Mild Traumatic Brain Injury Decreases Spatial Information Content and Reduces Place Field Stability of Hippocampal CA1 Neurons

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Abstract

Both clinical and experimental studies have reported that mild traumatic brain injury (mTBI) can result in cognitive impairments in the absence of overt brain damage. Whether these impairments result from neuronal dysfunction/alterd plasticity is an area that has received limited attention. In this study, we recorded activity of neurons in the cornu Ammonis (CA)1 subfield of the hippocampus in sham and mild lateral fluid percussion injured (mFPI) rats while these animals were performing an object location task. Electrophysiology results showed that the number of excitatory neurons encoding spatial information (i.e., place cells) was reduced in mFPI rats, and that these cells had broader and less stable place fields. Additionally, the in-field firing rate of place cells in sham operated, but not in mFPI, animals increased when objects within the testing arena were moved. Immunostaining indicated no visible damage or overall neuronal loss in mFPI brain sections. However, a reduction in the number of parvalbumin-positive inhibitory neurons in the CA1 subfield of mFPI animals was observed, suggesting that this reduction could have influenced place cell physiology. Alterations in spatial information content, place cell stability, and activity in mFPI rats coincided with poor performance in the object location task. These results indicate that altered place cell physiology may underlie the hippocampus-dependent cognitive impairments that result from mTBI.

Keywords: hippocampus; multi-electrode recording; object location task; place cells; parvalbumin-positive inhibitory neurons

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a considerable human health concern. According to the Centers for Disease Control, it has been estimated that ~2,800,000 people in the United States sustain a TBI each year, the majority of which can be classified as mild (a concussion) (www.cdc.gov/traumaticbraininjury/get_the_facts.html). Both clinical and experimental studies have shown that mild TBI can cause learning and memory dysfunction that can last for weeks to months after the injury. These deficits are often detected in the absence of overt hippocampal damage, suggesting that dysfunction of hippocampal neurons may underlie these impairments. Consistent with this premise, a few studies have reported reduced Schaffer collateral-cornu Ammonis (CA)1 long-term potentiation (LTP)—an electrophysiological correlate for synaptic plasticity and learning and memory in hippocampal slices prepared from brain-injured rats.¹ In addition to LTP changes, *in vivo* recording of local field potential (LFP) has shown reduced theta oscillation in brain-injured animals.² In a small study, mild injury did reduce the

number of CA1+CA3 place cells.³ Although these studies point to altered hippocampal physiology, it is unclear how mild TBI alters the activity of the CA1 neurons that are critical for hippocampus-dependent learning and memory.

In vivo recordings of neural activity in animals performing spatial learning and memory tasks have demonstrated that the firing of specific neurons in the CA1 subfield of the hippocampus is increased when an animal is placed within a new environment.^{4–8} These cells, referred to as “place cells,” display spatially localized firing patterns known as “place fields,” which are linked to cues/objects within the environment.⁹ These place fields are formed when an animal is introduced into a new environment, and the sum of the various place fields collectively forms a cognitive map. When an animal is returned to a familiar environment, the previously established place field map can be used to navigate the environment and also identify changes within it. Therefore, a failure to form stable place fields, and therefore a stable spatial map, can impair an animal’s ability to recognize the environment, and has been linked to learning and memory dysfunction.¹⁰ Consistent with

this, pharmacological and genetic manipulations that alter place cell function are associated with impaired hippocampus-dependent learning and memory.^{11,12} Therefore, an impairment of CA1 place cells' activity to form stable place fields may contribute to hippocampus-dependent learning and memory dysfunction observed after a mild TBI.

In the present study, we recorded hippocampal CA1 neuronal activity from sham and lateral mild fluid percussion injury (mFPI) animals while the animals were performing an object location task. These recordings were made during both the familiarization and novel location phases of the task. Our results indicate that CA1 place cells in sham animals form discrete and stable place fields. In contrast, we found that place cells in mFPI rats encoded broad place fields that were not stable across training and testing sessions. These altered place fields were associated with reduced numbers of parvalbumin (PV)-positive inhibitory neurons in the CA1 subfield and poor performance by the mFPI animals in the object location task.

Methods

Materials

All experimental procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the recommendations provided in the *Guide for the Care and Use of Laboratory Animals*. Protocols were designed to minimize pain and discomfort during the surgical procedures and recovery. A total of 28 male Sprague-Dawley rats (Envigo, 300–350 g) were used in these studies with 14 receiving a mFPI and 14 serving as sham-operated controls. For immunohistochemistry, antibodies to neuronal nuclei (NeuN) (cat# MAB377; 1:1000) and microtubule associated protein 2 (MAP2) (cat# AB5622; 1:1000) were purchased from Millipore (Burlington, MA), antibodies against parvalbumin (cat # ab11427; 1:1000) were obtained from Abcam (Cambridge, United Kingdom), and secondary antibodies coupled to Alexa Fluor (cat # A32723, A32732; 1:500) were purchased from Invitrogen (Carlsbad, CA). Bisbenzimidazole (Hoechst 33258; 0.05 µg/mL) was purchased from Sigma-Aldrich (St. Louis, MO). We implanted rats with 32 channel electrode arrays (Bio-Signal, Dallas, TX). A 32 channel unity gain headstage was plugged into the electrode array and a Blackrock microsystems data acquisition system (Salt Lake City, UT) was used to record spiking activity from individual CA1 neurons in the pyramidal cell layer. An infrared tracking system (Neuromotive, Blackrock microsystems) was included to enable real-time movement of the animals' head position during task performance.

Lateral mFPI and electrode implantation surgeries

Lateral FPI was delivered as described previously.^{13–16} Rats were deeply anesthetized using 5% isoflurane with a 1:1 air/O₂ mixture, and a surgical level of anesthesia was maintained using with 2.5% isoflurane with a 1:1 N₂O/O₂ mixture via a face mask. Rats were mounted on the stereotaxic frame, and a 4.8 mm diameter craniotomy was made in the right hemisphere (midway between bregma and lambda, with the medial edge ~1.0 mm from the midline) using a trephine. A hub (modified from a 20 gauge needle) was implanted into the burr hole and affixed to the skull by contact adhesive and dental cement. Once the assembly was secured, the rat was removed from anesthesia and connected via the hub to the fluid percussion device. Upon the rat regaining its toe pinch reflex, a single sterile saline pulse was delivered with a pressure of 2.0 atm over base room pressure (Custom Design & Fabrication, Richmond, VA). After injury, the hub and dental cement were removed, and the incision was closed by wound clips. In order to avoid unintentional contact with the dura or exposed brain, sham animals were anesthetized and received a scalp incision, but not the crani-

otomy or hub implantation. Animals' body temperature was maintained at 37°C during the surgery using a rectal thermometer coupled to a heating pad.

Following a 7 day recovery period, both sham and injured rats were implanted with 32 microwire arrays (BioSignal, Dallas, TX) directed at the dorsal CA1 subfield of the hippocampus. The electrode array was positioned 3.8 mm posterior to bregma and 2.0 mm lateral from the midline, and with a depth of 2.2 mm. To maximize the yield of these fixed arrays, recordings were made during surgery and electrode placement was adjusted to optimize visualization of sharp wave ripple (SWR) events and a maximal numbers of spikes. A topical analgesic and antibiotic ointment was applied for 7 days following implantation to minimize pain and the risk of infection.

Electrophysiological recordings

Recordings were performed on days 17–19 post-mFPI. Local field potentials were sampled at 10 kHz and digitally filtered (0.5–500 Hz). Spike data were sampled at 30 kHz and digitally filtered (250 Hz hi-pass filter), and thresholds were set to 50–80 µV. Spikes were sorted using a manual clustering program (Blackrock Offline Spike Sorter) based on peak amplitude, spike width, valley amplitude, area, and principal components 1–4. Principal component analysis extracts orthogonal basis vectors compiled from the covariance matrix of every waveform collected by an electrode in a session.¹⁷ Inter-spike interval histograms were built for each neuron. Neurons having an inter-spike interval of <2 ms were removed from analysis. Tracking data was sampled at 40 Hz with a resolution of ~0.2 cm.

Data analysis and place field quantification

Data analysis included only neurons with ≥50 recorded action potentials within a 5 min period.¹⁸ Neurons were considered putatively pyramidal neurons if they exhibited a peak latency of >400 µsec and a mean firing rate of <5 Hz.^{19,20} Neurons with a peak latency of <400 µsec and a mean firing rate of >5 Hz were classified as putative inhibitory neurons.¹⁹ The open field was segmented into 3 cm x 3 cm bins and the number of spikes in each bin was divided by the time spent (within that bin) to determine a firing rate. A heat map was generated for each recorded neuron's activity by combining the calculated firing rates for all of the individual spatial bins within the arena.

Spatial information (SI) is a measure of how unambiguously the cell's firing reflects the position of the animal. SI was calculated according to Skaggs and coworkers, using the equation:

$$SI = \sum_{i=1}^N p_i \left(\frac{R_i}{R} \right) \log_2 \left(\frac{R_i}{R} \right)$$

where p_i = the probability the rat was at spatial bin i (out of N bins), R_i = firing rate at spatial bin i , and R = overall firing rate of the place cell.⁷ Neurons with an SI of >0.99 are considered to be cells encoding potential place field information.

A place field was defined according to criteria outlined by Muller and coworkers²¹: a group of adjoining bins (sharing at least one side) with the average firing rate of each bin exceeding a threshold of 10% above the overall mean firing rate of each cell.^{21–23} The boundaries of the place field are defined as the bins around the place field that fall below the 10% threshold. The area of the place field was derived from the number of 9 cm² bins within the field. Fields with an area of <36 cm² (four adjacent bins) or >495 cm² bins (500 cm²) were excluded from analysis. Neighboring place fields with a gap of ≤6 cm were combined into a single field. In order to reduce the false positive rate associated with short times spent in specific bins, we used a 1 sec threshold for occupancy (within an individual bin). Once the field was established during the

familiarization phase of the task, we then measured the in-field firing rate of isolated place cells (the firing rate within the place field). The in-field firing rates and place field sizes/positions were compared between the familiarization and novel location testing phases to determine in-field firing rate changes and place field stability.

Place field stability

Place field stability was determined by comparing the in-field firing rates during the familiarization and testing periods using a Pearson's correlation.¹⁰ Comparing two rate maps containing bins [$x_1, x_2, \dots, x_N$ for map 1 (familiarization)] and [$y_1, y_2, \dots, y_N$ for map 2 (novel location)], the Pearson correlation coefficient (C) was calculated using the following equation:

$$C = 1/N \sum_{i=1}^N \left(\frac{x_i - \bar{x}}{\sigma_x} \right) \left(\frac{y_i - \bar{y}}{\sigma_y} \right)$$

where $\bar{x}$ and σ_x are the mean and standard deviation (SD) of [$x_1, x_2, \dots, x_N$] and $\bar{y}$ and σ_y are the mean and SD of [$y_1, y_2, \dots, y_N$], respectively. The correlation coefficient can range from 1 to -1, with a higher correlation between the two rate maps indicative of a highly stable place field.

Object location task

Seven days after implantation, rats were habituated to an empty open field arena (60 cm x 60 cm) with 30 cm opaque walls that were marked with cues for orientation. Cues in the maze, and items in the recording room remained constant across the experiment. Habituation was conducted over 4 days by allowing the rats to freely explore the arena for up to 30 min/day. Each behavioral session consisted of a 10 min familiarization (F) period, a 5 min inter-trial interval, and a 5 min testing period. During the familiarization period, two identical objects were placed in the maze, which the rat was allowed to freely explore. For the testing period, one of the two objects was moved to a novel location while the other remained in its original location. During both the familiarization and testing periods, the animals' location was recorded (via an overhead camera) and neuronal activity was continuously monitored. Between days, the objects were replaced so that new objects in novel positions were used for each familiarization period. Time spent exploring the two objects was measured from the overhead video by an experimenter unaware of the animal group designations. Novelty preference was defined as the difference between time spent exploring the objects in the novel and familiar locations divided by the total time spent exploring both objects. Scores ranged from 1 (exploration of only the object in the novel location) to -1 (exploration of only the object in the familiar location).

Immunohistochemistry

Groups of sham ($n=4$) and mFPI ($n=4$) animals were euthanized on day 17 post-injury by an overdose of sodium pentobarbital (100 mg/kg), and the position of the electrodes was marked by electrolytic lesion (30 μ A, 6–10 s). Rats were then transcardially perfused with ice-cold saline (300 mL) and post-fixed in phosphate buffered saline (PBS) containing 4% paraformaldehyde for at least 24 h. Brains were cryoprotected in 30% sucrose (in PBS) and 40 μ m thick coronal sections were prepared on a cryostat. Sections corresponding to the position of the electrode were stained with Cresyl violet and the positions of the electrodes were confirmed. For immunohistochemistry, free-floating sections were permeabilized in PBS containing 0.25% Triton-X100, and blocked in PBS containing 2% bovine serum albumin (BSA) and 2.5% normal goat serum for 2 h. Primary antibodies (in blocking buffer) were added and incubated overnight at room temperature (RT). After extensive

washing, sections were incubated in species-specific secondary antibodies conjugated to Alexa Fluor for 1 h at RT. After secondary antibody incubation, sections were washed in bisbenzimidazole solution for 5 min to stain cellular DNA, then rinsed in PBS. Sections were then slide-mounted, air-dried, and cover-slipped with Fluoromount G (Southern Biotech, Birmingham, AL). Immunoreactivity was visualized on a Zeiss UV microscope. Images were captured using a Retiga 6000 camera using QCapture Pro software (Qimaging, Surrey, BC) and pseudo-colored for presentation. Parvalbumin-positive cells within the CA1 subfield (restricted to the area above the inner blade of the dentate gyrus) were counted from two sections/animal. The area of the CA1 subfield was measured using Stereo Investigator and the number of cells/mm² was calculated for comparisons.

Statistical analysis

All data were subjected to a normality and equal variance test prior to performing statistical comparisons. Statistical comparisons of multi-electrode data from the same neurons' activity during the familiarization and novel location phases were made using a Student's two tailed paired samples t test. For comparison of the neurophysiological properties of neurons between sham and mFPI groups, the non-parametric Mann-Whitney U test (for data with unequal samples) was used. Cell counts were compared across the two groups using a two tailed Student's t test for unpaired variables. Results are presented as mean $\pm$ standard error of the mean (SEM), unless otherwise specified.

Results

mFPI reduces the number of neurons exhibiting place field activity

We performed recordings of CA1 neurons from sham ($n=4$) and mFPI ($n=5$) rats as they performed an object location task. Previous studies have demonstrated that this task requires the hippocampus, and alterations to hippocampal integrity have been shown to impair performance.²⁴ A timeline for the injury, electrode implantation, and cognitive testing is outlined in Figure 1A. On average, injured animals regain their response to toe pinch at 86 ± 7 sec, their tail pain reflexes by 137 ± 13 sec, and their righting response (ability to right themselves three consecutive times after being placed on their back) by 398 ± 10 sec. By comparison, sham-operated animals require only 16 ± 4 sec to regain their righting response (after subtraction of the tail pinch reflex to mimic the time at which injury was delivered). Figure 1B shows a graphical representation of the familiarization and testing phases of the object location task.

During training and testing, we recorded extracellular action potentials and used features of these action potentials, such as primary component 1 (PC1) and primary component 2 (PC2) (Fig. 2A), maximum and minimum voltages, peak latency, and full width and half minimum (FWHM) to isolate single units (Fig. 2B), which we classified as neurons. In sham animals, we identified 248 neurons, whereas in mFPI animals, 240 neurons were isolated. Previous studies have shown that CA1 neurons in the pyramidal cell layer are predominantly excitatory. Excitatory neurons were classified from the recorded neurophysiological data, as described previously using the criteria of a peak to valley distance (i.e., peak latency) of 400–800 μ sec and a firing rate of <5 Hz (Fig. 2C). Of the 248 neurons isolated from sham animals, 161 were classified as putative excitatory neurons (the blue inset box of Fig. 2C).¹⁹ Of these, 93 (52% of classified neurons) exhibited a SI score ≥ 0.99 bits/spike and were therefore classified as place cells (Fig. 2C). Seventeen

FIG. 1. Experimental timeline for surgery and object location task paradigm. **(A)** Timeline showing the time of injury, electrode implantation, and cognitive testing. Rats received either sham surgery or mild lateral fluid percussion injury (mFPI), and on day 7 post-injury, a 32 electrode array was stereotactically placed into the cornu Ammonis (CA)1 of the dorsal hippocampus ipsilateral to the injury. During the electrode placement, recordings were made to detect neurophysiological ripples of the CA1 pyramidal cell layer. **(B)** Rats were first habituated to an empty arena containing positional cues on the walls (20 min/day for 3 days). During habituation, the rats were exposed to the arena in the absence of any on-ground objects. During familiarization, rats were allowed to explore two identical objects for 10 min, after which they were placed in a holding box for a 5 min inter-trial interval. During testing, one of the objects was moved to a new location in the box and the animals were allowed to explore for 5 min. Electrophysiological recordings were made and infrared tracking of the head was performed during both the familiarization and testing periods.

(10% of classified neurons) neurons had a peak latency of $<400 \mu\text{sec}$ and firing rates of $>5 \text{ Hz}$ and were classified as inhibitory neurons.¹⁹

By comparison with the recordings in sham animals, of the 240 isolated neurons in FPI animals, of which 115 were classified as putative excitatory neurons, and only 51 (42% of classified neurons) of these neurons exhibited an SI score $\geq 0.99 \text{ bits/spike}$ (Fig. 2D, $\text{Max}=7.35$), there was a significant reduction in the proportion of place cells compared with that seen in sham operated controls ($\chi^2_{(3)}; p=8.30 \times 10^{-15}$). In addition, only seven (6% of classified neurons) exhibited a putative inhibitory interneuron phenotype.

mFPI decreases the number of parvalbumin-positive inhibitory neurons in the ipsilateral CA1 subfield

Based on the results presented, we questioned if the mFPI caused a loss of neurons that could account for the results we observed from neurophysiological recordings. In order to examine this, a separate group of animals was injured and euthanized 2 weeks post-mFPI to correspond to the start of the behavior experiments. Figure 3A shows representative brain sections from sham and mFPI rats fluorescently stained for nuclei using bisbenzimidazole. Figure 3B shows hippocampal sections immunostained for the neuron-specific marker NeuN. Consistent with previous reports, we did not observe any gross neuronal loss after mFPI, especially in the CA1 subfield in which the electrophysiological recordings were made

(Fig. 3C). Further, staining with an antibody to MAP2 showed no visible differences in dendritic architecture between sham and mFPI animals (Fig. 3B and C).

As described, only 3% of the isolated units in the mFPI rats had the firing characteristics of inhibitory neurons (compared with 7.5% in sham-operated rats). To specifically examine inhibitory neurons, immunohistochemistry was performed to examine the subclass of inhibitory neurons that expresses parvalbumin, as these neurons have been shown to project to axons and dendrites proximal to the cell soma of CA1 pyramidal neurons. In addition, these neurons have been shown to modulate the activity of a large population of pyramidal neurons, including place cells.^{25,26} Figure 3D shows representative images of parvalbumin immunoreactivity in the ipsilateral hippocampus of a sham and an mFPI rat. Quantification of the parvalbumin-positive cells was restricted to within the CA1 subfield (corresponding to the region of electrode implantation), as inhibitory neurons distal to the cell layer are unlikely to have been detected by the electrode arrays. Figure 3E shows that when the number of parvalbumin immunopositive cells in the CA1 subfield was counted, a significant decrease was observed as a result of mFPI ($t=-2.492, p=0.0343$).

mFPI reduces the stability of place fields

Figure 4A shows representative head-tracking maps for a sham and an mFPI animal during the familiarization and novel location phases of the object location task. Head tracking maps for individual sessions and neurophysiological spikes trains were synchronized to generate a heat map for each place cell. Figure 4B shows representative heat maps for two place cells from a sham and an mFPI rat. By comparison with the place fields observed in the sham-operated rats, those identified in the mFPI rat appear to be larger and less stable over the course of familiarization and testing. To quantify stability, a Pearson's correlation was calculated for each place cell by comparing its place fields between the familiarization and novel location phases. The mean Pearson's correlation of the place fields for the 93 place cells in shams was found to be 0.38 ± 0.02 . In contrast, the 51 identified place cells in the mFPI rats were found to be less stable (Pearson's Correlation -0.03 ± 0.05), resulting in a significant difference between sham and mFPI rats ($t_{(72)}=6.65, p<0.01$, Fig. 4C). Further, the place fields in the mFPI rats were found to be broader and less defined ($U=1152, p<0.00001$; Fig. 4D).

In-field firing rates of place cells from sham, but not injured, animals increase during the object location task

The exploration of a novel environment, or an environment in which a change has occurred, has been shown to lead to increased firing rates of CA1 place cells.^{27,28} To determine whether moving an object to a new location increases the firing rates of place cells, we compared the overall mean firing rates for CA1 place cells during the familiarization period and the novel location period. When the in-field firing rate of these neurons was examined, a significant increase in in-field firing rate was observed in sham-operated controls (Fig. 4E: $t_{(92)}=1.98; p<0.05$) that was not detected in rats receiving an mFPI ($t_{(51)}=0.48, p>0.05$). This change was specific to the firing rate, as displacing one of the objects did not affect other characteristics of the action potentials of place cells (i.e., means of the peak to valley latency, maximum voltage, minimum voltage, full width at half maximum, and area) in either sham or injured animals.

FIG. 2. Isolation and classification of neurons based on waveform and firing rate characteristics. **(A)** Principal components (PC1 and PC2) plot showing three isolated cluster clouds of waveforms of neuronal activity. **(B)** Average waveforms of the neural activity for each cluster are shown. Four parameters of the waveform are indicated, the minimum voltage (min), the maximum voltage (max), and two measures of width: the peak latency and full width at half maximum (FWHM). Example waveforms from two putative pyramidal neurons (green and blue) are shown. The multi-unit activity in pink was excluded from further analysis. **(C)** Scatterplot of the 248 neurons from sham animals ($n=4$) plotted by the peak latency on the ordinate and the firing rate on the abscissa. The neurons with a peak latency of 0.4 to 0.8 msec and a firing rate of <5 Hz are considered pyramidal neurons. The population of putative pyramidal neurons is indicated by the blue box, whereas inhibitory neurons are enclosed within the red box. Pie chart illustrating the distribution of identified cells types taken from within the two inset boxes is shown. Place cells were identified as indicated by having low firing rates, wide action potentials, and spatial information in bits/spike of ≥ 0.99 . **(D)** Scatterplot and pie chart of the 240 neurons identified in mild lateral fluid percussion injured (mFPI) animals.

mFPI impairs performance in the object location task

Previous studies have demonstrated that place cell activity and spatial map formation are required for hippocampus-dependent memories.^{11,29} As mFPI rats had reduced numbers of cells with SI, and these cells did not display stable place fields, we questioned if these changes are associated with poor performance in the object location task. Figure 5A shows that during the familiarization period, both sham and FPI rats explored the two objects equally, indicating no initial bias for the relative positions of the objects within the arena or the objects themselves (sham: $p=0.33$; FPI: $p=0.12$). When tested for their location memory, sham rats exhibited preference for the displaced object, spending significantly

more time exploring the object in the novel location than the one that remained in its original location (displaced object time: 35 ± 4.1 sec ($60.51 \pm 0.03\%$); familiar object time: 21.91 ± 3.69 sec, ($39.49 \pm 0.03\%$); $t=3.15$, $p<0.01$; Fig. 5B). In contrast, mFPI animals had impaired location memory as indicated by similar times (with a trend toward spending more time with the familiar object) exploring the objects in the familiar and novel locations (displaced object time: 18.87 ± 2.18 sec [$46.12 \pm 4.26\%$], familiar object time: 25.13 ± 4.2 sec [$53.88 \pm 4.26\%$] $t_{(14)}=1.64$; $p=0.06$).

Although we observed that sham-operated animals displayed a significant preference for the displaced object whereas mFPI rats did not, it is possible that the relatively small number of animals tested contributed to these results. To address this concern,

FIG. 3. Mild lateral fluid percussion injury (mFPI) causes loss of parvalbumin-positive inhibitory neurons in the ipsilateral cornu Ammonis (CA)1 subfield. To assess the histological consequences of mFPI, separate groups of rats ($n=3$ /group) received either a sham or mFPI surgery but were not implanted with electrodes. **(A)** Representative montaged photomicrographs of brain sections from a 14 day post-surgery sham and a 14 day post-injury mFPI animal stained with bisbinzimid (pseudo-colored green for maximum visibility). No overt damage or cell loss was observed, consistent with a mild injury magnitude. **(B)** Representative photomicrograph showing neuronal nuclei (NeuN) (top panels) and microtubule associated protein 2 (MAP2) (bottom panels) immunoreactivity in the dorsal, ipsilateral hippocampus of a sham and an mFPI rat. **(C)** Confocal images of NeuN and MAP2 immunostaining from the CA1 region (illustrated by the box in panel B) showing no overt cell loss or dendritic disruptions. **(D)** Representative photomicrograph showing parvalbumin immunoreactivity in the dorsal, ipsilateral hippocampus of a sham and an mFPI rat. **(E)** Quantification of the number of parvalbumin-positive cells in the pyramidal layer of the CA1.

additional, non-electrode-implanted sham and mFPI rats were tested in the object location task (Fig. 5C). As observed in our implanted rats, sham animals ($n=10$) spent significantly more percentage time exploring the displaced object (NL) as they did exploring the object in the familiar location (FL) ($t=-2.864$, $p=0.010$). Even with the increased group size, mFPI rats ($n=10$) were dysfunctional in their ability to perform the task, spending equivalent percentages of time exploring both NL and FL ($t=0.237$, $p=0.815$).

Discussion

Hippocampal place cell activity and place field stability have been shown to play an important role in learning and memory. In the present study, we made multi-electrode array recordings from the CA1 subfield of sham and mFPI rats and examined place cell firing properties. The results from these experiments revealed four key findings: (1) The number of CA1 pyramidal cells meeting the

criteria for a place cell was reduced in mFPI animals, (2) the place fields found in brain injured animals were broader and less stable than those detected in sham-operated controls, (3) immunohistochemical assessment of mFPI animals indicated a loss of parvalbumin-positive inhibitory interneurons within the pyramidal cell layer of the CA1, (4) during the object location task, in which an object was moved to a new location, the in-field firing rate of place cells in sham, but not mFPI, animals increased. Associated with these changes in place cell activity, mFPI animals performed poorly on the object location task, showing no preference for the object moved to a novel location. These findings may provide a neural correlate for the hippocampal-dependent learning and memory dysfunction seen after mild TBI.

The hippocampus is proposed to organize the encoding and representation of perceived space into a map, allowing the animal to navigate through space.^{4,30,31} In the object location task, ground level objects act as both egocentric cues (while the rat investigates the object), and as allocentric cues as the rat navigates the arena.

FIG. 4. Place fields in sham and mild lateral fluid percussion injured (mFPI) animals. **(A)** Head movement tracking traces from a sham and an mFPI rat during the familiarization and object location phases of the task. **(B)** The firing rate heat maps of two place cells from a sham and two place cells from an mFPI animal. The two place cells in the sham animal appear to be more constrained and stable. In contrast, the two place cells in the mFPI appear to remap to new areas during the object location phase of the task, indicating a lack of stability. The color bar below each heat map indicates the peak spatial firing rate for each neuron. **(C)** Mean Pearson's correlation between place fields recorded during the familiar and novel location phases. Place fields in the sham-operated group displayed higher stability than those detected in the mFPI group. **(D)** Mean place field size for all identified place cells in the sham or mFPI group. **(E)** The mean firing rate of place cells within the place field is significantly increased during the novel location phase of the task in the sham group, but not in the mFPI group. Data are presented as mean $\pm$ standard error of the mean (SEM); * $p < 0.05$.

FIG. 5. Fluid percussion injury (FPI) impairs object location recognition memory. **(A)** Behavioral performance of sham and mild FPI (mFPI) animals in the object location task. Percent of time spent exploring the locations of the two identical objects (F1 and F2) during familiarization is shown. Sham and mFPI animals spent equal time exploring both locations. **(B)** During memory testing, sham animals spent more time exploring the object in the novel location (NL) than in the familiar location (FL). In contrast, injured animals spent similar amounts of time investigating both FL and NL, indicating impaired memory. **(C)** Expansion of the group size by addition of a separate group of animals that were not implanted also resulted in significant exploration of the novel location in sham, but not mFPI, animals. Data are presented as mean $\pm$ standard error of the man (SEM); * $p < 0.05$.

This anchors the object in relationship to the other environmental cues used to form the spatial map. In rodents, exploration of an environment is necessary for generating this cognitive map, and the introduction of objects into familiar settings or the movement of the spatial arrangement of objects triggers new exploratory behavior.³² Using the criteria defined by Skaggs and coworkers to identify place cells, we examined the number and stability of cells exhibiting place activity in the ipsilateral hippocampus of sham and mFPI rats.⁷ Our results show that the percentage of pyramidal neurons with place fields is decreased after mFPI, and those displaying place activity had broader and less stable place fields.³ Although the number of cells exhibiting place activity was reduced, this did not appear to be caused by overall neuronal loss in the CA1 subfield (Fig. 3), as indicated by visual inspection at both low and high magnifications. However, we did observe that the number of parvalbumin-positive inhibitory neurons within the CA1 pyramidal cell layer was significantly reduced as a result of mFPI. Consistent with this, the number of units that were classified as inhibitory neurons was reduced from 10% in sham animals to 6.0% in mFPI rats. This observation is in line with previous studies that have reported loss of inhibitory neurons in the hilar region of the hippocampus after FPI,³³ as parvalbumin neurons have a broad influence on the activity of a large number of pyramidal neurons, and decreases in their numbers in the CA1 subfield could reduce the threshold for firing giving rise to broader place fields and less spatial information (SI) content. Optogenetic silencing of PV neurons in the Cornu Ammonis area 1 (CA1) demonstrated that these interneurons influence the beginning and decay of place cell activity.²⁵

Recently, optogenetically silencing PV+ neurons in mice increased the firing rate both within and out of the place field, and reduced spatial selectivity of pyramidal place cells.^{26,34} Consistent with this, when we relaxed the criteria for defining place cells from an SI score of 0.99–0.90, the number of cells meeting this criterion increased from 51 to 65 (53% of classified neurons) in the mFPI group, a percentage comparable with that seen in the sham controls (52% of classified neurons; Fig. 2C). Taken together, these findings suggest that mFPI reduces SI content of excitatory neurons rather than loss of place cells.

Zhao and colleagues previously showed that when animals are exposed to an identical arena on multiple trials, most place cells display stable place fields, giving rise to a correlation coefficient between 0.5 and 0.6 (on a scale of 1 to –1).¹¹ When we examined place cell stability using the object location task, we found that the place fields in sham animals had a correlation coefficient across sessions of 0.39. Although the reason for this reduced stability is not known, it has been reported that introducing novel spatial orientations (by moving objects or cues) within a testing arena can induce spatially localized remapping.³⁵ Thus, the lower across session stability observed in our sham animals (0.39) may have resulted from remapping caused by moving one of the objects to a novel location. By comparison, the place cells from mFPI rats were found to be highly unstable, resulting in a correlation coefficient across sessions of –0.03. Although the mechanism(s) underlying the lack of place cell stability after mFPI is not known, changes in glutamate and other neurotransmitter systems that are important for place cell stability have been reported to be altered after mFPI.³⁶ For example, previous studies have shown that FPI decreases *N*-methyl-D-aspartate (NMDA) receptor binding to radioactive ligands in the hippocampus.^{37,38} As disruption of NMDA receptors in the CA1 has been shown to reduce the stability of new place cell maps, mFPI-triggered decreases in glutamate signaling could have contributed to the instability of place cells in the mFPI animals that we observed.³⁶

Although these experiments demonstrate that place cell activity is disturbed after mFPI, several weaknesses must be acknowledged. One of the weaknesses of this study is that we employed a between-animal experimental design, rather than comparing place cells within animals pre- and post-injury. This approach was chosen because any movement of the brain tissue resulting from the impact force is likely to shift the position of the implanted electrodes, making it difficult to ensure that the same neurons' activities are being recorded pre- and post-injury. Another limitation is that we cannot ascribe the changes we observed in place cell firing and field stability as an intrinsic change within the place cells themselves. CA1 place cells receive input from CA3 pyramidal neurons (via the Schaffer collaterals) and the entorhinal cortex (via the perforant path), as well as modulatory inputs from structures such as the septal nucleus (cholinergic, GABAergic, ventral tegmental area [dopaminergic], and locus coeruleus [noradrenergic and dopaminergic]).^{39–41} Disrupted communication between these structures as a result of TBI may have contributed to the alterations in place cell activity that we observed. Finally, it is possible that the implantation of the electrodes themselves caused a “second” injury that contributed to reduced place cell stability in neurons already compromised as a result of mFPI. In future experiments, non-invasive imaging techniques using calcium sensors could be used to address this possibility.

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References

- Lyeth, B.G., Dixon, C.E., Hamm, R.J., Jenkins, L.W., Young, H.F., Stonnington, H.H., and Hayes, R.L. (1988). Effects of anticholinergic treatment on transient behavioral suppression and physiological responses following concussive brain injury to the rat. *Brain Res.* 448, 88–97.
- Paterno, R., Metheny, H., Xiong, G., Elkind, J., and Cohen, A.S. (2016). Mild traumatic brain injury decreases broadband power in area CA1. *J. Neurotrauma* 33, 1645–1649.
- 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.
- O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34, 171–175.
- Knierim, J.J., and McNaughton, B.L. (2001). Hippocampal place-cell firing during movement in three-dimensional space. *J. Neurophysiol.* 85, 105–116.
- Moser, E.I., Moser, M.B., and McNaughton, B.L. (2017). Spatial representation in the hippocampal formation: a history. *Nat. Neurosci.* 20, 1448–1464.
- Skaggs, W.E., McNaughton, B.L., Gothard, K., and Markus, E.J. (1993). An information-theoretical approach to deciphering the hippocampal code, in: *Proceeding Advances in Neural Information Processing Systems*, Morgan Kaufmann, San Francisco, CA, pp. 1030–1037.
- Ji, D., and Wilson, M.A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat. Neurosci.* 10, 100–107.
- Siegel, J.J., Neunuebel, J.P., and Knierim, J.J. (2008). Dominance of the proximal coordinate frame in determining the locations of hip-

- pocampal place cell activity during navigation. *J. Neurophysiol.* 99, 60–76.
10. Cuipek, S.M., Cheng, J., Ali, Y.O., Lu, H.C., and Ji, D. (2015). Progressive functional impairments of hippocampal neurons in a tauopathy mouse model. *J. Neurosci.* 35, 8118–8131.
 11. Zhao, R., Grunke, S.D., Keralapurath, M.M., Yetman, M.J., Lam, A., Lee, T.C., Sousounis, K., Jiang, Y., Swing, D.A., Tessarollo, L., Ji, D., and Jankowsky, J.L. (2016). Impaired recall of positional memory following chemogenetic disruption of place field stability. *Cell Rep.* 16, 793–804.
 12. Abel, T., Nguyen, P.V., Barad, M., Deuel, T.A., Kandel, E.R., and Bourchouladze, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* 88, 615–626.
 13. 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.
 14. Dietrich, W.D., Alonso, O., and Halley, M. (1994). Early microvascular and neuronal consequences of traumatic brain injury: a light and electron microscopic study in rats. *J. Neurotrauma* 11, 289–301.
 15. Reeves, T.M., Phillips, L.L., and Povlishock, J.T. (2005). Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. *Exp. Neurol.* 196, 126–137.
 16. 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.
 17. Lewicki, M.S. (1998). A review of methods for spike sorting: the detection and classification of neural action potentials. *Network* 9, R53–78.
 18. Yu, X., Yoganarasimha, D., and Knierim, J.J. (2006). Backward shift of head direction tuning curves of the anterior thalamus: comparison with CA1 place fields. *Neuron* 52, 717–729.
 19. Csicsvari, J., Hirase, H., Czurko, A., Mamiya, A., and Buzsaki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. *J. Neurosci.* 19, 274–287.
 20. Henze, D.A., Borhegyi, Z., Csicsvari, J., Mamiya, A., Harris, K.D., and Buzsaki, G. (2000). Intracellular features predicted by extracellular recordings in the hippocampus in vivo. *J. Neurophysiol.* 84, 390–400.
 21. Muller, R.U., Kubie, J.L., and Ranck, J.B., Jr. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *J. Neurosci.* 7, 1935–1950.
 22. Jung, M.W., Wiener, S.I., and McNaughton, B.L. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *J. Neurosci.* 14, 7347–7356.
 23. Mehta, M.R., Lee, A.K., and Wilson, M.A. (2002). Role of experience and oscillations in transforming a rate code into a temporal code. *Nature* 417, 741–746.
 24. Barker, G.R., and Warburton, E.C. (2011). When is the hippocampus involved in recognition memory? *J. Neurosci.* 31, 10721–10731.
 25. Royer, S., Zemelman, B.V., Losonczy, A., Kim, J., Chance, F., Magee, J.C., and Buzsaki, G. (2012). Control of timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition. *Nat. Neurosci.* 15, 769–775.
 26. Grienberger, C., Milstein, A.D., Bittner, K.C., Romani, S., and Magee, J.C. (2017). Inhibitory suppression of heterogeneously tuned excitation enhances spatial coding in CA1 place cells. *Nat. Neurosci.* 20, 417–426.
 27. Larkin, M.C., Lykken, C., Tye, L.D., Wickelgren, J.G., and Frank, L.M. (2014). Hippocampal output area CA1 broadcasts a generalized novelty signal during an object-place recognition task. *Hippocampus* 24, 773–783.
 28. Nitz, D. and McNaughton, B. (2004). Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *J. Neurophysiol.* 91, 863–872.
 29. Poucet, B., Hok, V., Sargolini, F., and Save, E. (2012). Stability and variability of place cell activity during behavior: functional implications for dynamic coding of spatial information. *J. Physiol. Paris* 106, 62–71.
 30. Danjo, T. (2019). Allocentric representations of space in the hippocampus. *Neurosci. Res.* [Epub ahead of print].
 31. O'Keefe, J., and Recce, M.L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* 3, 317–330.
 32. Clark, R.E., Zola, S.M., and Squire, L.R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J. Neurosci.* 20, 8853–8860.
 33. 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.
 34. McKenzie, S. (2018). Inhibition shapes the organization of hippocampal representations. *Hippocampus* 28, 659–671.
 35. Spiers, H.J., Hayman, R.M., Jovalekic, A., Marozzi, E., and Jeffery, K.J. (2015). Place field repetition and purely local remapping in a multicompartiment environment. *Cereb. Cortex* 25, 10–25.
 36. Kentros, C., Hargreaves, E., Hawkins, R.D., Kandel, E.R., Shapiro, M., and Muller, R.V. (1998). Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* 280, 2121–2126.
 37. Bales, J.W., Wagner, A.K., Kline, A.E., and Dixon, C.E. (2009). Persistent cognitive dysfunction after traumatic brain injury: a dopamine hypothesis. *Neurosci. Biobehav. Rev.* 33, 981–1003.
 38. Miller, L.P., Lyeth, B.G., Jenkins, L.W., Oleniak, L., Panchision, D., Hamm, R.J., Phillips, L.L., Dixon, C.E., Clifton, G.L., and Hayes, R.L. (1990). Excitatory amino acid receptor subtype binding following traumatic brain injury. *Brain Res.* 526, 103–107.
 39. Broussard, J.L., Yang, K., Levine, A.T., Tsetsenis, T., Jenson, D., Cao, F., Garcia, I., Arenkiel, B.R., Zhou, F.M., De Biasi, M., and Dani, J.A. (2016). Dopamine regulates aversive contextual learning and associated in vivo synaptic plasticity in the hippocampus. *Cell Rep.* 14, 1930–1939.
 40. 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.
 41. Kempadoo, K.A., Mosharov, E.V., Choi, S.J., Sulzer, D., and Kandel, E.R. (2016). Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. *Proc. Natl. Acad. Sci. U. S. A.* 113, 14,835–14,840.

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