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

Introduction: Traumatic brain injury (TBI) is linked to increased risk for a variety of neurodegenerative diseases including dementia, Alzheimer's disease, and chronic traumatic encephalopathy (CTE)[1–4]. However, there is a paucity of techniques available to study the progression of localized TBI and its impact on brain function at the cellular level. Specialized glial cells called astrocytes are activated in response to mild TBI and facilitate neural remodeling and repair[5–7]. However, the role of astrocytes in restoring neural function after TBI is largely *unknown*. *The long-term goal of this research is to implement an ex vivo precision model of TBI to understand the astrocytic and neuronal mechanisms underlying brain repair and function.*

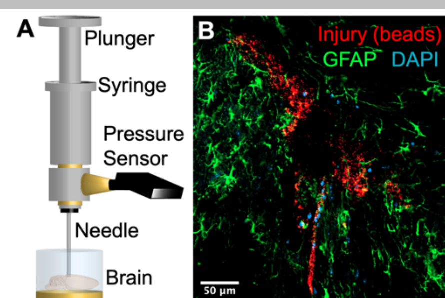

Fig.1 ***A*** NIC schematic. ***B*** Astrocytes (*green*) and visualization of NIC injury with fluorescent beads (*red*).

Needle-induced cavitation (NIC) is a technique recently adopted for studying soft tissue injury[8,9]. For NIC, a needle is inserted into an excised brain, pressurize fluid until a bubble injury occurs, and monitor pressure in real-time to relate NIC forces with the extent of injury (**Fig.1A**). Our current work demonstrates that NIC consistently results in tissue damage and astrocyte activation without complete tissue destruction (**Fig.1B**)[10]. Astrocyte activation leads to the secretion of glial fibrillary acidic protein (GFAP) and extracellular matrix (ECM) proteins, such as tenascin-C (TN-C), thrombospondin (TSP), and connective tissue growth factor (CTGF)[11]. After injury, ECM proteins promote inflammatory responses and neural repair[12,13].

The hippocampus is a brain region that regularly undergoes neurogenesis and is essential for learning, memory, and cognitive function, and injury to this region results in numerous neuropathologies[14]. ECM proteins contribute to synaptic function in the hippocampus[13], yet, how TBI impacts this function is *unknown.*

Interfacing Neuroscience and Engineering

Precise models for understanding how TBI impacts brain function and repair are lacking. There is a need to bridge specialized research approaches within engineering and neuroscience if we are to fully understand the complexities of TBI and its associated outcomes. This proposal uses an *ex vivo* model of TBI to fill that gap. Carey has optimized a precision model of TBI, incorporating fluid, solid, and thermodynamics to measure tissue damage. Carey can directly relate the forces associated with injury to extent of damage. However, elucidating the functional outcomes of NIC-induced TBI at the circuit level requires neurophysiological approaches. Dr. Roberts has expertise in live brain slice collection, slice electrophysiology, and the anatomical and physiological knowledge necessary to reliably answer these questions. Together, the applicants have completed proof-of-concept experiments justifying the feasibility of this proposal, including NIC-induced TBI (**Fig. 1**), DAPI cell staining of days *ex vivo* (DEV) 14 brain slice cultures with no injury (**Fig. 2B**), GFAP staining of DEV7 brain slice cultures with no injury, (**Fig. 2A**) and patch-clamp recordings from hippocampus neurons (**Fig. 3**). Combined, this proposal intersects engineering and neuroscience to advance approaches in understanding TBI.

Experimental Approach

Our *central hypothesis* is that NIC will promote astrocyte secretion of remodeling proteins (TN-C, TSP, and CTGF) near the site of injury, and alter synaptic function. We will test this with the following specific aims:

Aim 1: Determine short- and long-term astrocyte protein secretion in response to mild traumatic brain injury. We will approach this aim using a NIC brain injury model and immunohistochemistry (IHC) on cultured brain slices at Day *ex vivo* (DEV) 0, 7, and 14 post-NIC injury.

Aim 2: Determine the long-term impact of NIC on hippocampal function at the circuit level. We will approach this aim using *ex vivo* brain slice electrophysiology techniques with cultured slices from *Aim 1*.

Methods

Brain injury model: Group housed 6-8wk old male and female C57BL/6J mice are euthanized via decapitation. Brains are quickly removed and placed in a chilled and oxygenated NMDG cutting solution[15]. NIC is performed by inserting a 27G beveled needle into the brain and controlling the fluid flow rate of 250 μL/min with a syringe pump using a Hamilton 1mL glass syringe (**Fig. 1A**). The fluid is artificial cerebrospinal fluid (aCSF) containing 200 nm carboxylate functionalized latex beads and DAPI nuclear stain to locate the injury under a microscope after slicing. Pressure is measured in real time using an Omega pressure sensor.

Slice Cultures: After NIC, 3 mirrored sets (6 total) of 150μm coronal hippocampal slices are cut with a vibratome in NMDG cutting solution and transferred to tissue culture inserts with brain slice medium for incubation.

IHC Staining and Image Analysis: DEV0, 7, and 14 slices are fixed in 4% PFA and stained with GFAP or TN-C, TSC, and CTGF. Images are taken using a spinning disk confocal microscope and protein levels are quantified using ImageJ.

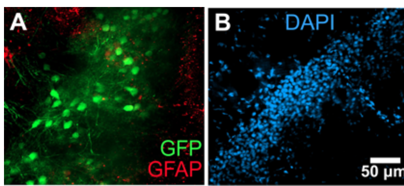

Fig.2 A Neurons (*green*) and GFAP (*red*) at DEV0. **B** Live cells (*blue*) at DEV14.

Electrophysiology: Cultured slices (*n*=3/mouse) are transferred to aCSF and placed under a microscope with gravity perfusion at 37°C. Recording pipettes (3-5MΩ) are filled with a K^+ based internal solution. 0.2% lucifer yellow (LY) is added to the pipette for posthoc identification of recorded neurons (**Fig. 3**). Resting membrane potential (RMP) is measured in the current (I) clamp configuration. The $GABA_A$ antagonist bicuculline (30µM) and Na^+ channel blocker tetrodotoxin (1µM) are added to the aCSF to isolate local miniature excitatory postsynaptic currents (mEPSC; V_{Hold}=-70mV). IV-relationship protocols are run with 10mV steps from -120 to 30mV.

Aim 1: Determine the short- and long-term astrocyte response to cavitation induced mild TBI

The *rationale* for this aim is that astrocytes respond to injury through initial activation and subsequently secrete remodeling proteins during the healing phase[5-7]. The accumulation of these proteins after mild injury may have long-term implications for multiple neurodegenerative diseases. Our preliminary data show that post-NIC, astrocytes are activated (GFAP, *green*) near the injury site (DAPI nuclear stain, *blue* & fluorescent beads, *red*) at DEV0 in *ex vivo* mouse brain exposed to NIC **(Fig. 1B).**

Study 1.1: We will perform SHAM control (no fluid pressure) or NIC on male and female *ex vivo* mouse brains (*n*=6 mice/group) near the hippocampus using pressures associated with mild TBI. We will then correlate NIC injury forces with the extent of macroscale tissue damage and astrocyte response. We will measure astrocyte activation at DEV0, 7, and 14 (*n*=3 slices/mouse) with GFAP staining in SHAM and NIC groups. This will determine regions of high and low astrocyte activity, and associated ECM protein levels.

Study 1.2: We will compare the ECM protein levels associated with NIC injury forces at DEV 0, 7, and 14 by performing IHC staining on brain slices for the ECM proteins: TN-C, TSP, and CTGF. These data will elucidate the progression of TN-C, TSP, and CTGF secretion during long-term brain remodeling.

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

The *rationale* for this aim is that hippocampal CA1 pyramidal (Pyr) neurons are critical for memory formation, and astrocytic ECM proteins such as TN-C mediate synaptic strength onto these neurons[13,16]. However, the impact of NIC injury on synaptic function in the hippocampus is *unknown*.

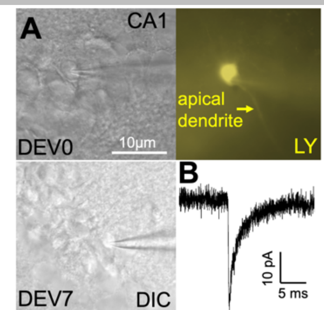

Fig.3 A Patch of CA1 Pyr neuron at DEV0 (*top*) and DEV7 (*bottom*), **B** EPSC from DEV0.

Study 2.1: Using the mirrored SHAM and NIC brain slices from *Aim 1* (*n*=3 slices/mouse) at DEV0, 7 and 14, we will implement patch-clamp electrophysiology to test how post-NIC injury impacts synaptic inputs and neural function of CA1-Pyr neurons. For this experiment we will measure the following parameters:

1) Cell properties including membrane resistance (R_{series}), capacitance, R_{input} (recording quality control), RMP, and action potential firing.
2) IV relationship: Inward/outward rectifying currents, I-density, and conductance.
3) Synaptic components of mEPSCs (amplitude, frequency, and decay tau).

Future Studies: Voltage-dependent calcium channels (VDCCs) mediate TN-C actions on synaptic plasticity. Future studies will incubate cultured NIC slices with the VDCC blocker nifedipine (10µM) added to the culture media and repeat the experiments in *Aim 1 & 2* to quantify how VDCC activation mediates the progression of synaptic function in the hippocampus after NIC induced TBI.

Impact on Career Development: To date, the primary applicant has obtained three travel grants to present their research at conferences. The co-applicant has obtained an SRS Small Research grant and two previous travel grants. However, the applicants have not had success obtaining substantial project funding at the graduate and postdoctoral level (respectively), which is critical for continuing their academic career. Successful completion of this proposal will result in three core outcomes for the applicants: 1) Bolster subsequent grant proposals with a robust preliminary dataset 2) Demonstrate to future grant reviewers the applicants' ability to obtain funding, and 3) Widen the scope of collaborations between engineering and neuroscience, broadening future work and publications by pairing data from this award with data collected under mentor funding from both applicants.

References: **1.** Shively S, et. al. *Arch Neurol*. 2012;69(10):1245-1251. doi:10.1001/archneurol.2011.3747 **2.** Ramos-Cejudo J, et al. *EBioMedicine*. 2018;28:21-30. **3.** Sivanandam TM, et. al. *Neurosci Biobehav Rev*. 2012;36(5):1376-1381. **4.** Stein TD, et. al. *Curr Pain Headache Rep*. 2015;19(10). **5.** Zhou Y, et. al. *Cell Commun Signal*. 2020;18(1):1-16. **6.** Jones EV, et. al. *Neural Plast*. 2014;2014. **7.** Burda JE, et. al. *Exp Neurol*. 2016;275(0 3):305-315. **8.** Zimberlin JA, et. al. *Soft Matter*. 2007;3(6):763. **9.** Barney CW, et. al. *PNAS*. 2020;117(17):9157-9165. **10.** Dougan CE, et. al. *bioRxiv*. Jan. 2022:2022.03.15.484522. **11.** Yonezawa, T. *et al. Glia.* 2010;58:755–767. **12.** Wiemann, S., et. al. *Biochem Soc Trans*. 2019;47:1651–1660. **13.** Geissler, M. et al. *J Neuro.* 2013;33:7742–7755. **14.** Shetty AK. *Epilepsy Behav*. 2016;38:117-124. **15.** Ting, J. T. et al. *JoVE.* 2018; e53825. **16.** Šekeljić, V. et. al. *Int J Biochem Cell Biol.* 2012;44:825–829.