Repeated mild traumatic brain injury blood-brain barrier permeability in adolescent female Wistar rats at 24 hours post injury.

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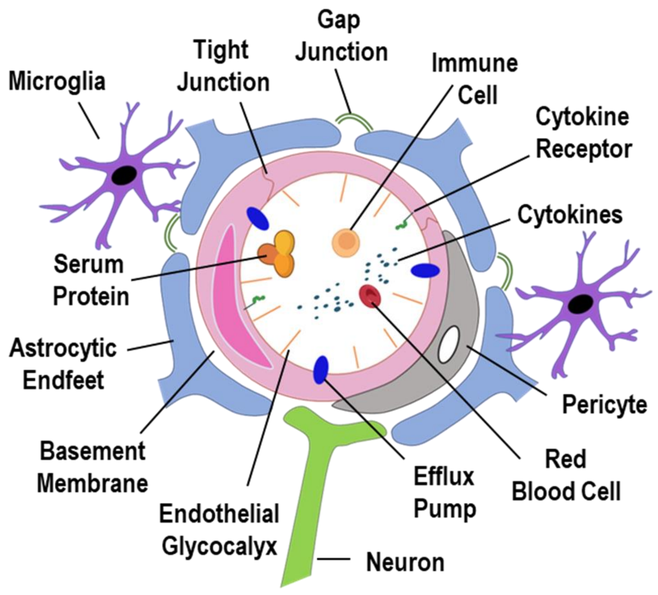
Introduction

- Adolescence is a key period for neurodevelopment and is associated with increased susceptibility to traumatic brain injuries (TBIs).
- Most TBIs are mild, but the damage from repeated mild TBIs (rmTBIs) can accumulate to levels similar to severe injury.
- The blood-brain barrier (BBB), which maintains the microenvironment of the brain, is disrupted by mild TBI.
- Here, we used Evans Blue Dye (EBD), which binds to endogenous serum albumin (normally excluded by the BBB) to assess BBB disruption.

We conducted this study to identify the timing of peak EBD extravasation following our model of rmTBI

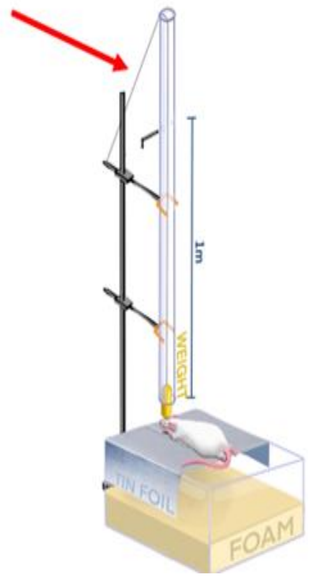
Blood Brain Barrier

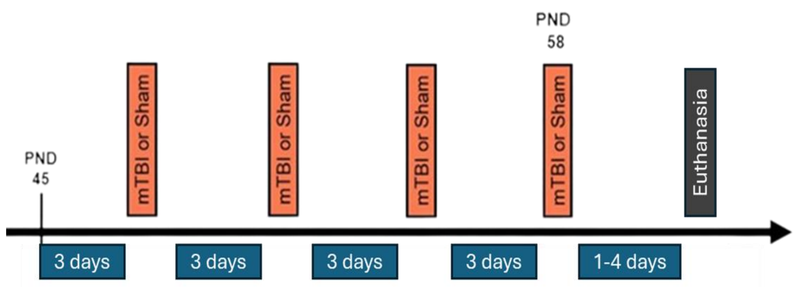
- Intricately linked cellular and molecular structure
- Maintains homeostasis in the microenvironment of brain
- Regulates transport of nutrients and solutes
- Albumin (65 kDa) is a large, endogenous protein that is normally excluded by the BBB and is this **an indicator of BBB permeability.**



Methods: TBI

- Adolescent female Wistar rats were anesthetized with isoflurane and received an injection of bupivacaine, a local anesthetic, into the scalp.
- Rats were placed upon a perforated foil sheet taped over top of a chamber containing a collection sponge.
- A 300g weight was dropped from a height of 1m, impacting the dorsal surface of the head at a force of ~2.94 Joules.
- The rat then fell through the foil sheet, landing on the sponge, and was immediately transferred to a recovery chamber and monitored until recovery.



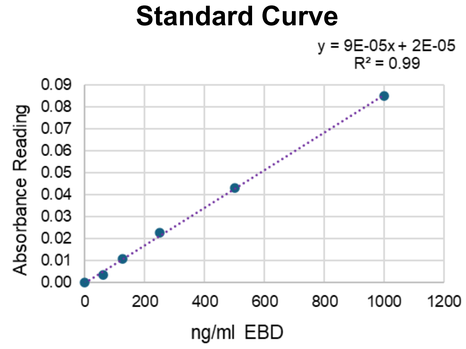


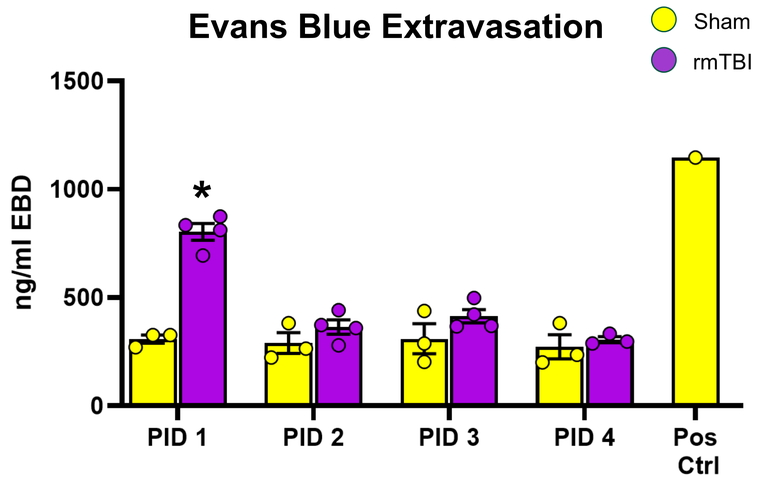
Methods: Evans Blue Dye

- Animals received either 4 rmTBIs or the sham procedure with a 96 hours between procedures.
- At 1-, 2-, 3-, or 4-days after last TBI, animals were deeply anesthetized with ketamine/xylazine and given an i.v. injection of Evans Blue Dye (EBD), which was allowed to circulate for 1h.
- Animals received a transcardial flush with heparinized saline.
- Brains were extracted, flash frozen, and stored at -80°C until processing
- Evans Blue was extracted.
- Samples were plated in triplicate in a 96-well plate with absorbance readings at 610nm.
- EBD content was quantified from a linear curve and expressed in ng/mg of brain tissue.

Results: Extravasation of EBD

- At 24h post-injury EBD extravasation was significantly increased
- EBD returned to sham levels by 48h post-injury





Conclusion

- We observed an increase in EBD in the brain tissues at 24 hours post-injury, suggesting a loss of BBB integrity.
- Ongoing experiments will increase the N and include male in order to analyze for sex differences.
- Ongoing experiments will examine whether animals that receive binge-like alcohol exposure in addition to rmTBI will show an increased, delayed, or prolonged disruption to the BBB to determine whether alcohol will exacerbate the negative effects of rmTBI.

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