The Effect of Mild Traumatic Brain Injury on Axon Initial Segment Plasticity

Siri Munnuluri, John Greer MD, PhD
Department of Neurosurgery, School of Medicine, Virginia Commonwealth University

Background

Traumatic brain injury (TBI) is a pressing global healthcare problem and has a substantial impact on society (Langlois et al. 2006). The majority of TBI worldwide are mild (mTBI) in nature (REF). Despite the mild nature of injury, over 50% of individuals sustaining a mTBI have deficits up to a year following injury. The structural basis for many of these chronic effects of mTBI remains unclear.

The axon initial segment (AIS) is a critical microdomain for action potential initiation and homeostatic plasticity (Jamann et al. 2021). Our lab has demonstrated alterations in AIS length following mTBI (Figure 1).

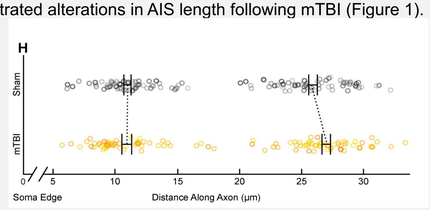


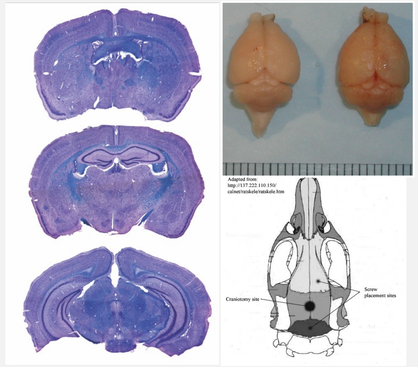
Figure 1

Mice placed in an enriched environment for threed hours demonstrate alterations in AIS structure in Layers 2/3 of the barrel cortex. This shortening results in a decrease in neuronal excitability resulting in homeostatic plasticity. This process is crucial for maintaining stable neural function by adjusting the strength of synaptic connections to prevent hyperexcitability or instability. When neural activity becomes excessively high (hyperexcitability), homeostatic mechanisms respond by shortening the AIS length of the neurons. This can occur post-enrichment. In contrast, when neuronal activity becomes excessively low (e.g. deprivation) the AIS elongates. Overall, homeostatic plasticity acts as a regulatory mechanism that adjusts synaptic strength and neuronal excitability to maintain a balanced level of network activity.

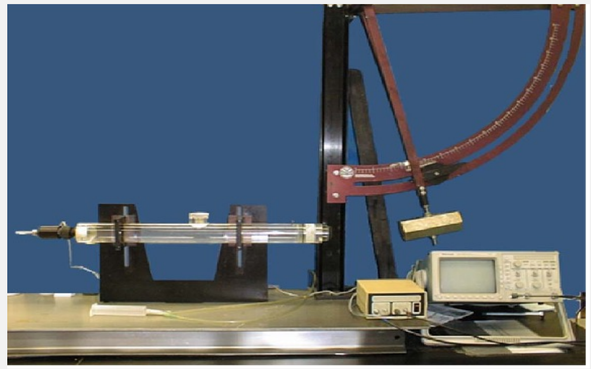
Abstract

This experiment aims to study how mild traumatic brain injury (mTBI) affects axon initial segment (AIS) plasticity. The hypothesis predicts that an enriched environment (EE) will induce elongation of AIS in Layers 2/3 of the barrel cortex and the whisker shaving and sensory deprivation will conversely result in shortening of the AIS in the contralateral barrel cortex. With exposure to EE and resultant increased synaptic input, the AIS of Layer 2/3 will elongate and neurons will become less excitable; whisker shaving, however, will decrease synaptic input upon Layer 2/3 and result in AIS shortening and increased excitability. To investigate the effect of mTBI upon AIS plasticity the difference in AIS length between the EE and deprived hemispheres (delta) was quantified; this value was compared between sham-injured control and mTBI animals. By doing so, the experiment seeks to understand if mTBI alters AIS plasticity similarly or differently compared to the Sham condition under enriched conditions. This study used a total of 4 βIV-spectrin mCherry mice (age: 3–7 months) assigned to either sham-injury (control) or mTBI (experimental) groups. To induce mTBI, a craniotomy was performed under anesthesia, exposing the skull. A fluid percussion device was then employed, applying controlled pressure (1.6 atm) to the exposed dura mater. Following the injury procedure, the whiskers of the mice were trimmed on the left side. 24 hours post-injury mice placed in an enriched environment for 3 hours. Following this, the mice were euthanized via perfusion to preserve brain tissue. The brain was carefully removed and sectioned using a microtome to obtain thin slices (50 microns). Image acquisition was performed routine fluorescent microscope. AIS profiles where then measured in Layers 2/3 of the barrel cortex. Average lengths on the EE side were subtracted from the deprived hemisphere. These values were then compared between sham and injured animals.

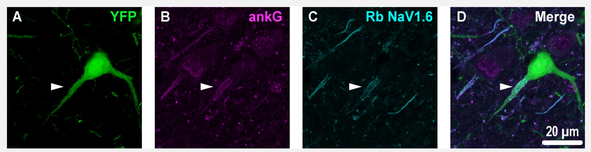
Testing and Methods



A 3mm craniectomy is made midway between bregma and lambda sutures. cFPI results in diffuse mild TBI without evidence of contusion or focal lesion as can be seen grossly and with the Nissl staining seen here.



The image above depicts the fluid percussion injury model. In order to model mTBI in the mice, we used midline central fluid percussion injury (cFPI). After recovery of the craniectomy, the mice were re-anesthetized and connected to the fluid percussion apparatus, a closed mechanical system. The pendulum was released, striking the piston in the fluid-filled cylinder to generate a mild pressure wave (1.6 atm) delivered onto the mice's dura. For the sham-injury mice, the same procedure was completed except for the pendulum's release.

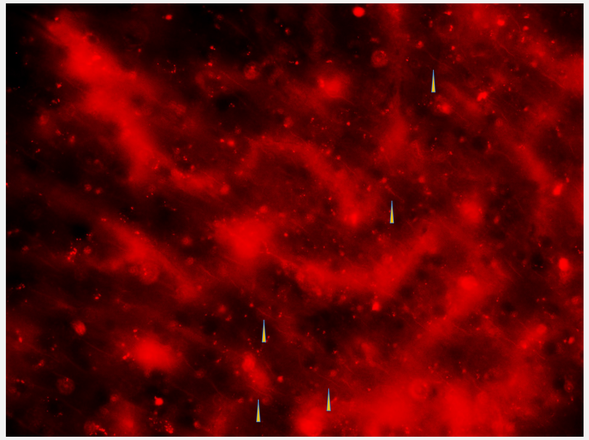


Arrow demonstrates AIS profile, in this case through immunostaining for Ankryin G and sodium channels. The length and the relative location along the axon of the AIS will be measured following sham and cFPI injury in animals with exposure to an enriched environment with one hemisphere deprived of stimulation by whisker shaving.

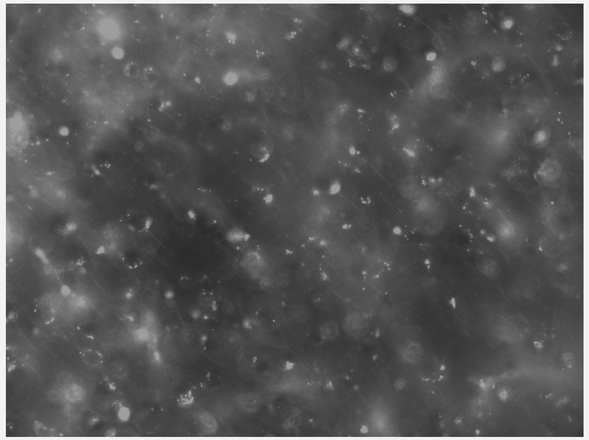
Results

Table of animals with injury and righting reflexes

Animal	Injury/Sham	Time Point	Injury Severity	Righting Reflex
1	cFPI	1d	1.55 atm	6:35
2	cFPI	1d	1.62 atm	7:12
3	cFPI	1d	1.53 atm	12:25
4	sham	1d	n/a	1:24



Beta-4-spectrin-mCherry expression in cFPI injured animal. Arrowheads indicate axon initial segments.



Beta-4-spectrin-mCherry expression in cFPI injured animal. Arrowheads indicate axon initial segments. (gray scale image)

Conclusion

- Unable to quantify AIS length due to excessive background immunofluorescence
- Beta-4-spectrin mCherry seems as though it is a reasonable model for visualizing AIS pathology with appropriate tissue preparation optimization
- Unclear whether mTBI affects AIS plasticity

Future Directions

- Optimize tissue perfusion protocol to minimize immunofluorescence, likely from poor saline flush prior to fixation
- Immunostaining with antibodies targeting mCherry to improve resolution of AIS above background
- Quantify AIS length in Layers 2/3

Acknowledgements

First, I would like to sincerely thank my PI, Dr. Greer for his invaluable assistance throughout this project. I would like to also acknowledge the MSIP program co-directors for the opportunity to do this program.

References

Jamann, N., Dannehl, D., Lehmann, N., Wagener, R., Thielemann, C., Schultz, C., Staiger, J., Kole, M. H. P., & Engelhardt, M. (2021, January 4). Sensory Input Drives Rapid Homeostatic Scaling of the Axon Initial Segment in Mouse Barrel Cortex. *Nature Communications*, *12*(1), 14. 10.1038/s41467-020-20232-x

Vascak, M., Sun, J., Baer, M., Jacobs, K. M., & Povlishock, J. T. (2017, June). Mild Traumatic Brain Injury Evokes Pyramidal Neuron Axon Initial Segment Plasticity and Diffuse Presynaptic Inhibitory Terminal LossFrontiers in Cellular Neuroscience | www.frontiersin.org. *Frontiers in Cellular Neuroscience*, *11*, 24. 10.3389/fncel.2017.00157