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Short-term neural and glial response to mild traumatic brain injury in the hippocampus

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ABSTRACT Traumatic brain injury (TBI) is an established risk factor for developing neurodegenerative disease. However, how TBI leads from acute injury to chronic neurodegeneration is limited to postmortem models. There is a lack of connections between in vitro and in vivo TBI models that can relate injury forces to both macroscale tissue damage and brain function at the cellular level. Needle-induced cavitation (NIC) is a technique that can produce small cavitation bubbles in soft tissues, which allows us to relate small strains and strain rates in living tissue to ensuing acute cell death, tissue damage, and tissue remodeling. Here, we applied NIC to mouse brain slices to create a new model of TBI with high spatial and temporal resolution. We specifically targeted the hippocampus, which is a brain region critical for learning and memory and an area in which injury causes cognitive pathologies in humans and rodent models. By combining NIC with patch-clamp electrophysiology, we demonstrate that NIC in the cornu ammonis 3 region of the hippocampus dynamically alters synaptic release onto cornu ammonis 1 pyramidal neurons in a cannabinoid 1 receptor-dependent manner. Further, we show that NIC induces an increase in extracellular matrix protein GFAP associated with neural repair that is mitigated by cannabinoid 1 receptor antagonism. Together, these data lay the groundwork for advanced approaches in understanding how TBI impacts neural function at the cellular level and the development of treatments that promote neural repair in response to brain injury.

SIGNIFICANCE Current models of mild traumatic brain injury (TBI) cannot relate injury forces to both macroscale tissue damage and brain function at the cellular level. We combine a microscale injury model in ex vivo brain slices while simultaneously recording glutamatergic inputs onto cornu ammonis 1 hippocampal pyramidal neurons. Postinjury examination of day 3 tissue regeneration by astrocytes allows us to connect acute neuronal signaling responses to potential chronic fibrosis after TBI. These studies provide a new tool for understanding the physiological and molecular responses to TBI and lay the groundwork for future experiments unraveling the synaptic mechanisms that mediate these responses seconds, minutes, and days following injury.

INTRODUCTION

1.5 million Americans are diagnosed with traumatic brain injury (TBI) every year, and according to the Centers for Disease Control and Prevention, 5.3 million more people currently live with disabilities caused by TBIs. Mild TBIs (mTBIs) account for approximately 80% of all TBI cases worldwide. Members of the military are especially at risk of cavitation-related blast-associated mTBIs (1–5). When the head is exposed to blast waves, the associated negative hydrostatic pressures can cause cavitation, the rapid expansion of a void within the brain. Additionally, sub-concussive impact forces associated with linear acceleration result in cavitation in brain phantoms (6). There is a lack of in vitro mTBI models that relate injury forces to both macroscale tissue damage and brain function at the cellular level, and in vivo studies often fail to capture the molecular nuances of the cellular response to injury (7). Needle-induced cavitation (NIC) is a technique that induces highly localized injury to ex vivo brain tissue by applying fluid pressure (8,9). In TBI-related cavitation, strain rates can reach as high

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as 10^3 s^{-1} during impact events, and NIC has the potential to span strain rates from the quasistatic regime (10^{-4} s^{-1}) to the ultrahigh strain rate regime (10^8 s^{-1}) (9). A better understanding of cavitation damage in brain can enable better treatment options for TBI. Blast wave experiments can cause both macroscale tearing of tissue and cellular damage observed as scarring at the boundaries between white and gray matter and at blood vessel/tissue interfaces (10,11). The cellular response leads to not only astrocyte-mediated glial scarring but also accumulation of astrocyte-secreted proteins during the wound-healing process (12,13).

Astrocytes are crucial responders to injuries throughout the central nervous system (CNS), and they have characteristic changes after injury, including astrogliosis, and changes in cell growth, size, and protein expression (14–16). Activated astrocytes secrete glial fibrillary acidic protein (GFAP) and extracellular matrix proteins such as tenascin-c (TNC) and connective tissue growth factor (CTGF), which promote inflammation and neural repair (17–22). It is essential to identify the spatial and temporal responses of astrocyte-secreted proteins to diagnose brain injury and determine potential pathways of neurodegenerative disease progression. Glial activation has been shown to contribute to the ongoing vestibulomotor deficits associated with high strain rate blast injury at the chronic stages (23). Although astrocyte-secreted extracellular matrix proteins are known contributors to synapse formation (24,25), how low-strain-rate cavitation acutely impacts synaptic function is unknown.

We have previously observed that NIC causes tissue damage along the hippocampus, a brain region critical for learning and memory formation (26). The hippocampus is particularly important in understanding the mechanisms of TBI pathologies due to its contribution to memory loss, increased risk of seizures, and neuroinflammation after TBI (27–30). However, many previous approaches use more generalized TBI models, such as mechanical-force weight drop, fluid percussion, and blast-induced injury (31). Injury to this region causes cognitive pathologies in humans and rodent models. However, how NIC impacts neural function at the cellular level is unknown, neither acute changes to synaptic function nor chronic astrocyte scarring. In the present study, we combined NIC in a brain slice with patch-clamp electrophysiology to investigate changes in excitatory signaling caused by the injury. We also aimed to identify potential mechanisms by which NIC impacts synaptic function, enhancing our understanding of the neural responses that follow TBI.

MATERIALS AND METHODS

Animals

Animal procedures and experiments were approved by the University of Massachusetts Amherst IACUC in accordance with the US Public Health Service Policy and NIH Guide for the Care and Use of Laboratory Animals. Brains were collected from 4- to 6-week-old male and female BALBc/nude or BALBc wild-type mice (Jackson Laboratories). Mice were anesthetized using isoflurane and euthanized by decapitation, and brains were immediately removed for NIC and organotypic brain slice preparation.

Organotypic brain slicing

Immediately after brain removal and/or ex vivo NIC, the forebrain was blocked in a 0°C–4°C NMDG cutting solution (mM): NMDG 92, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 30, sodium pyruvate 3, thiourea 2, HEPES 20, $MgSO_4$ 10, $CaCl_2$ 0.5, glucose 25, sucrose 20 (pH 7.4) with HCl (32). Forebrains were mounted individually, or as two brains adjacent to each other, and sectioned simultaneously at 300 μm with a sapphire knife (Delaware Diamond Knives, Wilmington, DE, USA) on a vibratome (Leica VS1200), yielding 3–5 hippocampal slices per mouse. Slices were then used for tissue culture or allowed to recover for patch-clamp electrophysiology experiments.

Tissue/organotypic slice culture

Slices were placed on tissue culture inserts (EMD Millipore, Burlington, MA, USA) and cultured at 37°C with 5% CO_2 for up to 14 days with slice media (33).

Brain slice electrophysiology

Slices were allowed to recover from slicing for 30 min at room temperature in recording artificial cerebrospinal fluid (aCSF) (mM) containing 124 NaCl, 3.7 KCl, 2.6 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgSO_4$, and 10 glucose and bubbled using 95% 02/5% CO2. For recording, slices were transferred to a perfusion chamber containing aCSF maintained at 34°C–37°C. Neurons were visualized with an Olympus BX51WI microscope. Recording electrodes were backfilled with internal solutions as follows: 125 mM K-gluconate, 10 mM KCl, 10 mM NaCl, 5 mM HEPES, 10 mM EGTA, 3 mM NaATP, and 0.25 mM NaGTP. Patch electrodes (3–5 MΩ) were guided to neurons with an MPC-200-ROE controller and MP285 mechanical manipulator (Sutter Instruments, Novato, CA, USA). Neurons were held at $V_{Hold} = -70$ mV for spontaneous excitatory postsynaptic current (sEPSC) recordings. Recordings were collected with a UPC-10 USB dual digital amplifier and Patchmaster NEXT software. One neuron was analyzed per slice to avoid confounds associated with repeated injury or drug application. All electrophysiology reagents were purchased from Sigma-Aldrich, and AM4113 was purchased from Tocris Bioscience.

NIC on ex vivo mouse brain slices

A custom pulled pipette (~5 μm diameter tip) backfilled with aCSF was inserted ~2–3 cell layers deep in the hippocampus and monitored with an Olympus BX51WI microscope. The NIC pipette was pressurized with the aCSF using a syringe pump (World Precision Instruments) at 5 μL/min until a bubble injury occurred at the tip of the needle observed by a drop or leveling off of pressure. Non-NIC control groups received a sham treatment by placing an NIC pipette on the brain slice without applying pressure with the syringe pump. Pressure was monitored in real time using a pressure sensor (Omega Engineering). Slices were then bisected along the midline, and the injured hemisphere was placed on tissue culture inserts (EMD Millipore) and cultured at 37°C with 5% CO_2 for 1–3 days in slice media (33).

Slice processing and conditioned medium collection

Day 0 and 3 NIC-injured and sham slices on culture inserts were removed from the incubator and transferred to a fresh 6-well plate and gently rinsed with 1× PBS to detach slices from the insert membrane. Slices were places

in 4% paraformaldehyde (Thermo Fisher Scientific) and placed in the 4°C fridge overnight. Conditioned media from injured and sham slices were syringe filtered (0.45 μm) (Fisher Scientific), flash frozen, and stored in a −80°C freezer.

Mouse brain immunohistochemistry

Fixed sham and injured slices were rinsed with 1× PBS (3×) and permeabilized and blocked with 0.3% (v/v) Triton X-100/in Intercept blocking buffer (LI-COR Biosciences, 927-70001, Lincoln, NE, USA) with 10% donkey serum (Abcam, ab7475) for 1 h at 4°C. Slices were rinsed in 1× PBS (3×) and stained with primary antibodies: GFAP (Abcam, ab7260), TNC rat (Invitrogen), and CTGF (Thermo Fisher Scientific, MA5-31420) diluted in Intercept blocking buffer at 1:250 overnight at 4°C. Slices were rinsed with 1× PBS (3×) and stained with secondary antibodies: donkey anti-rat 570 (Jackson ImmunoResearch Laboratories: 712-295-153, West Grove, PA, USA), donkey anti-goat 405 (Jackson ImmunoResearch Laboratories: 705-475-747), donkey anti-rabbit 647 (Jackson ImmunoResearch Laboratories: 711-605-152), and donkey anti-mouse 488 (Jackson ImmunoResearch Laboratories:715-545-150) at 1:400 for 1 h at 4°C. Slices were rinsed with 1× PBS (3×) and mounted on charged slides (Genesee Scientific, 29-107, Rochester, NY, USA) with Gelvatol and covered with 24 × 60 mm coverslips (Corning, 12-553-472, Corning, NY, USA) and sealed with nail polish (Sally Hansen, New York, NY, USA). Slides were imaged on a Spinning Disc Observer Z1 microscope (Carl Zeiss).

Statistical analysis

Electrophysiology data were analyzed in Patchmaster NEXT or converted with ABF Utility (Synaptosoft, Fort Lee, NJ, USA) for analysis in Clampfit (Molecular Devices) and/or MiniAnalysis (Synaptosoft). Data sets were tested for normality before statistical analysis. Unless otherwise noted, comparisons of data between conditions were done using a one-way ANOVA or a Kruskal-Wallis test for data sets that failed normality. Recordings from male and female mice were pooled for increased power, as there was no impact of sex on the experimental results (Fig. S1). Statistics were calculated with Prism 9 (GraphPad) and Python 3.11.

RESULTS

Precision model for studying TBI

NIC is a technique allowing for highly localized brain injury (8, 26). Here, we are the first to apply hippocampus-specific NIC to an organotypic brain slice with μm resolution while simultaneously using patch-clamp electrophysiology to continuously (sampling at μs resolution) measure synaptic responses before, during, and after injury in the hippocampus (Fig. 1, *a* and *b*). For this technique, we simultaneously placed an NIC injury pipette into the cornu ammonis (CA)3 region of the hippocampus and used whole-cell patch clamp to measure synaptic responses in CA1 pyramidal neurons (Fig. 1 *a*). The pressure in the NIC pipette was continuously measured until an instability occurred at a critical pressure (Fig. 1, *b–d*). Immediately following injury, the tissue closes around the ruptured injury cite, which can be localized through the inclusion of rhodium beads in the pipette (Fig. 1 *e*). The needle puncture was validated through microscopic imaging of the needle insertion process.

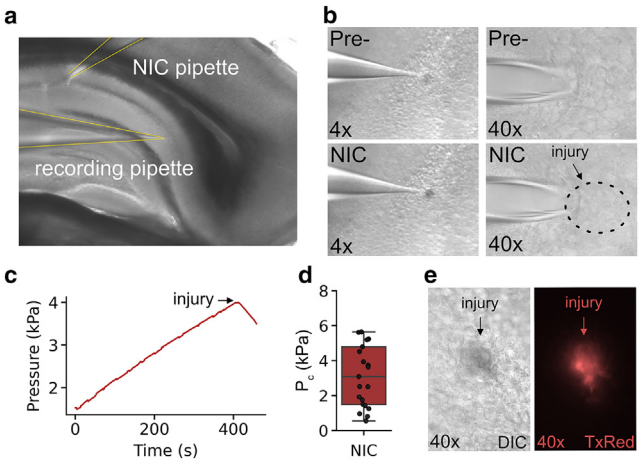

FIGURE 1 Characterization of novel organotypic slice NIC model for studying synaptic function following brain injury. (*a*) Image of simultaneous recordings of injury force and excitatory responses in CA1 hippocampal pyramidal neurons (*bottom*). (*b*) Live imaging just prior (*top*) and at exact time of injury (*bottom*) at 4× (*left*) and 40× (*right*) magnification. (*c*) Representative recording trace and (*d*) mean critical pressure (P_c) required to evoke an NIC injury event. (*e*) Image of injury site after NIC pipette removal (*left*) labeled with rhodium beads from injury pipette (*right*). Boxplot represents median and inner and outer (error bars) quartile ranges and is displayed with individual datapoints.

Ex vivo NIC transiently decreases glutamate release onto CA1 pyramidal neurons

It is unclear how synaptic function in the hippocampus is altered during and immediately following TBI. To better understand the circuit-level changes in the hippocampus immediately following TBI, an approach with high temporal resolution is needed. Here, we incorporated a novel approach to studying TBI (Fig. 1) and used whole-cell patch-clamp techniques to measure sEPSCs onto CA1 pyramidal neurons (Fig. 2 *a*). We found that in the 1–2 min period following NIC injury, the frequency of excitatory events onto CA1 pyramidal neurons was greatly reduced (Fig. 2, *a–d*). However, this transient decrease in sEPSC frequency was followed by a strong rebound in excitatory activity 5–10 min proceding the initial injury (Fig. 2, *b–d*). As measured by sEPSC amplitude, there were no indications that the injury induced immediate postsynaptic effects on excitatory transmission (Fig. 2 *e*). These data suggest that synaptic responses to brain injury are highly dynamic within the hippocampus and that a potential protective mechanism occurs directly at the time of injury. Further, we show that these changes are due to presynaptic inputs, likely projections from the CA3 Schaffer collaterals, and are not a postsynaptic response to injury.

CB1 receptor activation mediates synaptic release in response to brain injury

Cannabinoid receptor 1 (CB1R) is a known mediator of excitatory synaptic release, and previous work demonstrates

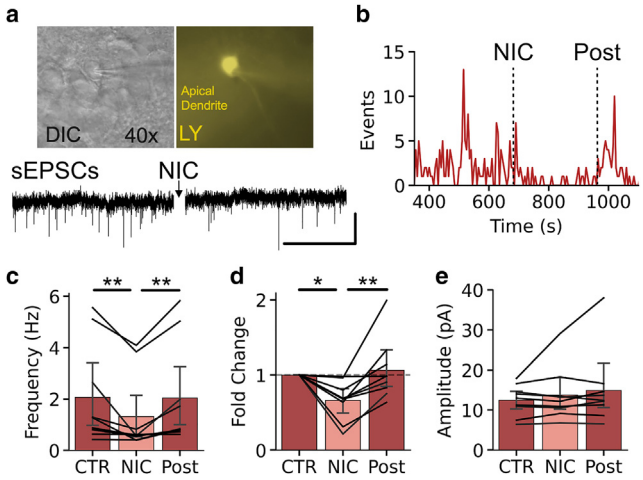

FIGURE 2 Decrease in glutamate release post-NIC. (*a*) Representative cell (*left*) with lucifer yellow (*right*) in patch pipette for visual identification of a CA1 pyramidal neuron and a representative trace of sEPSCs before and immediately following NIC injury (*bottom*). (*b*) Histogram in 10 s bins of average sEPSC events before, during, and after NIC. (*c*) Average sEPSC frequency ($F_{(2,16)} = 8.39$, $p = 0.003$), (*d*) frequency expressed as fold change ($F_{(2,16)} = 7.67$, $p = 0.005$), and (*e*) amplitude ($F_{(2,16)} = 1.15$, $p = 0.34$) before, immediately after, and 5–7 min post-NIC ($n = 9$ cells). Error bars represent ± SEM. One-way repeated measures ANOVA with Tukey post hoc analysis. *$p < 0.05$ and **$p < 0.01$.

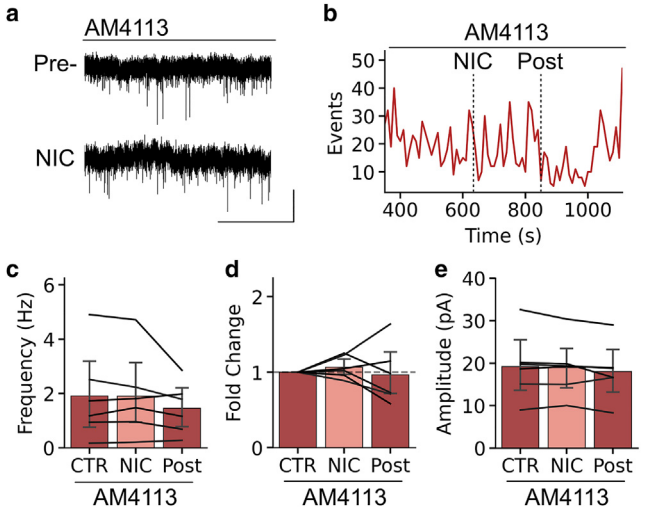

FIGURE 3 CB1R blockade inhibits transient decreases in synaptic transmission following NIC injury in the hippocampus. (*a*) Representative trace of sEPSC recording in the presence of CB1 antagonist AM4113 before and immediately post-NIC injury. (*b*) Histogram in 10 s bins of average sEPSC events with bath application of CB1R antagonist AM4113 (100 nM) before, during, and after NIC. (*c*) Average sEPSC frequency ($F_{(2,10)} = 1.81$, $p = 0.213$), (*d*) frequency expressed as fold change ($F_{(2,10)} = 0.366$, $p = 0.702$), and (*e*) amplitude ($F_{(2,10)} = 1.62$, $p = 0.246$) before, immediately after, and 5–7 min post-NIC ($n = 6$ cells). Error bars represent ± SEM. One-way repeated measures ANOVA.

that CB1R activation decreases excessive glutamate release in the hippocampus (34). We hypothesized that CB1R activation may mediate the transient decrease in glutamate release observed directly after NIC induced hippocampal injury. To test this, we repeated the experiments from Fig. 2 and measured sEPSCs in the presence of CB1R antagonist AM4113. Surprisingly, we found that pretreatment with the bath application of AM4113 (100 nM) was sufficient to block the immediate decrease in excitatory release following NIC (Fig. 3, *a–c*). There were also no apparent postsynaptic effects of excitatory signaling as measured by sEPSC amplitude (Fig. 3 *d*). Together, these data suggest that endocannabinoid (eCB) signaling is a core component in mediating synaptic function in response to brain injury.

Hippocampal astrocyte activation is higher 72 h postinjury

In response to injury, astrocytes are activated to varying degrees, with corresponding changes in gene expression, morphology, proliferation, and contribution to repair and remodeling (16). GFAP expression is an established measure of astrocyte activation. In the hippocampus injured region of mouse brain slices, astrocyte activation was higher than in control slices of brain tissue (Fig. 4, *a* and *b*). Astrocyte activation increased over the course of 72 h postinjury compared to day 0 injured slices (Fig. 4, *c* and *d*). This suggests that astrocytes have an acute response to NIC injury that continues for at least 72 h.

Extracellular matrix protein upregulation mitigated by CB1R antagonist

The matricellular protein families secreted protein acidic and rich in cysteine (SPARC), TNC, thrombospondin (TSP), and CCN (CYR61/CTGF/NOV) are upregulated in reactive astrocytes following injury or disease (15). To test this, we used immunohistochemistry to assess how several of these proteins changed following NIC. We expected post-injury acute astrocyte activation and increased activation and secretion of the extracellular proteins GFAP, TNC, and CTGF in the days following injury. Immunohistochemistry of day 0 slices indicated similar levels of TNC and CTGF with an increase in GFAP expression for NIC-injured versus sham slices (Fig. 5). Interestingly, after 72 h, TNC and CTGF levels decreased, but GFAP remained consistent with day 0 levels (Fig. 5). The addition of a CB1R antagonist at the time of injury decreased the upregulation of GFAP, but not CTGF or TNC proteins, at 72 h postinjury (Fig. 5).

DISCUSSION

Progress in understanding the immediate cellular sequelae of TBI has been hampered by a lack of tools. In this study, we aimed to address this by combining several technical approaches that revealed important new information about the short-term effects of TBI on hippocampal circuits. Ex

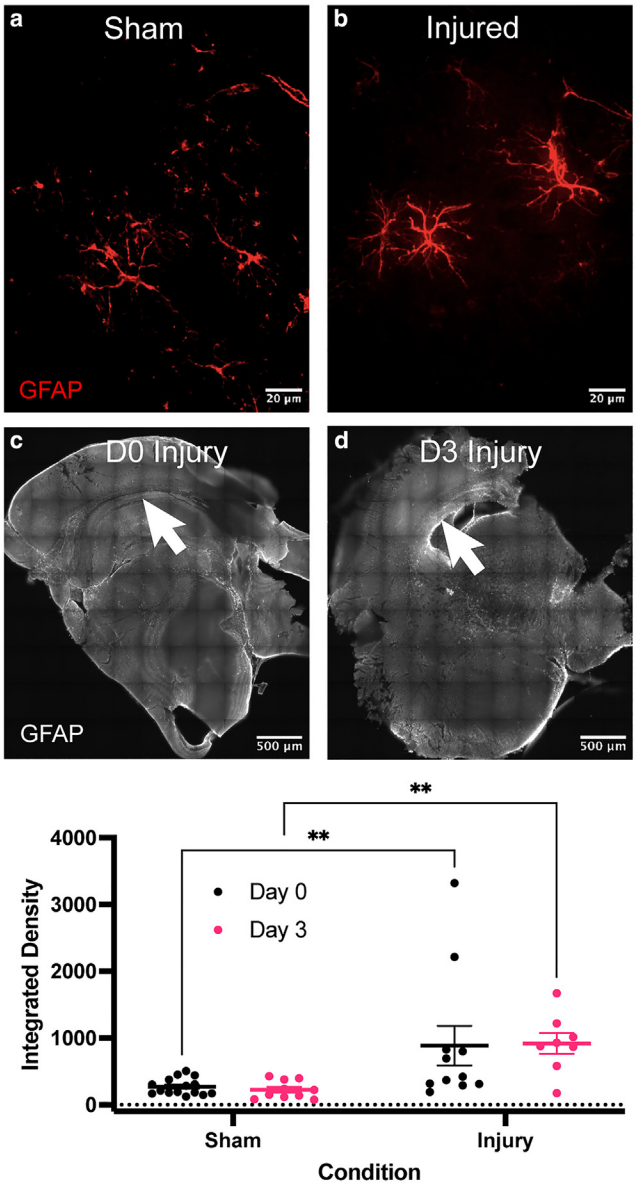

FIGURE 4 Astrocyte activation in NIC-injured brain slices. Representative astrocyte activation (GFAP, *red*) of (*a*) control and (*b*) NIC-injured brain slices at day 0. Representative images of astrocyte activation (GFAP, *white*) in the hippocampus (*white arrows*) injured region of brain slices at (*c*) day 0 (D0) and (*d*) day 3 (D3) postinjury. Scale bars represent 20 μm in (*a* and *b*) and 500 μm in (*c* and *d*). Error bars represent $\pm$ SEM. Two-way repeated measures ANOVA. $^{**}p < 0.01$.

vivo-cultured brain slices have been used to study neurological function, disease, and injury (35–40). These cultured or organotypic slices maintain cellular complexity and many of the 3D structural properties of brain tissue, which allows a more physiologically relevant approach to understanding the longer-term (more than 30 min) biochemical mechanisms associated with TBI. TBI is linked to increased risk for a variety of neurodegenerative diseases, including dementia, Alzheimer's disease, and chronic traumatic encephalopathy (41–44). Understanding the cavitation phenomenon will provide better understanding of the causation of these diseases and, therefore, may help scientists/engineers develop early detection methodologies, treatments, and prevention measures. This information will elucidate the role of CTGF and TNC in long-term brain remodeling, which might be important for understanding the link between TBI and neurodegenerative diseases such as Alzheimer's disease and epilepsy (45–49). These findings lay the groundwork for numerous future studies that can utilize this new technique to identify the mechanisms underlying TBI and neurodegenerative disease.

Although TBI is a risk factor for numerous neurological disorders, many of the underlying mechanisms of how TBI impacts neural function are unknown. Current models of TBI often lack reproducibility, have high mortality rates, and/or involve surgical techniques. Further, many of these techniques lack the ability to measure essential metrics such as diagnostic biomarkers and changes in neural function with high temporal resolution (50). NIC allows precise spatial control over hippocampal injury in mouse brain slices. Understanding how TBI impacts short-term (day 0–3) astrocyte responses and synaptic function lays the groundwork for advanced approaches in understanding how TBI impacts neural function and the development of treatments that promote TBI repair and prevent neurodegenerative disease.

The impact of cellular damage on hippocampal memory formation after TBI is often measured by changes in long-term potentiation at the synapse postinjury (51,52). However, current models of TBI lack the temporal and spatial resolution necessary for measuring synaptic function in real time. Conflicting reports suggest that glutamate levels and signaling either increase or decrease immediately following hippocampal injury (53–58). Current studies rely on techniques such as microdialysis and microarrays to measure extracellular glutamate levels on the order of minutes, hours, and days postinjury and indirectly infer how this may impact neural function. These studies have demonstrated that there is unregulated release of glutamate and a buildup of extracellular glutamate following TBI (59,60). However, other reports using magnetic resonance spectroscopy show a decrease in glutamate in the first few hours to days following TBI (61,62). This discrepancy is attributed to microdialysis measuring extracellular glutamate, while magnetic resonance spectroscopy measures both intra- and extracellular glutamate levels. However, neither approach addresses the functional synaptic responses immediately pre- and postinjury. By combining NIC with patch-clamp electrophysiology, we measured in real time that excitatory release is highly dynamic at the onset of injury.

Here, we show small-scale CA3 NIC injury clearly reduces excitatory glutamate release onto downstream CA1 pyramidal neurons for $\sim$1–2 min postinjury. We speculate that this may contribute to short-term memory loss of events

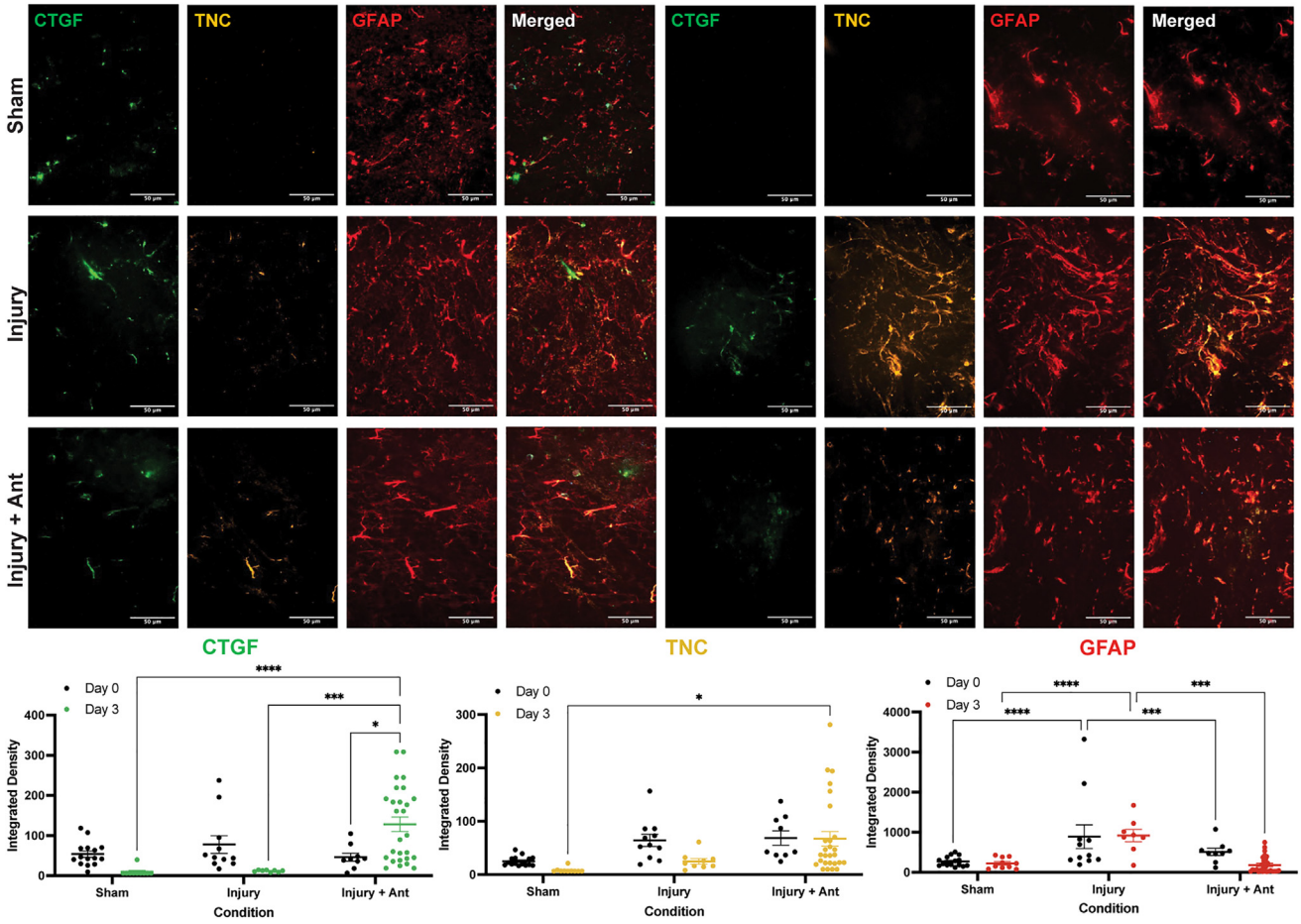

FIGURE 5 Day 0 vs. day 3 protein staining. Sham, injury, and injury + antagonist immunohistochemistry staining images for connective tissue growth factor (CTGF, *green*), tenascin-C (TNC, *orange*), GFP (*red*), and merged at day 0 vs. day 3. Scale bar: 50 μm. Scale bars represent 50 μm. Error bars represent $\pm$ SEM. Two-way repeated measures ANOVA. *$p < 0.05$, ***$p < 0.001$, and ****$p < 0.0001$.

leading up to TBI, such as after a concussion. This is followed by a marked increase in glutamate release several minutes after the injury event. Interestingly, excessive glutamate release in the hippocampus can lead to seizures, and up to 10% of concussion patients develop epilepsy following the injury event. Thus, NIC applied directly to an organotypic brain slice is an effective technique for measuring changes in neural function with high spatial and temporal resolution.

The CNS endocannabinoid (eCB) system is a network of neurotransmitters and receptors that regulates many physiological and cognitive processes. The eCB system is essential in maintaining the excitatory-inhibitory balance in the hippocampus, and excessive glutamate release results in the activation of CB1Rs, which serve as a negative feedback mechanism. There are two key cannabinoid receptors in the brain: CB1R and CB2R. In the brain, CB1R is primarily expressed on the presynaptic neuron and on astrocytes, while CB2R is expressed on microglia (61,62). In neurons, CB1R activation leads to a decrease in presynaptic glutamate release. The accumulation of eCBs in response to injury, anti-inflammatory effects, and their role in neurogenesis suggests that eCBs may contribute to a neuroregenerative response post-TBI (63). One consequence of eCB effects on astrocytes is the increase in cytosolic Ca^{2+} signals through cannabinoid (CB1) activation. In the context of TBI where increased neuronal firing and excitotoxicity are common, activation of the CB1R may mediate neurotransmitter release and provide a negative feedback mechanism in response to high levels of neural activity. Interactions between eCBs and neurons, astrocytes, and microglia promote anti-inflammatory and neuroprotective effects post-TBI (64,65). We show that the transient decrease in glutamate release following NIC is due to CB1R-mediated buffering at excitatory synapses. We observe that pretreatment with the CB1R antagonist AM4113 greatly attenuates the transient decrease in excitatory release following injury (Fig. 5). Additionally, CB1R inhibition increases basal glutamate release prior to injury. Future work is necessary to determine the exact mechanisms underlying the relationship between the eCB system and hippocampal function following injury.

eCB-mediated bidirectional communication between astrocytes and neurons has been demonstrated to significantly impact synaptic plasticity; however, the role of eCBs in astrocyte remodeling of brain tissue is largely unexplored. In some cases, astroglial CB1R antagonism reduces GFAP expression and promotes an anti-inflammatory state by simultaneously lowering levels of pro-inflammatory cytokines (66,67). eCB signaling acts on CB1Rs on astrocytes, increasing intracellular calcium and signaling back to neurons in a feedback loop to release glutamate, which might be important in remodeling (66,68). Surprisingly, when brain slices were treated with a CB1R antagonist prior to NIC, there were more reduced levels of astrocyte-secreted remodeling protein GFAP after 72 h than in NIC-injured slices without the antagonist. We speculate that either the antagonist interrupted the repair mechanism postinjury or that CB1 signaling after washing away the antagonist overcompensated and interrupted the remodeling process. Future work is necessary to determine the role of endocannabinoid-mediated effects on remodeling post-TBI.

Astrocytes make up ∼30% of the brain cell population. In addition to their many functions in the healthy CNS, astrocytes respond to CNS damage and disease through a process called astrogliosis or to changes in the molecular and functional levels in response to pathologies. Intermediate filaments are networks of long strands of proteins that provide mechanical support for cells. GFAP is the principal astrocyte intermediate filament protein in astrocytes, along with vimentin, synemin, and lamin (69). The upregulation of matricellular proteins by reactive astrocytes in response to injury is dependent on the type, location, and severity of insult. Given their role in remodeling the microenvironment surrounding regions of brain injury, astrocyte-secreted extracellular proteins may represent important therapeutic targets for CNS repair. GFAP, TNC, and CTGF are upregulated in the hippocampus injured region of mouse brain slices over sham slices after 72 h. Conversely, prolonged activation of astrocytes may result in long-term accumulation of extracellular proteins that may promote the progression of neurodegenerative diseases and/or increased risk for delayed epileptic episodes associated with TBI (70). Future work is necessary to determine whether the protein upregulation is reversible or if there is a link to acute mTBI and chronic neurodegenerative pathologies.

CONCLUSIONS

In this research, we developed a novel technique to perform NIC in an ex vivo brain slice while simultaneously recording glutamatergic inputs onto CA1 hippocampal pyramidal neurons. The high spatial and temporal resolution of this technique has allowed us to fill a major gap in knowledge in the understanding of how acute injury to the hippocampus alters glutamate release. There are conflicting data in the literature on whether acute injury to the hippocampus induces an increase or decrease in glutamate release. In isolating single cavitation injuries with high spatial resolution, we gain valuable insight into the cellular and physiological mechanisms underlying cavitation injury after mTBI. Based on the chosen NIC fluid flow rate (5 μL/min) and needle diameter (∼20 μm) for our experiments, the estimated strain rates during NIC were between 0.5 and 0.3 s^{-1}, which is below the threshold for mTBI: 60 s^{-1} (71). Using our newly developed technique, we established that NIC induces an immediate presynaptic reduction in glutamate release for the first 1–2 min, followed by a long-term increase in excitatory activity (5–10 min post injury). This suggests that conflicting reports in the literature are correct but did not have the temporal resolution to identify this mechanism. Using a CB1R antagonist, we show that an initial decrease in excitatory activity is mediated by an eCB feedback mechanism. We posit that this may be to protect against excessive excitatory activity immediately following acute injury to the hippocampus and that it leads to lower levels of protein remodeling postinjury. Although future studies are necessary to understand how these mechanisms impact neural function and behavior in vivo, these studies provide a new tool for understanding the physiological and molecular responses to TBI and lay the groundwork for future experiments unraveling the synaptic mechanisms that mediate these responses seconds, minutes, and days following injury.

SUPPORTING MATERIAL

Supporting material can be found online at https://doi.org/10.1016/j.bpj.2024.07.040.

AUTHOR CONTRIBUTIONS

C.E.D. and B.L.R. contributed to conceptual design, drafting the manuscript, data collection, analysis, and data interpretation. I.K. and S.R.P. contributed to conceptual design, data interpretation, and editing the manuscript.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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