1] years, completed LICI testing. The mean [SD] CDRS-R total score of these 14 MDD LICI youth was 59.0 [9.6]. Nineteen healthy controls (11 female) with a mean [SD] age of 13.9 [2.2] years completed LICI testing. The mean [SD] CDRS-R total score of these 19 healthy control LICI youth was 19.6 [1.7]. Two of these healthy controls who completed LICI testing were left handed.

AGE AND TMS MEASURES OF CORTICAL INHIBITION AND EXCITABILITY

In the overall sample of 46 youth, the Spearman partial correlations and the multiple linear robust regression, while adjusting for sex and CDRS-R total, revealed a significant negative linear relationship between age and MT in both the right ($r_s = -0.49$, raw $p = 0.0007$, FDR-adjusted $p = 0.007$; $\beta = -0.08$, 95% $CI = -0.12$ to -0.05, raw $p = 0.0001$, FDR-adjusted $p = 0.001$) and left ($r_s = -0.42$, raw $p = 0.004$, FDR-adjusted $p = 0.029$; $\beta = -0.05$, 95% $CI = -0.09$ to -0.02, raw $p = 0.0007$, FDR-adjusted $p = 0.004$) hemispheres. This significant negative relationship of age with MT, while adjusting for sex, was also observed in the sample of depressed youth in both the right ($r_s = -0.70$, raw $p = 0.0002$, FDR-adjusted $p = 0.002$; $\beta = -0.09$, 95% $CI = -0.13$ to -0.05, raw $p = 0.0001$, FDR-adjusted $p = 0.001$) and left ($r_s = -0.54$, raw $p = 0.007$, FDR-adjusted $p = 0.034$; $\beta = -0.05$, 95% $CI = -0.09$ to -0.02, raw $p = 0.003$, FDR-adjusted $p = 0.017$) hemispheres, but not in healthy controls. Note that lower MT values reflect increased excitability. We also present scatterplots of the log transformed MT values (left and right hemispheres) against age, with a fitted regression line and 95% confidence limits, by depressed youth and healthy controls (**Figures 1**, **2**). We note that the *post-hoc* results of the interaction of group with age indicated that the slope of the regression of MT on age was similar for both healthy controls and MDD patients in both the left ($p = 0.61$; **Figure 1**) and right ($p = 0.28$; **Figure 2**) hemispheres.

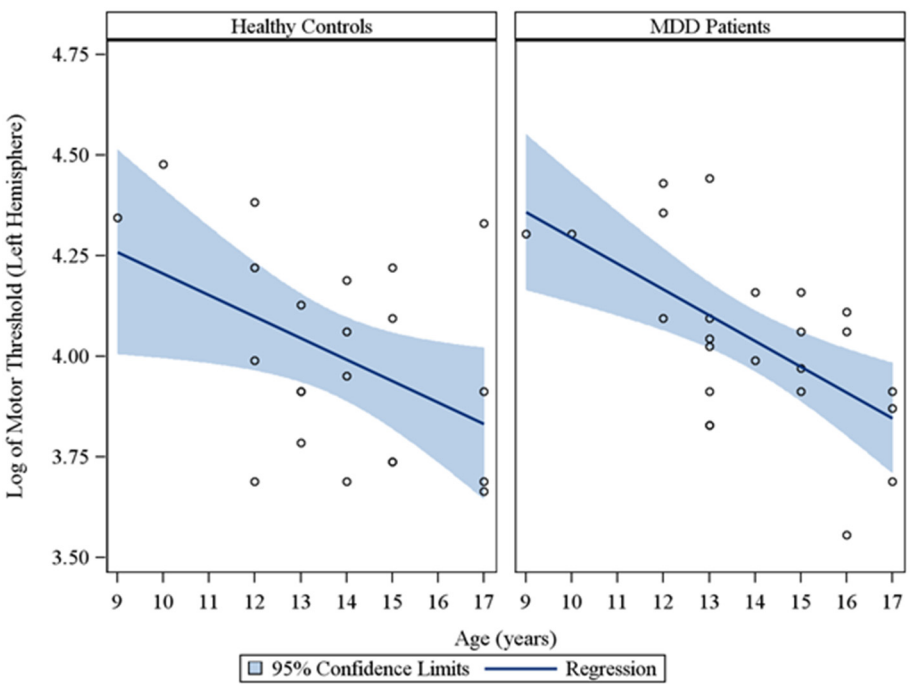


FIGURE 1 | Scatterplots of the log transformed MT values (left hemisphere) against age, with a fitted regression line and 95% confidence limits, by healthy controls and MDD patients. Note: The interaction of group with age indicated that the slope of the regression of motor threshold on age was similar for both healthy controls and MDD patients in the left hemisphere ($p = 0.61$).

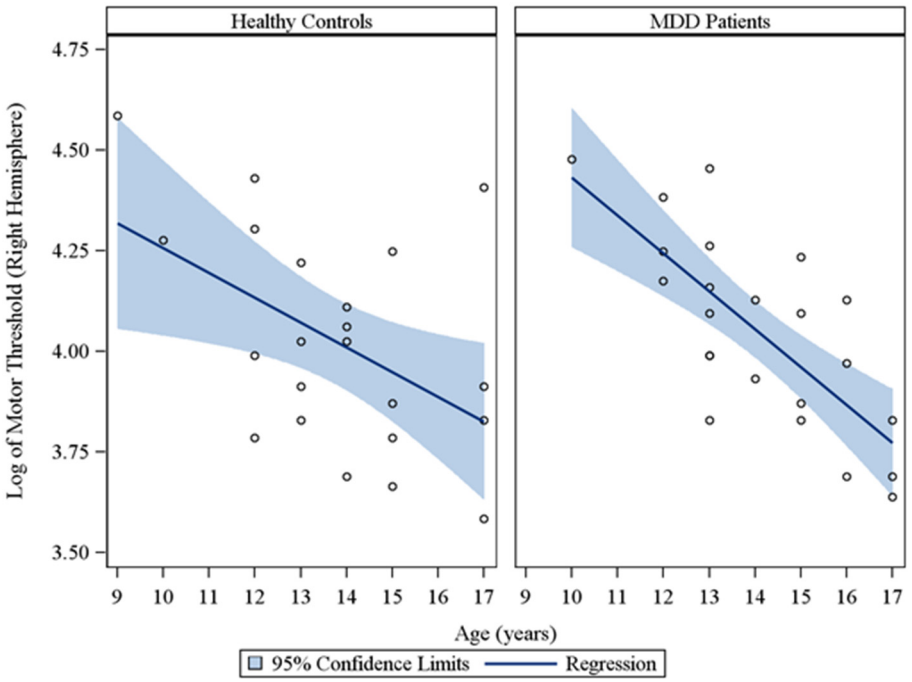


FIGURE 2 | Scatterplots of the log transformed MT values (right hemisphere) against age, with a fitted regression line and 95% confidence limits, by healthy controls and MDD patients. Note: The interaction of group with age indicated that the slope of the regression of motor threshold on age was similar for both healthy controls and MDD patients in the right hemisphere ($p = 0.28$).

Moreover, in the sample of the 33 participants who completed LICI testing, while adjusting for sex and CDRS-R total, age had a significant negative relationship with LICI (200 ms interval) in both the right ($r_s = -0.48$, raw $p = 0.01$, FDR-adjusted $p = 0.056$; $\beta = -0.24$, 95% $CI = -0.39$ to -0.09, raw $p = 0.001$, FDR-adjusted $p = 0.007$) and left ($r_s = -0.64$, raw $p = 0.0001$, FDR-adjusted $p = 0.002$; $\beta = -0.23$, 95% $CI = -0.32$ to -0.14, raw $p = 0.0001$, FDR-adjusted $p = 0.001$) hemispheres. This negative relationship between age and LICI (200 ms interval), while adjusting for sex, was also observed in depressed participants (Supplementary Table 5) in both the right ($r_s = -0.76$, raw $p = 0.006$, FDR-adjusted $p = 0.034$; $\beta = -0.35$, 95% $CI = -0.55$ to -0.15, raw $p = 0.0007$, FDR-adjusted $p = 0.004$) and left ($r_s = -0.92$, raw $p = 0.0001$, FDR-adjusted $p = 0.002$; $\beta = -0.25$, 95% $CI = -0.34$ to -0.16, raw $p = 0.0001$, FDR-adjusted $p = 0.001$) hemispheres and in healthy controls (Supplementary Table 6), but only in the left hemisphere ($r_s = -0.45$, raw $p = 0.05$, FDR-adjusted $p = 0.877$; $\beta = -0.19$, 95% $CI = -0.36$ to -0.02, raw $p = 0.02$, FDR-adjusted $p = 0.478$). Note that lower LICI values reflect increased inhibition. Scatterplots of the log transformed LICI (200 ms interval) values (left and right hemispheres) against age, with a fitted regression line and 95% confidence limits, by depressed youth and healthy controls are presented in **Figures 3**, **4**. We note that the *post-hoc* results of the interaction of group with age indicated that the slope of the regression of LICI-200 ms on age was similar for both healthy controls and MDD patients in the left hemisphere ($p = 0.14$; **Figure 3**), but significantly different in the right hemisphere ($p = 0.049$; **Figure 4**). A difference in slopes here (in the right hemisphere) can be interpreted as differences in the mean change in LICI-200 ms per 1-year increase in age between healthy controls and MDD patients. There were no significant relationships of age with SICI, CSP, or ICF. The correlation and regression results are presented in Tables 1–6 which are available in the Supplementary Material. Note that nontransformed data of these TMS measures from this cohort have been published previously (Croarkin et al., 2013).

DISCUSSION

This exploratory study builds on prior work examining the early developmental course of cortical inhibition and excitability measures. It is unique in examining these electrophysiological findings in the context of MDD. As expected, the findings suggest that younger children have high MTs which decrease with age. Of note, this was statistically significant in the depressed participant group, but not in the healthy control group. The current, developmental MT findings are congruent with prior work (Eyre et al., 1991). Garvey et al. demonstrated that bilateral MT measurements decreased with age and in three subjects with a mean age of 7.6 years (*SD* 0.5, range 7–7.8) the MT was unobtainable with maximum output of the stimulator (Garvey et al., 2003). Gilbert and colleagues also reported statistically significant, negative correlations between age and MT measures from the left hemisphere in 98 (49 with attention-deficit/hyperactivity disorder and 49 healthy control subjects) child participants who were 8–12 years of age (Gilbert et al., 2011). Moll and colleagues reported similar findings in 40 healthy control subjects who were 8–16 years of age (Moll et al., 1999). The present study is the first

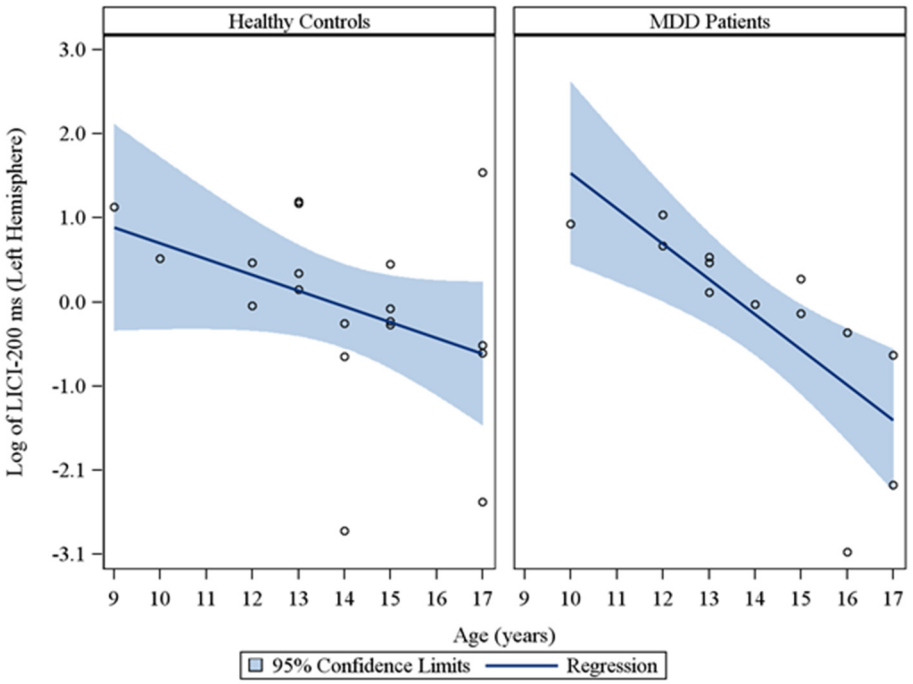


FIGURE 3 | Scatterplots of the log transformed LICI (200 ms interval) values (left hemisphere) against age, with a fitted regression line and 95% confidence limits, by healthy controls and MDD patients. Note: The interaction of group with age indicated that the slope of the regression of LICI-200 ms on age was similar for both healthy controls and MDD patients in the left hemisphere ($p = 0.14$).

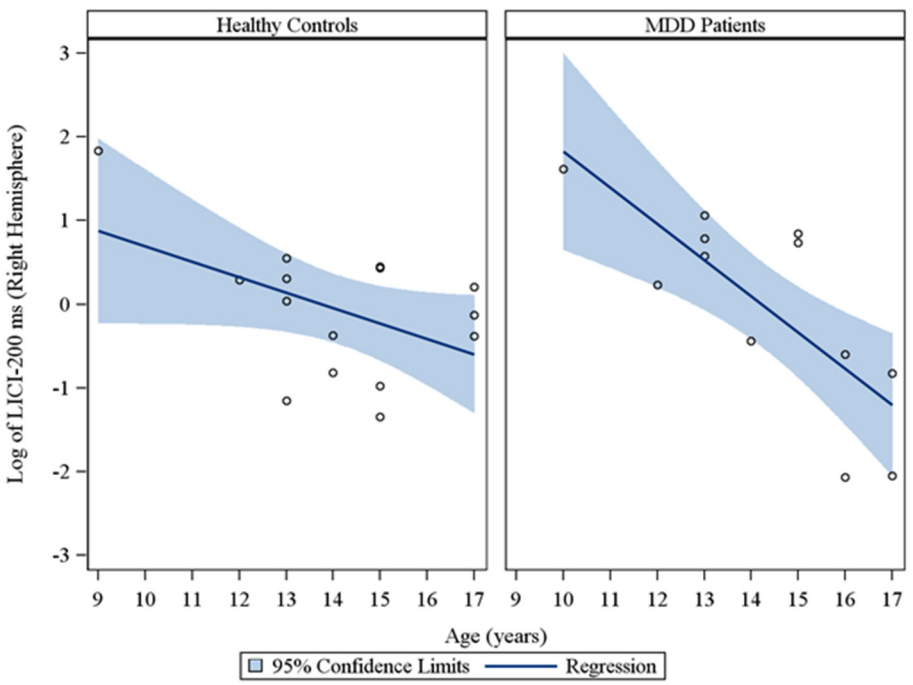


FIGURE 4 | Scatterplots of the log transformed LICI (200 ms interval) values (right hemisphere) against age, with a fitted regression line and 95% confidence limits, by healthy controls and MDD patients. Note: The interaction of group with age indicated that the slope of the regression of LICI-200 ms on age was significantly different for healthy controls vs. MDD patients in the right hemisphere ($p = 0.049$).

to note this pattern in a group of depressed and healthy control subject. This finding warrants further consideration and study. Perhaps, healthy control youth subjects reach an adult MT earlier, while depressed youth have a more delayed course. It is possible that differences in scalp to cortex distances could play a role in these age differences. Recent work demonstrates that brain-scalp differences are lower in young children and increase throughout childhood (Beauchamp et al., 2011). Based on this anatomical finding alone, it might be inferred that lower TMS current would be sufficient to induce motor activity in younger children and that younger children would have lower MTs. As this does not appear to be the case, age related differences are more likely due to neurodevelopmental changes.

The present CSP, SICI, and ICF findings are inconclusive. Similarly, findings from two prior studies also found no statistically significant relationships between age and CSP measures (Garvey et al., 2003; Gilbert et al., 2011). However, Moll and colleagues reported a correlation with age and CSP in healthy controls which suggests that cortical inhibition and $GABA_B$ mediated neurotransmission is potentiated with age (Moll et al., 1999). It is often noted that CSP measures have large interindividual variability in children and adolescents (Garvey and Mall, 2008). Further, hemispheric differences are also apparent (Garvey, 2008; Garvey and Mall, 2008; Croarkin et al., 2013). In general, our findings and two prior studies suggest that there are no SICI or ICF age dependent relationships in youth (Garvey et al., 2003; Gilbert et al., 2011). However, Mall and colleagues examined SICI in 50 healthy participants aged 6–34 years. In this case, children and adolescents had less SICI as compared to adults, which suggests that cortical inhibition and $GABA_A$ mediated neurotransmission develops throughout childhood (Mall et al., 2004). This effort did involve stimulation with a round rather than a figure-of-eight coil but otherwise its methodology was congruent with negative studies.

The current findings suggest that LICI inhibition increases with age youth. In particular, LICI (200 ms interval) had a statistically significant negative relationship with age in depressed youth, but not in healthy control participants. It is important to acknowledge the lack of a relationship may be attributable to low power, given the high degree of variability in the measure in general. Lower LICI ratios signify greater inhibition and increased $GABA_B$ mediated neurotransmission (Connors et al., 1988; McDonnell et al., 2006; Daskalakis et al., 2008). Prior work postulates that deficits in $GABA_A$ and $GABA_B$ mediated neurotransmission play a role in the pathophysiology of MDD (Bajbouj et al., 2006; Levinson et al., 2010; Croarkin et al., 2011). However, $GABA_B$ receptors are widely distributed throughout the brain presynaptically and postsynaptically with complex and diverse functioning (Benarroch, 2012). In some instances, facilitated GABA activity likely contributes to neuropsychiatric disease mechanisms. Heightened thalamic postsynaptic $GABA_B$ activity is thought to play a central role in the pathophysiology of petit mal seizures (Blumenfeld and McCormick, 2000; McCormick and Contreras, 2001). Other work suggests that that aberrant interplay between GABA and NMDA receptors is necessary for the genesis of hippocampal seizures (Bradford, 1995; Katsumori et al., 1998). Enhanced $GABA_B$ development in childhood could represent a vulnerability to or a consequence of mood disorders. Our findings are unique in that there are no other known published reports examining potential relationships of age with LICI measures in child and adolescent samples.

Longitudinal studies of cortical inhibition and excitability during childhood are warranted. The GABAergic and glutamatergic neurotransmitter systems have a complex and poorly understood maturation with which widely influences central nervous system development and functioning. For example, in prenatal and early infantile life GABA serves as an excitatory neurotransmitter and this is mediated primarily by the structure and fuction of GABA receptors (Ben-Ari et al., 1994; Leinekugel et al., 1999; Rakhade and Jensen, 2009). It is thought that density of $GABA_A$ receptors decreases from childhood to adulthood (Chugani et al., 2001) and that the subunit composition of $GABA_A$ receptors varies substantially during development (Duncan et al., 2010). Lower cortical inhibition early in life may reflect increased plasticity and optimum conditions for learning and development (Mall et al., 2004). Safe, noninvasive, *in vivo* measures afforded by TMS offer an important tool to probe the development of these neurotransmitter systems (Dayan et al., 2013).

LIMITATIONS

These discussion points and findings must be placed in the context of the limitations of the study. This was a *post-hoc* exploratory study, which was based on a small, age-restricted, sample. The parent study (Croarkin et al., 2013, 2014) from which the current findings are based was not designed to specifically address the question of age effects on TMS measures of cortical inhibition and excitability. Like previous studies that examined the effects of age on TMS measures, the current study design was cross-sectional. A longitudinal study would provide more insight into the pattern of findings regarding the impact of development over the lifespan. It is also unknown if alterations in cortical inhibition and excitability measures are deficiencies related to the burden of psychiatric illnesses or markers of risk. Moreover, a cross-sectional approach is limited as it has been demonstrated that TMS measures have wide inter-individual variability (Kiers et al., 1993; Cuypers et al., 2014). The present findings may relate to the ontogeny of GABA and glutamate neurotransmission or could be a result of developmental changes in anatomy, such as skull bone shape and thickness.

CONCLUSIONS

These findings provide additional information regarding the potential impact of age on TMS measures of cortical excitability and inhibition. Findings suggest that younger children have higher MTs which decrease with age and that this is more pronounced in depressed youth. These findings also suggest that LICI inhibition may increase with age and, like MTs, this is more pronounced in depressed youth. Present CSP, SICI, and ICF findings are inconclusive and warrant further study.

DISCLOSURES

Paul E. Croarkin has received research grant support from the National Institute of Mental Healthy, Brain and Behavior Research Foundation, National Alliance for Research on Schizophrenia and Depression (NARSAD) Great Neck, New

York; and Pfizer Inc., New York, NY. He has served as a site subprincipal or principal investigator (without additional compensation) for Eli Lilly and Co, Indianapolis, Indiana; Forest Laboratories, Inc., New York, NY; Merck and Co, Inc., Whitehouse Station, New Jersey; and Pfizer Inc.

Mustafa M. Husain has received research support from the National Institute of Mental Healthy, Stanley Medical Research Institute, Cyberonics, Inc., Pfizer, Inc., Neuronetics Inc., Magstim; has served as a consultant for AstraZeneca; served on speaker bureau for AstraZeneca, Bristol-Meyers-Squibb, and Abbott Laboratories.

Graham J. Emslie has received research support from the National Institute of Mental Health, Biobehavioral Diagnostic Inc., BioMarin, Duke University, Eli Lilly, Forest Laboratories, GlaxoSmithKline, Mylan, and Somerset; has served as a consultant for Alkermes, Inc., Allergan, NCS Pearson (previously BioBehavioral Diagnostics Inc.), Bristol-Myers Squibb, Eli Lilly, Forest Laboratories, GlaxoSmithKline, INC Research Inc., Lundbeck, Merck, Pfizer, Seaside Therapeutics, Shire, the Texas Department of State Health Services, University of Miami, Valeant, and Wyeth; and was on the Speakers Bureau for Forest Laboratories.

In the last 5 years, Zafiris J. Daskalakis received research and equipment in-kind support for an investigator-initiated study through Brainsway Inc. and a travel allowance through Merck. Zafiris J. Daskalakis has also received speaker funding through Sepracor Inc., AstraZeneca and served on the advisory board for Hoffmann-La Roche Limited and Merck and received speaker support from Eli Lilly.

Drs. Paul A. Nakonezny, Charles P. Lewis, Michael J. Zaccariello, John E. Huxsahl, and Betsy D. Kennard have no disclosures.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institute of Mental Health of the National Institutes of Health under Award Number K23MH100266. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This research was also supported by a NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation. The authors also acknowledge support from the Ontario Mental Health Foundation (OMHF), the Canadian Institutes of Health Research (CIHR), the Brain and Behavior Research Foundation and the Temerty Family and Grant Family and through the Centre for Addiction and Mental Health (CAMH) Foundation and the Campbell Institute.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnhum.2014.00669/abstract

REFERENCES

Bajbouj, M., Lisanby, S. H., Lang, U. E., Danker-Hopfe, H., Heuser, I., and Neu, P. (2006). Evidence for impaired cortical inhibition in patients with unipolar major depression. *Biol. Psychiatry* 59, 395–400. doi: 10.1016/j.biopsych.2005.07.036

Beauchamp, M. S., Beurlot, M. R., Fava, E., Nath, A. R., Parikh, N. A., Saad, Z. S., et al. (2011). The developmental trajectory of brain-scalp distance form birth through childhood: implications for functional neuroimaging. *PLoS ONE* 6:e24981. doi: 10.1371/journal.pone.0024981

Ben-Ari, Y., Tseeb, V., Raggozzino, D., Khazipov, R., and Gaiarsa, J. L. (1994). Gamma-Aminobutyric acid (GABA): a fast excitatory transmitter which may regulate the development of hippocampal neurones in early postnatal life. *Prog. Brain Res.* 102, 261–273. doi: 10.1016/S0079-6123(08)60545-2

Benarroch, E. E. (2012). GABAB receptors: structure, functions, and clinical implications. *Neurology* 78, 578–584. doi: 10.1212/WNL.0b013e318247cd03

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc. B Stat. Methodol.* 57, 289–300.

Blumenfeld, H., and McCormick, D. A. (2000). Corticothalamic inputs control the pattern of activity generated in thalamocortical networks. *J. Neurosci.* 20, 5153–5162. Available online at: http://www.jneurosci.org/content/20/13/5153.full.pdf+html

Bradford, H. F. (1995). Glutamate, GABA and epilepsy. *Prog. Neurobiol.* 47, 477–511. doi: 10.1016/0301-0082(95)00030-5

Chugani, D. C., Muzik, O., Juhász, C., Janisse, J. J., Ager, J., and Chugani, H. T. (2001). Postnatal maturation of human GABAA receptors measured with positron emission tomography. *Ann. Neurol.* 49, 618–626. doi: 10.1002/ana.1003

Connors, B. W., Malenka, R. C., and Silva, L. R. (1988). Two inhibitory postsynaptic potentials, and GABAA and GABAB receptor-mediated responses in neocortex of rat and cat. *J. Physiol.* 406, 443–468.

Croarkin, P. E., Levinson, A. J., and Daskalakis, Z. J. (2011). Evidence for GABAergic inhibitory deficits in major depressive disorder. *Neurosci. Biobehav. Rev.* 35, 818–825. doi: 10.1016/j.neubiorev.2010.10.002

Croarkin, P. E., Nakonezny, P. A., Husain, M. M., Melton, T., Buyukdura, J. S., Kennard, B. D., et al. (2013). Evidence for increased glutamatergic cortical facilitation in children and adolescents with major depressive disorder. *JAMA Psychiatry* 70, 291–299. doi: 10.1001/2013.jamapsychiatry.24

Croarkin, P. E., Nakonezny, P. A., Husain, M. M., Port, J. D., Melton, T., Kennard, B. D., et al. (2014). Evidence for pretreatment LICI deficits among depressed children and adolescents with nonresponse to fluoxetine. *Brain Stimul.* 7, 243–251. doi: 10.1016/j.brs.2013.11.006

Cuypers, K., Thijs, H., and Meesen, R. L. (2014). Optimization of the transcranial magentic stimulation protocol by defining a reliable estimate for corticospinal excitability. *PLoS ONE* 9:e86380. doi: 10.1371/journal.pone.0086380

Daskalakis, Z. J., Farzan, F., Barr, M. S., Maller, J. J., Chen, R., and Fitzgerald, P. B. (2008). Long-interval cortical inhibition from the dorsolateral prefrontal cortex: a TMS-EEG study. *Neuropsychopharmacology* 33, 2860–2869. doi: 10.1038/npp.2008.22

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

Duncan, C. E., Webster, M. J., Rothmond, D. A., Bahn, S., Elashoff, M., and Shannon Weickert, C. (2010). Prefrontal GABA(A) receptor alpha-subunit expression in normal postnatal human development and schizophrenia. *J. Psychiatr. Res.* 44, 673–681. doi: 10.1016/j.jpsychires.2009

Enticott, P. G., Kennedy, H. A., Rinehart, N. J., Tonge, B. J., Bradshaw, J. L., and Fitzgerald, P. B. (2013). GABAergic activity in autism spectrum disorders: an investigation of cortical inhibition via transcranial magnetic stimulation. *Neuropharmacology* 68, 202–209. doi: 10.1016/j.neuropharm.2012.06.017

Eyre, J. A., Miller, S., and Ramesh, V. (1991). Constancy of central conduction delays during development in man: investigation of motor and somatosensory pathways. *J. Physiol.* 434, 441–452.

Farzan, F., Barr, M. S., Hoppenbrouwers, S. S., Fitzgerald, P. B., Chen, R., Pascual-Leone, A., et al. (2013). The EEG correlates of the TMS-induced EMG silent period in humans. *Neuroimage* 83, 120–134. doi: 10.1016/j.neuroimage.2013.06.059

Farzan, F., Barr, M. S., Levinson, A. J., Chen, R., Wong, W., Fitzgerald, P. B., et al. (2010). Reliability of long-interval cortical inhibition in healthy human subjects: a TMS-EEG study. *J. Neurophysiol.* 104, 1339–1346. doi: 10.1152/jn.00279.2010

Fling, B. W., and Seidler, R. D. (2012). Fundamental differences in callosal structure, neurophysiologic function, and bimanual control in young and older adults. *Cereb. Cortex* 22, 2643–2652. doi: 10.1093/cercor/bhr349

Garvey, M. A. (2008). "TMS: neurodevelopment and perinatal insults," in *The Oxford Handbook of Transcranial Magnetic Stimulation*, eds E. M. Wassermann, C. M. Epstein, and U. Ziemann (Oxford: Oxford University Press), 337–355.

Garvey, M. A., and Gilbert, D. L. (2004). Transcranial magnetic stimulation in children. *Eur. J. Paediatr. Neurol.* 8, 7–19. doi: 10.1016/j.ejpn.2003.11.002

Garvey, M. A., and Mall, V. (2008). Transcranial magnetic stimulation in children. *Clin. Neurophysiol.* 119, 973–984. doi: 10.1016/j.clinph.2007.11.048

Garvey, M. A., Ziemann, U., Bartko, J. J., Denckla, M. B., Barker, C. A., and Wassermann, E. M. (2003). Cortical correlates of neuromotor development in healthy children. *Clin. Neurophysiol.* 114, 1662–1670. doi: 10.1016/S1388-2457(03)00130-5

Garvey, M. A., Ziemann, U., Becker, D. A., Barker, C. A., and Bartko, J. J. (2001). New graphical method to measure silent periods evoked by transcranial magnetic stimulation. *Clin. Neurophysiol.* 112, 1451–1460. doi: 10.1016/S1388-2457(01)00581-8

Gilbert, D. L., Isaacs, K. M., Augusta, M., Macneil, L. K., and Mostofsky, S. H. (2011). Motor cortex inhibition: a marker of ADHD behavior and motor development in children. *Neurology* 76, 615–621. doi: 10.1212/WNL.0b013e31820c2ebd

Heise, K. F., Zimerman, M., Hoppe, J., Gerloff, C., Wegscheider, K., and Hummel, F. C. (2013). The aging motor system as a model for plastic changes of GABA-mediated intracortical inhibition and their behavioral relevance. *J. Neurosci.* 33, 9039–9049. doi: 10.1523/JNEUROSCI.4094-12.2013

Katsumori, H., Minabe, Y., Osawa, M., and Ashby, C. R. Jr. (1998). Acute effects of various GABA receptor agonists and glutamate antagonists on focal hippocampal seizures in freely moving rats elicited by low-frequency stimulation. *Synapse* 28, 103–109. doi: 10.1002/(SICI)1098-2396(199801)28:1<103::AID-SYN12>3.0.CO;2-Y

Kaufman, A. S., and Kaufman, N. L. (2004). *Kaufman Brief Intelligence Test (KBIT-2), 2nd Edn*. Circle Pines, MN: American Guidance Service.

Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., et al. (1997). Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. *J. Am. Acad. Child. Adolesc. Psychiatry* 36, 980–988. doi: 10.1097/00004583-199707000-00021

Keel, J. C., Smith, M. J., and Wassermann, E. M. (2001). A safety screening questionnaire for transcranial magnetic stimulation. *Clin. Neurophysiol.* 112, 720. doi: 10.1016/S1388-2457(00)00518-6

Kiers, L., Cros, D., Chiappa, K. H., and Fang, J. (1993). Variability of motor potentials evoked by transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol.* 89, 415–423. doi: 10.1016/0168-5597(93)90115-6

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., et al. (1993). Corticocortical inhibition in human motor cortex. *J. Physiol.* 471, 501–519.

Leinekugel, X., Khalilov, I., McLean, H., Caillard, O., Gaiarsa, J. L., Ben-Ari, Y., et al. (1999). GABA is the principal fast-acting excitatory transmitter in the neonatal brain. *Adv. Neurol.* 79, 189–201.

Levin, O., Fujiyama, H., Boisgontier, M. P., Swinnen, S. P., and Summers, J. J. (2014). Aging and motor inhibition: a converging perspective provided by brain stimulation and imaging approaches. *Neurosci. Biobehav. Rev.* 43, 100–117. doi: 10.1016/j.neubiorev.2014.04.001

Levinson, A. J., Fitzgerald, P. B., Favalli, G., Blumberger, D. M., Daigle, M., and Daskalakis, Z. J. (2010). Evidence of cortical inhibitory deficits in major depressive disorder. *Biol. Psychiatry* 67, 458–464. doi: 10.1016/j.biopsych.2009.09.025

Liguz-Lecznar, M., Lehner, M., Kaliszewska, A., Zakrzewska, R., Sobolewska, A., and Kossut, M. (2014). Altered glutamate/GABA equilibrium in aged mice cortex influences cortical plasticity. *Brain Struct. Funct.* doi: 10.1007/s00429-014-0752-6. [Epub ahead of print].

Mall, V., Berweck, S., Fietzek, U. M., Glocker, F. X., Oberhuber, U., Walther, M., et al. (2004). Low level of intracortical inhibition in children shown by transcranial magnetic stimulation. *Neuropediatrics* 35, 120–125. doi: 10.1055/s-2004-815834

McCormick, D. A., and Contreras, D. (2001). On the cellular and network bases of epileptic seizures. *Annu. Rev. Physiol.* 63, 815–846. doi: 10.1146/annurev.physiol.63.1.815

McDonnell, M. N., Orekhov, Y., and Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp. Brain Res.* 173, 86–93. doi: 10.1007/s00221-006-0365-2

McGinley, M., Hoffman, R. L., Russ, D. W., Thomas, J. S., and Clark, B. C. (2010). Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. *Exp. Gerontol.* 45, 671–678. doi: 10.1016/j.exger.2010.04.005

Moll, G. H., Heinrich, H., Wischer, S., Tergau, F., Paulus, W., and Rothenberger, A. (1999). Motor system excitability in healthy children: developmental aspects from transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol. Suppl.* 51, 243–249.

Müller, K., Kass-Iliyya, F., and Reitz, M. (1997). Ontogeny of ipsilateral corticospinal projections: a developmental study with transcranial magnetic stimulation. *Ann. Neurol.* 42, 705–711. doi: 10.1002/ana.410420506

Oberman, L. M., Rotenberg, A., and Pascual-Leone, A. (2013). Use of transcranial magnetic stimulation in autism spectrum disorders. *J. Autism Dev. Disord.* doi: 10.1007/s10803-013-1960-2. [Epub ahead of print].

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

Paulus, W., Classen, J., Cohen, L. G., Large, C. H., Di Lazzaro, V., Nitsche, M., et al. (2008). State of the art: pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. *Brain Stimul.* 1, 151–163. doi: 10.1016/j.brs.2008.06.002

Poznanski, E. O., Cook, S. C., Carroll, B. J., and Corzo, H. (1983). Use of the children's depression rating scale in an inpatient psychiatric population. *J. Clin. Psychiatry* 44, 200–203.

Radhu, N., de Jesus, D. R., Ravindran, L. N., Zanjani, A., Fitzgerald, P. B., and Daskalakis, Z. J. (2013). A meta-analysis of cortical inhibition and excitability using transcranial magnetic stimulation in psychiatric disorders. *Clin. Neurophysiol.* 124, 1309–1320. doi: 10.1016/j.clinph.2013.01.014

Rakhade, S. N., and Jensen, F. E. (2009). Epileptogensis in the immature brain: emerging mechanisms. *Nat. Rev. Neurol.* 5, 380. doi: 10.1038/nrneurol.2009.80

Wasserman, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin. Neurophysiol.* 113, 1165–1171. doi: 10.1016/S1388-2457(02)00144-X

Ziemann, U. (2004). TMS and drugs. *Clin. Neurophysiol.* 115, 1717–1729. doi: 10.1016/j.clinph.2004.03.006

Ziemann, U., Chen, R., Cohen, L. G., and Hallett, M. (1998). Dextromethorphan decreases the excitability of the human motor cortex. *Neurology* 51, 1320–1324. doi: 10.1212/WNL.51.5.1320

Ziemann, U., Lönnecker, S., Steinhoff, B. J., and Paulus, W. (1996a). Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann. Neurol.* 40, 367–378. doi: 10.1002/ana.410400306

Ziemann, U., Rothwell, J. C., and Ridding, M. C. (1996b). Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.* 496(pt 3), 873–881.

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

Received: 29 May 2014; accepted: 11 August 2014; published online: 02 September 2014.

Citation: Croarkin PE, Nakonezny PA, Lewis CP, Zaccariello MJ, Huxsahl JE, Husain MM, Kennard BD, Emslie GJ and Daskalakis ZJ (2014) Developmental aspects of cortical excitability and inhibition in depressed and healthy youth: an exploratory study. Front. Hum. Neurosci. ***8****:669. doi: 10.3389/fnhum.2014.00669*

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

frontiers in
HUMAN NEUROSCIENCE

METHODS ARTICLE
published: 19 September 2014
doi: 10.3389/fnhum.2014.00739

Pediatric stroke and transcranial direct current stimulation: methods for rational individualized dose optimization

Bernadette T. Gillick[1]*, Adam Kirton[2], Jason B. Carmel[3], Preet Minhas[4] and Marom Bikson[4]

[1] Department of Physical Medicine and Rehabilitation, Program in Physical Therapy, University of Minnesota, Medical School, Minneapolis, MN, USA
[2] Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada
[3] Weill-Cornell Medical College, Burke Medical Research Institute, White Plains, NY, USA
[4] Department of Biomedical Engineering, The City College of New York of CUNY, New York, NY, USA

Edited by:
Lindsay M. Oberman, E. P. Bradley Hospital, USA

Reviewed by:
Alexander Rotenberg, Boston Children's Hospital, USA
Kate Hoy, Monash University, Australia

***Correspondence:**
Bernadette T. Gillick, Department of Physical Medicine and Rehabilitation, Program in Physical Therapy, University of Minnesota, Medical School, 420 Delaware Street SE, MMC 388, Minneapolis, MN 55455, USA
e-mail: gillick@umn.edu

Background: Transcranial direct current stimulation (tDCS) has been investigated mainly in adults and doses may not be appropriate in pediatric applications. In perinatal stroke where potential applications are promising, rational adaptation of dosage for children remains under investigation.

Objective: Construct child-specific tDCS dosing parameters through case study within a perinatal stroke tDCS safety and feasibility trial.

Methods: 10-year-old subject with a diagnosis of presumed perinatal ischemic stroke and hemiparesis was identified. T1 magnetic resonance imaging (MRI) scans used to derive computerized model for current flow and electrode positions. Workflow using modeling results and consideration of dosage in previous clinical trials was incorporated. Prior *ad hoc* adult montages vs. *de novo* optimized montages provided distinct risk benefit analysis. Approximating adult dose required consideration of changes in both peak brain current flow and distribution which further tradeoff between maximizing efficacy and adding safety factors. Electrode size, position, current intensity, compliance voltage, and duration were controlled independently in this process.

Results: Brain electric fields modeled and compared to values previously predicted models (Datta et al., 2011; Minhas et al., 2012). Approximating conservative brain current flow patterns and intensities used in previous adult trials for comparable indications, the optimal current intensity established was 0.7 mA for 10 min with a tDCS C3/C4 montage. Specifically 0.7 mA produced comparable peak brain current intensity of an average adult receiving 1.0 mA. Electrode size of 5×7 cm^2 with 1.0 mA and low-voltage tDCS was employed to maximize tolerability. Safety and feasibility confirmed with subject tolerating the session well and no serious adverse events.

Conclusion: Rational approaches to dose customization, with steps informed by computational modeling, may improve guidance for pediatric stroke tDCS trials.

Keywords: pediatrics, stroke, hemiparesis, modeling, transcranial direct current stimulation

INTRODUCTION

Transcranial direct current stimulation (tDCS) modulates human cortical excitability and improved motor outcomes in adults with and without neurologic diagnoses (Brunoni et al., 2012). For reasons of safety, cost, portability and potential promise for improved outcomes in children, we desired to investigate the application of tDCS in pediatric stroke. Considering the potential variability in dosing for the child's brain, due to difference in brain size and anatomy, the direct transition from adult dosing to the safe and effective dose in a child has not yet been established. The relationship between the dose of stimulation (defined as the externally controlled parameters) (Peterchev et al., 2012) and brain current flow can be complex, such that computational models are used in dose design (Bikson et al., 2012). While for adults there are generally adopted principles regarding directions of effect (anodal excites, cathodal inhibits) and dosing (10–20 min, 0.5–2.0 mA), emerging evidence suggests even minor dose or brain integrity changes can lead to opposite effects (Fritsch et al., 2010; Fricke et al., 2011; Sohn et al., 2012; Batsikadze et al., 2013; Hasan et al., 2013; Schabrun et al., 2013). Moreover, modeling and imaging studies suggest limitations in the conventional effects of anodal and cathodal stimulation (Datta et al., 2009; Antal et al., 2012, 2014; Peña Gómez et al., 2012; Wagner et al., 2012; Rahman et al., 2013).

In contrast to individual-specific cortical excitability testing using TMS, subject-specific titration of dose in tDCS is rare,

though probably equally important. When working with children, (even when adopting a technique such as tDCS, with a compelling safety record in adults) questions of safety gain new importance. Subject specific factors such as differences in brain size, water content, myelination, proximity of the brain to the skull and other characteristics of the developing brain may alter safety/tolerability and optimal dose (Minhas et al., 2012; Kessler et al., 2013). A broad review of available clinical reports integrated with computational models provides a basis to address this issue. This report describes the methods used in consideration of a pilot safety study applying a single-session of tDCS in a child with hemiparesis due to perinatal stroke and more broadly presents possible methodology for dose customization in pediatric populations (clinicaltrials.gov NCT01636661). A child who met the inclusion criteria for the pilot was identified, and the following methods were employed to establish safety and feasibility of the application of tDCS before the trial began. The description below identifies the methods in detail, while the trial itself is reported elsewhere (Gillick et al., in press). The study was approved by the University of Minnesota Institutional Review Board, and the Clinical and Translational Science Institute, Written and verbal parental consent and child assent was obtained. Modeling analysis of de-identified data at City College of New York of CUNY is IRB exempt.

COMPONENTS OF TRIAL DESIGN

At this time, a standardized tDCS dose has not been established, neither in adults or children. Understanding this, in order to determine tDCS parameters for this subject, we reviewed existing clinical experience and publications regarding current parameters of tDCS dose (including current polarity and intensity) from healthy adults and adults with stroke. Computational models were used to relate brain current flow in these cases to that in a child with stroke and resultant hemiparesis. Broadly, the goal was to use a stimulation paradigm that (1) produced the same brain current intensity (not necessarily same external dose) as in the adult cortex; and (2) targeted the motor cortices of the brain and the interactions between the hemispheres. However, a detailed methodological analysis, driven by rational trial design, and divergent approaches toward this aim must be selected balancing tradeoffs between innovation/conservatism, efficacy/safety, and putative non-monotonic dose response. Our process involved seven specific steps from concept to implementation: (1) gathering of subject-specific information; (2) formulating the desired clinical outcome; (3) considering constraints that may influence decisions; (4) defining brain current flow criterion; (5) investigating potential montages; (6) modeling montages to estimate brain current flow; and (7) determination of subject-specific dose. These stages are elaborated below and summarized in **Figure 1**.

SUBJECT-SPECIFIC INFORMATION

This pilot study focused on a 10-year-old child with a diagnosis of arterial perinatal ischemic stroke. The child had a normal perinatal history but presented in infancy with hemiparesis and was found to have a focal infarction (Kirton, 2013). Magnetic resonance imaging (MRI) confirmed a distal M1 segment of the middle cerebral artery stroke with involvement of peri-Rolandic regions of the right frontal and parietal lobes and centrum semiovale but sparing of the basal ganglia. Left hemiparesis was first noted at 4 months of age. At 10 years, the child had moderate hemiparetic cerebral palsy with a Manual Ability Classification System Scale Score (Eliasson et al., 2006, 2007) of II—"Handles Most Objects but with Somewhat Reduced Quality and/or Speed of Achievement". The lower extremity was less affected, spasticity was minimal, and she had not received any new rehabilitation treatments within 6 months. She was otherwise developmentally normal, did not have epilepsy, and was not taking any neuroactive medications. Informed consent was obtained as part of a pilot safety and feasibility study on the application of tDCS in children with congenital hemiparesis (ClinicalTrials.gov Identifier: NCT01636661).

DESIRED CLINICAL OUTCOME

Emerging evidence combining animal and human studies has defined models of developmental motor plasticity following perinatal stroke. These models suggest that inhibition of the non-lesioned hemisphere might enhance motor learning in the lesioned hemisphere, possibly via effects on excessive ipsilateral projections or disordered interhemispheric inhibition. Therefore, while the underlying neurophysiology is likely different, the strategy in adult stroke of inhibiting the non-lesioned hemisphere with non-invasive stimulation to enhance therapy may be applicable to children with perinatal stroke-induced hemiparesis (Hsu et al., 2012; Marquez et al., 2013). Studies in non-invasive brain stimulation and specifically in the use of repetitive transcranial magnetic stimulation (rTMS) have recently shown promising results in children with stroke, requiring further research to determine further clinical merit in this population (Kirton et al., 2008, 2010; Gillick et al., 2013).

CONSTRAINTS

DOSE

Given the volume of safety/efficacy data using a limited set of standard montages (Nitsche and Paulus, 2000; Brunoni et al., 2012), and because incremental changes may allow precise detection of potential adverse events, a historical approach of previous research and modeling was deemed appropriate. To ensure safety and to decrease the likelihood of side effects, adult protocols have typically utilized 0.2–2.0 mA with duration of stimulus $\leq$30 min (Rothwell, 2012; Batsikadze et al., 2013). However, we propose that when initiating studies in novel, potentially vulnerable populations (such as children) and/or injured brain (such as following stroke) approaches in adults should be re-examined using the latest available tools and scientific data to adjust for developmental variation in children. Furthermore, identification of possible risks and risk mitigation were identified (**Table 1**).

DEVICE

We used a Soterix Limited Total Energy (LTE) 1×1 tDCS unit which is limited to maximal current of 1.5 mA and 20 min maximum duration of stimulation. For this specific modeling, we first determined our current needs, and then the device model. The LTE also has a built-in sham stimulation mode,

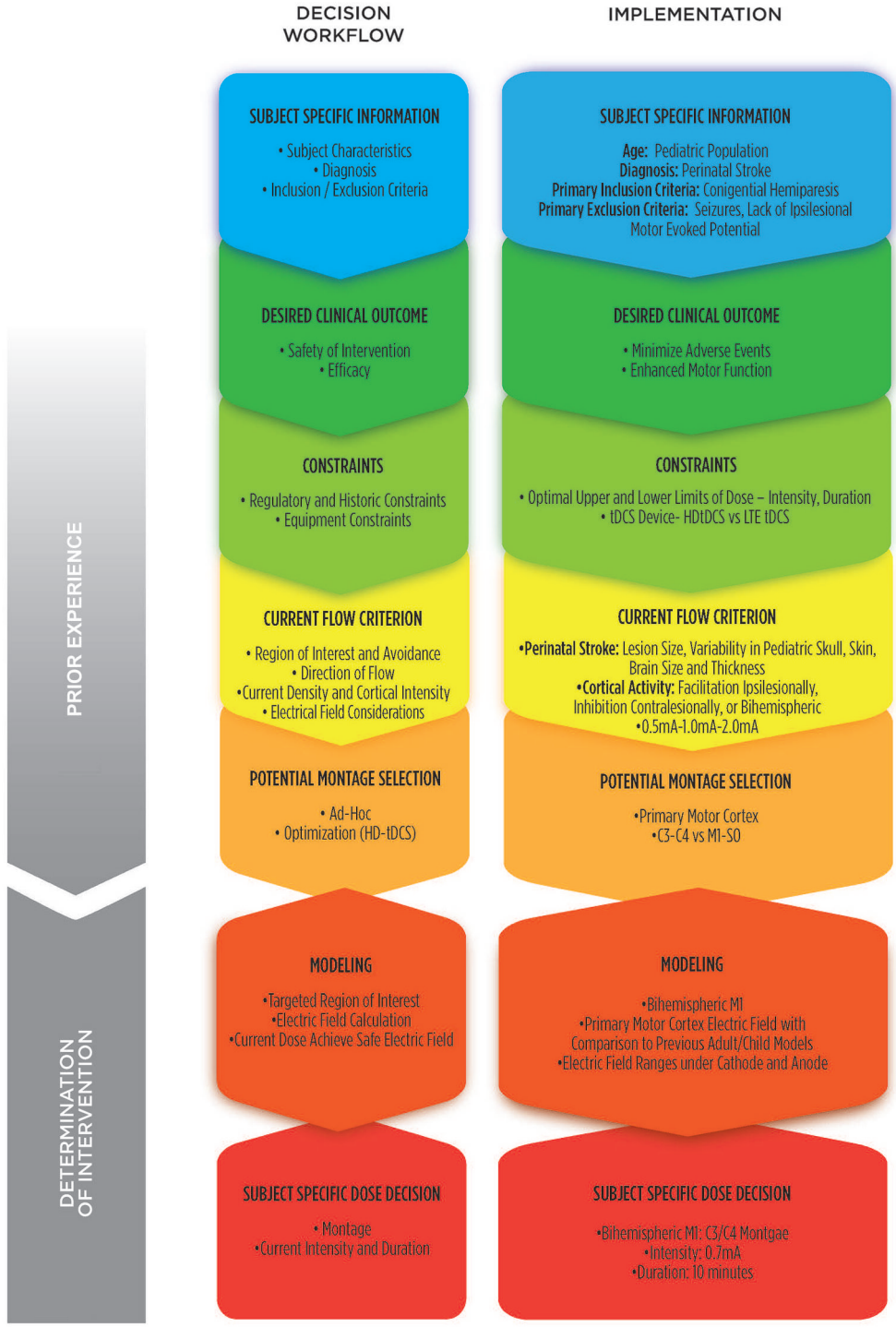


FIGURE 1 | Conceptual methodology for determination of dose. Theoretical 7-step compartmentalization of decision workflow (left column) and implementation considerations specific to this case study of a 10 year-old child with hemiparesis due to perinatal stroke (right column). Incorporation of prior experience in the dose decision process coupled with modeling allows value-determination of subject-specific dose decisions for implementation.

providing ramp-up and ramp-down stimulation at the beginning and end of the placebo session. During the 30–60 s period of time, the current is increased in order to reach the targeted dose and then discontinued. Two additional features reinforce safety. (1) An adaptive impedance monitoring feature provides continuous visual indication of electrode quality before and during

stimulation. If impedance increases, producing a typical high voltage, the device not only automatically reduces the current output in accordance with changes in resistance but also provides an alert; and (2) a current monitoring feature acts as an independent current meter and provides continuous visual indication of the instant output of the device. This component adds redundancy to the safety of device and confirms the current setting. Pediatric size EasyStraps were used to ensure proper contact with the skin for each individual. Tolerability was enhanced by the RELAX feature which allows, if enhanced discomfort is expressed, transient reduction in current without interrupting or aborting the trial. Once comfort is re-established, the current may then again be adjusted. The device is limited to two electrodes and used with 5 × 7 EasyPads providing further dose design constraints (i.e., number of electrodes, electrode size and maximum current).

CURRENT FLOW CRITERION

Transcranial direct current stimulation alters brain function by polarizing the brain (Nitsche et al., 2008; Rothwell, 2012). Generally, brain regions exposed to higher current densities would be more likely to be influenced and each brain region receives various current intensity depending on the electrode montage. It is precisely because brain current flow is not a simple function of electrode montage (e.g., current is not restricted to only under the electrodes) that current flow criterion is described in terms of brain targets for neuromodulation, rather than scalp electrode position. Moreover, the intensity of current reaching the brain for any given applied electrode current can vary significantly depending on the montage and subject anatomy. Thus while electrode dose is controlled at the head surface (current in mA provided and montage), current flow criterion indicates the desired current intensity at the brain target level (in terms of electric field in units of V/m or A/m^2).

With limited research in children, we recognized the need to re-examine adult-based practices including the effects of relatively high dose stimulation in adults. In deciding on desired brain electric field intensity it is typical to reference "gold-standard" experimentation where modulation of Transcranial Magnetic Stimulation (TMS)-motor evoked potentials (MEPs) by tDCS was quantified—with the strong caveat that TMS-MEP modulation and behavioral changes are only putatively linked. The M1-S0 montage conventionally used in these studies is shown to produce lasting TMS-MEP changes following several minutes of stimulation and with polarity-specific changes at intensities at 1 mA (Nitsche and Paulus, 2000)—where 1 mA corresponds to approximately 0.3 V/m of electric field in motor regions in adults (Datta et al., 2012).

Importantly, recent findings suggest that increasing tDCS current intensity may change the direction of these effects (Batsikadze et al., 2013). For example, in a study of 21 healthy adults, 2.0 mA cathodal (0.6 V/m brain electric field) tDCS for a duration of 20 min over the motor cortex resulted in enhancement of cortical excitability, *not* inhibition. An ongoing investigation of the application of tDCS in children and adolescents ages 10–18 reports that 1 mA anodal and cathodal stimulation over the motor cortex *both* produced an increase in the amplitude of the MEPs (Moliadze et al., 2013) which may reflect the higher brain current densities (e.g., ∼0.6 V/m) produced in children for the same total current (see Section Modeling below).

Though non-linear (non-monotonic) TMS-MEP dose response is observed at higher tDCS intensities, at least across "moderate" tDCS stimulation intensities response seems consistent, at least for healthy inactive motor cortex. A study of 14 healthy adults investigated anodal tDCS over the motor cortex for 10 min at three different intensities—0.8, 1.0 and 1.2 mA—and found no difference in modulation of cortical excitability or inhibition (Kidgell et al., 2013a). One can therefore speculate that approximately 0.3 V/m for several minutes is a reasonable approach. Changes are polarity dependent, with anode/cathode tending to produce increased/decreased TMS-MEP amplitude, respectively. Importantly, increasing intensity or duration does not necessary magnify effects and the direction of changes can reverse (e.g., 2 mA cathodal is excitatory). Our decisions were (1) to limit total current to 2 mA or less based on skin tolerability and to use electrodes validated for tDCS; and (2) to limit current further as required to match electric fields corresponding to approximately 1 mA in adult (current flow criterion).

POTENTIAL MONTAGE SELECTION

Potential Montage Selection involves a selection of candidate montage to explore further with computational modeling. This involves integration of prior clinical trials and modeling; (1) with the subject specific information, desired clinical outcome, constraints and current flow criterion (**Figure 1**).

For our study two candidate montages were explored leveraging computational models to target the primary motor cortex (M1): (1) a supraorbital montage (M1/SO); and (2) a bihemispheric montage using the International 10/20 EEG System designation (C3/C4). Considering the M1/SO montage, the cathode would have been placed over the contralesional motor cortex and anode over the ipsilesional supraorbital region with the intent to inhibit contralesional effects upon the ipsilesional cortex. The bihemispheric montage was considered with the cathode placed over the contralesional cortex to down-regulate excitability, and the anode positioned to facilitate excitation of the ipsilesional cortex. An individualized head model was developed based on the child's 1 mm^3 resolution T1-weighted MRI scans obtained from a Siemens Trio Scanner with a 12-element head coil using methods described previously (Datta et al., 2012, 2013; Marquez et al., 2013).

RESULTS

MODELING

Consistent with previous models in adult and children, use of two large electrodes produced diffuse current flow between and under the electrodes (Bikson et al., 2012; **Figure 2**). The current flow pattern produced in our subject was, in this sense, broadly consistent with the typical current flow patterns produced by the M1/SO and C3/C4 montages used in prior clinical trials. The peak electric field produced in the regions-of-interest (under the electrodes) as well as across the entire brain (as using two pads typically produces peak electric field between electrodes) was compared for this subject and adapted from prior adult and pediatric models (**Table 2**). As noted previously, there are large (several

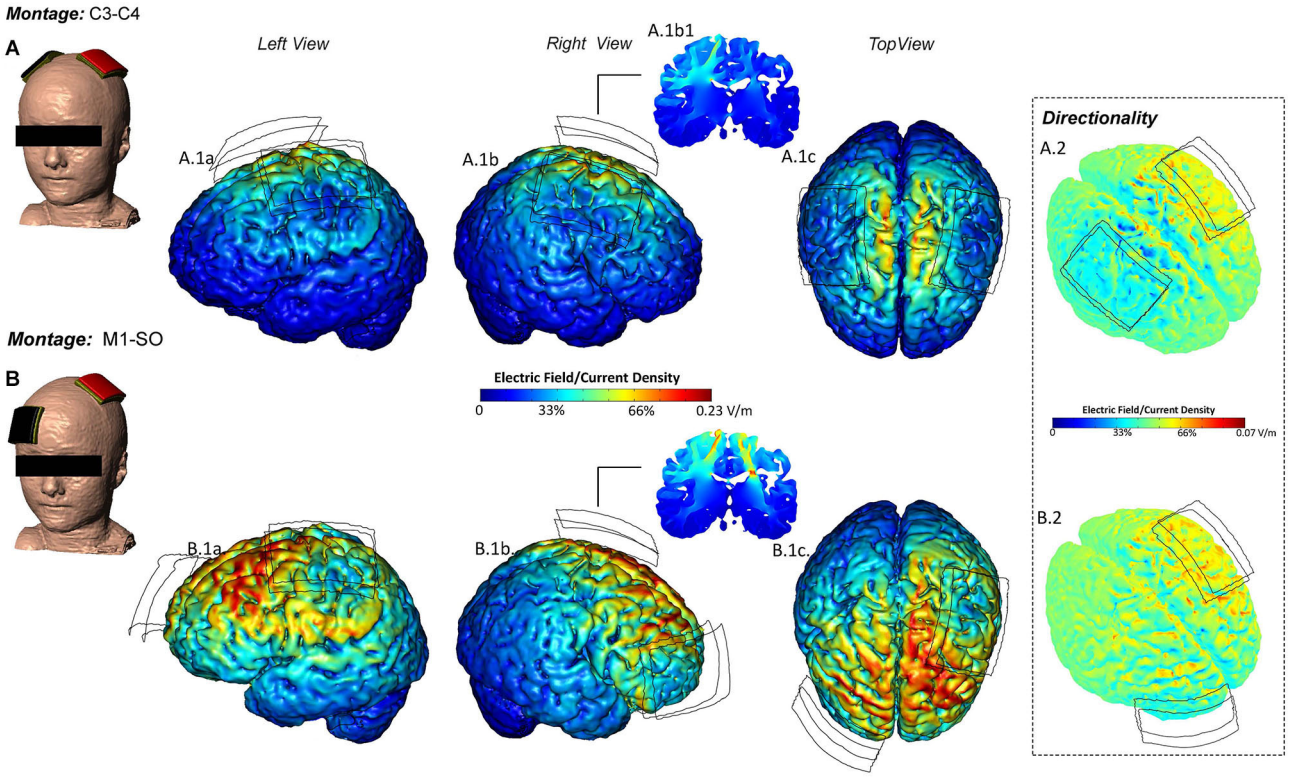


FIGURE 2 | Current flow predictions during tDCS in individual pediatric model for the M1-SO and Lateralized Temporal montages. *M1-SO-* The center of anode (red) was positioned on the motor strip and the cathode (black) was positioned over the contraletral supraorbital area. At 0.7 mA applied current, the peak electric field was 0.23 V/m. *C3-C4-* The center of anode (red) was positioned over the left temporal lobe and the cathode (black) was positioned contralateral to the anode (M-O). At 0.7 mA applied current, the peak electric field was 0.29 V/m. EF plots in the left, right and top views, are shown respectively **(A.1a–c, B.1a–c)**. Cross-sectional coronal electric field plots were taken from the center of the anode **(A.1.b1, B.1.b1)**. Directionality plots were also plotted. The red corresponds to current flowing inwards, the green corresponds to a net flow of zero, and the blue corresponds to current flowing outwards **(B.1–B.2)**.

fold) differences in peak electric fields even among adults (Datta et al., 2012; Edwards et al., 2013). The peak regional and global electric fields in our subject were comparable to those in previously modeled children, which are moderately higher than those in previously modeled adults (though comparable to the most sensitive adults). As modeled, application of 0.7 mA in our subject would produce peak electric field comparable to an average adult receiving 1.0 mA with a comparable distribution of current flow.

SUBJECT-SPECIFIC DOSE DECISION

Intensity and duration

After all prior experience, constraints, and modeling considerations, it was determined that for this single-session intervention a current intensity of 0.7 mA for 10 consecutive minutes would be most appropriate to accomplish the primary purpose of the pilot—establishing tDCS safety and feasibility in children. The determination of the model for this child was based on the adult model. Current flow models in the adult present with a wide range of variability. For example, the potential exists for (1) paradoxical stimulation under the cathode (Batsikadze et al., 2013); (2) typical montages stimulating between the electrodes; and (3) accessing deeper structures (Dasilva et al., 2012). The low intensity decided upon was lower than an adult equivalent of approximately 1.0 mA. The intent was to prevent "direction flipping" of stimulation while at the same time to rely on available recent adult safety data with a concomitant assumption of similarities in electrophysiology in response to stimulation in the brains of both an adults and children (Brunoni et al., 2011; Marquez et al., 2013). Considering the child's diagnosis of perinatal ischemic stroke, a challenge exists in attempts to incorporate into our calculations the lesion location and size as well as the variation in conductivity (cortex, cerebral spinal fluid) (Datta et al., 2009; Bikson et al., 2012). Incorporating neuromodulatory tools such as TMS as a locator for motor hotspots may provide additional knowledge regarding individual cortical excitability (Gillick et al., 2013).

Montage

Specific to the child with focal hemispheric lesion, a translational goal of the application of tDCS is to improve motor outcomes (Schlaug et al., 2008). Cathodal tDCS (M1-SO montage) has shown significant motor improvements in stroke, and specifically when coupled with rehabilitation (Nair et al., 2011). In children with language disorders, application of a similar montage-inferior frontal gyrus/contralateral SO montage was found to be safe and

Table 1 | Possible adverse events related to transcranial direct current stimulation (tDCS) and risk mitigation.

Study procedure	Anticipated risks	Risk mitigation
tDCS	Burn- Electrolysis	Ensure proper electrode contact with skin
tDCS	Stimulation in subjects with reduced sensation	Assess sensation, avoid placing electrodes over areas of decreased sensation
tDCS	Stimulation over broken skin, reduced resistance	Assess skin integrity, avoid placement of electrodes over recent shaving, skin defects
tDCS	Stimulation over conductive implants	Screen appropriately for exclusion criteria of implants
tDCS	Stimulation over a tumor which may alter metabolic activity	Screen appropriately for exclusion criteria of neoplasm.
tDCS	Threshold altering pharmacologic agent	Physician review of each medical record for determination of appropriateness for study inclusion.
tDCS	Itching, Tingling, Burning Sensation in the area of the electrodes	Ensure proper contact of surface electrodes with skin. Maintain current dosage within low-range of researched dosages. Ensure that electrode sponges are properly sanitized and that saline solution is appropriately employed.
tDCS	Headache	Ensure that headband securing electrodes is in proper placement, yet not to the level of impingement of scalp area. Maintain current dosage within low range of delivery.
tDCS	Pain- Neck, Scalp	Ensure that electrodes are in proper contact with skin and adjust head position as needed for comfort.
tDCS	Skin Redness	Ensure proper electrode position and proper level of moisture to even stimulation across the electrode
tDCS	Fatigue, Sleepiness	Screen for continuous effect at follow-up visit.
tDCS	Concentration or Mood changes	Evaluate cognitive status through physician examination and psychometric testing at three time points.

feasible. Although we found higher intensities within a range of comparable safety using an M1/SO montage (**Figure 2**), we decided upon a bihemispheric C3 contralesional cathodal/C4 ipsilesional anodal montage. Considering direct involvement of both hemispheres, we decided to investigate the safety and feasibility of tDCS intensity/application to the pediatric motor cortex bilaterally through an in-out lateralized pattern of activity between lesioned and non-lesioned hemispheres. This montage has been applied in both neurologically involved (e.g., stroke) and healthy adult populations to investigate enhancement of motor performance (Bolognini et al., 2011; Lefebvre et al., 2012; Kidgell et al., 2013b). We decided to maintain electrode sizes used in conventional (adult) trials (as opposed to "child size" electrodes), that combined with the use of low-voltage and reduced current intensity (0.7 mA) should enhance tolerability since current density at the electrode is low minimizing skin sensation and potential

Table 2 | Electrical field (EF) ranges and peaks, in volts per meter, for each modeled head, by montage.

		Montage		
		M1[A]–SO[C]	**Lateralized motor C3[A]–C4[C]**	**Modeled sponge size**
Child 1 (Normal Anatomy)	EF Range (C)	0.11–0.27	0.25–0.37	5×5 sponge pads
	EF Range (A)	0.14–0.30	0.26–0.44	
	EF Peak	0.33	0.44	
Child 2 (Normal Anatomy)	EF Range (C)	0.08–0.31	0.16–0.40	5×5 sponge pads
	EF Range (A)	0.18–0.44	0.19–0.40	
	EF Peak	0.44	0.40	
Child 3 Clinical Hemiparesis	EF Range (C)	0.05–0.28	0.05–0.23	5×7 sponge pads
	EF Range (A)	0.05–0.33	0.07–0.23	
	EF Peak	0.33	0.42	
Adult 1 (Normal Anatomy)	EF Range (C)	0.11–0.30		5×5 sponge pads
	EF Range (A)	0.11–0.30		
	EF Peak	0.36		
Adult 2 (Normal Anatomy)	EF Range (C)	0.08–0.28		5×5 sponge pads
	EF Range (A)	0.07–0.24		
	EF Peak	0.29		
Adult 3 (Normal Anatomy)	EF Range (C)	0.04–0.19	0.09–0.18	5×5 sponge pads
	EF Range (A)	0.07–0.20	0.05–0.21	
	EF Peak	0.23	0.21	

[A] denotes anode and [C] denotes cathode. Detailed descriptions of montages are contained in the text (Adapted from Kessler et al., 2013).

irritation. The stimulation was expected to be well tolerated given the total current (0.7 mA) selected to provide average adult brain electric fields (at 1.0 mA) and use of an electrode design validated for even higher intensities (up to 2.0 mA).

The goal of this study was to determine the intensity of stimulation and location of electrodes for this tDCS session in children with hemiparesis. After this integrative dose design consideration, including modeling, we determined that for this single-session intervention a current intensity of 0.7 mA in a bihemispheric C3 contralesional cathodal/C4 ipsilesional anodal montage for 10 consecutive minutes would be within historic safety limits based on generated brain electric fields to establish safety in tDCS application with translated to tolerable use in children. This bi-hemispheric montage over an M1-S0 montage was chosen to induce neuromodulation between the two hemispheres, lesioned and non-lesioned, with the potential to increase neuronal activity on the lesioned hemisphere and transiently inhibit over-activity of the nonlesioned hemisphere. The child tolerated this session well, with a sensation reported of mild, tolerable tingling under the electrodes within the first minute of stimulation which extinguished thereafter. The child reported no discomfort, nor did any adverse events occur (Gillick et al., in press).

DISCUSSION

The montage and parameters of dose represent a modification of historical adult methods facing the unknowns of pediatric stroke lesions and tDCS interventions. In summary, our aim was to design a tDCS protocol to determine an intervention applicable to pediatric stroke as there is evidence for efficacy of this application in adult stroke. We sought to determine this current intensity and montage allowing us to assess the electric field generation over the target region of M1. We used computational modeling and incorporation of past models to test the variation in parameters and decided upon those which supported safety and feasibility.

In progressing through a rational work-flow for montage selection, including assumptions about disease etiology and trade-offs in "best" montage design which require informed judgment based on integration across scientific and clinical tDCS literature we described our process. First, the study design started with safety as the primary objective. This requires a balance between minimizing dose (e.g., zero risk at zero dose) with maximizing dose to make results as relevant as possible for subsequent efficacy trials. This balance influences all subsequent decisions. Next, based on an assumption of asymmetric dysfunction we adopted a bicephalic ("lateralized") approach. Then, though adult trials have used 2.0 mA, we recognized that clinical neurophysiologic studies increase non-monotonic dose response with high (2.0 mA) cathodal stimulation becoming excitatory. Based on our assumption of disease etiology and adopting a conservative approach, we elected to *approximate* a 1.0 mA adult dose. We also elected a relatively low duration. However both the intensity and duration determined were still within a range expected to produce significant lasting changes in brain excitability. Thereafter, to approximate electric field produced in adults, we determined it was necessary to decide which electric fields since the distribution in the brain is variable (e.g., electric field in target, average electric field, peak electric field across the brain). Moreover, in doing so we assumed no specific difference in susceptibility of the pediatric (injured) brain from adult in regards to safety or efficacy, but erred on approximating a "low" adult dose (intensity, duration). Finally, with the above caveats we elected to approximate a montage found successful in adult trials while recognizing the montage produced diffuse electrode flow through much of the brain, including deep brain structures. Reinforcing this approach, modeling predicted that the pattern of current flow across our subject was comparable to that in previous studies with adult subjects with no significant distortion due to the presence of the lesion.

Although this method may not be ideal, i.e., an individual representation of the *optimal* electric field generated at a target, the design of the model for this child attempted to incorporate the knowledge of tDCS modeling in adults and modify to a brain in a child with congenital hemiparesis. The models assume that the damaged brain regions have similar conductivity of cerebral spinal fluid while the peri-lesional area has the conductivity of healthy brain (Bijsterbosch et al., 2012). However, increased precision could incorporate changes in conductivity considering resultant gliosis or other pathologic processes that accompany cerebral lesions (Ruohonen and Karhu, 2012; Huang et al., 2014).

True comparability among future pediatric studies can only be established if each tDCS protocol articulates the rationale behind its methods, as well as current intensity, electrode size, location and stimulation duration (Nitsche et al., 2008; Batsikadze et al., 2013). Assessment of physiologic outcomes, serial applications and the longitudinal effects in combination with rehabilitation should include a thorough accounting of safety and dosing parameters.

ACKNOWLEDGMENTS

Research reported was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health Award Number UL1TR000114 of the National Institutes of Health (NIH) to the University of Minnesota Clinical and Translational Science Institute (CTSI), and CTSI Biostatistical Design and Analysis Center as well as the Minnesota Medical Foundation and the Department of Defense (Air Force Research Lab), the Wallace H Coulter Foundation, and a NIH Award to The City College of New York. We thank Sally Jones for assistance in manuscript review and Katie Tobin for graphic design.

REFERENCES

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., Kovács, G., Chaieb, L., Cziraki, C., Paulus, W., and Greenlee, M. W. (2012). Cathodal stimulation of human MT+ leads to elevated fMRI signal: a tDCS-fMRI study. *Restor. Neurol. Neurosci.* 30, 255–263. doi: 10.3233/RNN-2012-110208

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

Bijsterbosch, J. D., Barker, A. T., Lee, K. H., and Woodruff, P. W. (2012). Where does transcranial magnetic stimulation (TMS) stimulate? modelling of induced field maps for some common cortical and cerebellar targets. *Med. Biol. Eng. Comput.* 50, 671–681. doi: 10.1007/s11517-012-0922-8

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

Bolognini, N., Vallar, G., Casati, C., Latif, L. A., El-Nazer, R., Williams, J., et al. (2011). Neurophysiological and behavioral effects of tDCS combined with constraint-induced movement therapy in poststroke patients. *Neurorehabil. Neural Repair* 25, 819–829. doi: 10.1177/1545968311411056

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., 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

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

Datta, A., Baker, J. M., Bikson, M., and Fridriksson, J. (2011). Individualized model predicts brain current flow during transcranial direct-current stimulation treatment in responsive stroke patient. *Brain Stimul.* 4, 169–174. doi: 10.1016/j.brs.2010.11.001

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

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

Datta, A., Zhou, X., Su, Y., Parra, L. C., and Bikson, M. (2013). Validation of finite element model of transcranial electrical stimulation using scalp potentials: implications for clinical dose. *J. Neural Eng.* 10, 036018. doi: 10.1088/1741-2560/10/3/036018

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

Eliasson, A. C., Krumlinde-Sundholm, L., Rösblad, B., Beckung, E., Arner, M., Ohrvall, A. M., et al. (2006). The manual ability classification system (MACS) for children with cerebral palsy: scale development and evidence of validity and reliability. *Dev. Med. Child Neurol.* 48, 549–554. doi: 10.1111/j.1469-8749.2006.tb01313.x

Eliasson, A. C., Krumlinde-Sundholm, L., Rösblad, B., Beckung, E., Arner, M., Ohrvall, A. M., et al. (2007). Using the MACS to facilitate comunication about manual abilities of children with cerebral palsy. *Dev. Med. Child Neurol.* 49, 156–157.

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

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Gillick, B. T., Feyma, T., Menk, J., Usset, M., Vaith, A., Wood, T., et al. (in press). Safety and feasibility of transcranial direct current stimulation in pediatric hemiparesis: a randomized, controlled pilot study. *Phys. Ther.*

Gillick, B., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., Thomas, W., et al. (2013). Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: a randomized controlled trial. *Dev. Med. Child Neurol.* 56, 44–52. doi: 10.1111/dmcn.12243

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

Hsu, W.-Y., Cheng, C.-H., Liao, K.-K., Lee, I.-H., and Lin, Y.-Y. (2012). Effects of repetitive transcranial magnetic stimulation on motor functions in patients with stroke: a meta-analysis. *Stroke* 43, 1849–1857. doi: 10.1161/strokeaha.111.649756

Huang, L., Wu, Z.-B., Zhuge, Q., Zheng, W., Shao, B., Wang, B., et al. (2014). Glial scar formation occurs in the human brain after ischemic stroke. *Int. J. Med. Sci.* 11, 344–348. doi: 10.7150/ijms.8140

Kessler, S. K., Minhas, P., Woods, A. J., Rosen, A., Gorman, C., and Bikson, M. (2013). Dosage considerations for transcranial direct current stimulation in children: a computational modeling study. *PLoS One* 8:e76112. doi: 10.1371/journal.pone.0076112

Kidgell, D. J., Daly, R. M., Young, K., Lum, J., Tooley, G., Jaberzadeh, S., et al. (2013a). Different current intensities of anodal transcranial direct current stimulation do not differentially modulate motor cortex plasticity. *Neural Plast.* 2013:603502. doi: 10.1155/2013/603502

Kidgell, D. J., Goodwill, A. M., Frazer, A. K., and Daly, R. M. (2013b). Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. *BMC Neurosci.* 14:64. doi: 10.1186/1471-2202-14-64

Kirton, A. (2013). Can noninvasive brain stimulation measure and modulate developmental plasticity to improve function in stroke-induced cerebral palsy? *Semin. Pediatr. Neurol.* 20, 116–126. doi: 10.1016/j.spen.2013.06.004

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

Kirton, A., Deveber, G., Gunraj, C., and Chen, R. (2010). Cortical excitability and interhemispheric inhibition after subcortical pediatric stroke: plastic organization and effects of rTMS. *Clin. Neurophysiol.* 121, 1922–1929. doi: 10.1016/j.clinph.2010.04.021

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

Marquez, J., van Vliet, P., McElduff, P., Lagopoulos, J., and Parsons, M. (2013). Transcranial direct current stimulation (tDCS): does it have merit in stroke rehabilitation? A systematic review. *Int. J. Stroke* doi: 10.1111/ijs.12169. [Epub ahead of print].

Minhas, P., Bikson, M., Woods, A. J., Rosen, A. R., and Kessler, S. K. (2012). Transcranial direct current stimulation in pediatric brain: a computational modeling study. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2012, 859–862. doi: 10.1109/embc.2012.6346067

Moliadze, V., Schmanke, T., Bassüner, S., Freitag, C., and Siniatchkin, M. (2013). "The effects of direct current stimulation on motor cortex excitability in children and adolescents," in *Abstract of Presentations from the International Conference on Non-Invasive Brain Stimulation.* March 19–21.

Nair, D. G., Renga, V., Lindenberg, R., Zhu, L., and Schlaug, G. (2011). Optimizing recovery potential through simultaneous occupational therapy and non-invasive brain-stimulation using tDCS. *Restor. Neurol. Neurosci.* 29, 411–420. doi: 10.3233/RNN-2011-0612

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

Peña Gómez, C., Sala Lonch, R., Junqué, C., Clemente, I. C., Vidal, D., Bargalló, N., et al. (2012). Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. *Brain Stimul.* 5, 252–263. doi: 10.1016/j.brs.2011.08.006

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

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., et al. (2013). Cellular effects of acute direct current stimulation: somatic and

synaptic terminal effects. *J. Physiol.* 591, 2563–2578. doi: 10.1113/jphysiol.2012.247171

Rothwell, J. C. (2012). Clinical applications of noninvasive electrical stimulation: problems and potential. *Clin. EEG Neurosci.* 43, 209–214. doi: 10.1177/1550059412444973

Ruohonen, J., and Karhu, J. (2012). tDCS possibly stimulates glial cells. *Clin. Neurophysiol.* 123, 2006–2009. doi: 10.1016/j.clinph.2012.02.082

Schabrun, S. M., Chipchase, L. S., Zipf, N., Thickbroom, G. W., and Hodges, P. W. (2013). Interaction between simultaneously applied neuromodulatory interventions in humans. *Brain Stimul.* 6, 624–630. doi: 10.1016/j.brs.2012.09.009

Schlaug, G., Renga, V., and Nair, D. (2008). Transcranial direct current stimulation in stroke recovery. *Arch. Neurol.* 65, 1571–1576. doi: 10.1001/archneur.65.12.1571

Sohn, M. K., Kim, B. O., and Song, H. T. (2012). Effect of stimulation polarity of transcranial direct current stimulation on non-dominant hand function. *Ann. Rehabil. Med.* 36, 1–7. doi: 10.5535/arm.2012.36.1.1

Wagner, S., Rampersad, S., Aydin, U., Vorwerk, J., Neuling, T., Herrmann, C. S., et al. (2012). Volume conduction effects in tDCS using a 1mm geometry-adapted hexahedral finite element head model. *Biomed. Tech.* 57(Suppl. 1), 329. doi: 10.1515/bmt-2012-4072

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

Received: 13 June 2014; accepted: 03 September 2014; published online: 19 September 2014.

Citation: Gillick BT, Kirton A, Carmel JB, Minhas P and Bikson M (2014) Pediatric stroke and transcranial direct current stimulation: methods for rational individualized dose optimization. Front. Hum. Neurosci. ***8****:739. doi: 10.3389/fnhum.2014.00739*

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

frontiers in
HUMAN NEUROSCIENCE

OPINION ARTICLE
published: 05 August 2014
doi: 10.3389/fnhum.2014.00600

Transcranial stimulation of the developing brain: a plea for extreme caution

Nick J. Davis*

Department of Psychology, Swansea University, Swansea, UK
**Correspondence: n.j.davis@swansea.ac.uk*

Edited by:
Peter G. Enticott, Deakin University, Australia

Reviewed by:
Lindsay M. Oberman, University of California San Diego, USA
Brian D. Earp, University of Oxford, UK

Keywords: TMS, tDCS, non-invasive, neuroethics, safety, paediatric

INTRODUCTION

Transcranial stimulation can be used to modulate the activity of the brain. Recent developments in our understanding of technologies such as transcranial magnetic or electrical stimulation have afforded reasonable grounds for optimism that techniques such as TMS or tDCS might be effective treatments for neurally-mediated disorders. Researchers have demonstrated encouraging benefits of TMS and tDCS in treating conditions such as tinnitus (Burger et al., 2011), depression (Arul-Anandam and Loo, 2009), and stroke (Nowak et al., 2010). Collectively these techniques are often referred to as "non-invasive brain stimulation" (NIBS), although I would argue that this term is not appropriate since in all cases energy is being transferred across the skull (Davis and van Koningsbruggen, 2013), and the use of this term may be misleading to the general public who are not aware of the documented risks associated with these procedures.

More recently it has been suggested that brain stimulation be used to treat neurological disorders in pediatric cases. A recent review by Vicario and Nitsche (2013a) identified a number of opportunities and challenges for the use of brain stimulation in children. Here I offer a plea for calm and for caution. The ethical stakes in clinical and research procedures with children are high enough that a conservative approach is warranted. Many of the ethical issues, relevant both to adult and child participants, have been touched on by other authors (e.g., Cohen Kadosh et al., 2012; Krause and Cohen Kadosh, 2013); however this paper will focus on the gaps in our knowledge that affect our ability to assess risk in translating brain stimulation procedures to pediatric cases.

There are a number of known risks associated with brain stimulation. Mild side-effects may include scalp tenderness, headache or dizziness, which are typically associated with the mechanism of delivery or with being immobilized in a chair or frame, and which may be underreported (Brunoni et al., 2011). More serious effects may include seizure, mood changes or induction of hyper- or hypomania. However, the risk of seizure is low, at around 0.1% of adult cases and around 0.2% of pediatric reports, although these figures may not reflect unreported off-label use of the techniques (Rossi et al., 2009). These more serious symptoms are largely associated with people who already possess a degree of susceptibility, such as people with a history of epilepsy (Davis et al., 2013). Adult brain stimulation is thought be reasonably safe when used within defined limits (see below), however here I wish to focus on a number of factors that complicate the translation of TMS and tDCS protocols to pediatric cases.

I will focus on the key unknowns in brain stimulation research:

1. The unknown effects of stimulation;
2. The unknown side-effects of stimulation;
3. The lack of clear dosing guidelines;
4. The lack of translational studies from adults to children.

I will set out these "known unknowns" in translating our knowledge about TMS and tDCS effects to clinical pediatric applications, and touch on the practical and ethical barriers to their widespread usage.

GAPS IN OUR KNOWLEDGE

THE UNKNOWN EFFECTS OF STIMULATION

It is thought that the effects of stimulation on the brain involve modulating the excitability of cortical areas near to the tCS electrode or to the TMS coil. However, there are considerable gaps in our knowledge of how this modulation is achieved and maintained. It is assumed that long term depression- or potentiation-like processes mediate a change in the resting potential of neurons (e.g., Fritsch et al., 2010), and it is likely that the induced electric currents induce plastic changes in neurotransmitter availability (Stagg et al., 2009; Stagg and Nitsche, 2011), but the biophysical mechanism for the induction of these processes from electric fields is obscure. It is not clear to what extent white matter is involved in mediating the effects of brain stimulation. Children are known to show less myelination in some brain regions than adults (Klingberg et al., 1999; Barnea-Goraly et al., 2005), and it is thought that non-uniformity in brain tissue has a large role in determining the spread of current (Shahid et al., 2013). It is even less clear to what extent glial cells are involved during brain stimulation, although it is known that many of the changes in brain structure that occur during childhood and adolescence are due to changes in glial density (Caviness et al., 1996). These architectonic differences between child and adult brains are likely to affect the spread of applied current through brain tissue, making it more

difficult to predict the electric field at, or away from, target brain areas.

THE UNKNOWN SIDE-EFFECTS OF STIMULATION

As well as the short-term effects of transcranial stimulation, we do not yet understand the effects of long-term use. It seems likely that repeated sessions of TMS or tCS lead to longer-lasting neural effects; these long-duration effects are what makes brain stimulation an attractive possibility for clinical treatment. However, no brain region exists in isolation, and researchers are only now beginning to understand the knock-on effects of modulating one brain area on other areas in the brain. For example, there is evidence that enhancing one aspect of cognition may be detrimental to other cognitive faculties, making neuromodulation a zero-sum intervention (Brem et al., 2014). Conversely, reduction in activation of a brain area may induce a paradoxical overall facilitation in function (Earp et al., 2014), through disinhibition in a network or through changes in neural noise. These notions suggest that we should be checking more widely for possible adverse effects of brain stimulation, since the resulting effect of stimulation may not be seen in the hypothesized behavior, but in behaviors governed elsewhere in a brain network. There is also the worrying possibility that electrical stimulation of the skull may induce or inhibit bone growth, an issue of particular importance in children whose cranial bones are not yet fused (Friedenberg et al., 1971, 1974). This latter possibility has not been explored in human volunteers in brain stimulation experiments.

THE LACK OF CLEAR DOSING GUIDELINES

It is currently not known how to determine the appropriate dose of stimulation to give to an individual person to achieve a given size of effect. At present our best knowledge in dose-setting comes from studies that model the electric and magnetic fields generated in stimulation, and attempt to relate these fields to physical effects on brain tissue. For example, the current applied between two tDCS electrodes placed on the scalp induces an electric field across the brain surface (Miranda et al., 2006). Modeling this electric field may in principle afford predictions of the behavioral effect of specified levels of current (e.g., Mendonca et al., 2011). However, there are known to be considerable differences in the modeled field between individuals, depending on such factors as fat deposits, cortical folding and skull thickness. Importantly, one recent modeling study suggests that the transmission of electric current to the brain is more efficient in children than in adults, implying that clinicians should be more conservative in dose-setting for children than for adults (Kessler et al., 2013). This latter study suggested that the same electric field magnitude at the brain surface might be achieved with half of the applied current in children compared to adults. However, it is interesting to note that TMS-induced motor potentials are generated at a higher TMS intensity in children than older people, possibly as a result of different levels of inhibitory processing in the cortex (Mall et al., 2004). While not a complete solution, developing individual MRI-derived models for dose prediction is likely to remain the most effective strategy for safe delivery of brain stimulation.

THE LACK OF TRANSLATIONAL STUDIES FROM ADULTS TO CHILDREN

It is a well-established principle that children should not be considered as "small adults" when testing medical interventions. A recent study suggested that most medical devices used in children are never tested in pediatric populations before approval (Hwang et al., 2014). I argued above that modeling studies can inform our ability to safely apply the correct level of dose in individual children. However, we are left with an ethical dilemma: how to judge the safety of a procedure in children without exposing children to the procedure's potential risks during testing? This is not an uncommon problem in vulnerable groups. For example, in order to be certain that a drug is safe for use in pregnancy, it must be tested on pregnant women (Chambers et al., 2008). In the case of drug testing in pregnancy, this requires that physicians monitor and report rare adverse effects. Brain stimulation is similarly associated with rare and subtle side-effects, although in this case the patient may not be aware of or able to report these adverse effects. I propose that a clear system be developed for recording adverse effects in people with limited capacity to report these effects.

WIDER ETHICAL CONCERNS

We have seen how incomplete knowledge of the effects of brain stimulation in adults and in children may entail risks when applied to children, and have seen that TMS and tCS are likely to be of use in treating neurally-mediated disorders. In younger patients, the most promising treatment targets are epileptic disorders, depression and chronic pain, where some benefits have been shown in adults (Eldaief et al., 2013). There is at present a small number of publications that support the use of brain stimulation in developmental cognitive conditions including autism (Oberman et al., 2013; Enticott et al., 2014), attention deficit-hyperactivity disorder (e.g., Bloch et al., 2010) or developmental dyslexia (e.g., Costanzo et al., 2013; Vicario and Nitsche, 2013b).

Recently researchers have suggested that brain stimulation might enhance performance, in domains such as mathematical ability (Snowball et al., 2013), sport (Davis, 2013), moral reasoning (Young et al., 2010) and vigilance (Nelson et al., 2014). The possibility exists that a child might take a dose of stimulation before sitting an exam or a driving test. As access to brain stimulation becomes more widespread, in particular an internet-based do-it-yourself movement ("DIY-tDCS"), it is increasingly likely that people will take the findings reported in scientific reports and in the press, and attempt to apply the same stimulation parameters without the safeguards of the lab or clinic (Fitz and Reiner, 2013). Researchers and clinicians therefore have an increased duty of caution in presenting our findings to a wider audience.

CONCLUSION

I have so far presented a somewhat negative view of the use of brain stimulation in younger people. In balance, I would add that based on the published literature, amounting to around 1000 pediatric cases, the protocols do not appear to expose patients to significantly enhanced risk of serious adverse effects. Adverse reactions have occurred, although generally these have been in patients who have an increased risk, such as in a case of rTMS

leading to a seizure in a patient with elevated blood alcohol levels (Chiramberro et al., 2013). Sessions of TMS and tDCS are reasonably well tolerated in studies that have reported subjective experience. Rajpakse and Kirton (2013) and Krause and Cohen Kadosh (2013) give comprehensive recent overviews of brain stimulation studies in children. When used with care, brain stimulation in children appears to be safe and well tolerated, at least over the range of expected effects that occur following stimulation.

I therefore hope to offer a positive conclusion. Transcranial stimulation will almost certainly play a large role in future treatment options for neurological disorders in children, including the developmental cognitive disorders listed above, for which there is some theoretical justification for optimism. Many of the disorders discussed here are disorders of plasticity; the hope is that maladapted communication between or within brain areas might be adjusted through the use of externally-applied stimulation. Certainly in adults TMS and tDCS are likely to be associated with fewer and less unpleasant side-effects than the neuroactive drugs that they are intended to replace, and brain stimulation is thought to be safe when used within known safety parameters (e.g., Green et al., 1997; Bikson et al., 2009; Rossi et al., 2009; Davis et al., 2013).

It is clear that a large amount still remains to be done in establishing safe use of brain stimulation for children. The major practical problems that remain are: safe dosing of stimulation for individual children; developing a framework for establishing informed consent in children and their guardians; and an efficient system for monitoring and reporting adverse effects during and following brain stimulation in minors. Researchers and clinicians should also be conscious that children and parents are increasingly technologically aware, and that headline-grabbing news related to brain stimulation could lead people to self-administer stimulation; this is already occurring, as a brief search of internet forums will reveal.

Brain stimulation is a powerful tool, and it is our duty to ensure that it is used responsibly in people who are most vulnerable. With scientific and practical developments, we can be confident that brain stimulation offers an opportunity to help those who have most to benefit.

REFERENCES

Arul-Anandam, A. P., and Loo, C. (2009). Transcranial direct current stimulation: a new tool for the treatment of depression? *J. Affect. Disord.* 117, 137–145. doi: 10.1016/j.jad.2009.01.016

Barnea-Goraly, N., Menon, V., Eckert, M., Tamm, L., Bammer, R., Karchemskiy, A., et al. (2005). White matter development during childhood and adolescence: a cross-sectional diffusion tensor imaging study. *Cereb. Cortex* 15, 1848–1854. doi: 10.1093/cercor/bhi062

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

Bloch, Y., Harel, E., Aviram, S., Govezensky, J., Ratzoni, G., and Levkovitz, Y. (2010). Positive effects of repetitive transcranial magnetic stimulation on attention in ADHD Subjects: a randomized controlled pilot study. *World J. Biol. Psychiatry* 11, 755–758. doi: 10.3109/15622975.2010.484466

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

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

Burger, J., Frank, E., Kreuzer, P., Kleinjung, T., Vielsmeier, V., Landgrebe, M., et al. (2011). Transcranial magnetic stimulation for the treatment of tinnitus: 4-year follow-up in treatment responders - a retrospective analysis. *Brain Stimul.* 4, 222–227. doi: 10.1016/j.brs.2010.11.003

Caviness, V., Kennedy, D., Richelme, C., Rademacher, J., and Filipek, P. (1996). The human brain age 7-11 years: a volumetric analysis based on magnetic resonance images. *Cereb. Cortex* 6, 726–736. doi: 10.1093/cercor/6.5.726

Chambers, C., Polifka, J., and Friedman, J. (2008). Drug safety in pregnant women and their babies: ignorance not bliss. *Clin. Pharmacol. Ther.* 83, 181–183. doi: 10.1038/sj.clpt.6100448

Chiramberro, M., Lindberg, N., Isometsä, E., Kähkönen, S., and Appelberg, B. (2013). Repetitive transcranial magnetic stimulation induced seizures in an adolescent patient with major depression: a case report. *Brain Stimul.* 6, 830–831. doi: 10.1016/j.brs.2013.02.003

Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, R108–R111. doi: 10.1016/j.cub.2012.01.013

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

Davis, N. (2013). Neurodoping: brain stimulation as a performance-enhancing measure. *Sports Med.* 43, 649–653. doi: 10.1007/s40279-013-0027-z

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

Davis, N., and van Koningsbruggen, M. (2013). 'Non-invasive' brain stimulation is not non-invasive. *Front. Syst. Neurosci.* 7:76. doi: 10.3389/fnsys.2013.00076

Earp, B., Sandberg, A., Kahane, G., and Savulescu, J. (2014). When is diminishment a form of enhancement? rethinking the enhancement debate in biomedical ethics. *Front. Syst. Neurosci.* 8:12. doi: 10.3389/fnsys.2014.00012

Eldaief, M., Press, D., and Pascual-Leone, A. (2013). Transcranial magnetic stimulation in neurology: a review of established and prospective applications. *Neurol. Clin. Pract.* 3, 519–526. doi: 10.1212/01.CPJ.0000436213.11132.8e

Enticott, P., Fitzgibbon, B., Kennedy, H., Arnold, S., 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

Fitz, N., and Reiner, P. (2013). The challenge of crafting policy for do-it-yourself brain stimulation. *J. Med. Ethics*. doi: 10.1136/medethics-2013-101458. [Epub ahead of print].

Friedenberg, Z., Roberts, P. J., Didizian, N., and Brighton, C. (1971). Stimulation of fracture healing by direct current in the rabbit fibula. *J. Bone Joint Surg.* 53, 1400–1408.

Friedenberg, Z., Zemsky, L., and Pollis, R. (1974). The response of non-traumatized bone to direct current. *J. Bone Joint Surg.* 56, 1023–1030.

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Green, R., Pascual-Leone, A., and Wassermann, E. (1997). Ethical guidelines for rTMS research. *IRB Ethics Hum. Res.* 19, 1–7. doi: 10.2307/3563539

Hwang, T., Kesselheim, A., and Bourgeois, F. (2014). Postmarketing trials and pediatric device approvals. *Pediatrics* 133, e1197–e1202. doi: 10.1542/peds.2013-3348

Kessler, S., Minhas, P., Woods, A., Rosen, A., Gorman, C., and Bikson, M. (2013). Dosage considerations for transcranial direct current stimulation in children: a computational modeling study. *PLoS ONE* 8:e76112. doi: 10.1371/journal.pone.0076112

Klingberg, T., Vaidya, C., Gabrieli, J., Moseley, M., and Hedehus, M. (1999). Myelination and organization of the frontal white matter in children: a diffusion tensor MRI study. *Neuroreport* 10, 2817–2821. doi: 10.1097/00001756-199909090-00022

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

Mall, V., Berweck, S., Fietzek, U., Glocker, F.-X., Oberhuber, U., Walther, M., et al. (2004). Low levels of intracortical inhibition in children shown by

transcranial magnetic stimulation. *Neuropediatrics* 35, 120–125. doi: 10.1055/s-2004-815834

Mendonca, M., Santana, M., Baptista, A., 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

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

Nelson, J., McKinley, A., Goloba, E., Warm, J., and Parasuraman, R. (2014). Enhancing vigilance in operators with prefrontal cortex transcranial direct current stimulation (tDCS). *Neuroimage* 85, 909–917. doi: 10.1016/j.neuroimage.2012.11.061

Nowak, D. A., Bösl, K., Podubeckà, J., and Carey, J. R. (2010). Noninvasive brain stimulation and motor recovery after stroke. *Restor. Neurol. Neurosci.* 28, 531–544. doi: 10.3233/RNN-2010-0552

Oberman, L., Rotenberg, A., and Pascual-Leone, A. (2013). Use of transcranial magnetic stimulation in autism spectrum disorders. *J. Autism Dev. Disord.* doi: 10.1007/s10803-013-1960-2. [Epub ahead of print].

Rajpakse, T., and Kirton, A. (2013). Non-invasive brain stimulation in children: applications and future directions. *Transl. Neurosci.* 4, 217–233. doi: 10.2478/s13380-013-0116-3

Rossi, S., Hallett, M., Rossini, P., Pascual-Leone, A., and Group, T. S. O. T. C. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Shahid, S., Wen, P., and Ahfock, T. (2013). Numerical investigation of white matter anisotropic conductivity in defining current distribution under tDCS. *Comput. Methods Programs Biomed.* 109, 48–64. doi: 10.1016/j.cmpb.2012.09.001

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

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

Stagg, C., Wylezinska, M., Matthews, P., Johansen-Berg, H., Jezzard, P., Rothwell, J., et al. (2009). Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *J. Neurophysiol.* 101, 2872–2877. doi: 10.1152/jn.91060.2008

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

Vicario, C., and Nitsche, M. (2013b). Transcranial direct current stimulation: a remediation tool for the treatment of childhood congenital dyslexia? *Front. Hum. Neurosci.* 7:139. doi: 10.3389/fnhum.2013.00139

Young, L., Camprodon, J. A., Hauser, M., Pascual-Leone, A., and Saxe, R. (2010). Disruption of the right temporoparietal junction with transcranial magnetic stimulation reduces the role of beliefs in moral judgments. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6753–6758. doi: 10.1073/pnas.0914826107

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

Received: 01 May 2014; accepted: 18 July 2014; published online: 05 August 2014.

Citation: Davis NJ (2014) Transcranial stimulation of the developing brain: a plea for extreme caution. Front. Hum. Neurosci. ***8****:600. doi: 10.3389/fnhum.2014.00600 This article was submitted to the journal Frontiers in Human Neuroscience.*

frontiers in
HUMAN NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 18 December 2014
doi: 10.3389/fnhum.2014.00953

Brain stimulation for treatment and enhancement in children: an ethical analysis

Hannah Maslen *, Brian D. Earp, Roi Cohen Kadosh and Julian Savulescu

Oxford Uehiro Centre for Practical Ethics and Oxford Martin School, University of Oxford, Oxford, UK

Edited by:
Peter G. Enticott, Deakin University, Australia

Reviewed by:
Markus Christen, University of Zurich, Switzerland
Nick J. Davis, Swansea University, UK

****Correspondence:***
Hannah Maslen, Oxford Uehiro Centre for Practical Ethics and Oxford Martin School, University of Oxford, Suite 8, Littlegate House, 16/17 St Ebbe's Street, Oxford OX1 1PT, UK
e-mail: hannah.maslen@philosophy.ox.ac.uk

Davis (2014) called for "extreme caution" in the use of non-invasive brain stimulation (NIBS) to treat neurological disorders in children, due to gaps in scientific knowledge. We are sympathetic to his position. However, we must also address the *ethical* implications of applying this technology to minors. Compensatory trade-offs associated with NIBS present a challenge to its use in children, insofar as these trade-offs have the effect of limiting the child's future options. The distinction between treatment and enhancement has some normative force here. As the intervention moves away from being a treatment toward being an enhancement—and thus toward a more uncertain weighing of the benefits, risks, and costs—considerations of the child's best interests (as judged by the parents) diminish, and the need to protect the child's (future) autonomy looms larger. NIBS for enhancement involving trade-offs should therefore be delayed, if possible, until the child reaches a state of maturity and can make an informed, personal decision. NIBS for treatment, by contrast, is permissible insofar as it can be shown to be at least as safe and effective as currently approved treatments, which are themselves justified on a best interests standard.

Keywords: brain stimulation, pediatric ethics, cogntive enhancement, functional trade-offs, autonomy

Davis (2014) has called for "extreme caution" in the use of non-invasive brain stimulation (NIBS) methods to treat neurological disorders in children. His focus is on transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), which, respectively, involve passing either an electro-magnetic field or a small direct current through the skull in order to modulate neuronal activity. To justify his position, Davis calls attention to four major issues, framed as "known unknowns" in the current literature:

(1) unknown effects of brain stimulation, and unknown mechanisms for producing those effects;
(2) unknown side-effects of stimulation (both short- and long-term);
(3) a lack of clear dosing guidelines; and
(4) a lack of translational studies from adults to children.

As Davis rightly points out, "children [cannot] be considered as 'small adults' when testing medical interventions" (p. 2). This is especially the case for interventions into the central nervous system, since a child's developing brain may respond differently to stimulation compared to that of an adult. Indeed, research shows that the brain continues to develop even after the age of majority (Sowell et al., 2003). Nevertheless, Davis balances his plea for caution with longer-term optimism. He argues that—when used with care—brain stimulation in children does appear to be safe and well-tolerated, and may even turn out to be "associated with fewer and less unpleasant side-effects than the neuroactive drugs [such stimulation is] intended to replace" (p. 3).[1]

We are sympathetic with Davis' argument (Cohen Kadosh et al., 2012). Put simply, caution and sustained clinical scrutiny are required, both for research into the effects of pediatric brain stimulation and for the application of such technology. Yet while further empirical studies into appropriate dosing, side-effects, and so on should allow for brain stimulation in children to be made generally safer (as well as more effective therapeutically), we must also address the gaps in our understanding of the *ethical* implications of applying this technology to minors.

In this article, we aim to contribute to such an understanding. To frame our discussion, we draw a distinction between the use of NIBS (but see Davis and van Koningsbruggen, 2013)[2] as a form of *treatment* for a recognized neurological disorder, and its

[1]Consistent with this perspective, a recent review concluded that there is at least preliminary evidence of a therapeutic potential for TMS and/or tDCS in children with conditions such as depression and autism spectrum disorder; however, it should be noted that many of the studies included in this review did not have adequate control groups, and should therefore be interpreted with care (Vicario and Nitsche, 2013).

[2]They argue that the term "non-invasive" "is inappropriate and perhaps oxymoronic, as it obscures both the possibility of side-effects from the stimulation, and the longer-term effects (both adverse and desirable) that may result from brain stimulation. [Moreover, the] tendency for the effects of [such stimulation] to spread from the target brain area to neighboring areas is in itself contrary to the definition of non-invasiveness" (p. 1). Indeed, this ability for (intended) effects of brain stimulation to have potentially adverse

use as a form of *enhancement* in healthy children. Although we have argued in previous work that the treatment/enhancement distinction tends to break down in the case of adults (see Earp et al., 2014), in the case of children, we suggest, it has greater normative force. This is because, we argue, the relative weights of (parental judgements of) beneficence vs. respect for autonomy *shift* as the decision pertains more to "enhancement" than to "treatment".[3,4]

The tension between these two factors arises because some interventions may involve compensatory trade-offs or functional losses, such as potential cognitive costs in the case of brain stimulation. When these trade-offs have the effect of limiting the child's future options, they pose a threat to his or her (future) autonomy. Whilst choosing to "treat" a child will sometimes be in his or her best interests *even if* it precipitates cognitive trade-offs, interventions intended to "enhance" may not be justified in this way. In the absence of a clear pathology, we suggest, greater relative weight should be placed on the child's (future) autonomy, at least in part because the certainty with which the parents can determine what would be in his or her best interests is likely to be significantly reduced.

Given this, we argue that brain stimulation for "enhancement"—insofar as it involves a more controversial weighting of benefits vs. risks and costs—should be delayed until the child has reached a state of maturity. In this way, she can make an informed, personal decision about the proposed intervention. Brain stimulation for "treatment", by contrast, is permissible insofar it can be shown to be at least as safe and effective as currently approved treatments (which are themselves justified on a best interests standard).

THE PERMISSIBILITY OF TREATING NEUROLOGICAL DISORDERS IN CHILDREN

To begin our discussion, we ask, what makes pediatric "treatment" permissible in general? By "treatment" we intend to call to mind such interventions as surgery to correct a heart defect, or the administration of antibiotics to address an infection. In these cases, a disease or deformity is present that threatens the child's well-being, and the treatment is the best available means (or a good-enough means) to mitigate that threat. Thus, although (a) the child cannot strictly consent to the intervention, (b) the intervention may carry considerable risk, and (c) it may involve even a gross intrusion into the child's bodily sphere, it is nevertheless considered to be morally permissible. Such an intervention is permissible because, and insofar as, it is in the child's best interests—all things considered (see Hope et al., 2008).

We can extend this reasoning to the case of brain stimulation. If a child is experiencing significant psychological and/or physical burdens due to a neurological disorder, the benefits of treatment with stimulation might very well be in the child's best interests in the sense just described. In fact, this could turn out to be the case *even if* some significant negative side-effects were generated, so long as the overall costs to the child (including the cost to autonomy) were outweighed by the benefits of performing the stimulation before an age of consent. On these grounds, it could be considered permissible, assuming that it were shown to be at least as safe and effective as other, more established treatment paradigms.[5]

ENHANCEMENT AND THE CHILD'S INTEREST IN AUTONOMY

What about the case of "enhancement"? Ethicists are divided on the question of whether parental enhancement choices are in the child's best interests and this is often framed in terms of a consideration of the child's interest in (future) autonomy, or self-determination. Some have argued that the enhancement of a child might lead her to feel unfree to pursue her own life-projects due to the fact that decisions about her traits and capacities have been chosen for her. In developing this argument, Habermas (2003, p. 50) has argued that, in the case of genetic enhancement (i.e., selecting for specific traits, such as intelligence), the parents' choices represent intentions and expectations relating to their child's life. Such expectations, he suggests, lead to the stifling of the child's freedom to develop in his or her own way.

Others have argued that enhancement technologies would not undermine autonomy, insofar as they increase the options available in an individual's choice set. For example, Bostrom (2005) claims that an enhanced child might "enjoy significantly *more* choice and autonomy in her life, if the modifications were such as to expand her basic capability set. Being healthy, smarter, having a wide range of talents, or possessing greater powers of self-control are blessings that tend to open more life paths than they block" (p. 212). Such an analysis tends to assume that enhancement has the overall effect of increasing *objective* opportunities, even if a child might *experience* her freedom as being constrained by parental expectations. However, as we will now discuss, in the case of brain stimulation, the assumption of "more choice" may sometimes be mistaken. The arguments

ramifications for other brain areas (and/or functions) is a key component of our ethical analysis.

[3]We do not suggest, of course, that there is a clear-cut, universally agreed-upon distinction between treatment and enhancement (see Maslen et al., 2014). Instead, we envision a sliding scale from interventions that are intended simply to sharpen a certain cognitive skill in a healthy child ("enhancement") to those intended to relieve a child of pain or another burden that significantly affects his or her ability to pursue the normal range of activities that children pursue ("treatment").

[4]Throughout this paper we invoke three overlapping considerations: the child's "developing autonomy" (her learning to be self-governing), the child's "future autonomy" (her prospects for pursuing the life plans that she will come to value as an adult) and the child's "self-determination" (the freedom for her actions to be "up to her"). All three are relevant and closely related in our discussion. However, we use the term "future autonomy" more prominently, as this denotes best the concern with preserving options for the child to evaluate herself, once she has sufficient capacity to make such assessments.

[5]However, note that the permissibility of parents' choosing the intervention would depend in part on what specific "negative side effects" might be incurred by the stimulation, as well as the magnitude of the risk. As we discuss later on, one high-risk side-effect of some kinds of brain stimulation is the diminishment of a non-targeted cognitive capacity. In this case, the persistence of symptoms due to the neurological condition would have to be worse—that is, more contrary to the child's interests—than the cognitive trade-offs incurred by the stimulation (alongside any other negative side-effects and costs) for the stimulation to be considered permissible on the best interests test.

we make in what follows are about objective, not subjective, curtailment(s) of freedom.

BRAIN STIMULATION AND COGNITIVE TRADE-OFFS

While early research into brain stimulation in healthy adults has focused on its potential to enhance cognitive functions, the cognitive costs that might be associated with such enhancement have largely been neglected. However, as Davis points out, no brain region exists in isolation. Indeed, there is evidence that enhancing one aspect of cognition may be detrimental to other cognitive faculties, making neuromodulation "a zero-sum proposition" (Brem et al., 2014; but see Luber, 2014). For example, it has been shown that enhancing cognitive performance on one task can be associated with poorer performance on a different cognitive task (Iuculano and Cohen Kadosh, 2013; Sarkar et al., in press).

It must be acknowledged that the evidence for such enhancement tradeoffs has thus far been obtained only from well-controlled laboratory experiments that have poor ecological validity. However, this preliminary evidence should alert us to the possibility of similar trade-offs that might occur in more ecologically valid settings. Laboratory experiments can help to demonstrate what would be theoretically expected, based on the cognitive function that is targeted and the brain regions that are stimulated. Crucially, such experiments suggest that it is theoretically likely that enhancement of one domain of cognition will sometimes come at the cost of impairment in another. Thus, any decision to enhance could be also a decision to impair. When this is coupled with the emerging probability of long lasting effects on the brain (see Snowball et al., 2013), a situation arises in which parents might inadvertently or even knowingly limit (at least some) future options for their children when they choose to enhance particular capacities at the expense of others.

For example, imagine a parent who has aspirations for her child to be the star of the school's quiz team. The parent encourages the child to memorize facts whilst her brain is stimulated to enhance long-term memory. However, as a result, the child's visuospatial working memory is impaired and her ability to quickly solve mental arithmetic problems suffers (see de Jongh et al., 2008 for a review of such trade offs with respect to pharmacological enhancements). Although the child performs well on general knowledge tests, she performs less well in mental arithmetic: mathematics-related pursuits are, to a certain degree, limited as a result of the intervention.

In this example, by choosing to enhance the child's long-term memory and, correspondingly, the ease with which activities employing this particular cognitive capacity can be pursued, the parent is also choosing to impair a different capacity, making the pursuit of activities involving visuospatial working memory more difficult. It is our contention that making these opportunity-limiting choices on behalf of the child may not be permissible. This is the case even if opportunities associated with the enhanced cognitive domain are increased. Parents cannot know what the child will grow up to value and so should not restrict opportunities based on what *they* want their child to pursue. Whilst there are many decisions that parents can make in the best interests of their child, which cognitive capacities are more valuable, we contend, is usually not one.

This argument applies most strongly to cases in which there is (roughly) a one-to-one trade-off. However, if a given enhancement intervention *substantially* increased function in one domain, while only *slightly* reducing function in another (as judged by a reasonable observer), then the decision would turn more heavily on considerations of what would be in the best interests of the child, overall. Such valuations are hard to make, and are likely to be highly subjective in many cases. The more subjective they are (that is, the less clear an "objective" observer would be about the relative weights to assign to the enhanced vs. diminished capacities), the more the decision about intervening should be left to the individual who must live with the consequences.

AT WHAT AGE CAN CHILDREN DECIDE TO BE "ENHANCED"?

Let us summarize our argument so far. First, when "enhancement" interventions involve a functional trade-off, the agent whose relevant capacities will be altered should usually be the one to make a decision about whether the intervention is desirable, all-things-considered. However, young children are unlikely to know which capacities they will value later in life, since their self-knowledge and ability to make and pursue long-term goals is yet to develop. Therefore, there is a problem in terms of a child's capacity to consent—that is, to fully understand what an intervention involves and what the material consequences will be. Moreover, there is a problem in terms of the child's limited insight into what she will value over time. At what point, then, can children make meaningful decisions regarding self-enhancement, taking into consideration the apparent risk of cognitive trade-offs?

To begin with, we should point out that a child's inability to provide informed consent does not make pediatric interventions impermissible *per se*. As we have already suggested, when it comes to treatment, at least, parents (or legal guardians) can legitimately make decisions in the best interests of the child. Similarly, when an intervention is carried out for purposes of medical research, a child's lack of capacity to consent is not necessarily prohibitive either. In these cases, clinicians or researchers must seek (and obtain) the child's *assent* to participate in the study (as well meet all other ethical requirements, see Caldwell et al., 2004).[6]

For minor interventions, then—such as venipuncture for the purposes of a study—a child's assent may be all that is needed. This is because the risks that are associated with such a

[6]What is assent? Although there are several different theories of assent, at its most basic, it involves agreement to or acceptance of the intervention. It is often argued that the requirements for assent are less cognitively demanding than for consent (see John et al., 2008; Waligora et al., 2014), such that individuals whose capacities to make informed judgments are still developing may nevertheless be able to meet them. In relation to assent for pediatric research, for example, Roth-Cline and Nelson suggest that the child should "understand why he or she is being asked to participate and what will be his or her experience if he or she decides to participate" (Roth-Cline and Nelson, 2013, p. 296). Precise age ranges vary, but a child's assent is thought to be (ethically) obtainable by approximately age five or six, depending upon the specific intervention being proposed (including its risk profile, etc.), and also adjusting for the child's individual stage of development.

procedure are either immediate and transitory (e.g., pain, stress, or discomfort) or rare (e.g., hemorrhage or infection), assuming that the intervention is properly performed. By contrast, the effects of brain stimulation for "enhancement" may have consequences that reach far into the child's future. Therefore, in order to evaluate the reasons one might have for refusing such an "enhancement" (such as a desire to leave one's cognitive functions intact), one must be capable of meaningful temporal self-projection. Yet such projection is usually not possible for very young children.[7]

It may be possible, however, for older children and/or adolescents. Accordingly, some scholars have suggested that genuine *consent* may be possible before an age of legal majority (typically 18), at least for certain kinds of "medical" interventions (see, e.g., Levy et al., 2003). For simple procedures with minimal risks, children as young as 10 may be capable of giving age-appropriate consent. As the risks increase, however, and as the need for temporal projection becomes more central to the decision-making process, a higher threshold for consent is required. In the case of "enhancement" decisions involving potential trade-offs, such as the impairment of a cognitive capacity, the threshold should be higher still.

This is for two reasons: first, as we have discussed, a child's brain is still developing, and in numerous ways that are not yet understood. Indeed, even adolescent and adult brains continue to develop. Nevertheless, and second, adolescents (and adults) have much greater insight—compared to very young children—into their own future values. It is this forward-looking capacity, we contend, that is especially important when making decisions about how to weigh the relative value of different cognitive functions; and younger children seem to lack this capacity. Therefore, in the case of pediatric enhancement involving long-term cognitive tradeoffs, we suggest that consent may be (ethically) obtainable by later adolescence, perhaps around the age of 16, but usually not earlier than this.

PARENTS' TRADITIONAL INFLUENCE ON CHILDREN'S SKILL DEVELOPMENT

A first response to our argument might be to point out that parents already make many (relatively unproblematic) decisions when, for example, they allow their children to take part in certain extra-curricular activities but not others. A parent might encourage her child to go to drama club instead of French lessons or to practice football rather than sing. However, there are important differences between these decisions and the sorts of cognitive trade-offs under discussion.[8]

First, developing a skill through participation in an extra-curricular activity does not directly impair the skills that would have been developed had a different activity been selected: practicing music, for example, does not directly impair the ability to speak French. The significance of this disanalogy with cognitive trade-offs will depend upon two things. First, the extent to which the failure to develop a capacity is comparable (in an opportunity-limiting sense) to directly impairing it: if, later in life, non-developed skills can more easily be developed than impaired skills, then the child retains more options. Second, the permanency of the impairment will be highly relevant: temporary enhancement may only result in temporary impairment. If impairment to a capacity subsides, or is compensated for, then it becomes equivalent to a non-developed capacity and the (moral) distance between traditional intervention and neuro-intervention decreases.

Thus, neuroscientific evidence regarding the permanency—and extent—of cognitive costs associated with brain stimulation will be essential to determining the permissibility of parental "enhancement" decisions. It will also be crucial to know how these effects differ between one-off vs. repeated interventions, as well as whether the sought-after benefit can be achieved later in life, when the (future) adult can decide for himself or herself. Such knowledge is currently lacking. Accordingly, we highlight the need for careful consideration of these variables, and conclude that "enhancements" involving significant long-term cognitive tradeoffs should be delayed until the individual to be affected can express a considered preference (i.e., adolescence).

CONCLUSION

Whilst adults are in a position to decide whether effect X is valuable enough (to them) to justify incurring impairment Y, children do not yet have the capacity or the life experience to make such trade-off decisions. They do not know what they will value when they grow up and nor do their parents. Whilst an intervention that improves X may count as an enhancement for the individual who does not care much about Y, another individual, valuing Y over X, will view the very same outcome as an impairment. In such cases—that is, cases in which the very status of an intervention's being an (overall) enhancement vs. an impairment is controversial—the weight of considerations should shift toward delaying the intervention until the individual who will actually be affected by it has sufficient capacity to decide. The more permanent and substantial the trade-off, the more this argument has force.

The gaps Davis identifies in the literature on brain stimulation suggest that we do not currently have enough evidence to properly assess the magnitude and permanency of any trade-offs and, consequently, that the caution he recommends is indeed warranted. However, we have suggested that even when science

[7]Therefore, a simple understanding of the immediate experience of the intervention would not be sufficient to make the enhancement intervention morally permissible. Indeed, the *acceptance* that is characteristic of child assent must be supplemented with a strong, considered *preference* in cases in which long-term trade-offs are under consideration.

[8]There will, of course, be examples of "hyperparenting" (see Sandel, 2009), which may in fact be as problematic as electing opportunity-limiting interventions involving direct intervention into a child's brain—and for similar reasons. This will be the case particularly when the child is made to spend a considerable amount of time developing a certain skill despite her sustained dissent. Whilst parents should encourage children to try different activities, and sometimes override dissent when a child is less-than-enthusiastic on a particular occasion, a child's long-term resistance to an extra-curricular activity renders parental force morally questionable at best, and morally impermissible at worst. This is due to the failure of such parental pressure to nurture the child's developing autonomy and its prevention of the child's pursuit of alternative extra-curricular options, which may become increasingly difficult to master as time goes on.

can tell us about the effects of brain stimulation in more detail, the permissibility of parental decision-making may remain limited in some cases in which the aim is only to "enhance" an intact cognitive capacity. In contrast, the treatment of atypical cognitive abilities using brain stimulation will be permissible insofar as the stimulation is (at least) as safe and effective as existing treatments in providing an overall benefit to the child.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust [086041/Z/08/Z]; the Oxford Martin School; and the Uehiro Foundation on Ethics and Education.

REFERENCES

Bostrom, N. (2005). In defence of posthuman dignity. *Bioethics* 19, 202–214. doi: 10.1111/j.1467-8519.2005.00437.x

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

Caldwell, P. H., Murphy, S. B., Butow, P. N., and Craig, J. C. (2004). Clinical trials in children. *Lancet* 364, 803–811. doi: 10.1016/S0140-6736(04)16942-0

Cohen Kadosh, R., Levy, N., O'Shea, J., Shea, N., and Savulescu, J. (2012). The neuroethics of non-invasive brain stimulation. *Curr. Biol.* 22, R108–R111. doi: 10.1016/j.cub.2012.01.013

Davis, N. J. (2014). Transcranial stimulation of the developing brain: a plea for extreme caution. *Front. Hum. Neurosci.* 8:600. doi: 10.3389/fnhum.2014.00600

Davis, N. J., and van Koningsbruggen, M. G. (2013). "Non-invasive" brain stimulation is not non-invasive. *Front. Syst. Neurosci.* 7:76. doi: 10.3389/fnsys.2013.00076

de Jongh, R., Bolt, I., Schermer, M., and Olivier, B. (2008). Botox for the brain: enhancement of cognition, mood and pro-social behavior and blunting of unwanted memories. *Neurosci. Biobehav. Rev.* 32, 760–776. doi: 10.1016/j.neubiorev.2007.12.001

Earp, B. D., Sandberg, A., Kahane, G., and Savulescu, J. (2014). When is diminishment a form of enhancement? Rethinking the enhancement debate in biomedical ethics. *Front. Syst. Neurosci.* 8:12. doi: 10.3389/fnsys.2014.00012

Habermas, J. (2003). *The Future of Human Nature.* Cambridge: Polity Press.

Hope, R. A., Savulescu, J., and Hendrick, J. (2008). *Medical Ethics and Law: The Core Curriculum.* London: Elsevier Health Sciences.

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

John, T., Hope, T., Savulescu, J., Stein, A., and Pollard, A. J. (2008). Children's consent and paediatric research: is it appropriate for healthy children to be the decision-makers in clinical research?. *Arch. Dis. Child.* 93, 379–383. doi: 10.1136/adc.2007.118299

Levy, M. D. L., Larcher, V., and Kurz, R. (2003). Informed consent/assent in children. Statement of the ethics working group of the Confederation of European Specialists in Paediatrics (CESP). *Eur. J. Pediatr.* 162, 629–633. doi: 10.1007/s00431-003-1193-z

Luber, B. (2014). Neuroenhancement by noninvasive brain stimulation is not a net zero-sum proposition. *Front. Syst. Neurosci.* 8:127. doi: 10.3389/fnsys.2014.00127

Maslen, H., Faulmüller, N., and Savulescu, J. (2014). Pharmacological cognitive enhancement-how future neuroscientific research could advance ethical debate. *Front. Syst. Neurosci.* 8:107. doi: 10.3389/fnsys.2014.00107

Roth-Cline, M., and Nelson, R. M. (2013). Parental permission and child assent in research on children. *Yale J. Biol. Med.* 86, 291–301.

Sandel, M. J. (2009). *The Case Against Perfection.* Cambridge, MA: Harvard University Press.

Sarkar, A., Dowker, A., and Cohen Kadosh, R. (in press). Cognitive enhancement or cognitive cost: trait-specific outcomes of brain stimulation in the case of mathematics anxiety. *J. Neurosci.*

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

Sowell, E. R., Peterson, B. S., Thompson, P. M., Welcome, S. E., Henkenius, A. L., and Toga, A. W. (2003). Mapping cortical change across the human life span. *Nat. Neurosci.* 6, 309–315. doi: 10.1038/nn1008

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

Waligora, M., Dranseika, V., and Piasecki, J. (2014). Child's assent in research: age threshold or personalisation? *BMC Med. Ethics* 15:44. doi: 10.1186/1472-6939-15-44

Conflict of Interest Statement: Roi Cohen Kadosh has filed a patent entitled "Apparatus for Improving and/or Maintaining Numerical Ability" (International Application PCT/GB2011/050211).

Received: 25 September 2014; accepted: 09 November 2014; published online: 18 December 2014.

Citation: Maslen H, Earp BD, Cohen Kadosh R and Savulescu J (2014) Brain stimulation for treatment and enhancement in children: an ethical analysis. Front. Hum. Neurosci. ***8****:953. doi: 10.3389/fnhum.2014.00953*

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