Published in final edited form as:

Brain Stimul. 2018 ; 11(6): 1306–1313. doi:10.1016/j.brs.2018.07.050.

Transcranial Magnetic Stimulation and Environmental Enrichment Enhances Cortical Excitability and Functional Outcomes after Traumatic Brain Injury

Samuel S. Shin^{a,b,e}, Vijai Krishnan^{b,e,f,g}, William Stokes^{b,e}, Courtney Robertson^c, Pablo Celnik^{a,d}, Yanrong Chen^{b,e}, Xiaolei Song^{b,e}, Hanzhang Lu^{b,e}, Peiying Liu^{b,e}, and Galit Pelled^{b,e,f,g,h,*}

^aDepartment of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

^bF.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA.

^cDepartment of Anesthesiology/Critical Care Medicine and Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

^dDepartment of Physical Medicine and Rehabilitation, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

^eRussell H. Morgan Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

^fDepartment of Biomedical Engineering, Michigan State University, East Lansing, Michigan, USA.

^gThe Institute of Quantitative Health Science and Engineering, Michigan State University, East Lansing, Michigan, USA.

^hDepartment of Radiology, Michigan State University, East Lansing, Michigan, USA.

Abstract

Background: Therapeutic strategies for TBI in the last three decades have failed to show significant benefit in large scale studies. Given the multitude of pathological mechanisms involved in TBI, strategies focusing on multimodality regimen have gained interest as promising future interventions.

* Corresponding Author: Galit Pelled, Ph.D., Professor, Biomedical Engineering, Radiology and Neuroscience, Chief, Division of Neuroengineering, Institute for Quantitative Health Science and Engineering, 775 Woodlot Drive, Room 2114, Michigan State University, East Lansing, MI 48823, Phone: 517-884-7464, Fax: 517-355-2288, pelledga@egr.msu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declaration of Interest

The authors of this manuscript have no conflict of interest to be declared.

Hypothesis: We hypothesized that combining noninvasive transcranial magnetic stimulation (TMS) with rehabilitative training in an environmental enrichment (EE) can facilitate post-traumatic brain injury (TBI) recovery in rats via cortical excitability and reorganization.

Methods: We subjected rats to controlled cortical impact, and then assigned them to one of four groups: 1. No treatments (TBI), 2. EE after injury (TBI+EE), 3. TMS for one week (TBI+TMS), and 4. TMS for one week combined with EE (TBI+TMS/EE). For TMS, a 10Hz repetitive TMS protocol was used.

Results: At 7 days, TBI+TMS and TBI+TMS/EE groups had significantly increased primary somatosensory cortex local field potential (LFP) compared to TBI and TBI+EE groups ($P<0.05$). Also, TBI+TMS/EE group had significantly improved performance on beam walk test compared to TBI group ($P<0.005$). At 6 weeks, there was significantly higher response in TBI+TMS/EE group compared to TBI+TMS for somatosensory cortex LFP ($P<0.05$), bicep motor evoked potentials (MEP) ($P<0.05$), challenge ladder test performance ($P<0.01$), and fMRI responses to tactile forepaw stimulation.

Conclusions: We demonstrate here for the first time the mechanism by which combined therapy using TMS and EE after TBI leads to functional improvement, possibly via cortical excitability and reorganization.

Keywords

Traumatic brain injury; TBI; Transcranial magnetic stimulation; TMS; environmental enrichment

Introduction

Traumatic brain injury (TBI) is one of the major causes of death and disabilities in the world. The past three decades of preclinical TBI research have led to extensive testing and design of pharmacological and other therapeutic strategies. Nevertheless, clinical trials have shown very limited long-term improvement in functional outcomes. The difficulty in finding an effective treatment modality has been attributed to the multiple and complex pathophysiological mechanisms that occur concurrently following injury. A major etiology underlying behavioral deficits after TBI has been identified as abnormal cortical function [1, 2]. Indeed, evidence suggest that cortical excitability is compromised in human patients [3, 4] and motor evoked potential had increased threshold following TBI [5, 6], suggesting compromised sensorimotor neural function.

Neural reorganization underlies recovery following brain injury, and rehabilitative training enhances this recovery [7–10]. Harnessing the brain's innate post-injury plasticity through non-invasive methods such as transcranial magnetic stimulation (TMS) has recently gained interest for use in functional and behavioral research as well as rehabilitation research after brain injury [11–16] and neurodegenerative disease [17–22]. Various studies showed promising results using TMS in humans [11, 19, 23], primates [24, 25], and rodents [15, 26, 27].

While it emerges that TMS may have the potential to guide plasticity and restore normal cortical excitability levels, the cellular mechanisms by which this occurs, and the optimal

TMS protocols for augmenting recovery have yet to be determined. We have recently investigated the immediate effects of high frequency, repetitive TMS (rTMS) on the activity of cortical neurons [28]. Whole-cell patch-clamp recordings in acute brain slices demonstrated that rTMS has an immediate and cumulative effect on neuronal activity and intracellular calcium levels, and that rTMS may augment neuronal responses when combined with a stimulus modality.

Capitalizing on these findings, we tested if TMS combined with another therapeutic strategy will facilitate long-term post-TBI recovery. We have combined TMS with environmental enrichment (EE) since the latter was shown to be beneficial in TBI by improving motor function and spatial memory as well as reducing histological markers of injury [29–31]. Similar multimodal approaches have shown to be beneficial in other pathologies such as Parkinson's disease [32] and stroke [13]. Although TMS combined with rehabilitation has been shown to improve function in human subjects [33–36], the mechanism of this synergistic effect has not been described. Understanding this mechanism will allow the scientific community to further refine the current rehabilitative strategies. As therapeutic options for TBI patients have shown limited development over the course of last two decades, we aimed to explore the therapeutic effects and the mechanism of using a combined modality with TMS and EE. We employed a comprehensive battery of measurements including behavioral test, functional MRI, and electrophysiology to determine if TMS combined with rehabilitative training has additive benefits in functional recovery and its underlying mechanism.

Materials and Methods

Animals

Ninety-seven Sprague-Dawley male rats (Harlan Laboratories) weighing 280–360 were subjected to the protocols of this study. All the procedures were approved by the guidelines set by the Johns Hopkins Animal Care and Use Committee. Animals were provided with food and water *ad libitum* and housed in cages of two rats (standard housing: ST) or grouped housing (environmental enrichment: EE). For the animals in EE group, 6–10 animals were housed together with wide variety of sensory stimuli as described in [29], with nesting materials, balls, ladders, and nibbling toys of various colors.

Surgery and Stimulation

Rats in all groups underwent controlled cortical impact as described previously [37]. They were initially anesthetized using 5% isoflurane and placed on a stereotaxic frame with nose cone providing 2% isoflurane in O₂. After confirming that they are deeply anesthetized (showing no signs of pain with tail pinch), skin incision was made and craniotomy was performed on the right hemisphere large enough to fit a 6mm impactor tip. TBI was performed with 2.5mm deformation depth delivered at 4m/s velocity using a 6mm cylindrical impactor tip with a flat surface. The incision was closed with silk sutures after injury and animals were allowed to recover in either ST or EE housing.

At 24 hours following CCI, animals were subjected to either TMS or sham stimulus. The TMS system equipped with a figure eight, 25 mm custom rodent coil (Magstim, Rapid2) was placed over the center of the head between lambda and bregma at approximately 5mm distance from the skin. The stimulus was delivered daily for 6 days with the following settings: 4 second cycles of 10 Hz stimuli, 26 second interval between cycles, and 7 cycles (total of 280 pulses per day, 1680 total stimuli). For sham stimulus, a recorded sound of TMS stimulus was played with the TMS probe placed at the same location. During the stimulation, rats were anesthetized with 2% isoflurane in O₂. For TMS sham stimulus, rats were randomly labeled with different numbers at the underside of their tail. Externally, the rats from different groups were indistinguishable given that they all had identical surgical wounds. The information on label number and corresponding experimental group was written on a piece of paper and kept away from the laboratory personnel performing the behavioral, evoked stimulation, and imaging experiments to prevent bias.

In total, there were 4 groups in this study: 1. Rats in EE that had TMS (EE + TMS), 2. Rats in EE that had sham stimulus (EE only), 3. Rats in standard housing that had TMS (TMS only), 4. Rats in standard housing that had sham stimulus (sham).

Beam Walking Test

Rats were placed on one end of a suspended, narrow wooden beam (1 cm wide, 100 cm long) 30cm above ground. The travel time (latency) from one end of the beam to the other was measured. Bright lights were used to motivate the animal to traverse the beam in order to reach the other end with a darkened goal box. At each session, maximum of 60 seconds were given for rats to traverse the beam. The rats were pre-trained to traverse the beam the day prior to injury, and latency was measured before the injury (pre). The latency to traverse the beam was measured starting 1 day after injury and for 5 consecutive days after injury. Beam walking sessions were done prior to TMS or sham stimulation sessions each day to avoid acute effects of TMS and anesthesia on behavior. Sham group was used in this experiment without any treatment such as TBI, TMS, or EE.

Challenge Ladder

All challenge ladder testing was conducted with a San Diego Instruments challenge ladder (Fig. 1). A 10.16 (l) × 5.08 (w) × 17.78 (h) cm clear platform connected to a 114.3 cm horizontal ladder with clear Plexiglas walls and rungs elevated 60.96 cm off of the floor. The ladder led to a 40.64 (l) × 20.32 (w) × 17.78 (h) covered black box. The ladder consisted of 144 rungs each separated by 1.27 cm with 2.54 cm walls on either side. The width of the ladder begins at 5.08 cm at the entry from the clear platform and gradually narrowed to a width of 3.81 on the side of the ladder leading to the covered black box. Digital sensors were attached to the ladder. A laser sensor on the challenge ladder by the clear platform began a timer on the challenge ladder control box when it detected movement at the start of the ladder. The timer stopped once the rat passed a second laser sensor 113.03 cm on the other side of the challenge ladder near the black box. A laser sensor detected any movement 2.54 cm under the ladder and recorded that movement as a “slip” on the challenge ladder control box.

Rats were placed in the black covered box for two minutes. Rats were then placed on the clear platform and given three training sessions walking the ladder from the platform to the covered black box. The difficulty of the task was then modified by removing 25 rungs to create 2.54 cm gaps in 25 places on the ladder. Rats were then tested three consecutive times. The mean speed and number of slips of the three trials of each rat were then analyzed.

MEP Assessment

Motor evoked potentials in response to TMS stimulation were measured in 2% isoflurane anesthetized rats. The TMS coil was placed over the head at the midpoint and between lambda and bregma. Electromyography electrodes (Natus, Pleasanton CA) were placed in bilateral biceps and TMS was performed at 100% capacity for 10 single stimuli. Spike2 software (CED, Cambridge, UK) was used to average 10 spike amplitudes for each rat. Amplitude was determined to be the height from one negative peak to highest peak.

Electrophysiological Recordings

Rats were anesthetized with urethane (1.25 g/kg body weight, intraperitoneal injection) prior to placing in a stereotaxic frame. One day prior to the electrophysiology signal recording, craniotomy was performed on the left hemisphere contralateral to the injury to expose the left S1 cortex. Two needle electrodes were then inserted into the right forepaw to deliver sensory tactile stimulus of 3 mA at 3 Hz for 10 s. The sensory stimuli induced local field potential in S1 was detected by 12-channel axial array microelectrode (FHC, Inc.) placed at the following coordinates (AP: 0.0 mm, ML: +3.6 mm, DV: -2.0 mm). The axial array had 12 electrode sites spaced at 150 μ m distance between each electrode. Local field potential was detected using Cambridge Electronic Design interface (Micro1401-3, CED, Cambridge, UK), with amplifiers (HiZ $\times$ 8, FHC, Inc.) sampling between 11 and 1 KHz and band-pass filtered between 0.1 and 300 Hz. After band pass filtering and filtering artifacts from TMS at initial 10 ms, we analyzed the magnitude value of the LFP that typically occurs between 10–20 ms. Data was analyzed using Spike2 software (version 7, CED) and Matlab (Mathworks, Natick, MA, USA).

Functional MRI

During fMRI measurements, rats were lightly anesthetized with dexmedetomidine (0.1 mg/kg/h, SC). Respiration rate, heart rate, rectal temperature, and partial pressure of oxygen were continuously monitored throughout all measurements (Starr Life Sciences, Pennsylvania, USA). BOLD fMRI responses to contralateral tactile limb stimulation were measured in an ultra-high field of an 11.7 Tesla/16 cm horizontal bore small-animal scanner (Bruker BioSpin, Rheinstetten, Germany). A 72 mm quadrature volume coil and a 15 mm-diameter surface coil were used to transmit and receive magnetic resonance signals, respectively. For BOLD fMRI, gradient echo, echo planar imaging was used with a resolution of $150 \times 150 \times 1000 \mu\text{m}$. Five 1 mm thick coronal slices covering S1 were acquired (effective echo time (TE), 11 ms; repetition time (TR), 1000 ms; bandwidth, 250 KHz; field of view (FOV), $1.92 \times 1.92 \text{ cm}$; matrix size, 128×128). A T2-weighted RARE sequence was used to acquire high-resolution anatomical images (TE, 10 ms; TR, 5000 ms; bandwidth, 250 KHz; FOV, $1.92 \times 1.92 \text{ cm}$; matrix size, 256×256) corresponding to the BOLD fMRI measurements. fMRI block design was used, and forepaw stimulation was

conducted in a similar way as described above. The software Statistical Parametric Mapping (SPM, University College London, UK) was used for analysis. Activation maps were obtained using the general linear model. Z-score statistics were thresholded for $Z > 2.3$, which corresponds to an effective significance of $P < 0.01$.

Statistics

For statistical analysis, JMP (SAS Institute, Cary, NC, USA) and SPSS (IBM Corporation, Armonk, New York, USA) was used. Amplitudes of LFPs and MEPs were compared using one way ANOVA with Dunnett's post hoc test in 4 group comparisons, and Student's T test for 2 group comparisons. Student's T Test was used for challenge ladder test comparing number of slips between 2 groups. For beam walking test data, repeated measures ANOVA was used. When ANOVA was used, the F statistics value was reported.

Results

TBI was performed in $n=92$ adult rats using the well-established controlled cortical impact (CCI) model [37]. Following TBI, rats were housed either in standard cages in groups of two or large cage that is environmentally enriched with sensory and social stimuli. Specifically, the environmentally enriched groups consist of 6–8 TBI rats that were housed in 2-level cages ($96 \times 51 \times 96$ cm) filled with balls, ladders, tubes and climbing toys. These modifications, compared to standard rodent laboratory housing, allowed exercise, play, exploration, and permitted animals some control over their environment [29]. Among the rats in each housing group, there were those that received no TMS or rTMS at 10 Hz making the total number of conditions in this study to be 4 [Fig. 1]. We then tested the short-term and long-term behavioral and neurophysiological impact of the different therapeutic strategies.

Electrophysiology (evoked- LFP measurements across the primary somatosensory cortex (S1)) and beam walk testing were performed within 1 week of CCI. To assess the effects of long-term additive therapeutic effects of TMS and EE, TBI+TMS and TBI+TMS/EE groups were studied for electrophysiology, MEPs, fMRI, and challenge ladder test at 6 weeks after CCI.

TMS and EE lead to behavioral improvement at both short-term and long-term following TBI

To assess for short-term behavioral benefit from TMS and EE following TBI, rats ($n=30$) were subjected to daily sessions of beam walk test. Beam walk tests were performed on days 1–5 after injury. There were significant between group differences with $F(4,30)=6.297$, $P=0.001$. The Sham group has significant difference from TBI group ($p=0.004$) showing that injury increased the latency of Beam Walk Test [Fig. 2A]. Both TBI+TMS and TBI+EE groups were also significantly different from the Sham group ($p<0.001$ and $p=0.025$ respectively) showing no significant improvement in motor function by either of the two treatments alone. However, there was no significant difference between the Sham group and TBI+TMS/EE group ($p=0.355$) showing that only the combined treatment lead to significant recovery of motor function. Over time, all of the three experimental groups (TBI+EE, TBI

+TMS, TBI+TMS/EE) had improvement in beam walk latency similar to the TBI group. This daily improvement in latency for TBI rats has previously been demonstrated [38, 39].

Since the short-term motor behavioral data shows similar walking latency by 5 days after injury, a more sensitive motor behavioral test was performed at 6 weeks to assess for long-term effects of TMS and EE: challenge ladder test. The number of falls from the ladder as the rat walks across a ladder was counted in each group. The TBI+TMS/EE and TBI+TMS groups were compared, resulting in a significant decrease in the number of slips from the ladder among TBI+TMS/EE rats compared to TBI+TMS rats (8.5 vs 2.7 mean number of slips respectively, $P<0.01$) [Fig. 2B]. Together, these results indicate that the combination of TMS with EE following TBI significantly improves short-term and long-term functional recovery.

TMS and EE enhances local field potentials in primary sensory cortex

At 1 week after TBI, the four groups were subjected to cortical electrophysiology measurements ($n=17$). For the electrophysiology experiment, one way ANOVA comparison of LFP amplitudes at layers 2–3 resulted in statistically significant difference between the groups: $F(3,13)=4.289$, $P=0.026$ [Fig. 3]. Post hoc analysis using ordered differences report showed that TBI+TMS/EE group had a significantly higher LFP amplitude associated with increases in cortical excitability compared to TBI group, at cortical layers 2–3 ($P=0.011$) and 4 ($P=0.021$). There was also a significant difference between TBI+TMS/EE and TBI+EE groups for both Layers 2–3 ($P=0.009$) and Layer 4 ($P=0.022$).

An effective rehabilitation regimen should not only improve function at the time of the intervention, but it also should elicit long lasting effects. To assess if the TMS and EE enhanced long-term neuroplasticity, we tested LFP in sensory cortex at 6 weeks following injury. Long-term LFP response was assessed in TBI+TMS/EE and TBI+TMS groups by measurement of evoked LFP amplitudes ($n=14$) [Fig. 4]. The comparison of sensory function in these two groups showed that there was a significantly higher LFP amplitude for TBI+TMS/EE group in layers 2–3 ($P=0.026$) [Fig. 4A] and a similar trend but no statistically significant difference in LFP amplitude in TBI+TMS/EE group compared to other groups in layer 4 ($P=0.088$) [Fig. 4B].

TMS and EE enhances long-term motor evoked potential

To further investigate the mechanism underlying long lasting enhanced recovery of motor function among TBI+TMS/EE group, the amplitude of the muscle contraction using electromyography for MEP response was assessed at 6 weeks [Fig. 5]. As MEP amplitude is a marker of corticospinal reorganization [40, 41], TBI+TMS and TBI+TMS/EE groups were compared for MEP amplitudes at 6 weeks after injury ($n=19$). Muscle contraction was generated by motor cortex stimulation using a single TMS pulse [42, 43], and MEP responses were measured in the forelimb contralateral and ipsilateral to the injury. The results show that forelimb contralateral to the injury showed no significant difference between TBI+TMS/EE and TBI+TMS only groups ($P=0.257$) [Fig. 5A]. However, the forelimb ipsilateral to the injury had significantly higher MEP amplitude in TBI+TMS/EE group compared to TBI+TMS group ($P=0.0296$) [Fig. 5B].

TMS and EE enhances sensory evoked fMRI signal

In order to visualize improvement in brain function in a non-invasive manner and to identify biomarkers indicative of neuroplasticity and rehabilitation, high-resolution information was acquired using an ultra-high field MRI. We measured the extent of S1 cortex activation in response to tactile forelimb stimulation by comparing evoked fMRI BOLD signals among TBI+TMS and TBI+TMS/EE groups (n=12) [Fig. 6].

Our results demonstrate that out of six rats treated only with TMS (TBI+TMS group), four rats had minimal S1 activation (<20 activated pixels), and two had a greater S1 activation (at 617 and 476 activated pixels). If compared across all rats, there was a trend but no significant difference between the two groups ($P>0.05$) with the mean activated pixels at 296 ± 98.8 vs 185.8 ± 115.5 for TBI+TMS/EE and TBI+TMS groups respectively [Fig. 6C]. However, when only the four rats with <20 pixels in the TBI+TMS group were compared to six rats in TBI+TMS/EE group, there was a significant difference ($P=0.046$) with TBI+TMS having lower mean number of pixels (5.5 ± 2.9) compared to TBI+TMS/EE (296.8 ± 98.8). This variability suggests that in some individuals TMS by itself may enhance robust performance. On the other hand for all of the six TBI rats in TBI+TMS/EE group, forelimb stimulation evoked an extensive S1 activity, indicative of restored cortical function.

Discussion

Given the complexity of mechanisms involved in TBI such as oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis among many others, one of the main criticisms has been that most of the therapeutic approaches so far have targeted one specific mechanism. This concept has been suspected as the reason for limited success in therapeutic attempts at TBI. Thus in the recent years, additive benefits of multimodal therapies targeting more than one mechanism at a time have been gaining popularity in the medical and scientific community [44–47].

Prior studies using combination of physical activity with pharmacological treatment or electrical stimulation after TBI or stroke have received much attention [48–50]. As demonstrated in post stroke upper limb hemiparesis studies, combined therapy using rTMS and exercise therapy leads to functional benefits [48, 49, 51]. However, mechanistic evidence for this benefit has not been elucidated thus far, and further research into the biological basis of this additive effect is needed to maximize the benefits of combining rehabilitation with neural stimulation. The current study demonstrates a potential mechanism in which combination of EE and TMS has additive benefits in sensorimotor function.

As demonstrated in the current study this additive improvement is present at both short-term and long-term after injury. The lasting benefit that is present even up to 6 weeks is a promising finding that shows this noninvasive stimulation paradigm using TMS combined with rehabilitation such as EE may be an upcoming therapeutic modality for recovery from TBI. The findings from this report have important implications in clinical applications for noninvasive neural stimulation for rehabilitation after brain injury.

Among TBI subjects, reduction in cortical excitability has been reported previously [3, 52]. While therapeutic modalities focusing on numerous biochemical mechanisms have been attempted, enhancing neural activity by periodic magnetic stimulation following injury has not been explored extensively in the literature. High frequency rTMS on cortical neurons can lead to immediate activation of voltage gated sodium channels and action potentials as assessed by whole-cell patch-clamp recordings [28]. In current study, we utilized high frequency rTMS for 7 days following TBI in order to increase the cortical activity following injury to aid in functional recovery. Since rehabilitation from neural injury is highly dependent on the protocol of noninvasive stimulation, we provided 10 Hz stimulus following injury given that prior reports demonstrate enhanced cortical activity at higher frequencies [15, 53]. Although the results of TMS protocols using different frequency have not been reported in the setting of TBI, prior studies showed that low frequency stimulation at 1 Hz has an inhibitory effect whereas high frequency stimulation (greater than 3 Hz) has an excitatory effect in stroke patients [53, 54].

Electrophysiological measurements from left S1 cortex were assessed since there was a significant injury with no viable S1 cortex on the right side. The dynamic changes in blood-oxygenation-level-dependent (BOLD) signals in fMRI have been correlated to LFP [55], and thus we used fMRI signals as another measure of sensory cortex excitability after TMS and EE. Prior data have shown that there is a significant decrease in the fMRI signals and LFP amplitudes even in the contralateral somatosensory cortex of TBI rats compared to control rats [56]. It should be noted that contra coup injury at the time of TBI can be more severe than the coup injury, and that there is a widespread neural injury even remotely away from site of cortical impact as previously demonstrated using silver stain [57]. Thus, severe contralateral hemispheric injury likely resulted at the time of initial impact leading to reduced somatosensory cortex and motor cortex excitability. The LFP and MEP were then shown to be enhanced by TMS, with even further enhancement using a combination of TMS and EE. Current evidence suggests that the mechanism of improved behavioral function on beam walk test and challenge ladder test may be due to the enhanced sensorimotor excitability as well as cortical reorganization.

There are several limitations in this study. For practical reasons, four groups were included in most parts of this study: TBI, TBI+EE, TBI+TMS, and TBI+TMS/EE, with the Beam Walk Test having Sham group additionally. The experiments were focused on addressing the following aims: 1. To test whether TMS, EE or combination of the two improves recovery after TBI compared to no treatment, 2. To understand the underlying mechanistic basis of such therapy. Additional groups such as uninjured rats treated with EE, TMS, or both TMS and EE were excluded for the purpose of this study, given that the aim of this report is focused on TMS and EE on the pathophysiology of TBI. Also, additional experiments looking into LFP at the side ipsilateral to the injury would be helpful in understanding the interhemispheric difference between the two S1 cortices, but there was no viable cortical tissue on this side after injury. A milder level of TBI can be performed in the future to study the interhemispheric differences and relationship.

Conclusion

The results from this study demonstrate mechanistic background and provide exciting outlook for a therapeutic strategy combining noninvasive magnetic stimulation and rehabilitation. Mechanistic knowledge gathered from the current study can be used to further optimize future therapies and direct areas of future research. For example, S1 cortex LFP may be a useful biomarker of functional recovery and various TMS protocols combined with EE can be tested to find ones that maximize LFP.

Furthermore, MEP enhancement after TMS and EE is a supporting evidence for corticospinal reorganization that may be associated with motor recovery. Combining this treatment with pharmacological agents that have shown strong evidence for motor recovery (e.g. serotonin selective reuptake inhibitors [58, 59]) following brain injury may further enhance functional gains. As brain imaging and our understanding of connectivity is continuing to improve [60], targeted stimulation of specific circuitry may be utilized for maximal recovery from injury. Moreover, whereas this study utilized only TBI model of acquired neural injury, spinal cord injury model and ischemic stroke model can be further explored as target disease entities for TMS in combination with rehabilitative training.

Acknowledgements

Funding: Galit Pelled is supported by NINDS R01NS072171, 5R01NS079288. Courtney Robertson is supported by NINDS R01NS092747.

References

- [1]. Johnstone VP, Shultz SR, Yan EB, O'Brien TJ, Rajan R. The acute phase of mild traumatic brain injury is characterized by a distance-dependent neuronal hypoactivity. *J Neurotrauma*. 2014;31(22):1881–95. [PubMed: 24927383]
- [2]. Allitt BJ, Iva P, Yan EB, Rajan R. Hypo-excitation across all cortical laminae defines intermediate stages of cortical neuronal dysfunction in diffuse traumatic brain injury. *Neuroscience*. 2016;334:290–308. [PubMed: 27530700]
- [3]. Fecteau S, Dickler M, Pelayo R, Kumru H, Bernabeu M, Opisso Salleras E, et al. Cortical Excitability During Passive Action Observation in Hospitalized Adults With Subacute Moderate to Severe Traumatic Brain Injury: A Preliminary TMS Study. *Neurorehabil Neural Repair*. 2015;29(6):548–56. [PubMed: 25505219]
- [4]. Bagnato S, Boccagni C, Sant'Angelo A, Prestandrea C, Rizzo S, Galardi G. Patients in a vegetative state following traumatic brain injury display a reduced intracortical modulation. *Clin Neurophysiol*. 2012;123(10):1937–41. [PubMed: 22560638]
- [5]. Moosavi SH, Ellaway PH, Catley M, Stokes MJ, Haque N. Corticospinal function in severe brain injury assessed using magnetic stimulation of the motor cortex in man. *J Neurol Sci*. 1999;164(2):179–86. [PubMed: 10402031]
- [6]. Chistyakov AV, Soustiel JF, Hafner H, Trubnik M, Levy G, Feinsod M. Excitatory and inhibitory corticospinal responses to transcranial magnetic stimulation in patients with minor to moderate head injury. *J Neurol Neurosurg Psychiatry*. 2001;70(5):580–7. [PubMed: 11309450]
- [7]. Jones TA, Adkins DL. Motor System Reorganization After Stroke: Stimulating and Training Toward Perfection. *Physiology (Bethesda)*. 2015;30(5):358–70. [PubMed: 26328881]
- [8]. Nishibe M, Urban ET, 3rd, Barbay S, Nudo RJ. Rehabilitative training promotes rapid motor recovery but delayed motor map reorganization in a rat cortical ischemic infarct model. *Neurorehabil Neural Repair*. 2015;29(5):472–82. [PubMed: 25055836]

- [9]. Nudo RJ. Rehabilitation: Boost for movement. *Nature*. 2015;527(7578):314–5. [PubMed: 26581287]
- [10]. Frost SB, Chen D, Barbay S, Friel KM, Plautz EJ, Nudo RJ. Effects of Forced Use on the Ventral Premotor Cortex Distal Forelimb Representation After Ischemic Infarct in Primary Motor Cortex. *PM R* 2016;8(9S):S158.
- [11]. Sung WH, Wang CP, Chou CL, Chen YC, Chang YC, Tsai PY. Efficacy of coupling inhibitory and facilitatory repetitive transcranial magnetic stimulation to enhance motor recovery in hemiplegic stroke patients. *Stroke*. 2013;44(5):1375–82. [PubMed: 23532011]
- [12]. Pisegna JM, Kaneoka A, Pearson WG, Jr., Kumar S, Langmore SE. Effects of non-invasive brain stimulation on post-stroke dysphagia: A systematic review and meta-analysis of randomized controlled trials. *Clin Neurophysiol*. 2016;127(1):956–68. [PubMed: 26070517]
- [13]. Bolognini N, Vallar G, Casati C, Latif LA, El-Nazer R, Williams J, et al. Neurophysiological and behavioral effects of tDCS combined with constraint-induced movement therapy in poststroke patients. *Neurorehabil Neural Repair*. 2011;25(9):819–29. [PubMed: 21803933]
- [14]. Celnik P, Paik NJ, Vandermeeren Y, Dimyan M, Cohen LG. Effects of combined peripheral nerve stimulation and brain polarization on performance of a motor sequence task after chronic stroke. *Stroke*. 2009;40(5):1764–71. [PubMed: 19286579]
- [15]. Lu H, Kobil T, Robertson C, Tong S, Celnik P, Pelled G. Transcranial magnetic stimulation facilitates neurorehabilitation after pediatric traumatic brain injury. *Sci Rep*. 2015;5:14769. [PubMed: 26440604]
- [16]. Guo T, Li H, Lv Y, Lu H, Niu J, Sun J, et al. Pulsed Transcranial Ultrasound Stimulation Immediately After The Ischemic Brain Injury is Neuroprotective. *IEEE Trans Biomed Eng*. 2015;62(10):2352–7. [PubMed: 25935023]
- [17]. Brys M, Fox MD, Agarwal S, Biagioni M, Dacpano G, Kumar P, et al. Multifocal repetitive TMS for motor and mood symptoms of Parkinson disease: A randomized trial. *Neurology*. 2016;87(18):1907–15. [PubMed: 27708129]
- [18]. Nguyen JP, Suarez A, Kemoun G, Meignier M, Le Saout E, Damier P, et al. Repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease. *Neurophysiol Clin*. 2017;47(1):47–53. [PubMed: 28161090]
- [19]. Bocci T, Hensghens MJ, Di Rollo A, Parenti L, Barloscio D, Rossi S, et al. Impaired interhemispheric processing in early Huntington's Disease: A transcranial magnetic stimulation study. *Clin Neurophysiol*. 2016;127(2):1750–2. [PubMed: 26547356]
- [20]. Spagnolo F, Coppi E, Chieffo R, Straffi L, Fichera M, Nuara A, et al. Interhemispheric balance in Parkinson's disease: a transcranial magnetic stimulation study. *Brain Stimul*. 2013;6(6):892–7. [PubMed: 23810506]
- [21]. Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. *J Physiol*. 1992;453:525–46. [PubMed: 1464843]
- [22]. Shin SS, Pelled G. Novel Neuromodulation Techniques to Assess Interhemispheric Communication in Neural Injury and Neurodegenerative Diseases. *Front Neural Circuits*. 2017;11:15. [PubMed: 28337129]
- [23]. Noh NA, Fuggetta G, Manganotti P, Fiaschi A. Long lasting modulation of cortical oscillations after continuous theta burst transcranial magnetic stimulation. *PLoS One*. 2012;7(4):e35080. [PubMed: 22496893]
- [24]. Mueller JK, Grigsby EM, Prevosto V, Petraglia FW, Rao H, 3rd, Deng ZD, et al. Simultaneous transcranial magnetic stimulation and single-neuron recording in alert non-human primates. *Nat Neurosci*. 2014;17(8):1130–6. [PubMed: 24974797]
- [25]. Tischler H, Moran A, Belevsky K, Bronfeld M, Korngreen A, Bar-Gad I. Changes in basal ganglia processing of cortical input following magnetic stimulation in Parkinsonism. *Neurobiol Dis*. 2012;48(3):464–73. [PubMed: 22885186]
- [26]. Barnes WL, Lee WH, Peterchev AV. Approximating transcranial magnetic stimulation with electric stimulation in mouse: a simulation study. *Conf Proc IEEE Eng Med Biol Soc*. 2014;2014:6129–32. [PubMed: 25571396]

- [27]. Andreou AP, Holland PR, Akerman S, Summ O, Fredrick J, Goadsby PJ. Transcranial magnetic stimulation and potential cortical and trigeminothalamic mechanisms in migraine. *Brain*. 2016;139(Pt 7):2002–14. [PubMed: 27246325]
- [28]. Banerjee J, Sorrell ME, Celnik PA, Pelled G. Immediate Effects of Repetitive Magnetic Stimulation on Single Cortical Pyramidal Neurons. *PLoS One*. 2017;12(1):e0170528. [PubMed: 28114421]
- [29]. Bondi CO, Klitsch KC, Leary JB, Kline AE. Environmental enrichment as a viable neurorehabilitation strategy for experimental traumatic brain injury. *J Neurotrauma*. 2014;31(10):873–88. [PubMed: 24555571]
- [30]. Monaco CM, Mattiola VV, Folweiler KA, Tay JK, Yelleswarapu NK, Curatolo LM, et al. Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury. *Exp Neurol*. 2013;247:410–8. [PubMed: 23333563]
- [31]. Cheng JP, Shaw KE, Monaco CM, Hoffman AN, Sozda CN, Olsen AS, et al. A relatively brief exposure to environmental enrichment after experimental traumatic brain injury confers long-term cognitive benefits. *J Neurotrauma*. 2012;29(17):2684–8. [PubMed: 22774771]
- [32]. Costa-Ribeiro A, Maux A, Bosford T, Tenorio Y, Marques D, Carneiro M, et al. Dopamine-independent effects of combining transcranial direct current stimulation with cued gait training on cortical excitability and functional mobility in Parkinson's disease. *J Rehabil Med*. 2016;48(9):819–23. [PubMed: 27608611]
- [33]. Harris-Love M. Transcranial magnetic stimulation for the prediction and enhancement of rehabilitation treatment effects. *J Neurol Phys Ther*. 2012;36(2):87–93. [PubMed: 22592064]
- [34]. Meehan SK, Dao E, Linsdell MA, Boyd LA. Continuous theta burst stimulation over the contralesional sensory and motor cortex enhances motor learning post-stroke. *Neurosci Lett*. 2011;500(1):26–30. [PubMed: 21683125]
- [35]. Boggio PS, Alonso-Alonso M, Mansur CG, Rigonatti SP, Schlaug G, Pascual-Leone A, et al. Hand function improvement with low-frequency repetitive transcranial magnetic stimulation of the unaffected hemisphere in a severe case of stroke. *Am J Phys Med Rehabil*. 2006;85(11):927–30. [PubMed: 17079967]
- [36]. Takeuchi N, Tada T, Toshima M, Chuma T, Matsuo Y, Ikoma K. Inhibition of the unaffected motor cortex by 1 Hz repetitive transcranial magnetic stimulation enhances motor performance and training effect of the paretic hand in patients with chronic stroke. *J Rehabil Med*. 2008;40(4):298–303. [PubMed: 18382826]
- [37]. Dixon CE, Clifton GL, Lighthall JW, Yaghai AA, Hayes RL. A controlled cortical impact model of traumatic brain injury in the rat. *J Neurosci Methods*. 1991;39(3):253–62. [PubMed: 1787745]
- [38]. Robinson SE, Foxx SD, Posner MG, Martin RM, Davis TR, Guo HZ, et al. The effect of M1 muscarinic blockade on behavior and physiological responses following traumatic brain injury in the rat. *Brain Res*. 1990;511(1):141–8. [PubMed: 2331611]
- [39]. Beaumont A, Marmarou A, Czigner A, Yamamoto M, Demetriadou K, Shirotani T, et al. The impact-acceleration model of head injury: injury severity predicts motor and cognitive performance after trauma. *Neurol Res*. 1999;21(8):742–54. [PubMed: 10596383]
- [40]. Hajela N, Mummidisetty CK, Smith AC, Knikou M. Corticospinal reorganization after locomotor training in a person with motor incomplete paraplegia. *Biomed Res Int*. 2013;2013:516427. [PubMed: 23484130]
- [41]. Topka H, Cohen LG, Cole RA, Hallett M. Reorganization of corticospinal pathways following spinal cord injury. *Neurology*. 1991;41(8):1276–83. [PubMed: 1866018]
- [42]. Luft AR, Kaelin-Lang A, Hauser TK, Buitrago MM, Thakor NV, Hanley DF, et al. Modulation of rodent cortical motor excitability by somatosensory input. *Exp Brain Res*. 2002;142(4):562–9. [PubMed: 11845251]
- [43]. Luft AR, Kaelin-Lang A, Hauser TK, Cohen LG, Thakor NV, Hanley DF. Transcranial magnetic stimulation in the rat. *Exp Brain Res*. 2001;140(1):112–21. [PubMed: 11500803]
- [44]. Developing Multimodal Therapies for Brain Disorders: Proceedings of a Workshop. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC)2016.

- [45]. Haefeli J, Ferguson AR, Bingham D, Orr A, Won SJ, Lam TI, et al. A data-driven approach for evaluating multi-modal therapy in traumatic brain injury. *Sci Rep*. 2017;7:42474. [PubMed: 28205533]
- [46]. Maegle M, Lippert-Gruener M, Ester-Bode T, Sauerland S, Schafer U, Molcanyi M, et al. Reversal of neuromotor and cognitive dysfunction in an enriched environment combined with multimodal early onset stimulation after traumatic brain injury in rats. *J Neurotrauma*. 2005;22(7):772–82. [PubMed: 16004580]
- [47]. Martino Cinnera A, Bonni S, Iosa M, Ponzo V, Fusco A, Caltagirone C, et al. Clinical effects of non-invasive cerebellar magnetic stimulation treatment combined with neuromotor rehabilitation in traumatic brain injury. A single case study. *Funct Neurol*. 2016;31(2):117–20. [PubMed: 27358225]
- [48]. Hara T, Abo M, Kakita K, Masuda T, Yamazaki R. Does a combined intervention program of repetitive transcranial magnetic stimulation and intensive occupational therapy affect cognitive function in patients with post-stroke upper limb hemiparesis? *Neural Regen Res*. 2016;11(12):1932–9. [PubMed: 28197189]
- [49]. Cha HG, Kim MK. Effects of strengthening exercise integrated repetitive transcranial magnetic stimulation on motor function recovery in subacute stroke patients: A randomized controlled trial. *Technol Health Care*. 2017;25(3):521–9. [PubMed: 28106573]
- [50]. Kline AE, Leary JB, Radabaugh HL, Cheng JP, Bondi CO. Combination therapies for neurobehavioral and cognitive recovery after experimental traumatic brain injury: Is more better? *Prog Neurobiol*. 2016;142:45–67. [PubMed: 27166858]
- [51]. Graef P, Dadalt ML, Rodrigues DA, Stein C, Pagnussat Ade S. Transcranial magnetic stimulation combined with upper-limb training for improving function after stroke: A systematic review and meta-analysis. *J Neurol Sci*. 2016;369:149–58. [PubMed: 27653882]
- [52]. Seeger TA, Kirton A, Esser MJ, Gallagher C, Dunn J, Zewdie E, et al. Cortical excitability after pediatric mild traumatic brain injury. *Brain Stimul*. 2017;10(2):305–14. [PubMed: 27916406]
- [53]. Emara TH, Moustafa RR, Elnahas NM, Elganzoury AM, Abdo TA, Mohamed SA, et al. Repetitive transcranial magnetic stimulation at 1Hz and 5Hz produces sustained improvement in motor function and disability after ischaemic stroke. *Eur J Neurol*. 2010;17(9):1203–9. [PubMed: 20402755]
- [54]. Kim YH, You SH, Ko MH, Park JW, Lee KH, Jang SH, et al. Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. *Stroke*. 2006;37(6):1471–6. [PubMed: 16675743]
- [55]. Thompson GJ, Merritt MD, Pan WJ, Magnuson ME, Grooms JK, Jaeger D, et al. Neural correlates of time-varying functional connectivity in the rat. *Neuroimage*. 2013;83:826–36. [PubMed: 23876248]
- [56]. Li N, Yang Y, Glover DP, Zhang J, Saraswati M, Robertson C, et al. Evidence for impaired plasticity after traumatic brain injury in the developing brain. *J Neurotrauma*. 2014;31(4):395–403. [PubMed: 24050267]
- [57]. Garman RH, Jenkins LW, Switzer RC, Bauman RA, 3rd, Tong LC, Swauger PV, et al. Blast exposure in rats with body shielding is characterized primarily by diffuse axonal injury. *J Neurotrauma*. 2011;28(6):947–59. [PubMed: 21449683]
- [58]. Pariente J, Loubinoux I, Carel C, Albucher JF, Leger A, Manelfe C, et al. Fluoxetine modulates motor performance and cerebral activation of patients recovering from stroke. *Ann Neurol*. 2001;50(6):718–29. [PubMed: 11761469]
- [59]. Chollet F, Tardy J, Albucher JF, Thalamas C, Berard E, Lamy C, et al. Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. *Lancet Neurol*. 2011;10(2):123–30. [PubMed: 21216670]
- [60]. Wu D, Zhang J. In vivo mapping of macroscopic neuronal projections in the mouse hippocampus using high-resolution diffusion MRI. *Neuroimage*. 2016;125:84–93. [PubMed: 26499812]

Highlights

Combined therapy with TMS and environmental enrichment is assessed for TBI recovery.
This significantly improves motor behavior function compared to either therapy alone.
Motor evoked potentials are enhanced for animals with combined therapy.
Somatosensory cortex local field potential and fMRI response are also enhanced.

Fig. 1.

Diagram of experimental protocol. After TBI, rats were divided into EE or standard housing. Within this division, they were further divided into TMS or no stimulation groups.

Fig. 2.

Behavioral recovery following TBI. The performance of the sham rat group is shown by a gray dotted line for comparison to the rest of the groups. Beam walk test demonstrating motor behavioral performance at days 1–5 (A) show significant improvement in TBI +TMS/EE group compared to TBI+TMS group or TBI group. * $p < 0.005$. Motor behavioral test was also performed using challenge ladder test at 6 weeks (B). Here, TBI+TMS/EE group had significantly lower number of falls showing motor functional improvement at long-term. † $p < 0.01$.

Fig. 3.

Local Field Potential (LFP) differences among TBI groups treated with various stimulation paradigm. The amplitude of LFP in Layer 2–3 (**A**) and Layer 4 (**B**) showed significantly higher values in TBI+TMS/EE group compared to either TBI or TBI+EE groups.

Representative LFP amplitudes are demonstrated for TBI (**C**), TBI+EE (**D**), TBI+TMS (**E**), and TBI+TMS/EE (**F**) * $p < 0.05$.

Fig. 4.

Amplitudes of LFPs in the S1 cortex contralateral to the side of injury 6 weeks after CCI.

Layers 2–3 (**A**) show significantly higher mean LFP for TBI+TMS/EE compared to TBI+TMS group. However, there was no statistically significant difference at layer 4 (**B**).

Example LFP heat map (**C**), (**D**) qualitatively demonstrates the higher degree of LFP in TBI+TMS/EE animals (**D**) compared to TBI+TMS animals (**C**) * $p < 0.05$.

Fig. 5.

Motor evoked potentials in left (**A**) and right (**B**) forelimbs at 6 weeks after injury. Black horizontal bars represent mean value for each group. * $p < 0.05$. There are significantly higher MEP amplitudes in the TBI+TMS/EE rats in the right (ipsilateral to injury) forelimbs compared to TBI+TMS rats as shown by representative MEP graphs for each group (**C**).

Fig. 6.
Comparison of functional MRI results of TBI+TMS/EE vs TBI+TMS groups at 6 weeks.
Activated somatosensory cortex are qualitatively shown for TBI+TMS/EE animal (A) and
TBI+TMS animal (B).