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Septohippocampal Neuromodulation Improves Cognition after Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) often results in persistent attention and memory deficits that are associated with hippocampal dysfunction. Although deep brain stimulation (DBS) is used to treat neurological disorders related to motor dysfunction, the effectiveness of stimulation to treat cognition remains largely unknown. In this study, adult male Harlan Sprague-Dawley rats underwent a lateral fluid percussion or sham injury followed by implantation of bipolar electrodes in the medial septal nucleus (MSN) and ipsilateral hippocampus. In the first week after injury, there was a significant decrease in hippocampal theta oscillations that correlated with decreased object exploration and impaired performance in the Barnes maze spatial learning task. Continuous 7.7 Hz theta stimulation of the medial septum significantly increased hippocampal theta oscillations, restored normal object exploration, and improved spatial learning in injured animals. There were no benefits with 100 Hz gamma stimulation, and stimulation of sham animals at either frequency did not enhance performance. We conclude, therefore, that there was a theta frequency-specific benefit of DBS that restored cognitive function in brain-injured rats. These data suggest that septal theta stimulation may be an effective and novel neuromodulatory therapy for treatment of persistent cognitive deficits following TBI.

Key words: cognition; deep brain stimulation; hippocampal theta oscillation; medial septal nucleus; traumatic brain injury

Introduction

There are over 5.3 million people in the United States living with persistent cognitive deficits as a result of traumatic brain injury (TBI).[1,2] The pathophysiology of learning and memory deficits after TBI is poorly understood, and current treatment options have had limited success. Currently, deep brain stimulation (DBS) is being investigated as a potential treatment paradigm for memory deficits due to Alzheimer's disease and epilepsy,[3,4] but it has not been explored for cognitive recovery in TBI patients.

The hippocampus plays a critical role in a variety of memory tasks, and it is particularly prone to injury after TBI. Normal hippocampal activity is dominated by oscillations in the theta frequency range (6–10 Hz),[5–12] which synchronize activity across distal brain regions involved in cognitive processing.[13,14] Hippocampal theta oscillations result from a bidirectional feedback loop with the medial septal nucleus (MSN; Fig. 1A), considered a ''pacemaker'' for theta oscillations.[15,16] A chemical lesion in the MSN reduces hippocampal theta activity and causes persistent learning and memory deficits. Theta frequency stimulation of intact septal projections entrains hippocampal oscillations and recovers cognitive performance.[8]

We previously demonstrated that lateral fluid percussion TBI in rats resulted in decreased hippocampal theta power and persistent deficits in spatial navigation memory tasks.[17,18] These deficits are particularly salient as spatial memory problems in rodents correlated with deficits in human episodic memory.[19–21] We next demonstrated that 7.7 Hz pre-stimulation of the MSN for 60 sec improved outcome on the Barnes maze spatial learning task.[18] Based on these data, we generated four new hypotheses related to optimizing a stimulation paradigm for treatment of TBI-induced cognitive disorders. We hypothesized that improved outcome from stimulation would be theta frequency-dependent. In addition, we hypothesized that a brief stimulation period would have lasting effects on hippocampal oscillations, and stimulation prior to a task would be superior to a continuous stimulation paradigm, as continuous fixed-frequency stimulation is not physiological. Finally, we hypothesized that the improvement in behavior was a recovery of function, not an overall enhancement or an indirect consequence of increased activity.

Methods

One hundred thirty-six adult male Harlan Sprague-Dawley rats (Harlan, Indianapolis, IN, USA; 300–350 g) underwent a fluid percussion TBI ($n = 82$) or sham ($n = 54$) injury. Separate groups were used for each of four analyses including baseline recordings (sham $n = 6$, TBI $n = 7$), assessment of stimulation duration (TBI

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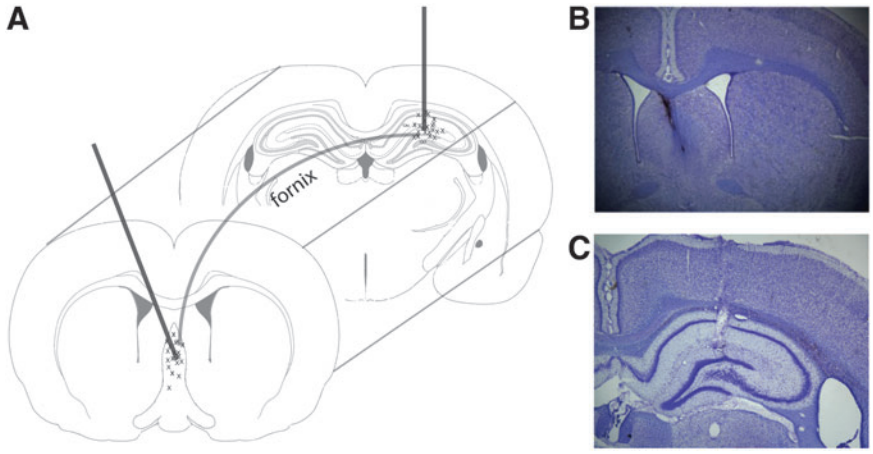

FIG. 1. **(A)** Atlas schematic of medial septal nucleus and hippocampal depth electrodes and typical sections showing representative electrode tracts (each ''x'' represents an estimated location of an electrode tip seen on cresyl violet staining). **(B)** Representative cresyl violet stain demonstrating the placement of medial septal stimulating/recording electrode. **(C)** Representative cresyl violet stain demonstrating placement of a hippocampal recording electrode. Color image is available online at www.liebertpub.com/neu

$n=11$), a stimulation-response evaluation (object exploration task; sham $n=19$, TBI $n=33$), and finally the Barnes maze spatial learning task (sham $n=29$, TBI $n=31$). Following surgery, animals were exposed to a 12-h light and dark cycle and monitored daily for changes in weight. Investigators were blind to sham versus TBI groups for all experiments. For stimulation studies, investigators were not blind to stimulation conditions during the collection of data. However, investigators were blind to both injury and stimulation conditions during all behavioral and electroencephalogram (EEG) analysis. All experiments were performed in compliance with the UC Davis IACUC protocol.

Lateral fluid percussion injury and electrode placement

Animals were anesthetized using 4% isoflurane in O_2/N_2O (1:2) carrier gas, and were then intubated and mechanically ventilated with 2% isoflurane to maintain surgical anesthesia. A circular 4.8 mm diameter craniectomy was made in the skull over the right hemisphere, leaving the dura intact, at a position of 3 mm lateral to the midline and midway between lambda and bregma (anterio-posterior [AP] $\sim$–4.5 mm).[22,23] A rigid plastic injury tube was placed into the craniectomy site, cemented to the skull using methyl methacrylate and cyanoacrylate adhesive and filled with sterile saline. Then a calibrated $\sim$0.10 mL pulse of sterile saline was rapidly injected (18 msec) through the injury tube onto the surface of the dura with a pressure of 2.12–2.15 atm to produce a moderate TBI in each rat.[22] After TBI, the intubated rat was then returned to the ventilator with isoflurane at $\sim$1% for maintenance during surgical placement of a twisted stainless steel, 0.005 inch diameter, three-channel bipolar electrode (Plastics One MS333-3-B-SPC; Plastics One, Roanoke, VA) spanning the CA3–CA1 dorsal hippocampal fissure with a ground wire wrapped around a surgical screw (AP –3.3 mm from bregma, lateral +2.0 mm from midline, 3.6 mm deep to skull; see Fig. 1A). An additional 2 mm craniotomy (–0.5 mm AP, 1.0 mm lateral) was made to allow access for implantation of a second chronic indwelling twisted parallel bipolar stimulating electrode into the MSN (–6.8 mm depth from the skull surface at 12.8 degrees to avoid the dural sinus).

Acute EEG recordings

On post-injury days (PID) 1–7, rats (sham=6, TBI=7) were placed in an enclosed translucent Plexiglas® cage, 25×45×50 cm for 10 min. An EEG was recorded with a pre-amplifier (Grass model 7P5B; Grass Technologies Inc., Quincy, MA) and polygraph driver amplifier (Grass model 7DAG). Data were collected with a computer acquisition system (Polyview16 v1.1; Astromed, Inc., West Warwick, RI).[17] EEG data were collected with a bandwidth ranging from 0.1 Hz to 500 Hz. Continuous video analysis of behavior (Logitech c920; Logitech, Newark, CA) was captured for the duration of the EEG recording. The EEG was analyzed for percentage of total time oscillating in the theta frequency range (6–10 Hz), ipsilateral septohippocampal phase coherence, and peak oscillatory frequency.

EEG analysis

"P_episode" percent time in theta analysis. Using an oscillatory detection method, p_episode, we determined the percent of recording time in which the EEG signal included significant oscillations in the 6–10 Hz theta frequency range[11,24–26] on PID 1–7. P_episode applies a power threshold, P_t, and a duration threshold, D_t, to determine the percent time in high power of the theta (6–10 Hz) frequency. Specifically, a background spectrum is calculated by fitting the observed power values to a χ^2 distribution using linear regression. The 95th percentile of this fit distribution in the theta frequency range was used to establish a power threshold, therefore excluding low power oscillations that may in fact be artifact. As previously published, the duration threshold was set to three cycles.[27] This means that there needed to be three consecutive peaks in the theta range for an oscillation to be detected. Based upon these parameters, the percent of recording time in which the values exceeded both power and duration thresholds was quantified.

Phase coherence estimation. To calculate coherence we used EEGLab[28] and custom-written code in MATLAB (Mathworks, Natick, MA). Phase coherence was estimated between the MSN and ipsilateral hippocampus in the 6–10 Hz frequency range using the pairwise phase consistency (PPC) index[29] on PID 1–7. The PPC index for each electrode pair was estimated by computing the relative phase angle difference between signals on each trial. The cosine was then computed between all pairwise (i.e., between trials) combinations of relative phases, and the PPC was taken as the mean of these cosine values. PPC values range from 0 to 1, with more positive values indicating greater phase coherence. We accounted for potential volume conduction confounds, which may artificially inflate phase coherence estimates, by removing relative phases at 0 degrees (±5 degrees) prior to calculating the PPC index.[29]

Fast Fourier Transform power analysis to assess peak oscillating frequency. One of the limitations of p_episode is that the analysis cannot distinguish small frequency differences ($\sim$1–2 Hz) in oscillations. Therefore, although it is a useful tool to assess theta across the 6–10 Hz range, it is not able to detect slight shifts in peak oscillatory frequency. Therefore, we used a power spectral frequency analysis performed with 0.1 Hz intervals to determine if injury caused a change in the peak oscillatory theta frequency on PID 3 and 7.

Object exploration task

On PID 5, rats (sham $n=19$, TBI $n=33$) were habituated to a white circular platform (1.8 m diameter). No objects were present on the platform and the rat was allowed to explore for 5 min. The following day animals were evaluated for object exploration. Two distinct objects, a pump soap dispenser and a soda can, were placed on the platform 68 cm apart. Animals were first brought into the room in an empty cage. They were subsequently connected to the EEG cables for 2 min prior to exposure to the objects on the platform. For the stimulation groups, stimulation was initiated in the second min in the cage, 1 min prior to introduction to the platform. Animals were separated into eight different stimulation groups: sham no stimulation ($n=8$), sham 7.7 Hz (80 μA, $n=5$), sham 100 Hz (80 μA, $n=6$), TBI no stimulation ($n=10$), TBI 7.7 Hz (20 μA, n=5; 80 μA, $n=6$; 200 μA, $n=5$), and TBI 100 Hz (80 μA, $n=7$). Initially, we compared 7.7 Hz stimulation across multiple stimulation amplitudes and identified 80 μA as the ideal stimulation intensity. Subsequently, we added additional sham stimulation as well as gamma stimulation test groups using the 80 μA stimulation intensity (with additional sham and TBI controls). The animals were placed on the platform equidistant between the two objects and behavior and EEG were observed for 5 min. Behavior was analyzed both for time interacting with objects as well as total distance traveled.

Barnes maze

The Barnes maze was performed as previously described by our lab.[18] Two trials per day were conducted with a 5-min inter-session interval on post-operative days 5–7. Six groups were evaluated on the Barnes maze including sham ($n=14$) and sham with 7.7 ($n=8$) or 100 Hz ($n=7$) stimulation, TBI ($n=15$), and TBI with 7.7 ($n=8$) or 100 Hz ($n=8$) stimulation. All stimulation during the Barnes maze was at 80 μA. At the start of each trial, animals were placed in the center of the Barnes maze apparatus under a dark box for 1 min. For animals in the stimulation groups, stimulation was initiated immediately prior to being placed in the box. After 1 min, the box was lifted and the animal was given 5 min to find a hidden escape box using four distinct spatial cues. If the animal did not find the escape box after 5 min, it was guided to it. During each trial, video-EEG was concurrently recorded. Latency to finding the escape hole and search strategies were analyzed for each trial. Search strategies were categorized as spatial, peripheral, and random. The spatial strategy was defined as the rat moving directly toward the escape hole, whereas the peripheral strategy indicated that the rodent follows the edge of the circular platform until it reached the escape hole. The random strategy was defined as a rat searching non-consecutive holes before reaching the escape hole.

Stimulation paradigm

The electrode in the MSN was connected to an isolated pulse stimulator (model 2100; A-M Systems, Sequim, WA), and electrical stimulation was delivered using the following stimulation parameters: 1 msec square wave pulses at a frequency of 7.7 or 100 Hz. To assess the effects of stimulus duration animals received 15, 30, or 60 sec of 80 μA stimulation. For the object exploration task, stimulation was initiated 1 min prior to animals being introduced to the behavioral apparatus and was terminated 6 min later immediately prior to returning animals to their home cages. Animals were stimulated at 7.7 (20, 80, or 200 μA) or 100 Hz (80 μA). For the Barnes maze task, 80 μA at 7.7 or 100 Hz stimulation was initiated 1 min prior to the first trial of each day. Stimulation was terminated at the completion of the second daily trial, immediately prior to returning the rat to its home cage.

Stimulation duration

A total of 11 rats receiving TBI were used to evaluate the effect of stimulus duration. On PID 3, animals were habituated to an opaque Plexiglas box ($1\times1\times0.5$ m). Habituation included two 5-min periods of exploring the empty box separated by 3 h. On PID 4 and 5, rats each received four 17-min trials, two per day, with each daily trial separated by 3 h. Animals were put in a start cage for 2 min. Animals received no stimulation or 15, 30, or 60 sec of stimulation immediately prior to being transferred to the Plexiglas box. Animals were randomized to stimulation condition with each animal exposed to each condition once over the four trials. For each trial, there were four objects in the box. On the first trial, they were four novel objects. For each subsequent trial, two novel objects were introduced in the place of the familiar objects. The two remaining familiar objects were moved to novel positions within the box. Objects included a plastic lemon, a plastic lime, three different sized (height and width) cans, a pump soap dispenser, and four different-shaped dog chew toys. The EEG was analyzed for percent of time oscillating in the theta frequency range during stimulation as well as percent of time oscillating in theta and septohippocampal phase coherence over the first 5 min following stimulation. A post hoc analysis confirmed that there was no effect of cumulative stimulation or repeated behavioral trials as data within a stimulation condition were similar regardless of trial number.

Histology

Rats were euthanized 15–20 days after injury by deep anesthesia and transcardial perfusion with 100 mL of 0.1 M sodium phosphate buffer saline (PBS, pH 7.4) followed by 50 mL of 4% paraformaldehyde (pH 7.4). Brains were extracted, stored overnight in 4% paraformaldehyde at 4°C, and subsequently placed in sucrose immersion for cryoprotection. Coronal sections were cut at 45 μm thicknesses using a sliding microtome (model 860, American Optical, Buffalo, NY). Serial sections starting at bregma and ending at –4.80 mm relative to bregma were saved in 24-well cell culture plates for histological staining. Sections in the vicinity of electrodes were mounted onto 0.1% gelatin-subbed slides, stained with cresyl violet to identify precise electrode placement.

Statistical analysis

SPSS statistics version 22 (IBM Corp., Armonk, NY) was utilized for all statistical evaluation. A paired-samples *t* test was used to compare percent time of theta oscillations at baseline relative to during stimulation. A repeat measures analysis of variance (ANOVA) was utilized to compare 1) daily percentage of time in theta recordings, 2) daily MSN-hippocampal coherence, 3) effect of stimulation on post-stimulation percent time and coherence of theta, and 4) Barnes maze latency. A Dunnett post hoc analysis was used to compare individual groups. A one-way ANOVA was utilized to compare 1) differences in peak oscillatory frequency, 2) differences among 15, 30, and 60 sec of stimulation, 3) object exploration times, and 4) total distance traveled during object exploration. Dunnett post hoc analysis was used to compare individual groups. A nonparametric binomial analysis was used to compare differences in search strategy, random as compared with spatial+peripheral, and among groups on the Barnes maze.

Performance of sham animals was used as the ''test proportion.'' Statistical significance was assigned to values $p < 0.05$. All data are presented as mean ± the standard error of the mean.

Results

Baseline physiological measurements

Over the first 7 days post-injury, theta oscillations were evaluated in the hippocampus and MSN in both sham ($n = 6$) and TBI ($n = 7$) rats. There was a significant difference in the percentage of time hippocampal theta oscillations were observed between sham and TBI rats ($F_{(1,11)} = 13.26$, $p < 0.005$; Fig. 2A); there was no difference in MSN theta oscillations ($F_{(1,11)} = 2.72$, $p = 0.13$; Fig. 2B). A comparison of septohippocampal theta coherence revealed a trend toward a difference between sham and TBI rats ($F_{(1,11)} = 3.17$, $p = 0.10$; Fig. 2C). In addition to percentage of time and coherence, we assessed whether there was a change in the peak oscillatory frequency on days 3 and 7. A significant change in peak theta frequency was observed in the hippocampus on PID 3 ($F_{(1,11)} = 10.75$, $p < 0.01$), and a trend toward a difference was observed at day 7 ($F_{(1,11)} = 3.5$, $p = 0.088$; Fig. 3).No difference in peak theta frequency was detected in the MSN on either PID 3 or 7.

Persistent effects of stimulation on oscillations

Previously, we demonstrated that 60 sec of 7.7 Hz MSN stimulation increased hippocampal theta power during stimulation and improved cognitive function on the Barnes maze task even after the stimulation was terminated.[18] We hypothesized, therefore, that there would be a stimulation duration-related enhancement of theta oscillations following termination of the stimulus. Similar to our previous data,[30] a significant increase in the percentage of time in theta oscillations was detected in the hippocampus relative to baseline during 15 sec of stimulation ($df = 10$, $t = -8.77$, $p < 0.001$), 30 ($df = 10$, $t = -9.38$, $p < 0.001$), and 60 ($df = 10$, $t = -5.86$, $p < 0.001$; Fig. 4A). Regardless of MSN stimulation time, percent time oscillating in the theta range during stimulation measured in the hippocampus was similar. Counter to our hypothesis, however, we did not observe a lasting change in either the hippocampal percentage of time in theta (Fig. 4B) or septohippocampal phase coherence (Fig. 4C), even with 60 sec of stimulation.

Dose and frequency response in the object exploration task

As our assessment of post-stimulation oscillations did not detect enduring changes in theta, we decided to evaluate a continuous stimulation paradigm to restore behavioral function. We also wanted to compare the specificity of stimulation frequency (i.e., theta as compared with gamma) for improving outcome. Prior to testing in the Barnes maze, performance on an object exploration task was evaluated to insure that continuous theta or gamma stimulation did not elicit significant side effects, particularly convulsions.

Initially, all TBI stimulation groups (0, 20, 80, and 200 μA of 7.7 Hz as well as 80 μA gamma stimulation) were compared with a sham control. A significant main effect of group on object exploration was detected ($F_{(5,34)} = 6.94$, $p < 0.001$; Fig. 5A). A Bonferroni post hoc analysis indicated that TBI rats with 0 μA ($p < 0.005$) and 20 μA ($p < 0.05$) of theta stimulation as well as TBI rats with 80 μA of 100 Hz gamma stimulation ($p < 0.05$) all explored objects significantly less than sham rats (Fig. 5A). In addition, TBI rats receiving 80 μA of theta stimulation explored objects significantly more than TBI rats with 0 μA ($p < 0.005$) and 20 μA ($p < 0.05$) of theta stimulation as well as TBI rats with 80 μA of gamma stimulation ($p < 0.05$; Fig. 5A). Importantly, there was no observed difference in distance traveled among groups (Fig. 5B), indicating that the change in behavior was not related to driving overall motor activity. In addition to TBI rats, sham theta and gamma stimulation

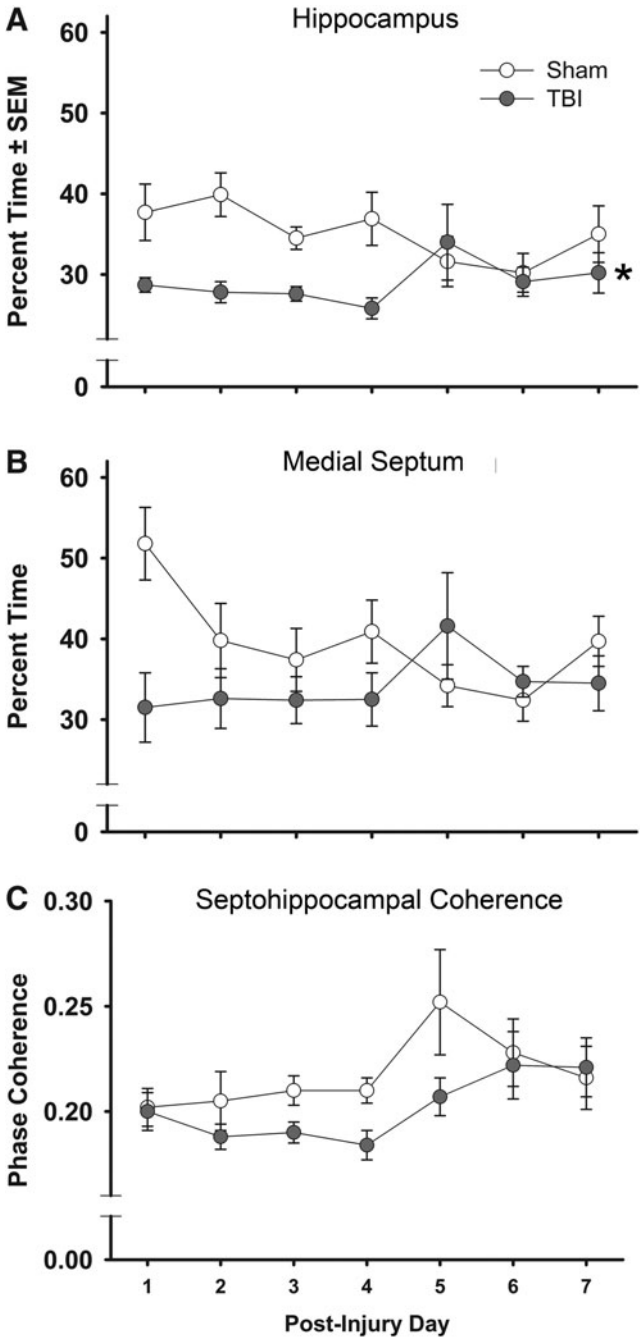

FIG. 2. **(A)** During the first 7 days after injury, there was a significant decrease in the hippocampal theta oscillations in TBI rats as determined by calculating the percentage of time theta oscillations are observed in the hippocampus. **(B)** There was no change in the percentage of time theta was observed in the MSN. **(C)** Septohippocampal phase coherence was not significantly changed over the first week following TBI. *$p < 0.05$. MSN, medial septal nucleus; TBI, traumatic brain injury.

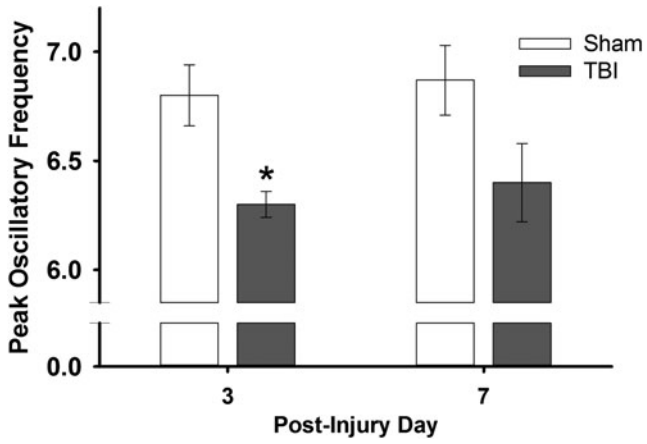

FIG. 3. There was a significant decrease in the peak oscillatory frequency of theta in the hippocampus on post-injury day 3 with a trend toward a decrease on post-injury day 7. *$p < 0.05$.

rats were also compared with sham controls. No effect of stimulation on object exploration (Fig. 5A) or distance traveled (Fig. 5B) was detected. Critically, none of the sham or TBI stimulated animals displayed any behavioral signs of hyperactivity or seizures.

Stimulation improves performance on the Barnes maze

Based on data observed on the object exploration task, we reduced the number of groups to be tested on the Barnes maze to sham and TBI controls as well as sham and TBI rats receiving 80 μA of 7.7 or 100 Hz stimulation. A repeated measures ANOVA comparing sham ($n = 14$), TBI ($n = 15$), TBI theta stimulation ($n = 8$), and TBI gamma stimulation ($n = 8$) identified a significant main effect of group on latency to finding the escape box ($F_{(3,41)} = 3.43$, $p < 0.05$; Fig. 6A). Post hoc analysis indicated that there was a significant difference in latency between sham and TBI rats ($p < 0.05$).

In addition to quantifying latency, we also analyzed search strategy. Over 3 days, sham animals used a random strategy 46% of the time, with 75% of the trials on day 1 being random and only 25% of the trials on day 3 (Fig. 6B). TBI rats ($p < 0.01$) and TBI rats receiving 100 Hz stimulation ($p < 0.01$) used significantly more random search strategies over the 3 days of testing. TBI rats receiving theta stimulation had a trend toward a reduction in random strategies relative to sham rats ($p = 0.078$). To test whether stimulation improved spatial learning in sham animals, the same sham animals used above ($n = 14$) were compared with sham rats receiving 7.7 ($n = 8$) or 100 Hz stimulation ($n = 7$). Analysis determined that there was a significant main effect of group on latency ($F_{(2,26)} = 10.47$, $p < 0.001$; Fig. 7A) with sham gamma rats performing significantly worse than sham ($p < 0.001$) and sham theta rats ($p < 0.005$). Although there was no difference in search strategy between sham animals and sham animals receiving 100 Hz stimulation (Fig. 7B), sham rats receiving 7.7 Hz stimulation used fewer random strategies relative to the sham controls ($p < 0.01$).

Discussion

Similar to our previous findings, we found that hippocampal theta oscillations were diminished over the first week following

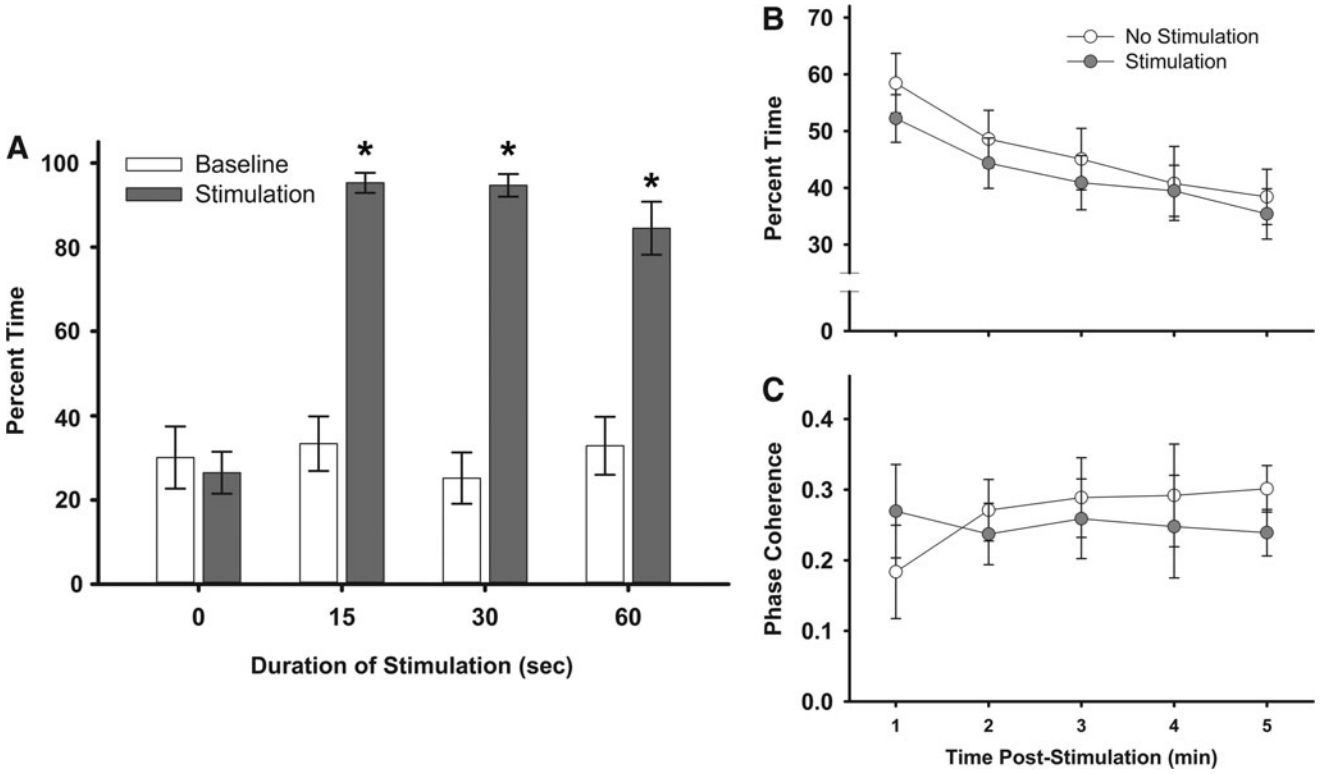

FIG. 4. **(A)** During stimulation, there was a significant increase in theta oscillations observed in the hippocampus as compared with baseline. There was no stimulation-duration effect on the percentage of time theta was observed among 15, 30, and 60 sec. **(B)** There was no lasting effect of stimulation on percentage of time or **(C)** septohippocampal coherence post-stimulation as both values returned to levels seen in animals that had not received stimulation. *$p < 0.001$.

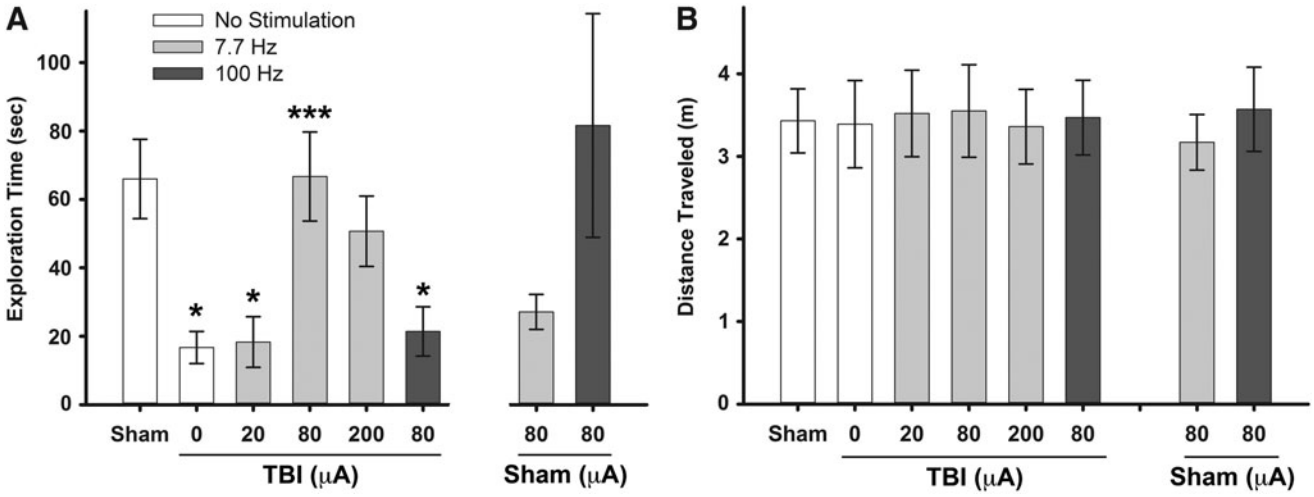

FIG. 5. **(A)** A range of stimulations were evaluated to determine an optimal frequency and intensity to improve performance in the absence of seizures. TBI rats had a significant decrease in object exploration as compared with sham rats. Similarly, TBI rats receiving 20 μA of 7.7 Hz or 80 μA of 100 Hz stimulation also explored objects significantly less than shams. TBI rats receiving 80 μA of 7.7 Hz stimulation explored significantly more than TBI rats receiving no stimulation. **(B)** Stimulation, regardless of frequency or intensity, did not influence an animal's total activity as measured by distance traveled. *$p < 0.05$ as compared with sham. ***$p < 0.05$ as compared with TBI without stimulation. TBI, traumatic brain injury.

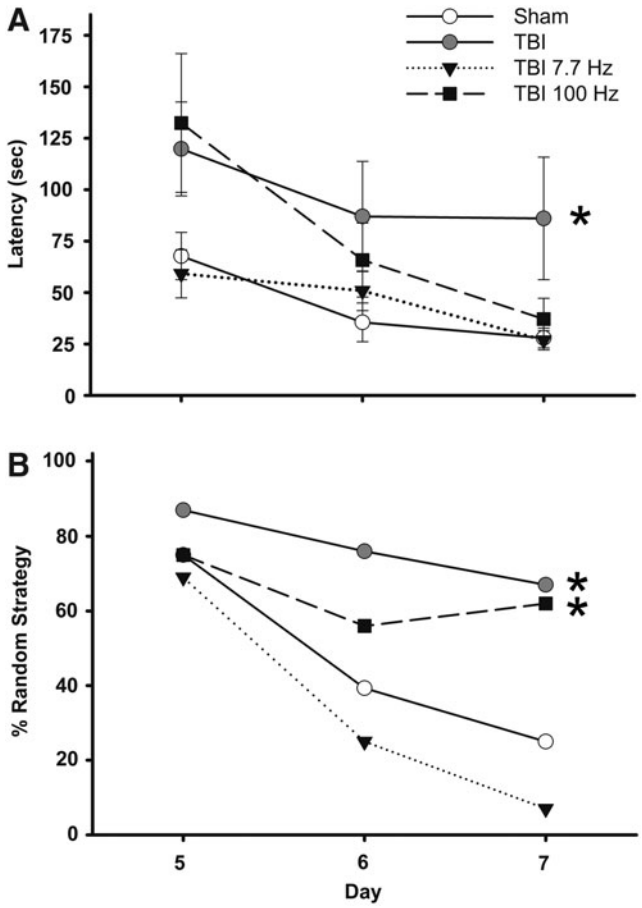

FIG. 6. **(A)** TBI rats had a significant increase in latency to finding the hidden escape box on the Barnes maze. **(B)** However, both TBI rats and TBI rats receiving 100 Hz stimulation demonstrated a significantly greater reliance on random search strategies to find the escape box relative to sham rats. *$p < 0.05$. TBI, traumatic brain injury.

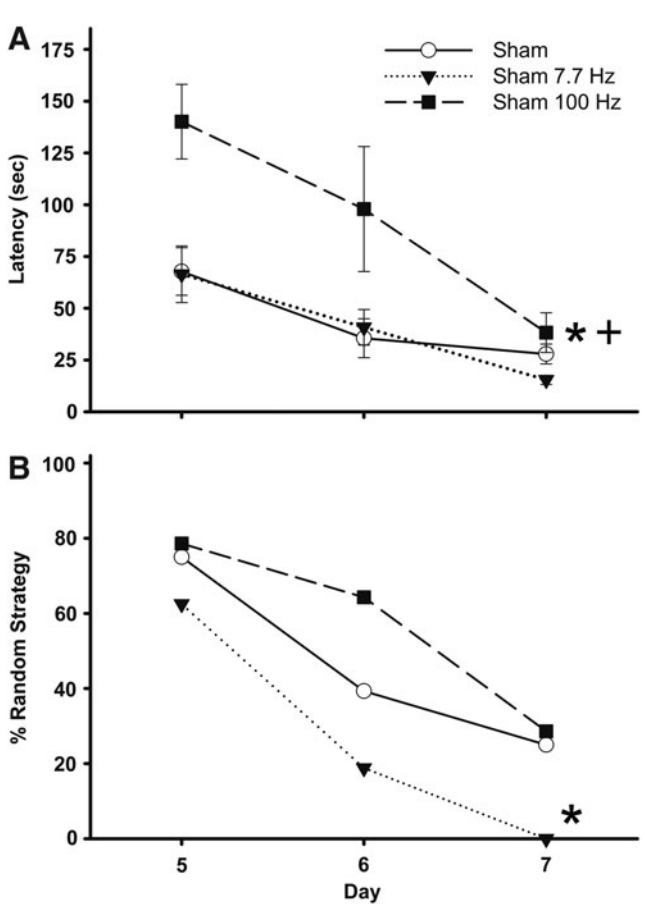

FIG. 7. **(A)** 100 Hz stimulation significantly impaired Barnes maze performance in sham rats compared with both sham and sham 7.7 Hz stimulated rats. **(B)** Whereas latency was similar between sham and sham 7.7 Hz animals, theta stimulated sham rats used significantly fewer random strategies to find the hidden escape box compared with sham rats. *$p < 0.05$.

TBI.[18] We now demonstrate that the percentage of time theta was observed in the MSN was unchanged compared with sham rats and, despite the reduction in hippocampal theta, there was not a significant change in the phase coherence of septohippocampal theta oscillations. In addition to time oscillating and coherence, we also observed that there was a significant shift in the peak oscillatory frequency of theta over the first week following injury in the hippocampus. These data suggest that there was a TBI-induced disruption of the septohippocampal circuit.

Previously, we demonstrated that 1 min of theta pre-stimulation significantly improved performance on the Barnes maze task.[18] One question that arose was whether there was a minimum amount of stimulation needed to entrain hippocampal theta. Therefore, we evaluated if 15, 30, or 60 sec of MSN stimulation would result in differential effects on hippocampal theta, specifically focusing on theta and theta phase coherence observed during and after stimulation. We found that stimulation of the MSN significantly increased hippocampal theta regardless of stimulation duration. However, regardless of stimulation duration, we did not observe a change in post-stimulation theta or septohippocampal phase coherence, with both returning to non-stimulated TBI levels within the first minute post-stimulation.

As we did not detect lasting effects of stimulation on percent time and coherence, we decided to evaluate if there was a benefit to continuous stimulation relative to pre-stimulation on outcome. A stimulation intensity-response curve demonstrated that 80 μA of stimulation, similar to our previous report, best restored function on an object exploration task. No animals, even at 200 μA, had evidence of seizure activity during stimulation. Using the 80 μA current, we also evaluated the effect of 100 Hz gamma stimulation on TBI rats as well as both 7.7 and 100 Hz stimulation in sham controls. Interestingly, 100 Hz stimulation did not improve object exploration in TBI rats and 7.7 Hz, but not 100 Hz, stimulation impaired object exploration in shams. These data support the hypothesis that frequency-specific theta stimulation can improve outcome in TBI rats, and the effect is restoring function not simply driving object exploration in all animals. In fact, analysis of total distance traveled (including exploration of the objects) did not find any difference in sham or TBI rats regardless of stimulation.

On the Barnes maze, TBI rats performed significantly worse than sham animals. Moreover, although search strategy improved in sham animals from 75% random on the first day to 25% random on the third day, over 50% of TBI rats continued to use random search strategies throughout the test. TBI rats receiving theta stimulation had latency and search strategy patterns similar to sham rats. Although TBI rats receiving gamma stimulation did not have a significantly different latency from shams, approximately half of these animals continued to use a random search strategy to find the escape box. Although theta stimulation of sham rats did not significantly change behavior, animals receiving gamma stimulation had significantly longer latencies to finding the escape box.

These data, therefore, demonstrate that 80 μA of continuous 7.7 Hz stimulation consistently improved behavioral function in TBI rats. Although 100 Hz stimulation rats did not have a significant change in Barnes maze latency, these animals did exhibit decreased object exploration and persisted in using random strategies on the Barnes maze. We therefore concluded that the benefit of stimulation is theta frequency-specific. In addition, there was no clear evidence that stimulation was driving object exploration or significantly improved spatial maze performance on the Barnes maze in sham animals. Therefore, we concluded that the effects of stimulation were restorative, rather than just enhancing overall function.

Effects of TBI on septohippocampal activity and cognition

Our results further support the theory that impairments in hippocampal theta oscillations and associated circuits affect cognitive performance during spatial navigation. Several lines of evidence support the theory that theta oscillations are necessary for navigation and spatial memory by generating a temporal organization of neuronal activity.[31–33] It is also known that retention and encoding have been associated with increased hippocampal theta.[34–37] In fact, Eakin and Miller have suggested that a TBI-induced decrement in theta activity disrupts hippocampal firing patterns important in working memory.[38]

One of the key findings of this article is that TBI led to a significant decrease in peak theta oscillatory frequency. Although the underlying mechanisms are poorly understood, previous research has demonstrated an upward phase shift in low frequency oscillations during successful performance in working memory tasks.[27,39] Therefore, a significant reduction in the peak oscillatory frequency of theta may play an important role in cognitive impairment in TBI animals.

Two types of hippocampal theta have been described: atropine-resistant (Type 1) and atropine-sensitive (Type 2). Type 1 theta oscillations are due to *N*-methyl-D-aspartate (NMDA) receptor-dependent input from the entorhinal cortex to the hippocampus associated with movement and voluntary behaviors.[40,41] Atropine-sensitive Type 2 theta oscillations are dependent on cholinergic projections from the basal forebrain and are associated with immobility and preparation for movement.[16,42] One of the concerns related to the observation that theta oscillations are depressed in the first week post-injury is that injured animals tend to be lethargic compared with sham controls; particularly in the first 72 h. Therefore, we also analyzed our EEG findings to assess whether theta was depressed during movement and/or rest. Analysis of five, 5-sec epochs of 100% movement or 100% rest indicated that oscillations were diminished at rest compared with movement. Also, it indicated that oscillations during movement or at rest were similar between sham and TBI rats (Fig. 8). Therefore, it is possible that the observed reduction in theta during the first days following injury was driven, at least in part, by differences in total activity.[43–45] However, it is also clear that during object exploration, total movement was similar between sham and TBI rats, yet TBI rats failed to explore the objects. Stimulation increased exploration without changing distance traveled. Furthermore, stimulation was clearly improving function on the Barnes maze. Therefore, it is critical that future research address how Type 1 and Type 2 theta are impacted by TBI as well as changes in specific neurotransmitter systems (e.g., cholinergic, glutamatergic, gamma-aminobutyric acid-ergic [GABAergic]) that play a key role in hippocampal theta oscillations and learning.

Potential mechanisms

Theta oscillations can be recorded throughout the limbic system, including the hippocampus, mammillary bodies, anterior nucleus of the thalamus, the anterior cingulate, and the pre-limbic cortex.[46,47] Loss of neurons in any of these regions could lead to disruptions in oscillations or function. There are considerable data demonstrating that lateral fluid percussion injury can lead to neuronal cell death in the cortex,[48–51] in the CA3 and dentate of the hippocampus,[52–54] as well as the thalamus.[48,49,51,55] In addition to cell death there is a considerable body of evidence that cholinergic and GABAergic neurotransmission can be altered following injury.

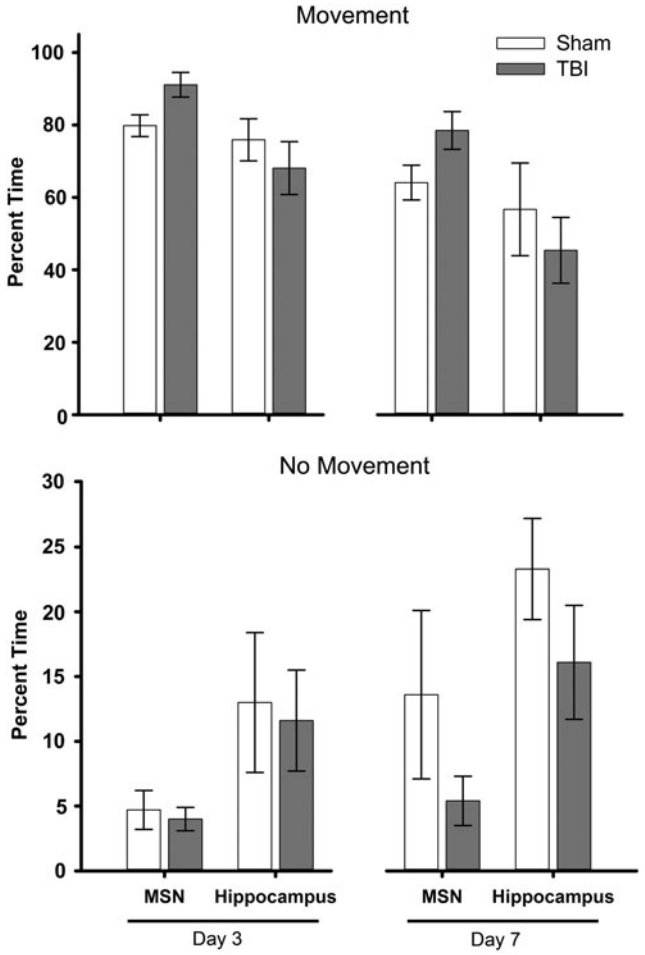

FIG. 8. To control for potential effects of lethargy, theta was analyzed during and compared between epochs of movement and rest. Clearly, there was an increase in theta when animals, regardless of injury, were moving as compared with resting. No differences were observed between sham or TBI on either day 3 or day 7 when comparing 5 sec of continuous activity versus rest. MSN, medial septal nucleus; TBI, traumatic brain injury.

For example, in the weeks following TBI, there is a reduction in the release of acetylcholine from the injured hippocampus[56,57] concomitant with spatial memory deficits.[58] Whereas choline aceyltransferase activity was decreased in the dorsal hippocampus acutely after injury, a significant increase in activity was detected in the medial septal area.[59,60] Further, there are changes in both muscarinic[61,62] and nicotinic[63,64] cholinergic receptor binding in the rat for weeks after injury. Similarly, TBI has chronic effects on the GABAergic system. In a model of paired-pulse stimulation, pronounced elevations in GABA levels were observed in the hippocampus contributing to increased inhibition for up to 2 weeks post-injury.[65] Over the first weeks post-injury, there were significant changes in several of the GABA-A receptor subunits in the hippocampus[66] that could be related to increases in GABA-A tonic inhibition.[67] Therefore, chronic changes in cell number as well as cholinergic and GABAergic dysfunction in surviving neurons could impair theta oscillations and ultimately impair cognitive function.

Analysis of theta oscillations using percentage of time

Traditionally, oscillations are quantified using power spectral density. The power of theta oscillations, in essence, is determined both by the total number of theta peaks as well as the amplitude of each peak. Buzsaki and colleagues demonstrated that theta power in the hippocampus is highly dependent on electrode placement within the lamina.[68] Specifically, slow wave oscillations were assessed throughout the hippocampus by advancing electrodes in 82.5 μm increments through the dorsal ventral axis. It was observed that theta power was highest near the soma of the interneurons in the striatum radiatum, with power decreasing as the electrode moved away from the radiatum, either toward the dentate gyrus or the CA1 of the hippocampus. However, the number of peaks and frequency of oscillations did not change along the dorsal-ventral axis. As power is a function of both the number of peaks as well as the amplitude, we decided to utilize a technique, p_episode,[11,25,26,69] that analyzes oscillations based on percentage of time, to quantify changes in theta following injury.

As described in the Methods section, p_episode establishes a power threshold, and a duration threshold to determine the percent time in high theta power during each analyzed epoch. Specifically, the amplitude of each oscillation was compared with the total background signal and a threshold (the 95th percentile of this fit distribution in the theta frequency was used to establish a power threshold for these data) was applied to reduce the possibility that noise was counted as an oscillation. In addition a minimum of three consecutive cycles of theta oscillations were required before the oscillations were quantified (as opposed to most analyses that will consider a single cycle an oscillation). This reduced the likelihood that random peaks were included in the analysis. The output of the analysis was the amount of time oscillations were observed compared with the total time analyzed (percentage of time). Compared with a traditional theta power analysis, p_episode reduces the likelihood of describing a change related to electrode position rather than condition. In addition, by applying strict power and duration criteria to the analysis, one is more likely to quantify only true oscillations.

Theta frequency stimulation of the MSN restores cognitive performance

Currently, clinical DBS paradigms utilize gamma stimulation. However, data presented here indicate that local field potentials in the hippocampus and those driven by the medial septum are predominantly within the theta frequency band, with only minimal gamma power (data not shown). Moreover, TBI resulted in decreased theta oscillations, and we have demonstrated that disruption of the septohippocampal circuit is involved in cognitive dysfunction. Therefore, we hypothesized that theta, and not gamma, frequency stimulation would result in optimal cognitive recovery. Stimulation at 100 Hz did not restore function in TBI rats, and when delivered to uninjured animals resulted in impaired performance on the Barnes maze. These data suggest that electrical neuromodulation of specific brain targets may be most beneficial when stimulation paradigms are similar to endogenous physiological EEG in the brain region of interest.

It is important to note that mean peak frequency of hippocampal oscillations in sham animals varied between 6.65 and 7.28 Hz, depending on behavior. In our study, a previously described stimulation frequency[8,18] was used that resulted in complete phase coherence between the MSN and hippocampus at 7.7 Hz. In fact, McNaughton and colleagues[8] ultimately found that using a strategy to modulate hippocampal theta based on recordings from the supramamillary nucleus, in essence bypassing an anesthetized MSN, was able to better restore cognitive function than fixed frequency

7.7 Hz stimulation. In a recent study evaluating epilepsy patients, Watrous and colleagues found that the frequency of coherent theta varied based on the type of cognitive task.[27] Specifically, theta was coherent at 1–4 Hz ranges when patients were asked to relate two objects based on spatial location and 7–10 Hz when asked to recall the temporal presentation of those objects. These data clearly suggest that, based on type of behavior, the optimal frequency varies. In fact, artificial theta stimulation of the fornix can impair encoding of contextual fear memory.[70] One thing to consider with TBI rats and patients is that there may not be a truly uninjured region, such as the supramammillary nucleus, to use as a reference to stimulate. Whereas we used the Barnes maze and object exploration task to evaluate for spatial learning and attention, respectively, future studies will benefit from more diverse learning tasks, as optimal frequencies may differ for varying cognitive processes.

In addition to oscillatory frequency within the theta range, the timing of theta activity in relation to a specific behavior may also be critical. For example, there is evidence in two intracranial studies that bursts of theta immediately prior to performing a behavioral task are most critical for encoding.[24,71] Similarly, in both of our stimulation studies, the stimulation was initiated in the min prior to an animal entering any of the learning apparatus. Ultimately, further research into potential stimulation paradigms, frequency, timing, and the cumulative effects of stimulation will improve the translatability of DBS to improve cognitive function following TBI.

In addition to local circuitry, MSN stimulation may also have effects on more distant brain regions that are involved in working memory and cognition. For example, rodent studies have demonstrated that CA1-prefrontal cortex theta coherence is important for distinguishing between correct and incorrect memory tasks,[72] and clinical studies have shown that memory formation in the human brain correlates with theta-frequency phase-locking within the hippocampus.[73] Coherence between remote areas of the brain presupposes functional connectivity. As axons are at risk during injury, additional research should be focused on tractography following TBI. Whereas current research has revolved around neuroprotection, further studies on the effects of TBI and/or stimulation on coherence between the hippocampus and other cortical or subcortical structures may elucidate other potential targets for neuromodulation.

Conclusion

Initially, we hypothesized that stimulation would be theta frequency-dependent and that effects would be restorative rather than generally enhancing overall behavior. Our results support that theta frequency stimulation significantly improved TBI rats compared with TBI controls, whereas similar results were not observed following gamma stimulation. Importantly, neither theta nor gamma stimulation improved performance in sham rats suggesting the stimulation was restoring function in injured rats rather than enhancing overall performance in all rats. We also hypothesized that pre-stimulation would be an optimal strategy compared with continuous stimulation as pre-stimulation would drive persistent changes in EEG and outcome. However, based on our current analyses we did not detect a lasting change in theta oscillations following a short stimulation.

Further, we observed that TBI rats receiving continuous theta stimulation performed as well as sham rats on the Barnes maze. DBS is an experimental therapy for enhancing cognitive performance, and recent clinical experiences suggest that modulation of limbic circuits may be effective in Alzheimer's disease and epilepsy.[3,4] Future studies will be needed to further evaluate the effects of pre-stimulation (or intermittent stimulation) as compared with continuous stimulation to improve cognition following TBI. However, these exciting new data continue to support the hypothesis that theta-frequency DBS is an exciting new treatment strategy to recover physiological hippocampal activity and improve quality of life in patients with persistent cognitive deficits following TBI.

Author Disclosure Statement

No competing financial interests exist.

References

1. Slovarp, L., Azuma, T., and Lapointe, L. (2012). The effect of traumatic brain injury on sustained attention and working memory. Brain Inj. 26, 48–57.
2. Palacios, E.M., Sala-Llonch, R., Junque, C., Roig, T., Tormos, J.M., Bargallo, N., and Vendrell, P. (2012). White matter integrity related to functional working memory networks in traumatic brain injury. Neurology 78, 852–860.
3. Laxton, A.W., Tang-Wai, D.F., McAndrews, M.P., Zumsteg, D., Wennberg, R., Keren, R., Wherrett, J., Naglie, G., Hamani, C., Smith, G.S., and Lozano, A.M. (2010). A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. Ann Neurol. 68, 521–534.
4. Suthana, N., Haneef, Z., Stern, J., Mukamel, R., Behnke, E., Knowlton, B., and Fried, I. (2012). Memory enhancement and deep-brain stimulation of the entorhinal area. N. Engl. J. Med. 366, 502–510.
5. Vertes, R.P. (2005). Hippocampal theta rhythm: a tag for short-term memory. Hippocampus 15, 923–935.
6. Winson, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science 201, 160–163.
7. Buzsaki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus 15, 827–840.
8. McNaughton, N., Ruan, M., and Woodnorth, M.A. (2006). Restoring theta-like rhythmicity in rats restores initial learning in the Morris water maze. Hippocampus 16, 1102–1110.
9. Mizumori, S.J., Barnes, C.A., and McNaughton, B.L. (1990). Behavioral correlates of theta-on and theta-off cells recorded from hippocampal formation of mature young and aged rats. Exper. Brain Res. 80, 365–373.
10. Cashdollar, N., Malecki, U., Rugg-Gunn, F.J., Duncan, J.S., Lavie, N., and Duzel, E. (2009). Hippocampus-dependent and -independent theta-networks of active maintenance. Proc. Natl. Acad. Sci. U. S. A.106, 20493–20498.
11. Ekstrom, A.D., Caplan, J.B., Ho, E., Shattuck, K., Fried, I., and Kahana, M.J. (2005). Human hippocampal theta activity during virtual navigation. Hippocampus, 15, 881–889.
12. Vanderwolf, C.H. (1969). Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr. Clin. Neurophysiol. 26, 407–418.
13. Paz, R., Bauer, E.P., and Pare, D. (2008). Theta synchronizes the activity of medial prefrontal neurons during learning. Learn. Mem. 15, 524–531.
14. O'Neill, P.K., Gordon, J.A., and Sigurdsson, T. (2013). Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion. J. Neurosci. 33, 14211–14224.
15. Nerad, L., McNaughton, N. (2006). The septal EEG suggests a distributed organization of the pacemaker of hippocampal theta in the rat. Eur. J Neurosci. 24, 155–166.
16. Mattis, J., Brill, J., Evans, S., Lerner, T.N., Davidson, T.J., Hyun, M., Ramakrishnan, C., Deisseroth, K., and Huguenard, J.R. (2014). Frequency-dependent, cell type-divergent signaling in the hippocamposeptal projection. J. Neurosci. 34, 11769–11780.
17. Fedor, M., Berman, R.F., Muizelaar, J.P., and Lyeth, B.G. (2010). Hippocampal theta dysfunction after lateral fluid percussion injury. J. Neurotrauma 27, 1605–1615.
18. Lee, D.J., Gurkoff, G.G., Izadi, A., Berman, R.F., Ekstrom, A.D., Muizelaar, J.P., Lyeth, B.G., and Shahlaie, K. (2013). Medial septal

nucleus theta frequency deep brain stimulation improves spatial working memory after traumatic brain injury. J. Neurotrauma 30:131–139.
19. Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res. 31, 47–59.
20. Eacott, M.J., Easton, A., and Zinkivskay, A. (2005). Recollection in an episodic-like memory task in the rat. Learn. Mem. 12, 221–223.
21. Leutgeb, S., Leutgeb, J.K., Barnes, C.A., Moser, E.I., McNaughton, B.L., and Moser, M.B. (2005). Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science 309, 619–623.
22. Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., and Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. J. Neurosurg. 67, 110–119.
23. McIntosh, T.K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H., and Faden, A.L. (1989). Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience 28, 233–244.
24. Addante, R.J., Watrous, A.J., Yonelinas, A.P., Ekstrom, A.D., and Ranganath, C. (2011). Prestimulus theta activity predicts correct source memory retrieval. Proc. Natl. Acad. Sci. U. S. A 108, 10702–10707.
25. Caplan, J.B., and Glaholt, M.G. (2007). The roles of EEG oscillations in learning relational information. Neuroimage 38, 604–616.
26. Caplan, J.B., Madsen, J.R., Raghavachari, S., and Kahana, M.J. (2001). Distinct patterns of brain oscillations underlie two basic parameters of human maze learning. J. Neurophysiol. 86, 368–380.
27. Watrous, A.J., Tandon, N., Conner, C.R., Pieters, T., and Ekstrom, A.D. (2013). Frequency-specific network connectivity increases underlie accurate spatiotemporal memory retrieval. Nat. Neurosci. 16, 349–356.
28. Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J. Neurosci. Methods 134, 9–21.
29. Vinck, M., van Wingerden, M., Womelsdorf, T., Fries, P., and Pennartz, C.M. (2010). The pairwise phase consistency: a bias-free measure of rhythmic neuronal synchronization. Neuroimage 51, 112–122.
30. Lee, D.J., Gurkoff, G.G., Izadi, A., Berman, R.F., Ekstrom, A.D., Muizelaar, J.P., Lyeth, B.G., and Shahlaie, K. (2013). Medial septal nucleus theta frequency deep brain stimulation improves spatial working memory following traumatic brain injury. J. Neurotrauma 30, 131–139.
31. Winson, J. (1974). Patterns of hippocampal theta rhythm in the freely moving rat. Electroencephalogr. Clin. Neurophysiol. 36:291–301.
32. Nyhus, E., and Curran, T. (2010). Functional role of gamma and theta oscillations in episodic memory. Neurosci. Biobehav. Rev. 34, 1023–1035.
33. Gupta, A.S., van der Meer, M.A., Touretzky, D.S., and Redish, A.D. (2012). Segmentation of spatial experience by hippocampal theta sequences. Nat. Neurosci. 15,1032–1039.
34. Sederberg, P.B., Kahana, M.J., Howard, M.W., Donner, E.J., and Madsen, J.R. (2003). Theta and gamma oscillations during encoding predict subsequent recall. J. Neurosci. 23, 10809–10814.
35. Jacobs, J., and Kahana, M.J. (2009). Neural representations of individual stimuli in humans revealed by gamma-band electrocorticographic activity. J. Neurosci. 29, 10203–10214.
36. Raghavachari, S., Kahana, M.J., Rizzuto, D.S., Caplan, J.B., Kirschen, M.P., Bourgeois, B., Madsen, J.R., and Lisman, J.E. (2001). Gating of human theta oscillations by a working memory task. J Neurosci.21: 3175–3183.
37. Lundqvist, M., Herman, P., and Lansner, A. (2011). Theta and gamma power increases and alpha/beta power decreases with memory load in an attractor network model. J. Cogn. Neurosci. 23:3008–3020.
38. Eakin, K., and Miller, J.P. (2012). Mild traumatic brain injury is associated with impaired hippocampal spatiotemporal representation in the absence of histological changes. J. Neurotrauma 29, 1180–1187.
39. Osaka, M., Osaka, N., Koyama, S., Okusa, T., and Kakigi, R. (1999). Individual differences in working memory and the peak alpha frequency shift on magnetoencephalography. Brain Res. Cogn. Brain. Res. 8, 365–368.
40. Buzsaki, G. (2002). Theta oscillations in the hippocampus. Neuron. 33, 325–340.
41. Giovannini, M.G., Rakovska, A., Benton, R.S., Pazzagli, M., Bianchi, L., and Pepeu, G. (2001). Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience 106, 43–53.
42. Vanderwolf, C.H. (1988). Cerebral activity and behavior: control by central cholinergic and serotonergic systems. Int. Rev. Neurobiol. 30, 225–340.
43. Geisler, C., Robbe, D., Zugaro, M., Sirota, A., and Buzsaki, G. (2007). Hippocampal place cell assemblies are speed-controlled oscillators. Proc. Natl. Acad. Sci. U. S. A.104, 8149–8154.
44. Jeewajee, A., Barry, C., O'Keefe, J., and Burgess, N. (2008). Grid cells and theta as oscillatory interference: electrophysiological data from freely moving rats. Hippocampus 18:1175–1185.
45. McFarland, W.L., Teitelbaum, H., and Hedges, E.K. (1975). Relationship between hippocampal theta activity and running speed in the rat. J. Comp. Physiol. Psychol. 88, 324–328.
46. Vertes, R.P., Albo, Z., and Viana Di Prisco, G. (2001). Theta-rhythmically firing neurons in the anterior thalamus: implications for mnemonic functions of Papez's circuit. Neuroscience 104, 619–625.
47. Aggleton, J.P., O'Mara, S.M., Vann, S.D., Wright, N.F., Tsanov, M., and Erichsen, J.T. (2010). Hippocampal-anterior thalamic pathways for memory: uncovering a network of direct and indirect actions. Eur. J Neurosci. 31, 2292–2307.
48. Hicks, R., Soares, H., Smith, D., and McIntosh, T. (1996). Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. Acta Neuropathol. 91, 236–246.
49. Hallam, T.M., Floyd, C.L., Folkerts, M.M., Lee, L.L., Gong, Q.Z., Lyeth, B.G., Muizelaar, J.P., and Berman, R.F. (2004). Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. J. Neurotrauma 21, 521–539.
50. Cortez, S.C., McIntosh, T.K., and Noble, L.J. (1989). Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. Brain Res. 482, 271–282.
51. Conti, A.C., Raghupathi, R., Trojanowski, J.Q., and McIntosh, T.K. (1998). Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. J. Neurosci. 18, 5663–5672.
52. Toth, Z., Hollrigel, G.S., Gorcs, T., and Soltesz, I. (1997). Instantaneous perturbation of dentate interneuronal networks by a pressure wave-transient delivered to the neocortex. J. Neurosci. 17, 8106–8117.
53. Lowenstein, D.H., Thomas, M.J., Smith, D.H., and McIntosh, T.K. (1992). Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. J. Neurosci. 12, 4846–4853.
54. Hicks, R.R., Smith, D.H., Lowenstein, D.H., Saint Marie, R., and McIntosh, T.K. (1993). Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. J. Neurotrauma 10, 405–414.
55. Bramlett, H.M., and Dietrich, W.D. (2002). Quantitative structural changes in white and gray matter 1 year following traumatic brain injury in rats. Acta Neuropathol. 103, 607–614.
56. Dixon, C.E., Bao, J., Long, D.A., and Hayes, R.L. (1996). Reduced evoked release of acetylcholine in the rodent hippocampus following traumatic brain injury. Pharmacol. Biochem. Behav. 53, 679–686.
57. Dixon, C.E., Bao, J., Johnson, K.M., Yang, K., Whitson, J., Clifton, G.L., and Hayes, R.L. (1995). Basal and scopolamine-evoked release of hippocampal acetylcholine following traumatic brain injury in rats. Neurosci. Lett. 198, 111–114.
58. Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., and Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. J. Neurosurg. 67, 110–119.
59. Gorman, L.K., Pang, K., Frick, K.M., Givens, B., and Olton, DS. (1994). Acetylcholine release in the hippocampus: effects of cholinergic and GABAergic compounds in the medial septal area. Neurosci. Lett. 166, 199–202.
60. Gorman, L.K., Fu, K., Hovda, D.A., Murray, M., and Traystman, R.J. (1996). Effects of traumatic brain injury on the cholinergic system in the rat. J. Neurotrauma 13, 457–463.
61. Lyeth, B.G., Jiang, J.Y., Delahunty, T.M., Phillips, L.L., and Hamm, R.J. (1994). Muscarinic cholinergic receptor binding in rat brain following traumatic brain injury. Brain Res. 640, 240–245.
62. Jiang, J.Y., Lyeth, B.G., Delahunty, T.M., Phillips, L.L., and Hamm R.J. (1994). Muscarinic cholinergic receptor binding in rat brain at 15 days following traumatic brain injury. Brain Res. 651, 123–128.

63. Verbois, S.L., Sullivan, P.G., Scheff, S.W., and Pauly, J.R. (2000). Traumatic brain injury reduces hippocampal alpha7 nicotinic cholinergic receptor binding. J. Neurotrauma 17, 1001–1011.
64. Verbois, S.L., Scheff, S.W., and Pauly, J.R. (2002). Time-dependent changes in rat brain cholinergic receptor expression after experimental brain injury. J. Neurotrauma 19, 1569–1585.
65. Reeves, T.M., Lyeth, B.G., Phillips, L.L., Hamm, R.J., and Povlishock, J.T. (1997). The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. Brain Res. 757, 119–132.
66. Gibson, C.J., Meyer, R.C., and Hamm, R.J. (2010). Traumatic brain injury and the effects of diazepam, diltiazem, and MK-801 on GABA-A receptor subunit expression in rat hippocampus. J. Biomed. Sci. 17, 38.
67. Mtchedlishvili, Z., Lepsveridze, E., Xu, H., Kharlamov, E.A., Lu, B., and Kelly, K.M. (2010). Increase of GABAA receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury. Neurobiol. Dis. 38, 464–475.
68. Buzsaki, G., Czopf, J., Kondakor, I., and Kellenyi, L. (1986). Laminar distribution of hippocampal rhythmic slow activity (RSA) in the behaving rat: current-source density analysis, effects of urethane and atropine. Brain Res. 365:125–137.
69. Watrous, A.J., Fried, I., and Ekstrom, A.D. (2011). Behavioral correlates of human hippocampal delta and theta oscillations during navigation. J. Neurophysiol. 105, 1747–1755.
70. Lipponen, A., Woldemichael, B.T., Gurevicius, K., and Tanila, H. (2012). Artificial theta stimulation impairs encoding of contextual fear memory. PLoS One 7, e48506.
71. Fell. J., Ludowig, E., Staresina, B.P., Wagner, T., Kranz, T., Elger, C.E., and Axmacher, N. (2011). Medial temporal theta/alpha power enhancement precedes successful memory encoding: evidence based on intracranial EEG. J. Neurosci. 31, 5392–5397.
72. Kleen, J.K., Wu, E.X., Holmes, G.L., Scott, R.C., and Lenck-Santini, P.P. (2011). Enhanced oscillatory activity in the hippocampal-prefrontal network is related to short-term memory function after early-life seizures. J. Neurosci. 31, 15397–15406.
73. Rutishauser, U., Ross, I.B., Mamelak, A.N., and Schuman, E.M. (2010). Human memory strength is predicted by theta-frequency phase-locking of single neurons. Nature 464, 903–907.

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