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Traumatic Brain Injury: Novel Methods for in Vitro and in Vivo Assessment of Drug Candidates

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Traumatic Brain Injury: Novel Methods for *in Vitro* and *in Vivo* Assessment of Drug Candidates

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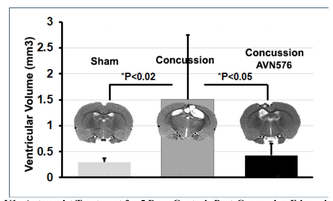
Introduction

- Traumatic Brain Injury (TBI) is the leading cause of death and disability for people under 35 and over 65 in the United States.
- There are currently no approved pharmaceutical treatments for TBI, which represents a major unmet need.
- Recent statistics show there were about 2.5 million TBI-related emergency department (ED) visits, 282,000 hospitalizations, and 56,000 deaths in the United States.
- The total economic burden of TBI in 2017, including direct and indirect medical costs, is estimated at $86 billion.
- If the injury is not fatal, the extent of swelling in the brain (edema) is strongly associated with long lasting damage due to cytotoxic edema. *In vivo* studies show moderate and severe TBI disrupts the Blood Brain Barrier (BBB) which causes a surge in arginine vasopressin (AVP) secretion, a hormone concerned with water permeability and ion homeostasis.
- The increased exposure of cells to vasopressin leads to dysregulation of AQP4, a water channel protein found in the brain, which is strongly implicated in cytotoxic edema.

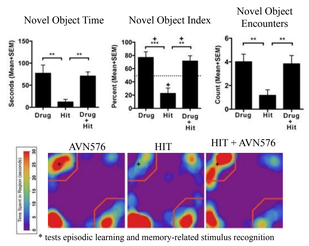
Background

. We previously found that five days of treatment with a highly selective V1a receptor antagonist that crosses the BBB:
  - significantly reduced cerebral edema
  - eliminated cognitive deficits



V1a Antagonist Treatment for 5 Days Controls Post-Concussion Edema in Lateral Ventricles

Figure 1



Cognitive Behavior: Novel Object Recognition*

Figure 2

Hypothesis

. Antagonism of V1a receptor will reduce or block the upregulation of AQP4, thus reducing post-TBI cerebral edema.

Summer 2018 Projects

1. *In vitro* regulation of AQP4 for the development of a rapid drug screening system

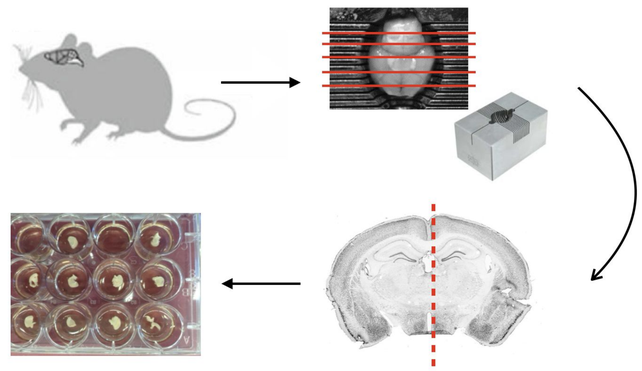
   Organotypic Culture
   - Mice were sacrificed and the brains were sectioned into 500 micrometer slices.
   - Slices were divided along the midline to the left and right halves.

   Western Blot
   - an analytical technique to detect specific proteins

2. Improved design and production of a momentum exchange device for inducing TBI *in vivo*

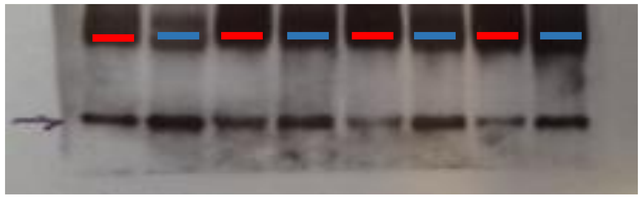
In Vitro Screening: Optimizing Conditions

Preparation of Brain Slices



Optimizing Culture Conditions for Western Blots: AQP4 expression in the presence or absence of serum-free media

30 KD AQP4



samples without serum samples with 20% serum

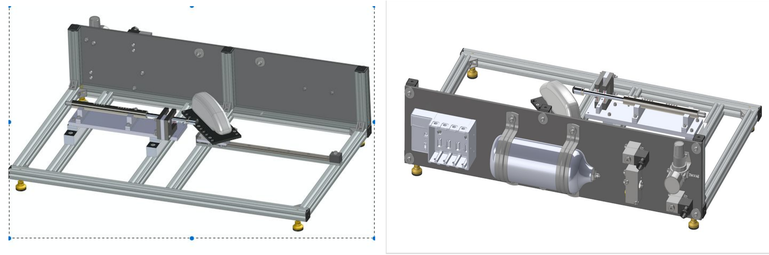
Results: Expression of AQP4 is higher in serum-containing conditions rather than serum-free conditions

Progress

- We have identified higher production of AQP4 in slices containing 20% serum than serum-free slices
- ~5 hour incubation time may be optimal for assessing AQP4 regulation

In Vivo

Momentum Exchange Model



- The momentum exchange device delivers a controlled impact to the head of an anesthetized rat. It was first developed by Viano and co-workers.
- This model is advantageous because after impact, it allows for movement of the head and neck. This mimics impact conditions in human TBI.
- Objective was to design an improved momentum exchange device. This was done in collaboration with mechanical engineers.
- Enhancements include:
  - Added degrees of freedom in the movement of animal after impact
  - Multi-axial accelerometry

Progress

- A prototype has of the momentum exchange device has been built

Future Directions

- Conduct time and dosage dependent experiments with multiple V1a antagonists in our organotypic culture conditions.
- Calibrate the momentum-exchange device using ballistic gels to simulate tissue impact.
- Screen V1a receptor antagonists *in vitro* for their effect on AQP4 and *in vivo* for effects on cerebral edema and cognitive functions.
- Test for TBI biomarkers including: Glial Fibrillary Acidic Protein (GFAP), Ubiquitin C-Terminal Hydrolase L1 (UCHL1), and S100B. These serum markers are currently used in clinical settings.
- Use the results to identify promising candidates for clinical development for the treatment of moderate and severe TBI.

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Acknowledgments

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