Published in final edited form as:

Behav Brain Res. 2014 October 15; 273: 123–132. doi:10.1016/j.bbr.2014.07.026.

Temporally-Patterned Deep Brain Stimulation in a Mouse Model of Multiple Traumatic Brain Injury

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Abstract

We report that mice with closed-head multiple traumatic brain injury (TBI) show a decrease in the motoric aspects of generalized arousal, as measured by automated, quantitative behavioral assays. Further, we found that temporally-patterned deep brain stimulation (DBS) can increase generalized arousal and spontaneous motor activity in this mouse model of TBI. This arousal increase is input-pattern-dependent, as changing the temporal pattern of DBS can modulate its effect on motor activity. Finally, an extensive examination of mouse behavioral capacities, looking for deficits in this model of TBI, suggest that the strongest effects of TBI in this model are found in the initiation of any kind of movement.

Keywords

Deep brain stimulation; traumatic brain injury; generalized arousal; mouse; central thalamus

1 Introduction

1.1 Traumatic Brain Injury

TBI (traumatic brain injury) is a common form of survivable brain injury. It can result, depending on severity, in a quickly healing concussion, sustained loss of consciousness, or death [1]. TBI is a contributing factor of 30.5% of all injury-related deaths in the United States [2,3]. An estimated 1.7 million TBIs occur in the US every year [2], resulting in 52,000 death, 275,000 hospitalization, and 1.4 million visits to emergency departments [2,3]. Due to the high prevalence and impact of such injuries, it is crucial to understand the

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consequences of TBI for the brain, and to learn how to prevent or reverse long-term functional impairment.

A host of animal models for TBI exist [4]. As most forms of TBI result from a blow to the head, we opted to use a multiple weight-drop model, which simulates a moderate closed head injury.

While large numbers of studies have imposed TBI on rats and mice, the great majority of those studies focused on consequent morphological, neurochemical and molecular damage in the brain, the latter studies including altered mRNA levels. Attempts at amelioration have usually focused on environmental manipulations. Examples of these would include the traditional ‘enriched environment’ [5] and the opportunity for exercise on running wheels [6]. With rare exceptions (see Discussion), deep brain stimulation (DBS) has not been tried with the intention of improving behavioral arousal in the manner that is currently being attempted with human patients [7]. The current study uses central thalamic DBS (successful with a human patient, see below) in conjunction with behavioral measures of motoric arousal, supplemented by other behavioral assays.

1.2 Previous Research on DBS to Alter Arousal in Humans

Deep brain stimulation (DBS) involves implantation of stimulating electrodes placed into specifically targeted brain regions, and has been used to ameliorate symptoms of epilepsy and psychiatric disorders. A recent study has indicated that DBS can be effective for patients in a minimally conscious state (MCS) [8]. The target of stimulation in that study was the nonspecific nuclei of the central thalamus (CT). This region is uniquely poised for neuromodulation of the injured brain due to its widespread neuroanatomical projections to the basal ganglia and cerebral cortical regions [9]. While patients in MCS may retain the thalamo-cortical connections needed to support cerebral activation, they lack sufficient innervation from the arousal systems to sustain awareness [10]. Therefore, DBS of the CT in a severely injured brain could approximate this missing arousal input, allowing the CT to support cerebral activity and cognition [10].

1.3 Significance of DBS Temporal Patterns and Neural Coding

It has been proposed that information can be encoded in the temporal patterns of neural spike trains [11–13], a hypothesis well supported in literature describing temporally-patterned neural responses [14–24], information theoretic analyses of temporally-patterned responses [15,23,25,26], physiological explanations for the presence of temporal patterns [27–29], and neural network models that utilize temporal patterns [30,31]. In addition, a small number of direct experiments examined the response of a neural system to temporally-patterned inputs [16,32].

Arousal systems are likely to depend on nonlinear dynamics [33] to ensure adequate lability, enabling them to amplify small perturbations in order to produce fast responses to salient stimuli, as is the case for several biological phenomena [34]. Despite the potential utility of non-linear stimulation, conventional DBS commonly utilizes fixed frequencies. We hypothesized that temporally-patterned DBS might be more effective for increasing arousal, as compared to fixed frequency stimulation. We therefore chose a simple deterministic

chaotic source, the logistic equation, to produce temporal patterns for use in DBS. A true random number generator that is internally independent was chosen as a control [35].

2. Methods

2.1 Animals and Materials

Experiments used C57BL/6J mice bred in house, at 6–9 weeks of age. Mice were singly-housed on a 12:12 hour light/dark cycle with lights on at 6 pm, with food and water available *ad libitum*. Mice were implanted bilaterally with monopolar electrodes (PlasticsOne) in the central thalamus (anterior/posterior: -1.70 mm, lateral: ± 1.00 mm, depth: -3.00 mm, coordinates relative to Bregma) with ground wires placed on the surface of the skull [36]. Surgical and injury procedures were done under ketamine/xylazine anesthesia (80/12 mg/kg). Analgesia (flunazine 5 mg/kg) was given twice daily for 2 days after all survival surgeries as well as after injury. All animal procedures were approved by the Rockefeller Institutional Animal Care and Use Committee.

2.2 Traumatic Brain Injury Model

To ensure that motor activity deficits lasted the length of the experiment, we used a multiple TBI model. On the day of injury, mice were anesthetized and placed under the weight drop apparatus. As small pointed 20 g weight was dropped from a height of 25 cm onto the right side of the mouse's skull up to 5 times. Less than 5 drops were used when the animal's behavior indicated that the injury was severe (for example, through altered posture or temporary seizure). Mice were visually inspected for signs of hemorrhage and skull fracture as both of these indicated injuries of higher morbidity that were likely fatal. Analgesia was administered as described above.

2.3 Neurological Severity Screen

A Neurological Severity Screen (NSS) [37] was used as a general indicator of neurological deficits after injury. Two hours after TBI, mice were placed in a circular open maze with a single small exit, and four tests were scored: 1) ability to exit circle within three minutes, 2) spontaneous investigation of environment (seeking behavior), 3) ability to walk straight, and 4) presence of acoustic startle (freezing or flinching in response to a sudden loud clap). The mouse was then picked up by the tail and the reflexive hind limb flex was scored. The more difficult function tasks followed. Mice were placed on flat and round beams (0.5 cm width or diameter) and scored on their ability to perch on the beams (all four feet touching the beam) for at least 10 seconds. Finally, mice were placed on a simple platform with 3 cm, 2 cm, and 1 cm wide beams, each 30 cm long, and tested on whether they were able to walk across to get to another platform. Each test is scored pass-fail: failing receives a score of 1, succeeding a score of 0. For each mouse, scores on all 10 tests were summed to produce an overall score; normal mice receive an overall score of 0 and mice with the most severe deficits receive an overall score of 10.

2.4 Motor Activity Observation

In addition to NSS, motor activity for each mouse was observed. In the 3D home cage monitoring system (Accuscan Instruments), two data measures were recorded: horizontal

activity, which consists of beam breaks in the horizontal plane, thought to represent fidgeting; and total distance, which includes non-repeating beam breaks in the horizontal plane, thought to represent ambulation. Mice were placed into the home cage monitors 5 days prior to injury, and motor activity data were collected from this point until the end of the experiment. To determine the rate of recovery, one set of mice (n=12) were left alone and observed for 14 days post-injury. A second set of mice (n=13) was implanted with electrodes bilaterally in the central thalamus two days post-injury and allowed to recover from surgery for an additional 4–6 days. After recovery, these mice were stimulated for several epochs (as described below) for one day.

To calculate gross indications of deficit, daily activity was recorded as a sum of motor activity within 24 hours, normalized to pre-injury baseline activity, grouped by baseline, post-injury, or post-surgery (only stimulated mice), and averaged across mice. To check for characteristic day-night activity patterns, daily activity was subdivided into two 12 hour sums, normalized to average total daily baseline activity, grouped by baseline, post-injury, or post-surgery (only in stimulated mice), and averaged across mice. For analyses on the effects of stimulation, activity data directly surrounding the stimulation (10 minutes before, 10 minutes during, and 10 minutes after stimulation) were analyzed.

2.5 Stimulation Characteristics

2.5.1 General Parameters—Stimulation was performed using a four channel programmable stimulus generator (Multi Channel Systems). Stimulation consisted of symmetric biphasic (negative phase first) square wave pulses with a total pulse width of 200 μ s (Table 1). Stimulation was always constant current, and amplitude and frequency of stimulation used were 150 μ A and 125 Hz. Stimulation epochs were 10 minutes of continuous stimulation every 4 hours over the course of 1 day.

2.5.2 Temporal Patterns—Temporal patterns used in experiments are listed in Table 1. Two methods of generating temporal patterns were chosen to compare with conventional fixed frequency stimulation. One pattern generation method, based on the logistic equation, was chosen subsequent to our hypothesis that nonlinear dynamics may be important in the control of CNS arousal systems [33]. The second pattern generation method, based on a true random number generator [35], was chosen as a patterned control; based on independent true random numbers, this random temporal pattern was used to give perspective on the internally structured chaotic temporal pattern.

The generation of alternative temporal patterns is described as follows. The logistic equation, $X_n = R X_{n-1} (1 - X_{n-1})$, where X is the output at time n , has a constant modifier, R , that creates chaotic output at certain values. Output of the logistic equation was calculated to two or three thousand iterations with initial conditions to ensure chaotic behavior of the equation ($R = 3.90$ and $X_0 = 0.2$). The true random number generator used to generate temporal patterns takes atmospheric noise measurements to generate three thousand uniformly distributed numbers [35]. To ensure that depolarization block did not occur, a minimum interpulse interval (IPI) was defined as 0.3 ms, and chaotic and random sequences were scaled to meet that minimum. For both sequences of numbers, a consecutive set of

numbers was found such that the number of elements in the set divided by the sum of the scaled output equaled the desired average frequency. In this manner, four temporal patterns were defined: 1) 10 pulses from the logistic equation output, named 'Nonlinear1,' 2) 50 pulses from the logistic equation output, named 'Nonlinear2,' 3) 50 pulses from the logistic equation output, named 'Chaotic,' and 4) 50 pulses from the true random number generator output, named 'Random.' To ensure the only difference in a mouse's response to stimulation was the temporal patterning of pulses, all patterns in a single experiment were identical with respect to pulse shape, pulse duration, amplitude, average frequency, and stimulation duration.

2.6 Other behavioral tests

The experimenters and scorers in all behavioral tests were blind to whether the experimental animals had received TBI. Most behavioral tests were performed under dim red light. For tests performed in the white light (elevated plus maze and light-dark transition assay), illumination intensity was 500 lux.

2.6.1 Parental Care Assay—One day prior to the test, female TBI and control mice were given cotton balls, to allow them to make nests. The following dark cycle, 4 pups were placed into each cage away from the nest, and the mice were filmed to determine their reaction to the P2–P6 pups. The pups were removed and returned to their mothers after 15 minutes. To test whether TBI mice had difficulty learning how to handle pups, the experiment was repeated for 3 consecutive days, each time with new pups. Mice were scored according to how many seconds from the introduction of the last pup they retrieved each of the pups and brought them back to the nest, as well as the total time spent retrieving, licking and interacting with the pups. If the mice failed to retrieve any of the pups within the allotted time, they were assigned a score of 900 (15 minutes) for this particular pup. Mice were tested 1 month after TBI.

2.6.2 Elevated Plus Maze—The elevated plus maze assay was performed as previously described [38]. The movements of the mice were tracked with ANYmaze software (Stoelting), and the number of entries and residence time in each area of the maze were recorded.

2.6.3 Light-dark transition assay—The light-dark transition assay was based on previously published behavioral tests [39]. Light-dark transitions were scored with JWatcher software.

2.6.4 Pheromonal spatial learning—The pheromonal spatial learning assay was performed as previously described [40] two months post-TBI. Each trial was recorded, and residence time in each area of the enclosure was analyzed using ANYmaze (Stoelting).

2.6.5 Spontaneous alternation in the T-maze—Mice were placed inside the start arm of a 3-arm T-maze at the beginning of each block of trials. The start arm was blocked by a door, which was opened after the mouse was placed in the maze. The mouse was then allowed to freely choose either the right or the left arm. Once the mouse selected an arm, the

other arm was blocked. The mouse was returned to the start arm after 1 minute of exploration, and allowed to rest for 1 minute. The procedure was then repeated 6 times. Each mouse was tested once a day for 5 days. The test was conducted during the dark phase of the light-dark cycle under red illumination. The maze was cleaned between mice and between blocks of trials. Mice were tested 1 month post-TBI.

2.6.6 Partition test—The experimental mouse and the first stimulus mouse were inserted into the opposite sides of a cage that was partitioned down the middle with wire mesh. They were then allowed to habituate to each other for 12 hours. At the beginning of the test, which was conducted in the dark under red illumination, the mice were filmed for 5 minutes, to obtain a baseline level of activity. The stimulus mouse was then replaced with a new stimulus mouse, unfamiliar to the experimental animal. Their interaction was again filmed for 5 minutes. Finally, the unfamiliar stimulus mouse was removed, and the familiar stimulus mouse was re-introduced into the cage. The first 5 minutes of the interaction between the stimulus mouse and the experimental mouse were then filmed. The amount of time that the experimental mouse spent approaching the partition and interacting with the stimulus mice in each trial was scored using JWatcher freeware. Mice were tested 2 months post-TBI.

2.6.7 Social discrimination—The social discrimination assay was conducted as previously described [41]. The video was analyzed using JWatcher. The time the mouse spent in each region of the chamber, as well as the total length of social and non-social investigation of the mesh cylinders during each trial was determined.

2.7 Statistical Analyses

Both parametric and non-parametric statistical tests were used to avoid the bias inherent in the assumptions of parametric tests. Multiple-factor ANOVA tests were used in addition to Friedman and Kruskal Wallis tests to analyze the effects of injury and surgery on daily motor activity as well as the effects of DBS in CT, light phase, and temporal pattern of stimulation on motor activity in the small time frame analyzed. Post-hoc analyses were done using t-tests, Wilcoxon matched-pair signed ranks test, and Mann Whitney U tests, as appropriate. P-values of 0.05 or less were considered statistically significant.

2.8 Brain Tissue Processing

After motor activity data were collected, mice were euthanized following deep anesthesia, and their brains were dissected and freshly frozen. Fresh frozen brain tissue was sliced on a cryostat at 30 μ m. To histologically confirm electrode placement, brain tissue slices from stimulated mice were processed using a standard acetylcholinesterase stain. All stimulated mice included in statistical analyses were confirmed to have, at minimum, a unilateral hit in the CT.

3 Results

3.1 Mouse Model of Multiple TBI

3.1.1 Acute injury assessment—Each injured mouse underwent a NSS 2 hours post injury, as shown in the timeline of experiments (Fig. 1). The test is summarized briefly in Table 2. In this set of mice ($n=12$), NSS scores ranged from 3–6 with an average overall score of 4.75 ± 0.28 . This average score is significantly different from 0 ($t_{11} = 17.05$, $p < 0.001$; signed rank = 0, $p < 0.001$). These data show that neurological deficits can be generated by this mouse model of traumatic brain injury, multiple TBI.

In addition to the NSS, some injured mice were also sacrificed for 2,3,5-triphenyltetrazolium chloride staining. This staining method is useful for detecting decreased blood flow to multiple brain regions. Infarcted regions show up pale. We were able to detect decreased blood flow in regions throughout the brain, likely due to diffuse blood vessel damage from the blows (Supplementary Figure 1).

3.1.2 Motor Activity Deficits—In addition to neurological deficits, multiple TBI can also generate deficits in the motor aspect of arousal. Motor activity was summed over 24 hours, and these sums were normalized to the average activity of a 5 day baseline observed prior to injury. Normalized daily activity was averaged across mice ($n=12$), and this average timeline can be found in Fig. 2.A. Significant deficits in two measures of motor activity were observed on the day after injury: horizontal activity ($t_{11} = -3.47$, $p < 0.01$; signed rank = 6, $p < 0.01$) and total distance ($t_{11} = -4.39$, $p < 0.001$; signed rank = 5, $p < 0.01$). These significant deficits lasted for several days after injury.

3.1.3 Recovery without Intervention—As this is a closed head injury, it is expected that the injured mice will begin to heal and recover functionality over the course of the experiment. As expected, deficits decrease and motor activity approaches pre-injury baseline levels at 11–14 days post TBI. In one of the two data measures, horizontal activity, deficits were significantly different from baseline up to day 12 using non-parametric statistics (signed rank = 21, $p < 0.05$) or up to day 11 using parametric t-tests ($t_{11} = -2.89$, $p < 0.01$). With total distance, deficits were significantly different from baseline up to day 14 for non-parametric (signed rank = 14, $p < 0.05$) or up to day 13 for parametric t-tests ($t_{11} = -3.18$, $p < 0.01$). These data show that multiple TBI can generate motor activity deficits that last 11–14 days post injury.

3.1.4 Nocturnal Behavior Pattern—In addition to overall locomotion, activity over the course of the light/dark cycle was observed to determine if multiple TBI hindered dark-light behavior. Normalized 12 hour data were grouped into baseline and post injury categories and then averaged across animals. These averages can be found in Fig. 3.A–B. Notice that even after injury, activity in the dark is increased over activity in the light, i.e., nocturnal behavior pattern is preserved.

3.2 Deep Brain Stimulation following Multiple TBI

The deficits caused by the multiple injury model of TBI are long-lasting enough for experiments that include electrode implantation surgery and subsequent recovery from surgery. With a brain injury model that results in sufficiently long-lasting deficits, the effects of deep brain stimulation on brain injured mice can be tested. We therefore analyzed the effect of DBS on injured mice.

3.2.1 Neurological and Motor Activity Deficits In Stimulated Mice—Neurological and motor activity deficits exhibited by this set of mice after TBI is shown in Table 3 and Fig. 2.C–D, respectively. The range of NSS overall scores in these animals was 3–10, and the average overall score was 7.3 ± 0.8 (Table 3, $n=13$). This average score is significantly different from 0 ($t_{12} = 12.73$, $p<0.001$; signed rank = 0, $p<0.001$). To determine motor activity deficits, daily summed and normalized data were grouped as follows: baseline (BL), post injury (PostTBI), and post surgery, but before stimulation (PostSg). These grouped data were averaged across mice ($n=13$). Averaged deficits can be seen in Fig. 2.C–D. Motor activity deficits were observed both PostTBI and PostSg. Horizontal activity was significantly lower than baseline, both PostTBI ($t_{18} = -9.66$, $p<0.001$; $z = -3.82$, $p<0.001$) and PostSg ($t_{75} = -7.38$, $p<0.001$; $z = -6.20$, $p<0.001$). Likewise, total distance traveled was significantly lower than baseline both PostTBI ($t_{18} = -10.88$, $p<0.001$; $z = -3.82$, $p<0.001$) and PostSg ($t_{75} = -18.51$, $p<0.001$; $z = -7.51$, $p<0.001$). Notice that the magnitude of motor activity deficit in these animals is larger than in mice shown in Fig. 2A.

For mice recovering from TBI without further intervention, activity deficits immediately after injury were 70% and 50% of baseline for motor activity and horizontal activity, respectively. In injured mice that had been implanted with electrodes, activity deficits were 55% and 40% of baseline, respectively. This is to be expected, as the batch of mice that underwent electrode transplantation surgery had a higher average NSS score immediately PostTBI, indicating a higher severity of injury, than the first group of mice we analyzed. It is logical to expect higher motor activity deficits with higher severity of injury.

In addition to neurological and motor activity deficits, these mice ($n=13$) also display preserved nocturnal behavior pattern (Fig. 3C–D). Again, 12 hour summed and normalized data were grouped into baseline, post injury, or post surgery categories and then averaged across animals (Fig. 3C–D). As with the mice that were allowed to recover without intervention, activity in the dark is increased over activity in the light, i.e., dark-light behavior is preserved.

3.2.2 Effects of Deep Brain Stimulation—DBS has been shown to increase arousal as measured by motor activity in intact mice [36,42,43]. We tested the effects of DBS on brain injured mice. Mice were implanted with electrodes bilaterally in the central thalamus (A: -1.70 mm, L: ± 1.00 mm, D: -3.00 mm). Only animals with at least one histologically confirmed hit were included in the statistical analyses. A diagram of electrode placements can be found in Fig. 4.

Motor activity, as measured by horizontal activity and total distance, increased during and after stimulation in brain injured mice. Data presented in Fig. 5 represent sums of activity 10

minutes before, 10 minutes during, and 10 minutes after stimulation. The activity during and after stimulation was normalized to activity before stimulation. These normalized sums were then averaged across mice ($n=13$, 4–6 stimulations per mouse) and analyzed to determine the effects of the stimulation, light phase of stimulation, and temporal pattern of stimulation. An overall effect of stimulation is detectable by multi-factor ANOVA in both the horizontal activity measure ($F_{2,415} = 3.26$, $p<0.05$, Fig. 5.A) and total distance measure ($F_{2,342} = 3.69$, $p<0.05$, Fig. 5B). There was no overall effect of light phase (Fig. 5C–D) or temporal pattern of stimulation (Fig. 5E–F). ANOVA analyses did uncover a significant interaction effect between temporal pattern and stimulation on horizontal activity ($F_{4,415} = 4.20$, $p<0.01$). The effect of stimulation on horizontal activity but not on light-dark activity cycles was confirmed using a non-parametric analysis. The balanced Friedman test uncovered a significant effect of stimulation on horizontal activity ($\chi^2 = 17.67$, $p<0.001$) and total distance ($\chi^2 = 22.97$, $p<0.001$). While these data are not completely congruous with previous stimulation data collected in intact mice[36], they do replicate the findings that DBS can increase motor aspects of arousal and that temporal pattern of stimulation can alter its effectiveness.

In post-hoc analyses, the effect of stimulation is confirmed and the interaction effect between the temporal pattern and stimulation, as seen in horizontal activity is explained. Using t-tests and Mann-Whitney U tests, horizontal activity is increased during ($t_{140} = 2.29$, $p<0.05$; $z = -3.03$, $p<0.001$) and after ($t_{139} = 2.44$, $p<0.05$; $z = -4.54$, $p<0.001$) stimulation, as opposed to before stimulation. The total distance is also increased during ($t_{105} = 2.58$, $p<0.05$; $z = -4.06$, $p<0.001$) and after ($z = -4.58$, $p<0.001$) stimulation, as opposed to before stimulation. Comparing temporal patterns of stimulation, horizontal activity is increased much more after chaotic stimulation, as opposed to fixed ($t_{48} = -2.54$, $p<0.05$) and random ($t_{50} = -2.61$, $p<0.05$) using Bonferroni-corrected t-tests. While this was not confirmed by non-parametric tests, these data suggest that the temporal pattern of stimulation can alter the magnitude of the effect DBS in CT.

In addition to normalized averaged data, raw motor activity of one illustrative injured and stimulated mouse is shown in Fig. 6. The three stimulations represented in this figure occurred during the dark phase of the light cycle. Notice the very little movement registered by the total distance data measure. This is much less than similar plots of uninjured stimulated mice[36]. As total distance is a measure of ambulation, it is possible that these injured mice are moving (as evidenced by horizontal activity), but have decreased motivation to walk within their cages thereby staying in one limited area. This has implications for the high variability inherent in these data (see Discussion).

3.3 Other behavioral tests

In order to characterize long-term behavioral alteration that may have occurred in mice post-TBI, we performed behavioral tests of parental care, social recognition, spatial learning [40] [44,45], and anxiety [41] (see Figure 1). We found no difference in social recognition, spatial learning, or parental care (Supplementary figures 2, 3 and 4, respectively). In the elevated plus maze, a measure of anxiety in mice, a statistically significant difference ($p=0.03$ by Student's t-test, $n=12$ control and 14 TBI animals) were observed in the percent

of time the mouse chose to enter the closed and open parts of the maze after entering the center. Average total entries into all sections of the maze were 48 ± 1 for TBI mice and 49 ± 2 for controls (Supplementary figure 4A–B). However, the significance of this phenotype for anxiety is debatable, especially given that no significant differences were found between TBI and control mice in the light-dark transition assay (Supplementary figure 4C).

4 Discussion

4.1 Major findings

We have used a modified closed head injury model, multiple TBI, to test the effect of DBS on arousal problems resulting from mild to moderate TBI. This injury model results in acute neurological and motor activity deficits lasting 11–14 days, while circadian rhythms are unaffected.

As the mice retained their ability to initiate movement after TBI, we were able to directly test behavioral effects of DBS to the CT. Using this model, we investigated the impact of chaotic parameters in DBS on motoric arousal. In these animals, chaotic stimulation increased horizontal activity more than either fixed or random stimulation. Thus, temporal parameters of stimulation can alter the magnitude of the effect of DBS in TBI mice.

4.2 Technical caveats

Regarding interpretation of the NSS, we note that the it was measured very shortly after TBI, and long before thalamic DBS. The NSS was used simply to assure ourselves that the TBI was indeed effective in rendering a deficit as a neurologist would define it, and thus cannot be used in this case to assess the effectiveness of stimulation for recovery.

Home cage motor activity data are inherently variable, especially with brain injured mice. Sources of variability include endogenous changes over the course of a single day, over the course recovery from injury, and differences between individuals. Inter-mouse variability can be caused by baseline arousal differences between mice, as well as differences in injury severity, injury locus, and electrode placement. This multifaceted variability can make it difficult to uncover trends or statistical differences between treatment groups. To mitigate some of this inherent variability, the effect of stimulation was analyzed within a very small timeframe surrounding that stimulation. To compensate for individual differences, the animal's activity during and post stimulation was normalized to the behavior prior to stimulation.

In a particularly vexing complication, there was absolutely no movement in one quarter of all stimulations, either before, during or prior to the stimulation. These non-responding events were likely caused by the decreased motivation to walk in injured mice. They had to be removed from analysis because they were non-informative.

Further, we note that despite prolonged attempts to measure post-TBI deficits in tasks requiring varying degrees of cognitive sophistication, by and large results in those tasks showed no difference between TBI and control mice. We cannot rule out the possibility that other methodological approaches to these tasks or other types of cognitive tasks would have

yielded positive proofs of extensive TBI effects. It is also possible that cognitive deficits are present in the first two weeks after TBI. However, as the outputs of behavioral analysis rely on normal motor function, and initiation of movement is decreased in mice up to 14 days post-TBI, we were not able to conduct cognitive tests at this earlier time point.

4.3 Comparison to the clinic

While one well-controlled clinical trial using DBS for TBI has been completed [8] very little is published in the pre-clinical or clinical literature on the use of this neurological intervention with brain injury. As well as the crucial clinical studies, more research on the effects of DBS in brain injury models in laboratory animals is needed to define optimal stimulation parameters and to analyze mechanisms. The data presented here suggest that deficits caused by our model of closed head injury (weight drop) can be ameliorated by DBS of the CT. It could also be beneficial to determine if DBS can improve deficits seen in other types of brain injury.

It is understood that from a clinical point of view, invasive neurosurgical procedures such as DBS will be justified only in patients who are chronically ill and are not recovering on their own. Such a situation was reported in a successful DBS intervention for a patient in a minimally conscious state [8]. By and large, the rodent literature has been slow to produce conditions that perfectly match chronic dysfunctions of human patients. An exception is the work of Lee et al, in which DBS of the septum using a theta-band frequency temporarily improved hippocampal theta activity and even was associated with increased performance in the Barnes maze, if the DBS was timed appropriately [46]. Likewise, Carballosa Gonzalez et al reported that long-term low-frequency stimulation (8 Hz) of the serotonergic cell groups of the midbrain improved the rate memory acquisition for a hidden platform in the Morris water maze [47].

We note that our current results speak not to a particular cognitive ability but instead to a much deeper function: motoric arousal, which means the ability to initiate *any* behavior. The absence of this function is the condition of all patients in persistent vegetative states, and in MCS, except during brief episodes of sporadic movements and apparent awareness.

4.4 Outlook

Our relatively mild model of rodent TBI allowed us to directly evaluate the behavioral consequences of various patterns of stimulation in DBS. The most promising conclusion from these data is that DBS of the CT can be used to increase the motoric aspect of arousal in a mouse model of multiple TBI. The data collected here suggest, further, that temporal pattern of DBS can make a difference in magnitude of effect. These results are promising enough to suggest that DBS can help an injured brain maintain arousal.

Since variability among animals was a factor in our experiments, it seems clear that optimization of rodent TBI models to make them more uniform may be in order. The model we chose for closed head injury was intended to mimic damage from motor vehicle and motorcycle accidents, for instance, but blast injuries, as suffered from improvised explosive devices, may provide a more severe and more constant form of TBI.

Our choice of the central thalamus as the site for DBS permits us to suggest that during these experiments DBS functions by ‘replacing’ missing or deficient neural inputs to the thalamus. It is likely that further analyses of the normal inputs from arousal systems to the CT, combined with testing of multiple DBS modes in various injury models, will eventually result in more effective approaches to DBS in the clinical setting. Next generation DBS-like techniques may therefore be applicable to alleviating the symptoms of mild to moderate brain injuries, and even to psychiatric problems such as depression. Understanding the nature of initial inputs to CT, as well as the most effective methods to replace them, will become paramount, as treatment of nervous system disorders with electrical stimulation becomes more common.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was funded by NIH 5R01 NS067249 to D. Pfaff with N. Schiff. Helpful discussions with Nicholas Schiff, Keith Purpura and Khatuna Gagnidze are gratefully acknowledged.

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Highlights

- Mice with traumatic brain injury have decreased motor activity
- Deep brain stimulation was used to increase motor activity in brain injured mice
- Stimulation was delivered to the central thalamus
- The temporal parameters of the stimulation influenced the activity increase

Timeline of experiments

Fig 1.

Timeline of Experiments. Three groups of mice (2 sets of males and 1 set of females) underwent TBI induction, followed either by electrode implantation and stimulation, or behavioral experiments. Due to possibility of the motoric limitations in TBI mice, which could affect behavioral measures, all behavioral experiments were initiated at least a month after TBI administration. Surgery for electrode implantation and behavioral testing were performed during the time window when the GA deficits, resulting from TBI, persist.

Fig. 2.

Motor Activity Deficits following Multiple TBI. (A) Normalized average daily activity (A) for two motor activity measures (horizontal activity — blue; total distance — green) up to 14 days after injury, represented as mean $\pm$ s.e.m. of $n=12$ mice. * $p<0.05$; ** $p<0.01$. BL — baseline. (B–C) Normalized, grouped, average daily activity for horizontal activity (B) and total distance (C), represented as mean $\pm$ s.e.m. of $n=13$ mice that were later stimulated. * $p<0.001$. Each mouse was initially yielded baseline (BL) measurements, then underwent TBI to yield PostTBI data, and finally, underwent surgery for implantation of DBS electrodes to yield post surgery (PostSg) data. Note that PostSg refers to data collected **after** electrode implantation but **before** any thalamic stimulation.

Fig. 3.

Preserved Nocturnal Behavior Pattern following Multiple TBI. Normalized average dark-light behavior for horizontal activity (A) and total distance (B), represented as mean $\pm$ s.e.m. of $n=12$ mice. (C–D) Preserved Nocturnal Behavior Pattern following Multiple TBI, Stimulated mice. Normalized, grouped, average dark-light behavior for horizontal activity (C) and total distance (D), represented as mean $\pm$ s.e.m. of $n=13$ mice that were later stimulated. Each mouse was initially yielded baseline (BL) measurements, then underwent TBI to yield post TBI data, and finally, underwent surgery for implantation of DBS electrodes to yield post surgery (PostSg) data. Note that PostSg refers to data collected **after** electrode implantation but **before** any thalamic stimulation.

Fig. 4.

Electrode Placements in Multiple TBI, Stimulated mice. Coronal section of mouse brains at Bregma -1.94 mm and zoomed in image of thalamus for reference (A). Placements are represented as red dots on coronal sections at Bregma -1.94 mm (B), -2.06 mm (C), and -2.30 mm (D). Placements of n=13 mice. Diagrams adapted from Paxinos[48].

Fig. 5. DBS following Multiple TBI increases Motor Aspect of Arousal. Response to stimulation in horizontal activity (A) and total distance (B). Differential response to stimulation in the dark and light in horizontal activity (C) and total distance (D). Differential response to temporal patterns of stimulation in horizontal activity (E) and total distance (F). Data are presented as mean $\pm$ s.e.m. of n=13 mice (4-6 stimulations per mouse). Data represent 10 minutes before, 10 minutes during, and 10 minutes after stimulation. Data during and after stimulation are normalized to data before stimulation. * $p < 0.05$ compared to before stimulation, ^ $p < 0.05$ compared to other temporal patterns.

Fig. 6. Raw motor activity of one illustrative injured and stimulated mouse with three different temporal patterns of stimulation: A) fixed, B) random, and C) chaotic. The three stimulations represented in this figure occurred during the dark phase of the light cycle.

Table 1

Temporal Patterns of Stimulation. Lists of interpulse intervals (μsec) for each temporal pattern used.

Nonlinear1	Nonlinear2	Chaotic	Random
11660	32260	12900	7300
29780	3440	1380	4460
13840	12000	4800	14180
31880	29860	11940	8820
7100	11580	4640	12940
21880	29400	11760	12960
30120	13020	5200	11300
12840	30840	12340	2320
31060	8420	3360	9880
9880	24500	9800	9480
	24940	9980	11200
	24080	9640	1520
	25700	10280	7240
	22560	9020	6220
	28120	11240	2560
	16620	6640	11200
	32320	12920	13660
	3160	1260	7800
	11120	4440	9080
	28840	11540	8620
	14660	5860	3160
	31900	12760	1700
	4720	1880	14300
	15800	6320	1800
	32260	12900	10280
	3400	1360	10600
	11920	4760	3760
	29780	11920	10220
	11820	4720	3820
	29660	11860	7020
	12200	4880	4240
	30080	12040	13140
	10880	4360	14400
	28520	11400	5860
	15560	6220	4440
	32200	12880	5540
	3600	1440	6000
	12520	5000	5620
	30380	12160	8920

Nonlinear1	Nonlinear2	Chaotic	Random
	9900	3960	11600
	27080	10840	13240
	19340	7740	3600
	31440	12580	7520
	6360	2540	13580
	20060	8020	5380
	30900	12360	10200
	8180	3280	5660
	24020	9600	11000
	25820	10320	5360
	22300	8920	5240

Table 2

Neurological Severity Screen following Multiple TBI. NSS tests are scored pass (0, normal behavior) or fail (1, abnormal behavior). The overall score is a sum for each test resulting in a score of 0 (normal) to 10 (high severity of injury). The range of overall scores (i.e., number of tests failed) was 3–6 and the overall score average was 4.75 ± 0.28 .

Neurological Tests (in the order of performance)	Number of mice that Failed	Total of mice Tested
Exit from 30 cm circle within 3 minutes	4	12
Straight walk	0	12
Startle reflex	4	12
Seeking behavior	0	12
Hind limb flex	0	12
Flat beam balance for 10 seconds	4	12
Round beam balance for 10 seconds	10	12
3 cm beam walk within 3 minutes	11	12
2 cm beam walk within 3 minutes	12	12
1 cm beam walk within 3 minutes	12	12

Table 3

Neurological Severity Screen following Multiple TBI, later stimulated. NSS tests are scored pass (0, normal behavior) or fail (1, abnormal behavior). The overall score is a sum for each test resulting in a score of 0 (normal) to 10 (high severity of injury). The range of overall scores (i.e., number of tests failed) was 3–10 and the overall score average was 7.3 ± 0.8 .

Neurological Tests (in the order of performance)	Number of mice that Failed	Total of mice Tested
Exit from 30 cm circle within 3 minutes	9	14
Straight walk	11	14
Startle reflex	10	14
Seeking behavior	3	14
Hind limb flex	2	14
Flat beam balance for 10 seconds	13	14
Round beam balance for 10 seconds	14	14
3 cm beam walk within 3 minutes	12	14
2 cm beam walk within 3 minutes	13	14
1 cm beam walk within 3 minutes	13	14