Determining the Effects of Neuronal Proximity to Blood Vessels on Cell Membrane Permeability Following Repetitive Traumatic Brain Injuries

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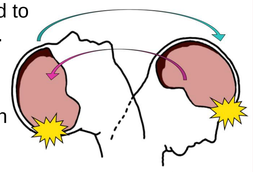
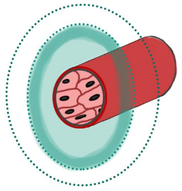
INTRODUCTION

A traumatic brain injury (TBI) is a disruption in the normal function of the brain that can be caused by a violent blow or jolt to the head [1]. Our research focused on inertial-loading TBI, in which the brain is damaged due to acceleration/deceleration forces of the brain within the skull. Repetitive TBI can lead to vascular deformities and long-term deficits in brain tissue.



Inertial injury damages the brain

Due to the difference in density between blood vessels and brain parenchyma, we believe the structural discontinuities caused by the presence of blood vessels in the brain may create mechanical stress points for brain tissue surrounding the vasculature. Characterizing the damage to the brain's vasculature can provide insight to the contribution of blood vessels to the pathology of repetitive TBI.



Perivascular domain surrounding blood vessel

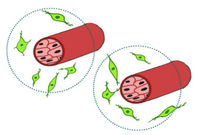
We define the junctions between blood vessels and surrounding brain tissue as the perivascular domain. We further suggest that the neural cells surrounding blood vessels in perivascular domains are most vulnerable to damage post-TBI, specifically to cell membrane permeability. Inertial-loading injuries damage cells by shearing the cell membrane and thus disrupting the neuron's normal functions.

The objective of our study is to determine whether neurons in perivascular domains, or physically near mid-sized blood vessels, are preferentially vulnerable to plasma membrane damage following repetitive TBI.

[1] *Traumatic Brain Injury and Concussion*. Centers for Disease Control and Prevention, 28 July 2020.

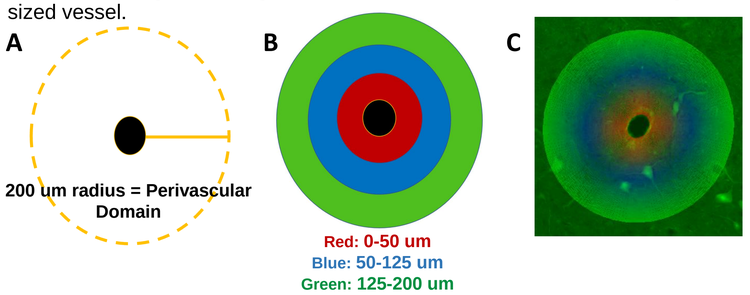
HYPOTHESIS

Neuronal vulnerability to plasma membrane damage following repetitive TBI is a function of proximity to mid-sized blood vessels.



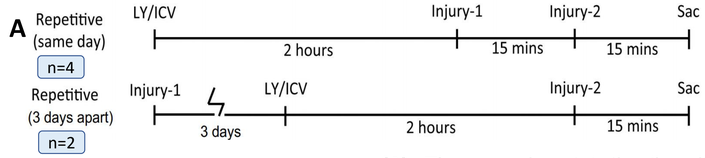
DEFINING THE PERIVASCULAR DOMAIN

We designed a macro plugin to represent the perivascular domain. **(A)** The program outlined the shape of each blood vessel and radially expanded the perimeter to create an annulus in the shape of the vessel with a 200-micron radius. We defined this 200-micron increased perimeter as the perivascular domain, creating a radius greater than twice the diameter of an average mid-sized vessel.



(B) Within the perivascular domain, we had 3 concentric zones indicated by different colors. The purpose of these color zones was to determine if neurons within the perivascular domain were more prevalent depending on their distance from the vessels. **(C)** Image of LY+ neurons in the midbrain with perivascular macro overlaid on top.

INJURY METHODS

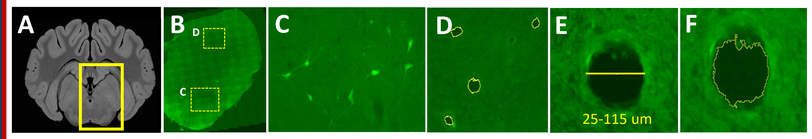


(A) The experimental timeline illustrates Lucifer Yellow (LY) dye administration and injury time in the repetitive injury model. **(B)** Prior to the injury, LY dye was bilaterally infused in throughout the interstitial space. **(C)** Anesthetized swine on a bite plate are rotated in a sagittal direction to induce a closed-head diffuse TBI.

[1–2] Cullen DK, Harris JP, Browne KD, et al. A Porcine Model of Traumatic Brain Injury via Head Rotational Acceleration. *Methods Mol Biol*. 2016.

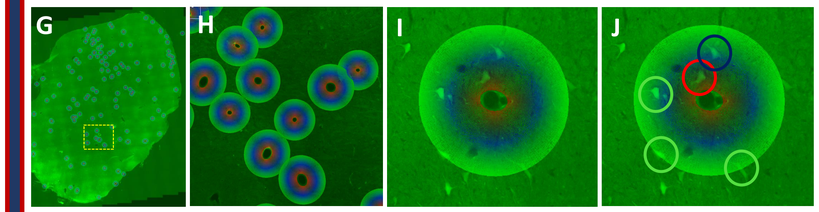
IMAGE ANALYSIS METHODS

(A-C) LY+ permeabilized cells in the midbrain region were identified based on neuronal shape, size, and the presence of processes. **(D-E)** We used an image software on ImageJ to identify mid-sized blood vessels with diameters of 25 to 115 microns. **(F)** The program outlined each individual vessel to measure the diameter and area, determining the eligible vessels as regions of interest.



CATEGORIZING PERMEABILIZED CELLS BY COLOR ZONE

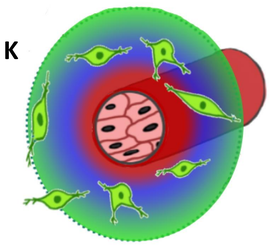
(G) We analyzed archived porcine midbrain tissue and overlaid the color zone annuluses over each mid-sized blood vessel in the regions of interest to visualize the perivascular domains. **(H-J)** To determine if neurons more proximal to vessels are preferential to damage, LY+ permeabilized cells within the perivascular domains were manually counted and categorized by the color zone in which they lie.



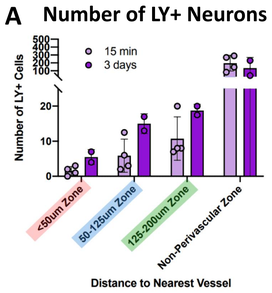
CALCULATING DENSITY OF PERMEABILIZED CELLS WITHIN EACH COLOR ZONE

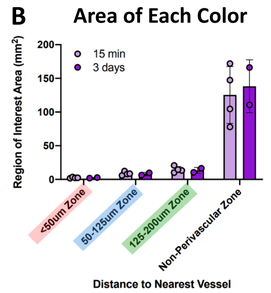
(K) Schematic of a blood vessel surrounded by the color zones of the perivascular domain macro program overlaid, showing permeabilized cells within the annulus.

$$Cell\ Density = \frac{Number\ of\ Cells\ in\ ROI}{Area\ of\ Color\ Zone}$$



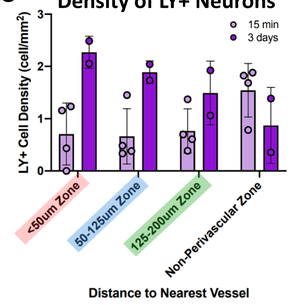
TIMING BETWEEN REPETITIVE INJURIES MAY AFFECT NEURONAL SUSCEPTIBILTY AROUND BLOOD VESSELS

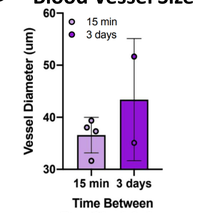




(A-B) For both injury groups, there was a positive correlation with LY+ neuron count and increasing distance away from the vessels.

(C) Cell density is greater in the non-perivascular domains compared to each individual color zone for the 15-minutes-between-injuries group. In contrast, the 3-days-between-injuries group demonstrates a negative correlation between cell density and vessel proximity. These findings were surprising, so we analyzed blood vessel size to assess the pathology further, **(D)** however, there was no significant difference in vessel diameter between injury groups.





CONCLUSIONS

- We developed a semi-automated platform that can identify mid-sized blood vessels, identify permeabilized neurons, and establish perivascular zones.
- Neurons in perivascular domains did not show significant preferential vulnerability to membrane disruptions following diffuse TBI using this image analysis methodology.
- Blood vessel size was not statistically altered between injury groups.
- Timing between injuries may be a mediating variable in neuronal susceptibility within perivascular zones.

FUTURE DIRECTIONS

- Redefine the perivascular domain with improved cerebrovascular anatomical justification.
- Stain for secondary injury markers to determine the physiological effects of the repetitive injury model to brain parenchyma and cerebral vasculature.
- Refine the image analysis software to produce more discrete color layers for a more objective analysis.
- Increase the sample size of porcine subjects.

Applying this to understanding TBI pathology could inform new ways to treat repetitive TBI.

ACKNOWLEDGMENTS

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