Diminished Dentate Gyrus Filtering of Cortical Input Leads to Enhanced Area CA3 Excitability after Mild Traumatic Brain Injury

Kaitlin A. Folweiler,^{1–3} Sandy Samuel,^{1,2} Hannah E. Metheny,^{1,2} and Akiva S. Cohen^{1–3}

Abstract

Mild traumatic brain injury (mTBI) disrupts hippocampal function and can lead to long-lasting episodic memory impairments. The encoding of episodic memories relies on spatial information processing within the hippocampus. As the primary entry point for spatial information into the hippocampus, the dentate gyrus is thought to function as a physiological gate, or filter, of afferent excitation before reaching downstream area Cornu Ammonis (CA3). Although injury has previously been shown to alter dentate gyrus network excitability, it is unknown whether mTBI affects dentate gyrus output to area CA3. In this study, we assessed hippocampal function, specifically the interaction between the dentate gyrus and CA3, using behavioral and electrophysiological techniques in *ex vivo* brain slices 1 week following mild lateral fluid percussion injury (LFPI). Behaviorally, LFPI mice were found to be impaired in an object-place recognition task, indicating that spatial information processing in the hippocampus is disrupted. Extracellular recordings and voltage-sensitive dye imaging demonstrated that perforant path activation leads to the aberrant spread of excitation from the dentate gyrus into area CA3 along the mossy fiber pathway. These results suggest that after mTBI, the dentate gyrus has a diminished capacity to regulate cortical input into the hippocampus, leading to increased CA3 network excitability. The loss of the dentate filtering efficacy reveals a potential mechanism by which hippocampal-dependent spatial information processing is disrupted, and may contribute to memory dysfunction after mTBI.

Keywords: area CA3; dentate gyrus; LFPI; mTBI; object-place recognition memory; perforant path

Introduction

MILD TRAUMATIC BRAIN INJURY (mTBI) leads to an array of long-lasting cognitive symptoms, notably including episodic memory dysfunction.¹ In the healthy brain, episodic memory relies on cortical input of spatial information to the hippocampus for memory encoding and consolidation. Experimental mTBI models have specifically demonstrated that hippocampal disruption is associated with spatial memory impairments.^{2–6} The dentate gyrus is poised to receive incoming signals from layer II/III entorhinal cortex via perforant path axons and to transmit signals to the Cornu Ammonis (CA3) region of the hippocampus via the mossy fiber axonal pathway.^{7,8} Synaptic transmission through this di-synaptic pathway plays a critical role in the processing of spatial information received by the hippocampus, as well as in pattern separation and completion.^{9–18}

The dentate gyrus (DG) is thought to act as a physiological gate, or filter, of afferent input into the hippocampus, limiting afferent cortical excitation to area CA3.¹⁹ This filtering property is dis-

tinctly exhibited by the sparse, action potential firing of dentate granule cells in response to perforant path stimulation.^{20–22} Sparse granule cell activation is likely the result of both the low intrinsic membrane excitability of granule cells, as well as robust GABAergic inhibition from interneurons within the DG network.^{23–27} As such, the putative dentate filter may be functionally important for discerning relevant spatial information for further hippocampal memory processing and diminishing irrelevant inputs.

Lateral fluid percussion injury (LFPI) is a well-characterized and commonly used rodent model of brain injury that reproduces key features of human TBI, including neuronal cell loss, gliosis, ionic perturbation, and memory deficits.^{28–30} After LFPI, the DG experiences a net increase in network excitability associated with an imbalance of excitatory and inhibitory synaptic transmission onto dentate granule cells.^{4,31–38} Although post-traumatic alterations in individual DG cell types have been well characterized, it remains unknown how these circuit alterations affect the net output of the DG to area CA3. In this study, we investigated the physiological filtering ability of the DG to limit afferent excitation to area CA3 using a

¹Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania.

²Department of Anesthesiology, Perelman School of Medicine, and ³Neuroscience Graduate Group, University of Pennsylvania, Philadelphia, Pennsylvania.

mouse model of LFPI. We initially confirm spatial novelty detection impairments in a hippocampal-dependent behavioral task at 1 week after LFPI. To evaluate DG filtering efficacy, we used electrophysiological techniques in acute brain slices to assess network excitability in the DG and area CA3 in response to perforant path stimulation. The results demonstrate that afferent excitation not only generates a hyperexcitable shift in the DG network response after LFPI, but also causes aberrant propagation of excitation to CA3 via the mossy fiber pathway. From these data, we conclude that synaptic transmission through the DG to area CA3 is altered after LFPI, indicating a diminished filtering capacity of the DG in the injured brain.

Methods

Mice

Experiments were performed on 6–8-week-old male C57/BL6 mice (Jackson Laboratory, Bar Harbor, ME; IMSR Cat# JAX:000664, RRID: IMSR_JAX:000664). All experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Children's Hospital of Philadelphia and the guidelines established by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Separate cohorts of animals were used for behavioral and electrophysiological experiments because of the large number of animals necessary for electrophysiological experiments.

Surgical procedures

Animals were anesthetized with a mixture of ketamine (100–200 mg/kg) and xylazine (0.06–0.16 mg/kg) via intraperitoneal injection. Once fully anesthetized, animals were placed in a stereotaxic frame (Stoetling, Wood Dale, IL). The scalp was incised and pulled away to fully expose the right parietal bone. An ultra-thin Teflon disk, with the outer diameter equal to the inner diameter of a trephine was glued to the skull with Vetbond (3M, St. Paul, MN) between lambda and bregma, and between the sagittal suture and the lateral ridge over the right hemisphere. Guided by the Teflon disk, a trephine was used to perform a 3 mm diameter craniectomy over the right parietal area. Following craniectomy, a Luer-lock needle hub (3 mm inner diameter) was secured above the skull opening with Loctite superglue and dental acrylic, filled with saline and capped. Lastly, animals were removed from stereotaxis, placed on a heating pad until fully recovered from anesthesia, and then returned to their respective home cages.

LFPI

Twenty-four hours following craniectomy, animals were placed under isoflurane anesthesia (2% oxygen in 500 mL/min) in a chamber, and respiration was visually monitored until animals reached a surgical plane of anesthesia (one respiration per 2 sec). At this point, animals were removed from isoflurane, and the needle hub was refilled with saline and connected to the FPI device (Department of Biomedical Engineering, Virginia Commonwealth University, Richmond) via high-pressure tubing. The animal was placed onto a heating pad on its left side, and upon resumption of normal breathing pattern but before sensitivity to stimulation, the injury was induced by a 20 msec pulse of saline onto the intact dura. The pressure transduced onto the dura was monitored with an oscilloscope, with injury severity ranging between 1.4 and 1.6 atm. Immediately after injury, the hub was removed from the skull, and the animal was placed in a supine position to assess righting reflex. After righting, the animal was subjected to inhaled isoflurane to suture the scalp. Animals were allowed to recover on a heating pad until mobile, at which point they were returned to their home cages. Sham animals underwent all surgical procedures including attachment to the FPI device, with exclusion of the actual fluid pulse.

Object-place recognition memory behavioral task

One week after LFPI or sham surgery, hippocampal-dependent spatial information processing was assessed with an object-place recognition memory task adapted from Oliveira and colleagues.³⁹ The task consisted of a respective training and testing day, spanning a total of 2 days. Mice were handled for 5 min each day for 4 days prior to training. During the training day, mice first received one 6 min habituation trial followed by three identical 6 min training trials with 3 min inter-trial intervals in their home cage. Habituation consisted of 6 min in a rectangular open field box (30.5 × 51 × 30.5 cm) with visual cues placed on each of the arena walls in the absence of objects. Subsequent training trials took place in the same box, but now with three identical, complex objects positioned diagonally in the box. Corner objects were placed 3 inches away from each wall. Mice were allowed to freely explore the environment and the objects for the duration of each trial. Twenty-four hours from the first training trial, mice were placed back in the box for a single 6 min testing trial. The three objects were again present, but one of the outer two objects was now displaced to a novel spatial location (Fig. 1A). Time spent exploring the displaced and non-displaced objects was measured in the third training trial and testing trial. The initial diagonal orientation of the objects, as well as the testing object selected for displacement, was counterbalanced

FIG. 1. Lateral fluid percussion injury (LFPI) animals show impairment in object-place recognition task. (A) The task consisted of familiarization training with three identical objects, each in a distinct spatial location. In the testing trial, one object was displaced to a novel location. (B) The normalized difference in the time spent exploring the displaced and non-displaced objects in testing and training was calculated as a discrimination index (i.e., positive index scores signify more time spent exploring the object in testing). If the animal showed a preference for the displaced object in the test trial over the non-displaced objects, this was considered a demonstration of spatial novelty detection. (Sham, displaced vs. non-displaced objects, $p=0.0004$; LFPI, displaced vs. non-displaced objects, $p=0.5970$; Sham displaced vs. LFPI displaced, $p=0.0019$; $n=10$ animals for each condition). Discrimination index calculation as follows: Discrimination index = $[(\text{Object} \times \text{interaction time}_{\text{testing}} / \text{Total Interaction time}_{\text{testing}}) \times 100] - [(\text{Object} \times \text{interaction time}_{\text{training}} / \text{Total Interaction time}_{\text{training}}) \times 100]$.

between mice. Testing was always performed in the morning at the end of the dark phase of a 12 h light–dark cycle.

Behavioral analysis

All testing and training sessions were videotaped and analyzed by an experimenter blinded to the injury conditions of the animals. Object interaction times were independently calculated by both manual observation of video recordings, and automated open-source behavioral software developed by Patel and colleagues.⁴⁰ The results of the manual and automated behavioral analysis were then subsequently averaged together. Total object interaction time, as well as time spent with each object, were recorded for each trial. Each animal's exploratory response to the spatial object displacement was measured with a normalized discrimination index.³⁹ The discrimination index was calculated by subtracting the percent of total interaction time a mouse spent exploring the respective object category (i.e., displaced and the non-displaced), in the testing trial from the percent of total interaction time exploring the same object category in the third training trial. Therefore, a positive index percentage value indicated that the animal spent more time with that object in testing than in training. Criteria for what was considered as object interaction was limited to the times when mice were facing and sniffing the objects within a 2 cm radius and/or touching them; sitting on the objects did not count as an exploration interaction. The first 2 min in the third training and testing trials were used for analysis, as preliminary testing in naïve mice showed a significant decrease in total object interaction time after the first 2 min of each trial. Animals that did not explore the objects for more than 3 sec during training or testing were excluded from analysis.

Slice preparation

All electrophysiological experiments were performed on days 6–8 after LFPI or sham surgery. Slice preparation was performed as previously described.⁴¹ Briefly, animals were anesthetized with isoflurane, the brain was dissected out and placed in ice-cold oxygenated (95% O₂/5% CO₂) sucrose-containing artificial cerebrospinal fluid (aCSF) containing (in mM): sucrose 202, KCl 3, NaH₂PO₄ 2.5, NaHCO₃ 26, glucose 10, MgCl₂ 1, and CaCl₂ 2. The hemisphere contralateral to the craniectomy was removed prior to vibratome slice cutting. Hippocampal–entorhinal slices (HEC) slices 350 μ m thick were cut on a vibratome (VT1200S, Leica Microsystems, Buffalo Grove, IL). HEC slice orientation was used in an effort to maximally preserve intact perforant path and mossy fiber pathway axons. In a subset of slices ($n=2$ slices per group), the hilus was transected with a scalpel at the terminal ends of DG stratum granulosum immediately following vibratome slicing to sever mossy fiber axons traveling to area CA3. Slices were transferred to 33–37°C oxygenated (95% O₂/5% CO₂) control aCSF containing (in millimolars): NaCl 130, KCl 3, NaH₂PO₄ 1.25, NaHCO₃ 26, glucose 10, MgCl₂ 1, and CaCl₂ 2. Slices were allowed to incubate and equilibrate for at least 60 min before recording. Voltage-sensitive dye (VSD) imaging and extracellular field potential recordings were performed in an interface chamber with a flow rate of 2.0 mL/min and maintained at 27–30°C.

Extracellular field recordings

Electrodes for recording field excitatory post-synaptic potentials (fEPSPs) were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL; #IB150F-4), pulled to a tip resistance of 2–6 M Ω when filled with aCSF. fEPSPs were recorded from an electrode placed in the suprapyramidal blade of the DG middle stratum moleculare. Stimulating electrodes were non-concentric bipolar (World Precision Instruments, Sarasota, FL; #ME12206) and placed at the apex of the stratum moleculare. Electrical stimuli

were 100 μ s in duration. Field potential input–output relationships (50–500 μ A stimulation, 50 μ A increments, 8 sec inter-stimulus interval) were performed prior to VSD recordings. Field potential recordings were also recorded simultaneously with VSD recordings. The inter-stimulus interval for field potentials recorded during the VSD trials was 20 sec. This duration was necessary to accommodate every other trial subtraction method used for VSD analysis. Simultaneous VSD and field recordings were performed at 200 μ A, 300 μ A, and 400 μ A stimulation intensities in aCSF.

In a separate experiment, extracellular recordings of orthodromic population spikes from the stratum granulosum of the dentate infrapyramidal blade and CA3 stratum pyramidale were recorded simultaneously. A non-concentric bipolar electrode was again placed at the apex of the dentate stratum moleculare to stimulate afferent perforant path fibers from the entorhinal cortex. Paired-pulse stimulation (100 msec inter-stimulus interval), each pulse 100 μ sec in duration, was used to elicit population spikes in both the DG stratum granulosum and CA3 stratum pyramidale, and, subsequently, fEPSPs in the CA3 stratum lucidum and stratum radiatum (Fig. 2). A paired-pulse protocol was chosen in order to increase the probability of eliciting a population spike in CA3 rather than a single stimulation (preliminary data, not shown). Field potential input–output relationships (20–500 μ A stimulation, 8 sec inter-stimulus interval) were performed for each cell layer. Recordings were obtained with an Axoclamp 900 A amplifier and pClamp10 data acquisition software (Molecular Devices, Sunnyvale, CA), filtered at 2 kHz. Population

FIG. 2. Anatomy of the dentate gyrus and area Cornu Ammonis [CA3] in a hippocampal–entorhinal cortex (HEC) brain slice. The perforant path (PP) is composed of axons from layer II of the entorhinal cortex, which traverse the subiculum and enter the stratum moleculare (StM) of the dentate gyrus (i.e., molecular layer [ML]), to synapse onto distal dendrites of granule cells. Granule cell bodies are located in stratum granulosum (StG; i.e., granule cell layer). Granule cell axons pass through the dentate hilus (HIL) forming the mossy fiber pathway (MF). Mossy fibers synapse onto CA3 pyramidal cell proximal dendrites in the stratum lucidum (StL) of area CA3. Pyramidal cell bodies are situated in stratum pyramidale (StP; i.e., pyramidal cell layer). Pyramidal cell axons project into the stratum oriens (StO). Pyramidal cells additionally send recurrent associational/commissural (A/C) axon collaterals to stratum radiatum (StR) where they synapse onto other pyramidal cells, forming the CA3 autoassociational network. In all experiments described, a nonconcentric bipolar stimulating electrode was placed in the dentate ML at the apex to stimulate perforant path fibers (Stim).

spike amplitude was measured as the amplitude of the first negative deflection overriding the field EPSP waveform.⁴² Field potential data were analyzed using pClamp10 and custom-written MATLAB scripts.

Voltage-sensitive dye imaging acquisition

Dye stock solutions of di-3-ANEPPDHQ (Invitrogen) were prepared at a concentration of 20 mg/mL in ethanol and stored at -20°C . Dye working solutions were prepared at a concentration of 67 $\mu\text{g/mL}$ by diluting dye stock solution 1:300 in aCSF on the day of recording. Slices were stained for 16 min, and afterwards rinsed thoroughly with aCSF before being transferred to the recording chamber. Stained slices were then washed for another 15–20 min prior to commencing VSD recording. As in extracellular field recordings, a non-concentric bipolar electrode was placed at the apex of the stratum moleculare to elicit electrical stimulation. The dye was excited by seven high-power green LEDs (Luxeon Rebel LXML-PM01-0100, Philips) coupled to a 535 ± 25 nm bandpass filter and 565 nm dichroic mirror. Emitted VSD fluorescence was isolated with a 610 nm long-pass filter and recorded at 0.5 kHz with a fast video camera with 80×80 pixel resolution (NeuroCCD, Redshirt Imaging, Decatur, GA) through a reverse-lens macroscope with a 50 mm f/1.3 M46 lens (Dark Invader). Each camera pixel imaged a $25 \mu\text{m} \times 25 \mu\text{m}$ region of tissue. The fluorescence light source was triggered 230 msec prior to the acquisition of the fluorescence signal to allow the light onset emission transient to stabilize, and the electrical stimulus was delivered 170 msec after commencing fluorescence data acquisition. All VSD recordings were 13 trials, 1.0 sec in duration (1000 samples), with a 20 sec interval between electrically stimulated trials. Each stimulated trial was followed 10 sec later, by a non-electrically stimulated trial that was used to subtract photo-bleaching from the active signal. All VSD figures and statistics reported here are from recordings at 200 μA stimulation, as this elicited an approximately half maximal field potential response, while providing a high enough signal-to-noise ratio for VSD analysis.

Voltage-sensitive dye imaging analysis

Initial processing of VSD signals was performed as described previously.^{41,43} All VSD measurements were recorded as fractional change in fluorescence values ($\Delta F/F$), which were calculated as follows: fluorescence values for each pixel in each trial were normalized according to the average fluorescence in the pixel during a 64 msec window immediately preceding the electrical stimulus. Then, the average from the corresponding pixel in the non-electrically stimulated trial was normalized and subtracted from the individual electrically stimulated trials to correct for photo-bleaching. VSD recordings were filtered in x and y spatial coordinates by convolution with a 5 pixels $\times$ 5 pixels Gaussian filter ($\Sigma = 1.2$ pixels), and in time by convolution with a five sample median filter. No additional filtering was applied to any of the images or analysis of VSD data. For VSD videos and representative movie frames, the $\Delta F/F$ for each pixel for the given sample (1 msec) is displayed as pseudocolor superimposed onto the image of the brain slice, where warm colors (i.e., red and orange) indicate depolarization and cool colors (i.e., blue and purple) indicate hyperpolarization. Time points selected for the representative movie frames correlate to fluorescence peaks analyzed in the regional average line plots. Peak maps were generated by pseudo-coloring and plot the maximum $\Delta F/F$ value at each pixel regardless of time for that slice.

Raster plot construction, analysis, and statistics were performed using the MATLAB VSD analysis toolbox.⁴⁴ To begin raster construction, regions of interest (anatomical regions) were defined for segmentation. In these experiments, the anatomical boundaries of the DG stratum moleculare, stratum granulosum and hilus, as well as the CA3 stratum pyramidale, radiatum/lucidum, and oriens, were clearly

visible and used to draw region boundaries. The DG strata granulosum and moleculare were subdivided into suprapyramidal and infrapyramidal blades, as these blades have been found to have different circuit properties and connections.⁴⁵ There was no clear visual division between the subregions CA3a,b, or c; therefore, approximate geometric divisions were made consistently across slices to include the regions closest to the dentate, putatively CA3b/c.

Regions were then split into 100 μm segments along the rostrocaudal axis, numbering segments in ascending order from the rostral to caudal ends of each region. Average $\Delta F/F$ values were calculated for each spatiotemporal site and plotted as pseudocolor in the distance from stimulating electrode versus time raster plots. To account for slight variability in region of interest size when creating average raster plots, all individual slice raster plots were stretched or compressed to have the same number of segments. That number was set as the mean number of segments from the individual slices. VSD recordings were made in a maximum of two slices per animal; therefore, $\Delta F/F$ values for each anatomical region of interest were averaged to create a raster plot for each animal for group analysis. Statistical comparison of group raster plots was performed at each individual spatiotemporal site using a permutation test ($t = 1000$). Permutation is a nonparametric test that randomly resamples data to generate a null distribution describing variability in the data. Significant differences ($\alpha < 0.05$) were registered at sites where injury versus sham groupings explained the variability in the data. The p values generated are displayed in pseudocolor on the p value raster plots. Multi-segment averages were calculated for each anatomical region by averaging the mean $\Delta F/F$ value for each signal to generate a regional average, and were plotted as line plots (i.e., multi-segment regional average line plots). Line plots show the average $\Delta F/F$ (dark line) surrounded by ellipses marking the standard error of the mean (shaded surrounding). The initial fast depolarization peak (max $\Delta F/F$ between 0 and 35 msec), was used for analysis, as it roughly corresponds to the time scale of synaptic transmission. The segments selected for line plots were the same between conditions and consistent by region.

Statistical analysis

All statistical analyses and calculations were performed using MATLAB and/or GraphPad Prism. *A priori* power calculations were performed using G*Power based on variability from similar previous experiments.⁴⁶ The spatial object recognition task discrimination index was analyzed with one way ANOVA with *post-hoc* Tukey's multiple comparisons test. Student's t tests were used to compare total object interaction times in training and testing, respectively. All statistical tests for fEPSP data were conducted using Mann–Whitney U tests, or two way repeated measures ANOVA with Sidak's multiple comparison test in order to test for injury effect and stimulation intensity effect. VSD data were analyzed spatiotemporally with the permutation test described by Bourgeois and coworkers where applicable.⁴⁴ Statistical significance is represented here as *: $p < 0.05$, $p < 0.01^{**}$, $p < 0.001^{***}$. N values represent number of animals in each condition, with a maximum of two slices per animal. For physiological experiments (fEPSP and VSD) when multiple brain slices from a single animal were used, data from individual brain slices generated from the same animal were averaged, yielding a single animal value for each given measure. The value generated was used in group analysis and statistics, based on the animal n . Data in the figures are presented as group means $\pm$ SEM.

Results

LFPI diminishes object-place recognition memory

To evaluate the effect of mTBI on the ability to detect spatial change within an environment (i.e., spatial novelty), we utilized an object-place recognition memory task based on a previously

established paradigm.³⁹ In this version of the task, all three objects were identical, and the spatial change consisted of the displacement of one object to a novel location within the chamber during the testing trial while keeping constant the locations of the remaining two objects (Fig. 1A). There was no difference in total object interaction time between the two groups during training (Sham: 8.14 ± 1.12 sec, LFPI: 8.07 ± 0.82 sec, $p = 0.95$, n.s.) or testing (Sham: 9.56 ± 0.74 sec, LFPI: 9.70 ± 1.05 sec, $p = 0.91$, n.s.). During the testing trial, sham animals spent significantly more time interacting with the displaced object than with the non-displaced objects (Fig. 1B; displaced object mean discrimination index score, 22.46%; non-displaced objects mean discrimination index score, -10.17%, $p < 0.001$; $n = 10$ animals). Conversely, there was no significant difference in the time spent between displaced and non-displaced objects for the LFPI animals (displaced mean discrimination index score, -5.88%; non-displaced mean discrimination index score, 2.82%; $p = 0.5970$, n.s.; $n = 10$ animals). Comparing between groups, LFPI animals spent less time with the displaced object than did sham

(Sham: mean discrimination index score, 22.46%; LFPI: mean discrimination index score, -5.88%, $p < 0.01$). There was no difference in the discrimination index scores of the non-displaced objects between the groups (Sham: mean discrimination index score, -10.17% LFPI: mean discrimination index score, 2.82%; $p = 0.26$, n.s.).

Mild LFPI causes an increase in DG network excitability

Next, we investigated DG physiological function to determine if alterations in this region may contribute to the behavioral deficits observed. In order to confirm that the DG experienced a net shift in network excitability as seen in previous studies, we recorded fEPSPs from the DG stratum moleculare in response to perforant path stimulation (Sham, $n = 7$ animals; LFPI, $n = 8$ animals; two slices per animal). fEPSP responses were recorded over a range of stimulation intensities (50–300 μ A) to generate input/output (I/O) curves (Fig. 3A). The I/O curve of the DG shows that as stimulation

FIG. 3. Increase in dentate gyrus net excitability. (A) Extracellular input/output (I/O) curves recorded from the stratum moleculare in response to incremental perforant path stimulation from 50 to 300 μ A. (Lateral fluid percussion injury [LFPI], $n = 8$; Sham, $n = 7$; two way repeated measures ANOVA, [injury effect] $F[1,13] = 8.694$, $p = 0.0113$; [stimulus intensity effect] $F[3,39] = 23.48$, $p < 0.0001$; [interaction] $F[3,39] = 4.099$, $p = 0.0127$). Multiple comparisons show a statistically significant shift in the field excitatory post-synaptic potentials [fEPSP] response to the upper range of stimulation intensities shown (Sidak's multiple comparisons test, 200 μ A, $p = 0.0042$; 300 μ A, $p = 0.0038$). (B) Representative fEPSP traces from Sham and LFPI slices at 200 μ A. (C) Representative voltage-sensitive dye (VSD) movie frames from a Sham and LFPI slice at respective time points after 200 μ A perforant path stimulation. Raster plots for the (D) stratum granulosum and (E) stratum moleculare show the group average fluorescence signals for 100 μ m segments along the longitudinal axis at each sample time point during the 200 μ A trial. (F) Multi-segment regional average line plots from the stratum granulosum demonstrate the fast depolarizing peak of the fluorescence signal in slices from the LFPI group (red) versus sham controls (green). Sham mean, 0.001594 ± 0.0002656 ; LFPI mean, 0.002254 ± 0.0001531 , $p = 0.0369$; Sham $n = 11$, LFPI $n = 9$ animals. (G) Raster indicating the statistical difference between the Sham and LFPI group rasters at each spatiotemporal point in the trial with representative pseudocoloring of bootstrapped p values (shades of purple indicate significance of $p < 0.05$ or more).

intensity increases, there is an increase in the fEPSP slopes from LFPI animals (two way repeated measures ANOVA, [injury effect] $F[1,13]=8.694$, $p<0.05$; [stimulus intensity effect] $F[3,39]=23.48$, $p<0.0001$; [interaction] $F[3,39]=4.099$, $p<0.05$). Multiple comparisons show a statistically significant shift in the fEPSP response to the upper range of stimulation intensities (Sidak's multiple comparisons test, 200 μA , $p<0.01$; 300 μA , $p<0.01$).

To visualize the spatiotemporal characteristics of the DG physiological response to perforant path stimulation, we performed VSD imaging. VSD is an advantageous tool for measuring population neuronal activity by recording changes in transmembrane potential with high spatial and temporal resolution. VSD dyes absorb into cell membranes where they emit fluorescence as a function of the transmembrane voltage. Changes in this fluorescence ($\Delta F/F$) are linearly proportional to changes in membrane voltage, and in hippocampal slices, the voltage-sensitive dye di-3-ANEPPDHQ emits fluorescence for which $1 \times 10^{-4} \Delta F/F$ equals a roughly 1 mV change in transmembrane voltage.⁴⁷ All $\Delta F/F$ values are visually represented by pseudo-coloring of spatial regions where warm colors (i.e., red and orange) indicate depolarization and cool colors (i.e., blue and purple) indicate hyperpolarization, as exhibited by representative video frames from sham and LFPI slices in Figure 3C (Supplementary videos; see online supplementary material at <http://www.liebertpub.com>).

In order to interpret group data, VSD videos were used to create regional raster plots and multi-segment regional average line plots for analysis. Rasterization of anatomical regions is a way to average $\Delta F/F$ for 100 μm wide segments within each region, thus quantifying the spatial response of the VSD signal in raster plots. The DG was separated into five regions for analysis, the suprapyramidal and infrapyramidal branches of the stratum moleculare and stratum granulosum, respectively, and the hilus. Raster plots from the suprapyramidal stratum granulosum and stratum moleculare, shown in Figure 3 D and E demonstrated enhanced and prolonged depolarization throughout this layer. Statistical differences for each spatiotemporal point between the sham and LFPI group rasters are shown in the p value raster plot generated by permutation testing (Fig. 3G).

Whereas rasters provide a way to measure the spatial response of the signal, multi-segment regional average line plots provide a way to measure the fast depolarizing peak of the fluorescence signal in each region. The multi-segment regional average line plot from the stratum granulosum revealed a significantly higher fast-depolarizing peak in LFPI slices (all $\Delta F/F$ values are reported in $[10^{-4}]$ notation: Sham $[\Delta F/F]$: 15.9 ± 26.6 , LFPI $[\Delta F/F]$: 22.5 ± 1.5 , $p<0.05$; Sham, $n=11$, LFPI, $n=9$). Subsequent analyses of line plot fast-depolarizing peaks showed that all cell layers of the DG showed enhanced depolarization in the LFPI group versus sham (for fast depolarizing peak $\Delta F/F$ values from each region, see Table 1).

Mild LFPI leads to spread of excitation through the DG into area CA3

Another advantage of VSD is that it can detect the spatial propagation of activity from one region to another. To assess the functional efficacy of the DG as a filter of perforant path input, we examined the spread of the VSD signal into area CA3. Representative VSD peak maps revealed that perforant path activation led to an aberrant spread of depolarization into area CA3 in slices from LFPI animals (Fig. 4D). Multi-segment regional average line plots also indicate a significant increase in the fast depolarizing peaks in all area CA3 cellular layers from LFPI slices, compared with Sham (Table 1; Sham $n=6$, LFPI $n=6$).

TABLE 1. FAST DEPOLARIZING PEAK (FDP) FRACTIONAL CHANGE IN FLUORESCENCE $\Delta F/F$ VALUES FROM VOLTAGE-SENSITIVE DYE FLUORESCENCE IN RESPONSE TO PERFORANT PATH STIMULATION

	<i>FDP $\Delta F/F$ values ($\times 10^{-4}$)</i>				
	<i>Sham</i>		<i>LFPI</i>		<i>P</i>
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>	<i>value</i>
Dentate gyrus					
St. molecular					
Suprapyramidal blade	23.5	3.9	33.9	3.7	0.070
Infrapyramidal blade	30.4	3.8	31.6	4.5	0.840
St. granulosum					
Suprapyramidal blade	15.9	26.6	22.5	1.5	0.030
Infrapyramidal blade	16.0	1.9	26.7	2.8	0.007
Hilus	13.7	2.3	21.8	2.1	0.019
Area CA3					
St. pyramidale	4.9	0.73	11.7	2.6	0.033
St. radiatum/lucidum	5.1	0.68	9.9	2.1	0.050
St. oriens	4.7	0.61	8.3	1.3	0.029

Sham and lateral fluid percussion injury (LFPI) group means and standard error of the mean (SEM) are reported for each anatomically defined cellular layer. The dentate gyrus (DG) molecular and granule cell layers (st. moleculare and st. granulosum) were divided based on their anatomical position in either the suprapyramidal or infrapyramidal blade, respectively. DG, Sham $n=11$, LFPI $n=9$ animals; CA3, Sham $n=6$, LFPI $n=6$ animals.

Next, we asked if the spread of depolarization to area CA3 could translate to a functional increase in action potential firing. To test this possibility, we simultaneously recorded extracellular population spikes in the DG stratum granulosum and area CA3 stratum pyramidale while stimulating the perforant path. In order to reliably examine population spiking activity in CA3, paired-pulse stimulation (100 ms inter-stimulus interval) was used. Figure 5 shows I/O curves of population spike amplitudes in response to the second stimulation. Interestingly, I/O curves recorded from the DG stratum granulosum in response to the second pulse were not different between sham and LFPI animals (two-way repeated measures ANOVA; injury factor, $F[1,11]=0.3743$, $p=0.5531$), however, I/O curves from the first pulse showed that LFPI animals had larger population spike amplitudes as stimulation intensity increased (Sham $n=7$, LFPI $n=6$, stimuli 350–500 μA two-way repeated measures ANOVA; stimulus intensity factor, $F[9,99]=33.63$, $p<0.0001$; injury factor, $F[1,11]=5.089$, $p<0.05$; interaction, $F[9,99]=4.432$, $p<0.0001$, data not shown). In the subset of slices with CA3 population spikes, I/O curves demonstrated an increase in population spike amplitude for LFPI slices (Fig. 5E; two-way repeated measures ANOVA; injury factor, $F[1,10]=5.594$, $p<0.05$; stimulus intensity factor, $F[9,90]=9.900$, $p<0.0001$; interaction: $F[9,90]=5.900$, $p<0.0001$). In slices where the hilus was transected, CA3 population spikes were not observed, confirming that CA3 population spike activity was the result of afferent excitation from DG mossy fibers as opposed to indirect stimulation of another afferent pathway (n =two slices per condition).

Next, we hypothesized that enhanced CA3 population spike activity could arise from two possibilities: an increase in synaptic transmission from mossy fibers located in the CA3 stratum lucidum,^{48–50} or alterations in the CA3 autoassociational network marked by net synaptic transmission from recurrent CA3 associational/commissural (A/C) synapses onto other CA3 neurons

FIG. 4. Perforant path stimulation leads to aberrant propagation of depolarization into area CA3. Group raster plots from (A) Sham and (B) lateral fluid percussion injury (LFPI) slices. (C) Raster plot of the statistical significance comparing the groups at each spatiotemporal point in the trial with representative pseudocoloring of the bootstrapped p values (shades of purple indicate significance of $p < 0.05$ or more). (D) Heat maps of representative Sham (left) and LFPI (right) slices demonstrate the spread of depolarization into area CA3 after injury. (E) Multi-segment regional average line plots from the CA3 stratum pyramidale depicts the increase in the fast depolarizing peak of the fluorescence signal in the LFPI group (red) versus sham (green); (all $\Delta F/F$ values in 10^{-4} notation). Sham mean, 4.9 ± 0.73 ; LFPI mean, 11.7 ± 2.62 , $p = 0.0329$; $n = 6$ animals per condition.

in the stratum radiatum.^{8,51} To test these alternative hypotheses, we again stimulated the perforant path using the same paired-pulse stimulation, and placed recording electrodes in the stratum lucidum and stratum radiatum, respectively. I/O curves from LFPI animals in response to the second stimulation were observed to have significantly larger fEPSP slopes in the stratum lucidum (Fig. 6A and C, Sham, $n = 4$; LFPI, $n = 4$; stimuli, 20–500 μA ; two way repeated measures ANOVA; injury factor, $F[1, 135] = 108.2$, $p < 0.0001$; stimulus intensity factor: $F[24, 135] = 10.25$, $p < 0.0001$; interaction, $F[24, 135] = 3.426$, $p < 0.0001$). Interestingly, there was no difference in the I/O curves from fEPSPs in the stratum radiatum between sham and LFPI (Fig. 6B and D, Sham, $n = 4$; LFPI, $n = 4$; stimuli, 20–500 μA , two-way repeated measures ANOVA; injury factor, $F[1, 135] = 1.419$, $p = 0.2356$; stimulus intensity factor, $F[24, 135] = 5.209$, $p < 0.0001$; interaction, $F[24, 135] = 1.276$, $p = 0.1922$).

Discussion

In this study, we examined the ability of the DG to regulate cortical input to the hippocampus 1 week after LFPI in mice. To first confirm hippocampal-dependent behavioral deficits and as-

sess animals' ability to detect spatial novelty within their environment, we tested mice in a spatial memory object-place recognition task. Whereas sham animals could discriminate the spatial object displacement, we found that LFPI mice could not. Total object interaction times during either training or testing trials were not significantly different between sham and LFPI, indicating that the observed deficit in spatial object discrimination was not caused by a decrease in overall exploratory behavior after injury.

When the physiological function of the DG was assessed by stimulating cortical inputs in *ex vivo* brain slices, we found that the DG experienced increased network excitability, as indicated by higher levels of VSD fluorescence signals throughout all cellular layers, and larger fEPSP slopes in the stratum moleculare. Subsequently, we observed that perforant path activation in LFPI animals caused aberrant spread of depolarization into downstream area CA3. This was associated with greater population spike amplitudes in the CA3 stratum pyramidale and suggests enhanced action potential firing in this region. In addition, we observed that increased CA3 population spike activity was accompanied by larger fEPSPs in the CA3 stratum lucidum, indicating augmented synaptic

FIG. 5. Increase in CA3 population spike activity after lateral fluid percussion injury (LFPI). Population spike traces from Sham (top) and LFPI (bottom) slices recorded simultaneously in (A) the dentate stratum granulosum and (B) the CA3 stratum pyramidal in response to paired-pulse stimulation (100 ms intervals) in the perforant path. (C) Hilar transection eliminated CA3 population spike activity in both sham and injured slices. (D) Input/output (I/O) curves for 50–500 μ A perforant path stimulation show a significant change in dentate gyrus (DG) population spike amplitude after LFPI in the first stimulation pulse (two way ANOVA, * p < 0.05), but are saturated at the second pulse shown in (E) (two way ANOVA, n.s., p = 0.5531). (F) Second stimulation demonstrated a marked increase in CA3 population spike amplitude (two-way ANOVA, **** p < 0.0001).

FIG. 6. Lateral fluid percussion injury (LFPI) leads to increased synaptic efficacy in the CA3 stratum lucidum but not the stratum radiatum. Representative field excitatory post-synaptic potentials (fEPSP) traces from Sham (top) and LFPI (bottom) slices in response to paired-pulse stimulation (100 ms interstimulus-interval) of the perforant path at 400 μ A in (A) CA3 stratum lucidum and (B) CA3 stratum radiatum. Input/output (I/O) curves of fEPSP slopes from 20 to 500 μ A in response to the first stimulation pulse recorded in (C) stratum lucidum and (D) stratum radiatum.

efficacy at mossy fiber synapses. Interestingly, enhanced excitability was not observed in the CA3 stratum radiatum, which suggests that autoassociational activity caused by CA3 A/C axon collaterals was not likely affected. In summary, our main finding that aberrant spread of afferent excitation from DG to area CA3 has functional consequences on CA3 network excitability is both novel and significant in understanding how synaptic transmission between localized circuits can lead to hippocampal disruption after mTBI.

DG hyperexcitability reflects a loss of excitatory/inhibitory (E/I) balance within the network. Departure from the endogenous state of E/I balance can cause network dysfunction, and ultimately manifest in behavioral impairments. After TBI, E/I imbalance has been observed in both the DG and area CA1 (reviewed in the study by Paterno and colleagues).⁶ The DG has intrinsically low network excitability (i.e., sparse granule cell firing), which is thought to confer its putative filtering property. Because of this low excitability under normal conditions, perturbations to the network tends to shift E/I balance toward hyperexcitability. This hyperexcitable shift within the DG is also seen in other neurological disorders, such as epilepsy and Alzheimer's disease, where it is associated with behavioral cognitive dysfunction.^{52–61} Our data demonstrate that mTBI causes E/I imbalance in the DG, leading to the diminished ability to regulate cortical input and behavioral dysfunction.

The results of our behavioral assessment substantiate an impairment in anterograde memory, recapitulating what is observed clinically in mTBI patients and experimentally in rodents after mild to moderate LFPI.^{4,30,62–70} Further, alterations in object-place recognition memory and spatial novelty detection confirm that spatial information processing—as one component of episodic memory—is disrupted in our model of mTBI. These data are consistent with object-place recognition memory impairments seen in other TBI models 1–2 weeks post-injury;^{63,71,72} however, they are in contrast to one study using a mild controlled cortical impact (CCI) model, in which no spatial object recognition deficits were observed.⁷³ In previous behavioral studies, spatial novelty detection has been shown to rely on the DG and CA3 hippocampal subregions.^{74,75} In a similar object exploration paradigm in rats, lesions of either DG or CA3 led to a decrease in the exploration of the displaced objects compared with controls, whereas CA1 lesions did not produce significant deficits.⁷⁴ Another study, using the same task as that utilized in this study, found that chemogenetically silencing hyperactive dentate granule cells in a mouse model of epilepsy was able to restore behavioral spatial novelty detection, further implicating the role of the DG in spatial information processing.⁷⁶ The DG–CA3 pathway has also been implicated in pattern separation; that is, the differentiation of similar, overlapping cortical input patterns, theorized to be a necessary computational step in episodic memory encoding.^{9–12,14–18,77,78} Recently, a study by Kim and colleagues demonstrated that pattern separation in an object-based recognition memory task is impaired after CCI in mice.⁷⁹ Although we do not provide direct evidence to support a causal link between diminished object-place recognition memory and DG–CA3 post-traumatic dysfunction in this study, these previous findings suggest that disruption to this pathway may underwrite the observed spatial novelty deficits after TBI, and should be explored in future experiments.

In addition to the hippocampus, other brain regions that contribute to object-place recognition memory may also be affected after LFPI. The hippocampus has been shown to functionally in-

teract with the perirhinal cortex and medial prefrontal cortex during object-in-place memory tasks in rodents.^{80–85} Additionally, the lateral entorhinal cortex and fornix play a role in recognition of object-context associations; that is, whether familiar objects are in a familiar or novel environment.^{83,84,86,87} Physiological, morphological, and histological studies have shown that these regions have alterations in TBI models and human patients.^{88–91} Further, alterations to the contralateral hippocampus may also contribute to the behavioral results observed in this study. Previously, our laboratory has found fewer healthy neurons and DG network hyperexcitability in the contralateral hippocampus 7 days after LFPI.⁹² Although the present study only examines DG physiological function in the hemisphere ipsilateral to the injury site, our previous work would suggest a similar network excitability shift in the contralateral DG as well. Anatomically, the bilateral dentate gyri are commissurally connected by glutamatergic and GABAergic interneuron projections.^{93–99} Therefore, it is possible that dysfunction of interneuron subtypes may affect commissural communication between dentate gyri and coordination of behavior after injury.

In examining the underlying physiology, we observed that the DG experienced an increase in network excitability as visualized by higher VSD fluorescence signals and increased fEPSP slopes in LFPI slices. These data corroborate previous work from our laboratory and others, which demonstrates increased DG network excitability with extracellular field recordings.^{4,32–34,36–38} VSD imaging provides additional information about the spatiotemporal spread of perforant path activation, and its enhancement of activity throughout the DG–CA3 network after injury. In sham control slices, excitation was predominantly limited to the DG anatomical region. However, in LFPI slices, perforant path activation led to the propagation of depolarization through the DG hilus—the location of the mossy fiber pathway—into area CA3. Further, the results of extracellular recordings in the CA3 stratum pyramidale demonstrated larger amplitude perforant pathway-evoked population spikes from LFPI slices (Fig. 5F), which suggests that upstream DG network perturbations can have functional consequences downstream in area CA3. These results are the first to describe aberrant propagation of perforant path input from the DG into area CA3, and provide insight into how the flow of information between hippocampal subregions can be affected by brain injury.

Our goal in utilizing a paired-pulse stimulation protocol was to reliably evoke population spikes in area CA3. Mossy fiber–CA3 synapses are known to be “conditional detonators,” whose neurotransmitter release depends on a granule cell firing pattern.¹⁰⁰ Single DG granule cell action potentials generally fail to discharge a CA3 pyramidal neuron; however, trains of granule cell action potentials reliably and effectively discharge CA3 cells. It has been previously shown that granule cells increase the number of action potentials fired in response to perforant path stimulation 1 week after LFPI.^{31,32} In response to the first stimulation pulse, DG population spikes had larger amplitudes after LFPI (Fig. 5D). However, the second stimulation pulse elicited no difference in DG population spikes between sham and LFPI slices (Fig. 5E). We believe that this lack of effect in the second DG population spike is most likely the result of maximal signal saturation in the hyperexcitable DG of LFPI slices.

Diminished DG filtering of cortical excitability to CA3 could also affect synaptic plasticity within the circuit, and contribute to learning and memory disruption. In area CA1, we previously showed an inability to induce and maintain long-term potentiation

(LTP) at the Schaffer collateral synapse at 7 days after LFPI.¹⁰¹ In separate study, we were able to restore contextual fear conditioning behavior, as well as CA1 network excitability using a dietary therapy; however, we found that the dietary therapy did not restore LTP in CA1 (unpublished observations).¹⁰² Recently, Titus and colleagues were able to reinstate CA1 LTP and contextual fear conditioning behavior with the use of a phosphodiesterase inhibitor after FPI.¹⁰³ Taking the results of these studies together, it seems that behavior restoration after injury can be achieved with or without LTP in CA1. Therefore, the role of CA1 LTP in recovery of hippocampal-mediated behavior after injury remains unclear.

Loss of LTP at the Schaffer collateral synapse does not necessarily relate to loss of LTP in the DG, as synaptic potentiation in these regions has different mechanisms, and depends on which DG synapse type is being examined. At the perforant path-granule cell synapse, loss of LTP has been demonstrated at 7 days after injury.^{104–107} Within the DG, LTP involving hilar excitatory circuitry (i.e., mossy cells) has been shown to modulate gating of cortical input into the DG via an *N*-methyl-D-aspartate (NMDA)-independent pre-synaptic mechanism.^{108,109} One week after LFPI, mossy cells were shown to be hyperexcitable and to exert aberrant excitation onto granule cells.³¹ Therefore, mossy cell dysfunction could play a major part in DG hyperexcitability and diminished filtering efficacy, as well as disruption of synaptic plasticity mechanisms.

Our results demonstrating enhanced synaptic efficacy in the CA3 stratum lucidum suggests that mossy fiber synaptic transmission is more pronounced after injury. The effect of TBI on LTP at the mossy fiber–CA3 pyramidal cell synapse has yet to be examined. LTP at this synapse is unique from that of other hippocampal synapses: it is NMDA receptor independent, and involves cyclic adenosine monophosphate (AMP)-activated pre-synaptic calcium influx, resulting in enhanced neurotransmitter release.¹¹⁰ We would hypothesize that enhanced mossy fiber excitatory transmission after LFPI would increase the probability of opening pre-synaptic voltage-gated calcium channels, likely leading to increased transmitter release. However, whether this activates the long-term molecular machinery necessary for sustained synaptic potentiation remains to be seen. In mouse models of epilepsy, which also exhibit a hyperexcitable DG network phenotype, LTP is induced at the mossy fiber–CA3 pyramidal cell synapse, similar to high-frequency stimulation of mossy fiber axons.^{111–113} The role of aberrant induction of LTP at this synapse could have major implications for learning and memory disruption. Future experiments are warranted in order to elucidate the maintenance of LTP at the mossy fiber–CA3 pyramidal cell synapse after LFPI.

Within the CA3 network, no significant change in fEPSPs was observed in the stratum radiatum, the site of CA3 pyramidal cell recurrent A/C axon collateral synapses. This finding suggests that injury may not necessarily affect the intrinsic excitability or synaptic output of CA3 pyramidal neurons within the network; however, further work is needed to directly support this hypothesis. A previous study in a closed head injury model found no changes in the intrinsic membrane properties of CA3 pyramidal neurons.¹¹⁴ However, it is well established that CA3 neurons are vulnerable to cell loss following TBI.^{4,33,115–118} Although further investigation of post-traumatic alterations within area CA3 itself is needed, the results of this study specifically demonstrate that in response to afferent excitation, mossy fiber transmission from the DG is a major source of CA3 network hyperexcitability.

The most parsimonious explanation of our data is that CA3 excitability is caused by electrical stimulation of the perforant path

branch projecting to the DG (a stimulating electrode was placed in the DG stratum moleculare), which then relays information to CA3 via the mossy fiber pathway. Intracellular labelling studies show that entorhinal cortex neurons with axons in the perforant path have collaterals that reach both the dentate gyrus and CA3.^{7,119} In the VSD data, we do not observe depolarizing spatiotemporal propagation along the CA field stratum lacunosum moleculare where perforant path axons travel directly to area CA3. Additionally, spread of depolarization into CA3 is evident through the DG hilus, where mossy fiber axons project. In slices with a hilar transection, no population spike activity was evident in area CA3 (Fig. 5C). Therefore, our data strongly suggest that the majority of stimulus-induced excitation in CA3 was orthodromic firing from DG granule cells, as opposed to antidromic firing of perforant path axons directly projecting to area CA3.

There are considerable mechanistic possibilities to explain the post-traumatic disruption of DG filtering function and subsequent CA3 hyperexcitability. After FPI, the DG experiences overt cell death, including loss of many interneuron subtypes.^{4,31–33} Within the DG, neuronal loss may contribute to E/I imbalance by the disappearance of synaptic connections. Our laboratory and others have shown a decrease in the frequency of inhibitory synaptic currents onto dentate granule cells, reflecting a potential loss of GABAergic synapses.^{4,32} Prior studies have also reported alterations in synaptic inputs onto other dentate cell types, such as hilar interneurons and semilunar granule cells, which may be the result of reorganization of structural network connections.^{31,37,120} Physiologically, surviving neurons in the DG may experience shifts in intrinsic membrane properties or synaptic inputs, resulting in E/I imbalance at the cellular level. For example, a decrease in the potassium-chloride transporter protein KCC2 occurs at 7 days post-LFPI, leading to reduced GABA_A-mediated inhibition onto granule cells.¹²¹ The complex DG interneuron network has also been shown to experience shifts in intrinsic excitability and synaptic inputs after TBI.^{4,31–34,120,122,123} Thus, an imbalance in excitatory/inhibitory neurotransmission throughout the DG after injury most likely contributes to the breakdown of DG filter function.

At the cellular level, TBI has been shown to cause alterations in ion channel function. Previous studies in *in vitro* injury models have shown that disruptions in voltage-gated sodium channel function after TBI lead to aberrant activation of voltage-gated calcium channels and increased calcium influx.^{124–126} Changes to voltage-gated channels could affect action potential propagation and initiation at the axon initial segment, as is seen in cortical neurons.¹²⁷ Action potential propagation can be functionally evaluated by measuring pre-synaptic fiber volley amplitude. Previously, we have shown that there is no change in the fiber volley amplitude of perforant path axons into the DG.⁴ Therefore, we do not believe that our model contains net physiological changes in the voltage-gated sodium channel properties of DG afferents. However, as mossy fiber synaptic transmission from the DG to area CA3 was elicited di-synaptically in this study—as designed to assess dentate network filtering function—we do not know if fiber volley amplitudes of mossy fibers are changed after injury. Additionally, modulation of mossy fiber release probability or membrane properties in CA3 neurons may also contribute to the observed downstream hyperexcitability. Future studies would benefit from examining changes in synaptic efficacy within the DG–CA3 di-synaptic pathway, as well as from regulation by interneuron subtypes within both hippocampal subregions.

Conclusion

In conclusion, the results of this study show that (1) behavioral spatial information processing is impaired after mTBI, and (2) the neuronal circuit within the DG has a diminished physiological “filtering” function, allowing too much excitation into area CA3 via the mossy fiber pathway. These novel findings expand on the current mechanisms of hippocampal circuit dysfunction after experimental mTBI and provide a physiological correlate of post-traumatic deficits in spatial information processing for encoding into memory. Forthcoming studies aim to identify mechanisms of posttraumatic DG dysfunction in order to restore DG filter function, and ultimately, the effects on episodic memory impairment.

Acknowledgments

We thank Drs. Sanghee Yun and Brian Johnson for constructive suggestions on previous drafts of the manuscript. The grant sponsor was NIH/ National Institute of Child Health and Human Development (NICHD); the grant number is R37 HD059288 (A.S.C.)

Author Disclosure Statement

No competing financial interests exist.

References

1. Tsirka, V., Simos, P., Vakis, A., Vourkas, M., Arzoglou, V., Syrmos, N., Stavropoulos, S., and Micheloyannis, S. (2010). Material-specific difficulties in episodic memory tasks in mild traumatic brain injury. *Int. J. Neurosci.* 120, 184–191.
2. Zohar, O., Rubovitch, V., Milman, A., Schreiber, S., and Pick, C.G. (2011). Behavioral consequences of minimal traumatic brain injury in mice. *Acta Neurobiol. Exp. (Wars)* 71, 36–45.
3. Tweedie, D., Rachmany, L., Rubovitch, V., Lehmann, E., Zhang, Y., Becker, K.G., Perez, E., Miller, J., Hoffer, B.J., Greig, N.H., and Pick, C.G. (2013). Exendin-4, a glucagon-like peptide-1 receptor agonist prevents mTBI-induced changes in hippocampus gene expression and memory deficits in mice. *Exp. Neurol.* 239, 170–182.
4. Witgen, B.M., Lifshitz, J., Smith, M.L., Schwarzbach, E., Liang, S.L., Grady, M.S., and Cohen, A.S. (2005). Regional hippocampal alteration associated with cognitive deficit following experimental brain injury: a systems, network and cellular evaluation. *Neuroscience* 133, 1–15.
5. Milman, A., Rosenberg, A., Weizman, R., and Pick, C.G. (2005). Mild traumatic brain injury induces persistent cognitive deficits and behavioral disturbances in mice. *J. Neurotrauma* 22, 1003–1010.
6. Paterno, R., Folweiler, K.A., and Cohen, A.S. (2017). Pathophysiology and treatment of memory dysfunction after traumatic brain injury. *Curr. Neurol. Neurosci. Rep.* 17, 52.
7. Witter, M.P. (2007). The perforant path: projections from the entorhinal cortex to the dentate gyrus. *Prog. Brain Res.* 163, 43–61.
8. Witter, M.P. (2007). Intrinsic and extrinsic wiring of CA3: indications for connectional heterogeneity. *Learn. Mem.* 14, 705–713.
9. Marr, D. (1971). simple memory: a theory for archicortex. *Philos. Trans. R. Soc. London. Ser. B, Biol. Sci.* 841, 23–81.
10. McNaughton, B.L., and Morris, R.G.M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci.* 10, 408–415.
11. O'Reilly, R.C., and McClelland, J.L. (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus* 4, 661–682.
12. Treves, A., and Rolls, E.T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus* 4, 374–391.
13. Leutgeb, J.K., Leutgeb, S., Moser, M.-B., and Moser, E.I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966.
14. Bakker, A., Kirwan, C.B., Miller, M., and Stark, C.E.L. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science* 319, 1640–2.
15. Gilbert, P.E., Kesner, R.P., and Lee, I. (2001). Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1. *Hippocampus* 11, 626–636.
16. Hunsaker, M.R., Rosenberg, J.S., and Kesner, R.P. (2008). The role of the dentate gyrus, CA3a,b, and CA3c for detecting spatial and environmental novelty. *Hippocampus* 18, 1064–1073.
17. McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M. A., and Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science* 317, 94–9.
18. Nakashiba, T., Cushman, J.D., Pelkey, K.A., Renaudineau, S., Buhl, D.L., McHugh, T.J., Barrera, V.R., Chittajallu, R., Iwamoto, K.S., McBain, C.J., Fanselow, M.S., and Tonegawa, S. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell* 149, 188–201.
19. Hsu, D. (2007). The dentate gyrus as a filter or gate: a look back and a look ahead. *Prog. Brain Res.* 163, 601–613.
20. Andersen, P., Holmqvist, B., and Voorhoeve, P.E. (1966). Entorhinal activation of dentate granule cells. *Acta Physiol. Scand.* 66, 448–460.
21. Alger, B.E., and Teyler, T.J. (1976). Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. *Brain Res.* 110, 463–480.
22. Teyler, T.J., and Alger, B.E. (1976). Monosynaptic habituation in the vertebrate forebrain: The dentate gyrus examined in vitro. *Brain Res.* 115, 413–425.
23. Coulter, D.A., and Carlson, G.C. (2007). Functional regulation of the dentate gyrus by GABA-mediated inhibition. *Prog. Brain Res.* 163, 235–243.
24. Staley, K.J., Otis, T.S., and Mody, I. (1992). Membrane properties of dentate gyrus granule cells: comparison of sharp microelectrode and whole-cell recordings. *J. Neurophysiol.* 67, 1346–1358.
25. Fricke, R. A. and Prince, D.A. (1984). Electrophysiology of dentate gyrus granule cells. *J. Neurophysiol.* 51, 195–209.
26. Scharfman, H.E. (1992). Differentiation of rat dentate neurons by morphology and electrophysiology in hippocampal slices: granule cells, spiny hilar cells and aspiny “fast-spiking” cells. *Epilepsy Res. Suppl.* 7, 93–109.
27. Williamson, A., Spencer, D.D., and Shepherd, G.M. (1993). Comparison between the membrane and synaptic properties of human and rodent dentate granule cells. *Brain Res.* 622, 194–202.
28. 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.
29. 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.
30. Smith, D.H., Okiyama, K., Thomas, M.J., Claussen, B., and McIntosh, T.K. (1991). Evaluation of memory dysfunction following experimental brain injury using the Morris water maze. *J. Neurotrauma* 8, 259–269.
31. Santhakumar, V., Bender, R., Frotscher, M., Ross, S.T., Hollrigel, G.S., Toth, Z., and Soltesz, I. (2000). Granule cell hyperexcitability in the early post-traumatic rat dentate gyrus: the “irritable mossy cell” hypothesis. *J. Physiol.* 524 Pt 1, 117–34.
32. 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, 8106–17.
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. Santhakumar, V., Ratzliff, A.D.H., Jeng, J., Toth, Z., and Soltesz, I. (2001). Long-term hyperexcitability in the hippocampus after experimental head trauma. *Ann. Neurol.* 50, 708–717.
35. Hunt, R.F., Scheff, S.W., and Smith, B.N. (2009). Posttraumatic epilepsy after controlled cortical impact injury in mice. *Exp. Neurol.* 215, 243–252.
36. Hunt, R.F., Scheff, S.W., and Smith, B.N. (2010). Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J. Neurophysiol.* 103, 1490–500.
37. Gupta, A., Elgammal, F.S., Proddutur, A., Shah, S., and Santhakumar, V. (2012). Decrease in tonic inhibition contributes to increase in

- dentate semilunar granule cell excitability after brain injury. *J. Neurosci.* 32, 2523–2537.
38. Butler, C.R., Boychuk, J. A., and Smith, B.N. (2015). Effects of rapamycin treatment on neurogenesis and synaptic reorganization in the dentate gyrus after controlled cortical impact injury in mice. *Front. Syst. Neurosci.* 9, 163.
 39. Oliveira, A.M.M., Hawk, J.D., Abel, T., and Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn. Mem.* 17, 155–60.
 40. Patel, T.P., Gullotti, D.M., Hernandez, P., O'Brien, W.T., Capehart, B.P., Morrison, B., Bass, C., Eberwine, J.E., Abel, T., and Meaney, D.F. (2014). An open-source toolbox for automated phenotyping of mice in behavioral tasks. *Front. Behav. Neurosci.* 8, 349.
 41. Johnson, B.N., Palmer, C.P., Bourgeois, E.B., Elkind, J.A., Putnam, B.J., and Cohen, A.S. (2014). Augmented inhibition from cannabinoid-sensitive interneurons diminishes cal output after traumatic brain injury. *Front. Cell. Neurosci.* 8, 435.
 42. Neuberger, E.J., Abdul Wahab, R., Jayakumar, A., Pfister, B.J., and Santhakumar, V. (2014). Distinct effect of impact rise times on immediate and early neuropathology after brain injury in juvenile rats. *J. Neurosci. Res.* 92, 1350–1361.
 43. Palmer, C.P., Metheny, H.E., Elkind, J.A., and Cohen, A.S. (2016). Diminished amygdala activation and behavioral threat response following traumatic brain injury. *Exp. Neurol.* 277, 215–226.
 44. Bourgeois, E.B., Johnson, B.N., McCoy, A.J., Trippa, L., Cohen, A.S., and Marsh, E.D. (2014). A toolbox for spatiotemporal analysis of voltage-sensitive dye imaging data in brain slices. *PLoS One* 9, e108686.
 45. Amaral, D.G., Scharfman, H.E., and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog. Brain Res.* 163, 3–22.
 46. Faul, F., Erdfelder, E., Lang, A.-G., and Buchner, A. (2007). G*Power 3.1 manual. *Behav. Res. Methods* 39, 175–191.
 47. Ang, C.W., Carlson, G.C., and Coulter, D. A. (2006). Massive and specific dysregulation of direct cortical input to the hippocampus in temporal lobe epilepsy. *J. Neurosci.* 26, 11,850–11,856.
 48. Soriano, E., and Frotscher, M. (1993). Spiny nonpyramidal neurons in the CA3 region of the rat hippocampus are glutamate-like immunoreactive and receive convergent mossy fiber input. *J. Comp. Neurol.* 333, 435–448.
 49. Frotscher, M. (1985). Mossy fibres form synapses with identified pyramidal basket cells in the CA3 region of the guinea-pig hippocampus: a combined Golgi-electron microscope study. *J. Neurocytol.* 14, 245–259.
 50. Acsády, L., Kamondi, A., Sík, A., Freund, T., and Buzsáki, G. (1998). GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J. Neurosci.* 18, 3386–3403.
 51. Henze, D.A., Urban, N.N., and Barrionuevo, G. (2000). The multifarious hippocampal mossy fiber pathway: a review. *Neuroscience* 98, 407–427.
 52. Nenov, M.N., Laezza, F., Haidacher, S.J., Zhao, Y., Sadygov, R.G., Starkey, J.M., Spratt, H., Luxon, B.A., Dineley, K.T., and Denner, L. (2014). Cognitive enhancing treatment with a PPAR γ agonist normalizes dentate granule cell presynaptic function in Tg2576 APP mice. *J. Neurosci.* 34, 1028–1036.
 53. Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.-Q., Kreitzer, A., Finkbeiner, S., Noebels, J.L., and Mucke, L. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's Disease. *Neuron* 55, 697–711.
 54. Putcha, D., Brickhouse, M., O'Keefe, K., Sullivan, C., Rentz, D., Marshall, G., Dickerson, B., and Sperling, R. (2011). Hippocampal hyperactivation associated with cortical thinning in Alzheimer's disease signature regions in non-demented elderly adults. *J. Neurosci.* 31, 17,680–17,688.
 55. Bejanin, A., Viard, A., Chételat, G., Clarys, D., Bernard, F., Pélerin, A., de La Sayette, V., Eustache, F., and Desgranges, B. (2012). When higher activations reflect lower deactivations: a PET Study in Alzheimer's Disease during encoding and retrieval in episodic memory. *Front. Hum. Neurosci.* 6, 107.
 56. Dengler, C.G., and Coulter, D.A. (2016). Normal and epilepsy-associated pathologic function of the dentate gyrus. *Prog. Brain Res.* 226, 155–178.
 57. Krook-Magnuson, E., Armstrong, C., Bui, A., Lew, S., Oijala, M., and Soltesz, I. (2015). *In vivo* evaluation of the dentate gate theory in epilepsy. *J. Physiol.* 593, 2379–2388.
 58. Heinemann, U., Beck, H., Dreier, J.P., Ficker, E., Stabel, J., and Zhang, C.L. (1992). The dentate gyrus as a regulated gate for the propagation of epileptiform activity. *Epilepsy Res. Suppl.* 7, 273–80.
 59. Lothman, E.W., Stringer, J.L., and Bertram, E.H. (1992). The dentate gyrus as a control point for seizures in the hippocampus and beyond. *Epilepsy Res. Suppl.* 7, 301–313.
 60. Marucci, G., Rubboli, G., and Giulioni, M. (2010). Role of dentate gyrus alterations in mesial temporal sclerosis. *Clin. Neuropathol.* 29, 32–35.
 61. Hester, M.S., and Danzer, S.C. (2013). Accumulation of abnormal adult-generated hippocampal granule cells predicts seizure frequency and severity. *J. Neurosci.* 33, 8926–8936.
 62. Lee, D.J., Gurkoff, G.G., Izadi, A., Seidl, S.E., Echeverri, A., Melnik, M., Berman, R.F., Ekstrom, A.D., Muizelaar, J.P., Lyeth, B.G., and Shahlaie, K. (2015). Septohippocampal neuromodulation improves cognition after traumatic brain injury. *J. Neurotrauma* 32, 1822–1832.
 63. Gurkoff, G.G., Gahan, J.D., Ghiasvand, R.T., Hunsaker, M.R., Van, K., Feng, J.-F., Shahlaie, K., Berman, R.F., Lyeth, B.G., and Folkerts, M.M. (2013). Evaluation of metric, topological, and temporal ordering memory tasks after lateral fluid percussion injury. *J. Neurotrauma* 30, 292–300.
 64. Zhang, Y., Chopp, M., Meng, Y., and Zhang, Z.G. (2015). Cerebrolysin improves cognitive performance in rats after mild traumatic brain injury. *J. Neurosurg* 122, 843–855.
 65. Hylin, M.J., Orsi, S. a, Zhao, J., Bockhorst, K., Perez, A., Moore, A.N., and Dash, P.K. (2013). Behavioral and histopathological alterations resulting from mild fluid percussion injury. *J. Neurotrauma* 30, 702–715.
 66. Fedor, M., Berman, R.F., Muizelaar, J.P., and Lyeth, B.G. (2010). Hippocampal θ dysfunction after lateral fluid percussion injury. *J. Neurotrauma* 27, 1605–1615.
 67. Pierce, J.E.S., Smith, D.H., Trojanowski, J.Q., and McIntosh, T.K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience* 87, 359–369.
 68. 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.
 69. Wu, A., Ying, Z., and Gomez-Pinilla, F. (2009). Vitamin E protects against oxidative damage and learning disability after mild traumatic brain injury in rats. *Neurorehabil. Neural Repair* 24, 290–298.
 70. Rutland-Brown, W., Langlois, J.A., Thomas, K.E., and Xi, Y.L. (2006). Incidence of traumatic brain injury in the United States, 2003. *J. Head Trauma Rehabil.* 21, 544–548.
 71. Beamer, M., Tummala, S.R., Gullotti, D., Kopil, K., Gorka, S., Ted Abel, B., Bass, C.R.D., Morrison, B., Cohen, A.S., and Meaney, D.F. (2016). Primary blast injury causes cognitive impairments and hippocampal circuit alterations. *Exp. Neurol.* 283, 16–28.
 72. Darwish, H., Mahmood, A., Schallert, T., Chopp, M., and Therrien, B. (2014). Simvastatin and environmental enrichment effect on recognition and temporal order memory after mild-to-moderate traumatic brain injury. *Brain Inj.* 28, 1362–1301.
 73. Chen, Y., Mao, H., Yang, K.H., Abel, T., and Meaney, D.F. (2014). A modified controlled cortical impact technique to model mild traumatic brain injury mechanics in mice. *Front. Neurol.* 5, 100.
 74. Lee, I., Hunsaker, M.R., and Kesner, R.P. (2005). The Role of Hippocampal Subregions in Detecting Spatial Novelty. *Behav. Neurosci.* 119, 145–153.
 75. Kesner, R.P., Taylor, J.O., Hoge, J., and Andy, F. (2015). Role of the dentate gyrus in mediating object-spatial configuration recognition. *Neurobiol. Learn. Mem.* 118, 42–48.
 76. Kahn, J., Yue, C., Takano, H., and Coulter, D. (2016). Dentate granule cell hyperactivity contributes to cognitive comorbidities in temporal lobe epilepsy, in: *The 46th Annual Meeting of the Society for Neuroscience*. Program No. 594. 27, San Diego, CA.
 77. Leutgeb, J.K., Leutgeb, S., Moser, M.-B., and Moser, E.I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966.
 78. Kesner, R.P. (2013). Role of the hippocampus in mediating interference as measured by pattern separation processes. *Behav. Processes* 93, 148–154.

79. Kim, D., Nishida, H., An, S.Y., Shetty, A.K., Bartosh, T.J., and Prockop, D.J. (2016). Chromatographically isolated CD63⁺ CD81⁺ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. *Proc. Natl. Acad. Sci.* 113, 170–175.
80. Barker, G.R.I., and Warburton, E.C. (2011). When is the hippocampus involved in recognition memory? *J. Neurosci.* 31, 10,721–10,731.
81. Barker, G.R.I., Bird, F., Alexander, V., and Warburton, E.C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex TL - 27. *J. Neurosci.* 27, 2948–2957.
82. Hannesson, D.K. (2004). Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *J. Neurosci.* 24, 4596–4604.
83. Bussey, T.J., Duck, J., Muir, J.L., and Aggleton, J.P. (2000). Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. *Behav. Brain Res.* 111, 187–202.
84. Ennaceur, A., and Aggleton, J.P. (1997). The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behav. Brain Res.* 88, 181–193.
85. Kesner, R.P., and Ragozzino, M.E. (2003). The role of the prefrontal cortex in object-place learning: a test of the attribute specificity model. *Behav. Brain Res.* 146, 159–165.
86. Langston, R.F., and Wood, E.R. (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus* 20, 1139–1153.
87. Wilson, D.I.G., Langston, R.F., Schlesiger, M.I., Wagner, M., Watanabe, S., and Ainge, J.A. (2013). Lateral entorhinal cortex is critical for novel object-context recognition. *Hippocampus* 23, 352–366.
88. Bolton, A.N., and Saatman, K.E. (2014). Regional neurodegeneration and gliosis are amplified by mild traumatic brain injury repeated at 24-hour intervals. *J. Neuropathol. Exp. Neurol.* 73, 933–947.
89. Card, J.P., Santone, D.J., Gluhovsky, M.Y., and Adelson, P.D. (2005). Plastic reorganization of hippocampal and neocortical circuitry in experimental traumatic brain injury in the immature rat. *J. Neurotrauma* 22, 989–1002.
90. Tate, D.F., and Bigler, E.D. (2000). Fornix and hippocampal atrophy in traumatic brain injury. *Learn. Mem.* 7, 442–446.
91. Ekolle Nnode-Ekane, X., Kharatishvili, I., and Pitkänen, A. (2017). Unfolded maps for quantitative analysis of cortical lesion location and extent after traumatic brain injury. *J. Neurotrauma* 34, 459–474.
92. Tran, L.D., Lifshitz, J., Witgen, B.M., Schwarzbach, E., Cohen, A.S., and Grady, M.S. (2006). Response of the contralateral hippocampus to lateral fluid percussion brain injury. *J. Neurotrauma* 23, 1330–1342.
93. Buckmaster, P.S., Wenzel, H.J., Kunkel, D.D., and Schwartzkroin, P.A. (1996). Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *J. Comp. Neurol.* 366, 271–292.
94. Goodman, J.H., and Sloviter, R.S. (1992). Evidence for commissurally projecting parvalbumin-immunoreactive basket cells in the dentate gyrus of the rat. *Hippocampus* 2, 13–21.
95. Scharfman, H.E., and Myers, C.E. (2012). Hilar mossy cells of the dentate gyrus: a historical perspective. *Front. Neural Circuits* 6, 106.
96. Deller, T., Nitsch, R., and Frotscher, M. (1995). Phaseolus vulgaris - leucoagglutinin tracing of commissural fibers to the rat dentate gyrus: evidence for a previously unknown commissural projection to the outer molecular layer. *J. Comp. Neurol.* 352, 55–68.
97. Frotscher, M., Seress, L., Schwerdtfeger, W.K., and Buhl, E. (1991). The mossy cells of the fascia dentata: a comparative study of their fine structure and synaptic connections in rodents and primates. *J. Comp. Neurol.* 312, 145–163.
98. Zimmer, J. (1971). Ipsilateral afferents to the commissural zone of the fascia dentata, demonstrated in decommisurated rats by silver impregnation. *J. Comp. Neurol.* 142, 393–416.
99. Berger, T.W., Semple-Rowland, S., and Bassett, J.L. (1981). Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. *Brain Res.* 224, 329–336.
100. Henze, D. a, Wittner, L., and Buzsáki, G. (2002). Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. *Nat. Neurosci.* 5, 790–795.
101. Schwarzbach, E., Bonislawski, D.P., Xiong, G., and Cohen, A.S. (2006). Mechanisms underlying the inability to induce area CA1 LTP in the mouse after traumatic brain injury. *Hippocampus* 16, 541–550.
102. Cole, J.T., Mitala, C.M., Kundu, S., Verma, A., Elkind, J.A., Nissim, I., and Cohen, A.S. (2010). Dietary branched chain amino acids ameliorate injury-induced cognitive impairment. *Proc. Natl. Acad. Sci. U. S. A.* 107, 366–371.
103. Titus, D.J., Sakurai, A., Kang, Y., Furones, C., Jergova, S., Santos, R., Sick, T.J., and Atkins, C.M. (2013). Phosphodiesterase inhibition rescues chronic cognitive deficits induced by traumatic brain injury. *J. Neurosci.* 33, 5216–5226.
104. Zhang, B., Chen, X., Lin, Y., Tan, T., Yang, Z., Dayao, C., Liu, L., Jiang, R., and Zhang, J. (2011). Impairment of synaptic plasticity in hippocampus is exacerbated by methylprednisolone in a rat model of traumatic brain injury. *Brain Res.* 1382, 165–172.
105. Zhang, B.-L., Chen, X., Tan, T., Yang, Z., Carlos, D., Jiang, R.-C., and Zhang, J.-N. (2011). Traumatic brain injury impairs synaptic plasticity in hippocampus in rats. *Chin. Med. J. (Engl.)* 124, 740–5.
106. White, E.R., Pinar, C., Bostrom, C.A., Meconi, A., and Christie, B.R. (2016). Mild traumatic brain injury produces long-lasting deficits in synaptic plasticity in the female juvenile hippocampus. *J. Neurotrauma* 34, 1111–1123.
107. Yamashita, S., Hasuo, H., Tokutomi, T., Shigemori, M., and Akasu, T. (2011). Edaravone attenuates impairment of synaptic plasticity in granule cell layer of the dentate gyrus following traumatic brain injury. *Kurume Med. J.* 58, 47–58.
108. Wright, B.J., and Jackson, M.B. (2014). Long-term potentiation in hilar circuitry modulates gating by the dentate gyrus. *J. Neurosci.* 34, 9743–9753.
109. Hashimoto-dani, Y., Nasrallah, K., Jensen, K.R., Chávez, A.E., Carrera, D., and Castillo, P.E. (2017). LTP at hilar mossy cell-dentate granule cell synapses modulates dentate gyrus output by increasing excitation/inhibition balance. *Neuron* 95, 928–943.e3.
110. Nicoll, R.A., and Schmitz, D. (2005). Synaptic plasticity at hippocampal mossy fibre synapses. *Nat. Rev. Neurosci.* 6, 863–876.
111. Ben-Ari, Y., and Gho, M. (1988). Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurones induced by kainic acid. *J. Physiol.* 404, 365–384.
112. Cherubini, E., Neuman, R., Rovira, C., and Ben Ari, Y. (1988). Epileptogenic properties of the mast cell degranulating peptide in CA3 hippocampal neurones. *Brain Res.* 445, 91–100.
113. Anderson, W.W., Swartzwelder, H.S., and Wilson, W.A. (1987). The NMDA receptor antagonist 2-amino-5-phosphonovalerate blocks stimulus train-induced epileptogenesis but not epileptiform bursting in the rat hippocampal slice. *J. Neurophysiol.* 57, 1–21.
114. Griesemer, D., and Mautes, A.M. (2007). Closed head injury causes hyperexcitability in rat hippocampal CA1 but not in CA3 pyramidal cells. *J. Neurotrauma* 24, 1823–1832.
115. Chen, Y., Constantini, S., Trembovler, V., Weinstock, M., and Shohami, E. (1996). An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits. *J. Neurotrauma* 13, 557–568.
116. Tang, Y.P., Noda, Y., Hasegawa, T., and Nabeshima, T. (1997). A concussive-like brain injury model in mice (I): impairment in learning and memory. *J. Neurotrauma* 14, 851–862.
117. Golarai, G., Greenwood, A.C., Feeney, D.M., and Connor, J.A. (2001). Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. *J. Neurosci.* 21, 8523–8537.
118. Baldwin, S.A., Gibson, T., Callihan, C.T., Sullivan, P.G., Palmer, E., and Scheff, S.W. (1997). Neuronal cell loss in the CA3 subfield of the hippocampus following cortical contusion utilizing the optical disector method for cell counting. *J. Neurotrauma* 14, 385–398.
119. Tamamaki, N., and Nojyo, Y. (1993). Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure— injection of neurobiotin. *Hippocampus* 3, 471–480.
120. Hunt, R.F., Scheff, S.W., and Smith, B.N. (2011). Synaptic reorganization of inhibitory hilar interneuron circuitry after traumatic brain injury in mice. *J. Neurosci.* 31, 6880–6890.

121. Bonislawski, D.P., Schwarzbach, E.P., and Cohen, A.S. (2007). Brain injury impairs dentate gyrus inhibitory efficacy. *Neurobiol. Dis.* 25, 163–169.
122. Howard, A.L., Neu, A., Morgan, R.J., Echegoyen, J.C., and Soltesz, I. (2007). Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. *J. Neurophysiol.* 97, 2394–2409.
123. Tsuda, S., Hou, J., Nelson, R.L., Wilkie, Z.J., Mustafa, G., Sinharoy, A., Watts, J. V, Thompson, F.J., and Bose, P.K. (2016). Prolonged hippocampal cell death following closed-head traumatic brain injury in rats. *Neuroreport* 27, 724–729.
124. Wolf, J.A., Stys, P.K., Lusardi, T., Meaney, D., and Smith, D.H. (2001). Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. *J. Neurosci.* 21, 1923–1930.
125. Iwata, A., Stys, P.K., Wolf, J.A., Chen, X., Taylor, A.G., Meaney, D.F., and Smith, D.H. (2004). Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. *J. Neurosci.* 24, 4605–4613.
126. Yuen, T.J., Browne, K.D., Iwata, A., and Smith, D.H. (2009). Sodium channelopathy induced by mild axonal trauma worsens outcome after a repeat injury. *J. Neurosci. Res.* 87, 3620–3625.
127. Vascak, M., Sun, J., Baer, M., Jacobs, K.M., and Povlishock, J.T. (2017). Mild traumatic brain injury evokes pyramidal neuron axon initial segment plasticity and diffuse presynaptic inhibitory terminal loss. *Front. Cell. Neurosci.* 11, 157.

Address correspondence to:

Kaitlin A. Folweiler, BS

Department of Anesthesiology and Critical Care Medicine

Children's Hospital of Philadelphia

3615 Civic Center Boulevard

Abramson Research Center, Room 816-H

Philadelphia, PA 19104-4399

E-mail: kfolw@pennmedicine.upenn.edu