# Impact of traumatic brain injury on glial and neural function in the hippocampus

**Carey E. Dougan[1*], Brandon L. Roberts[2*]**, Ilia Karatsoreos[2], Shelly R. Peyton[1]

[1]Department of Chemical Engineering, University of Massachusetts, Amherst, MA 01003, USA
[2]Department of Psychological and Brain Sciences, University of Massachusetts, Amherst, MA 01003, USA

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## Introduction

Traumatic brain injury (TBI) is an established risk factor for developing neurodegenerative disease[1-4]. However, **there is a lack of TBI models** that relate injury forces to both macroscale tissue damage and brain function at the cellular level. Needle-induced cavitation **(NIC) is a technique that induces highly localized injury** to *ex vivo* brain tissue by applying fluid pressure[5,6]. We have previously observed that **NIC causes tissue damage along the hippocampus**, a brain region critical for learning and memory formation[7-9]. Injury to this region causes cognitive pathologies in humans and rodent models. However, **the impact of NIC at the cellular level is *unknown***. NIC related injury activates specialized glial cells called astrocytes[10-12]. We hypothesize that NIC induced astrocyte activation will lead to secretion of the signaling and remodeling proteins: tenascin-c (TNC), thrombospondin (TSP), and connective tissue growth factor (CTGF) into the extracellular matrix (ECM)[8-13]. Although astrocyte secreted ECM proteins are known contributors to synapse formation[13,15], **how NIC impacts synaptic function is *unknown***. We hypothesize that NIC will disrupt synaptic function in the hippocampus in the weeks following injury. Here we propose to combine a range of engineering and neuroscience techniques to test these hypotheses. Understanding how TBI impacts short- and long-term astrocyte responses and synaptic function is essential in determining the underlying mechanisms that relate acute brain injury with neurodegenerative disease. **This collaboration 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.

## Interfacing Engineering and Neuroscience

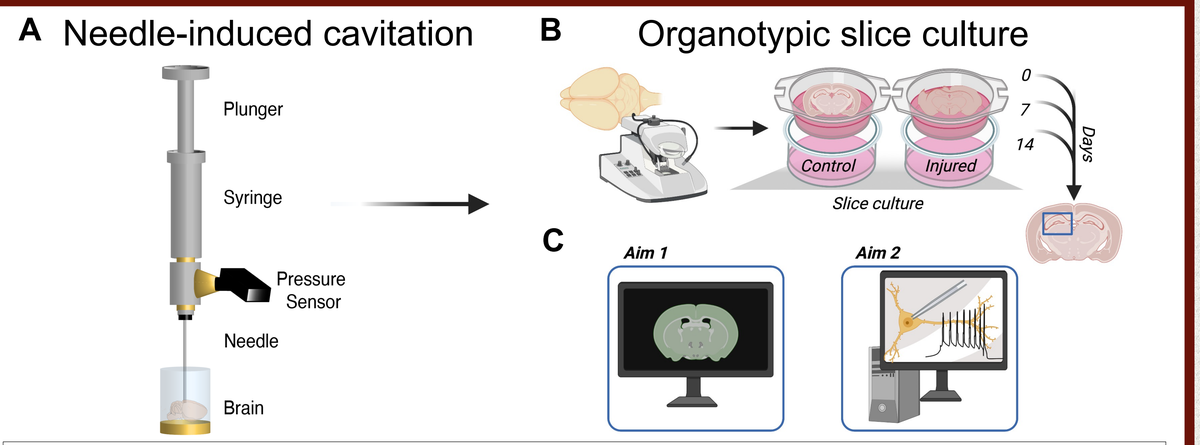


**Figure 1.** Visual representation of the proposed work using ***A,*** needle-induced cavitation as a model of TBI to investigate the acute and chronic impact of tissue damage to hippocampal function. ***B,*** NIC-treated brains are immediately processed using a vibratome and placed on inserts in culture media for investigation at DEV 0, 7 and 14. ***C,*** Illustration of approach using imaging analysis in **Aim 1** (*left*) to determine the impact of TBI on astrocyte activation and secretion of signaling proteins, and **Aim 2** (*right*)

## Aim 1: Determine short- and long-term astrocyte protein secretion in response to mild traumatic brain injury


**Figure 2.** ***A,*** Live and dead hippocampus cells after organotypic slice culture 7 days *ex vivo* (DEV). ***B,*** Live hippocampus cells (DAPI) after slice culture at DEV14. ***C,*** Astrocyte activation (GFAP, red) with no injury on GFP (green) mice at DEV0. ***D,*** Astrocyte activation (GFAP, green) near injury (beads, red and DAPI, blue) at DEV0.

## Aim 2: Determine the long-term impact of NIC on hippocampal function at the circuit level

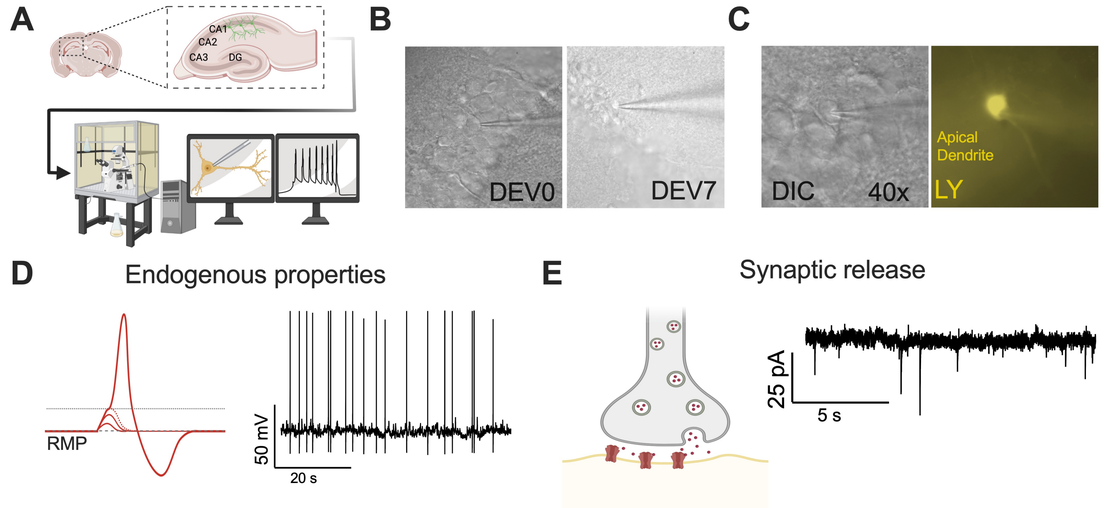


**Figure 3.** ***A,*** Illustration of hippocampal slice and pyramidal neurons in the CA1 region utilized for whole-cell patch clamp. ***B,*** Image of CA1 region with patch pipette at DEV0 (left) and DEV5 (right), ***C,*** Representative cell (*left*) with lucifer yellow (*right*) in patch pipette for visual identification of pyramidal neuron. ***D,*** Diagram of an action potential and resting membrane potential (RMP; *left*) and representative recording of action potential firing (*right*). E, Illustration of excitatory synaptic release (*left*) and representative recording of sEPSCs (*right*).

## Detailed Methods

**Mice**. Animal procedures and experiments were approved by the University of Massachusetts Amherst IACUC in accordance with the U.S. Public Health Service Policy and NIH Guide for the Care and Use of Laboratory Animals. Organotypic brain slices are from male and female 8-16 week-old C57BL/6J wild-type mice (Charles River) that were group-housed under a 12/12-hr light/dark (LD) cycle, with food and water available and libitum.

**Needle-induced cavitation (NIC).** 6-8 week-old male and female Balbc mice were euthanized, and brains immediately removed and stored in ice cold 1X Hanks' Balanced Salt Solution for ~20 minutes prior to injury. A 27 G needle was inserted 3 mm deep using an actuator into one hemisphere of the mouse brain. Water with far red fluorescent beads and DAPI was pressurized using a syringe pump at 250 µL/min until a bubble injury occurred at the tip of the needle. Pressure was monitored in real time using a pressure sensor. The brains were immediately fixed in 4% PFA prior to cryoslicing, immunochemistry, and imaging.

**Organotypic Slices.** Mice were euthanized by decapitation and brains were immediate removed. The forebrain is then blocked in a 0-4ºC NMDG cutting solution (mM): 92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 3 sodium pyruvate, 2 thiourea, 20 HEPES, 10 MgSO4, 0.5 CaCl2, 25 glucose, 20 sucrose, pH 7.4 with HCl. Forebrains were mounted adjacent to each other and sectioned simultaneously with a sapphire knife (Delaware Diamond Knives, Wilmington, DE) yielding ~3 mPFC slices (12-250-µm) per mouse. Slices were then placed on tissue culture inserts and cultured at 37°C with 5% $CO_2$. Media was changed once a week. Propidium iodide was added for 30 minutes into cell culture media before DEV7 slices were fixed in 4% PFA. GFAP (1:500) staining was performed on DEV0 without and with NIC injury before imaging on a confocal spinning disk microscope.

**Electrophysiology.** Slices were allowed to recover for 30 min at RT in recording artificial cerebrospinal fluid (aCSF) (mM): 124 NaCl, 3.7 KCl, 2.6 NaH2PO4, 26 NaHCO3, 2 CaCl2, 2 MgSO4, 10 glucose, and bubbled using 95% 02/5% C02. For recording, slices were transferred to a perfusion chamber containing aCSF maintained at 34-37°C. Neurons were visualized with a Zeiss Axoskop 2. Recording electrodes were back-filled with internal solutions as follows (mM): Current-clamp and sEPSCs; 125 K-gluconate, 10 KCl, 10 NaCl, 5 HEPES, 10 EGTA, 3 NaATP and 0.25 NaGTP. sIPSCs; 140 CsCl, 5 MgCl2, 1 BAPTA, 10 HEPES, 3 NaATP, and 0.25 NaGTP. EPSCs were recorded in the presence of bicuculline (30 µM). Miniature (m) EPSCs had the addition of the voltage-gated sodium channel blocker tetrodotoxin (TTX; 1 µM). Patch electrodes (3-5MΩ) were guided to neurons with an MPC-200-ROE controller and MP285 mechanical manipulator (Sutter Instruments, Novato, CA). Recordings were collected with a UPC-10 USB dual digital amplifier and Patchmaster NEXT software (HEKA, Holliston, MA). Only neurons with holding currents not exceeding 100pA at $V_H$= -70mV for a 10-min control period (input resistance > 70 MΩ), a series resistance < 50MΩ, and drift <10% were studied further. Reagents were obtained from Tocris Cookson, Cayman Chemical, and Sigma Aldrich.

**Statistics.** Electrophysiology data was analyzed in Patchmaster NEXT or converted with ABF Utility (Synaptosoft) for analysis in Clampfit (Molecular Devices) and/or MiniAnalysis (Synaptosoft). Datasets were tested for normality before statistical analysis. Comparison of effects is done using one-way ANOVA or Kruskal-Wallas test for datasets that failed normality. Two-way ANOVA is used for comparison of current-voltage IV relationships. Statistics were calculated with Prism 9 (Graphpad).

## References

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## SUPPORT

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## Future Directions

- Correlate NIC injury forces with macroscale tissue damage and astrocyte response by quantifying astrocyte activation (GFAP)
- Compare ECM protein levels at acute and long-term injury phase to elucidate the progression of TNC, TSP, and CTGF in brain remodeling
- Determine how NIC impacts synaptic plasticity in the hippocampus immediately after injury and during injury recovery
- Determine the mechanism by which tanycytic ECM proteins alter neural function at the physiological level