Short Chain Fatty Acid Supplementation Improves Neurocognitive Outcomes After Traumatic Brain Injury In Mice

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ABSTRACT

Over 3 million Americans sustain a traumatic brain injury (TBI) annually with significant long-term morbidity resulting in motor, cognitive and behavioral deficits. Evolving evidence suggests that post-TBI disruption in the gut microbiome may play a role in these long-term neurocognitive outcomes. In particular, post-TBI depletion of commensal bacteria responsible for fermenting dietary fiber into short-chain fatty acids (SCFA) is an increasing area of interest. Severe TBI or sham-injury was induced in 15-week-old (n=32) male C57BL/6 mice via controlled cortical impact. The short-chain fatty acids acetate, butyrate, and propionate vs. vehicle were added to the drinking water post-injury. 45-days post-TBI or sham-injury, mice underwent neurocognitive testing with open-field testing and cued fear conditioning to assess learning, memory, and anxiety. Data analyzed using one-way ANOVA and Tukey's multiple comparison test. TBI mice supplemented with SCFA post-injury displayed preservation of normal anxiety-like behavior than vehicle-treated TBI mice as measured by time spent in the center region of the open field (19.75 ± 6.1% time vs. 29.9 ± 4.9% time, p=0.004) **(Figure 4).** In addition, we observed significant preservation of associative learning and memory in SCFA treated TBI mice as compared to the vehicle-treated TBI mice as measured by cued fear conditioning (68.44s ± 12.99% time freezing vs. 29.90 ± 17.77 % time freezing, p=0.0053) **(Figure 5).**

INTRODUCTION

Trauma is the leading cause of death and disability in patients between the ages of 1-44 with TBIs contributing to a nearly a third of them. Presently, approximately 2% of the U.S. population are afflicted with disabilities and behavior deficits as a result of a TBI. Despite promising preclinical data, clinical trials have failed to produce effective therapies for this highly morbid injury process.

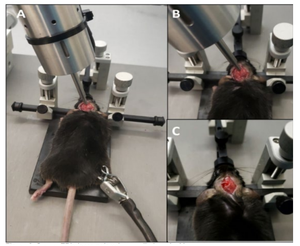
Recent studies have shown a bidirectional communication between the brain and the gut via the gut-brain axis post injury. Injury has been shown to deplete of the microbiota responsible for fermenting dietary fiber into SCFA. SCFA play a role in modulating the immune system. This metabolites signaling plays a role in blood-brain barrier (BBB) permeability, microglial functionality, and neurogenesis.

RESEARCH OBJECTIVES

Our research objective was to see if dietary supplementation with short-chain fatty acids results in a neurocognitive difference of learning, memory and anxiety in animals post-injury. To further these Aims. **We hypothesized that dietary supplementation with short-chain fatty acids would attenuate neurocognitive impairment after traumatic brain injury.**

METHODS

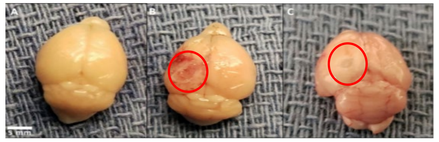
Figure 1. Severe TBI via Murine Model



(Schwulst et al, JoVE 2019)

(A) The grounding cable is clipped to the mouse's hind region and the impacting tip is lowered onto the dura mater. This is the zero point. (B) The impacting tip is retracted, a 2 mm depth of injury is dialed into the stereotaxic frame, and the impact is applied. (C) After the CCI is applied, the impacting tip is rotated out of the field and the mouse is recovered from the stereotaxic frame

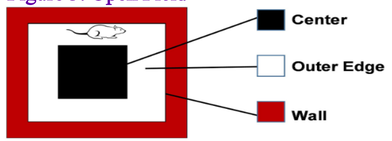
Figure 2. Murine Model Exhibit Severe TBI



(Schwulst et al, JoVE 2019)

(A) Brain from a 12-week-old naïve mouse. (B) Brain from a 12-week-old mouse 24 h after injury (C) Brain from a 12-week-old mouse 7 days after injury

Figure 3. Open Field

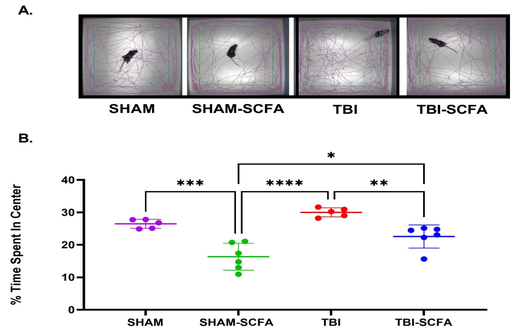


(Islaam, et al Experimental Neurology 2021)

Open field testing (OF) was an additional test for an anxiety-like phenotype in mice as well as for a generalized assessment of exploratory behavior. Anxiety is determined by the percent time spent and total distance traveled within the box .

RESULTS

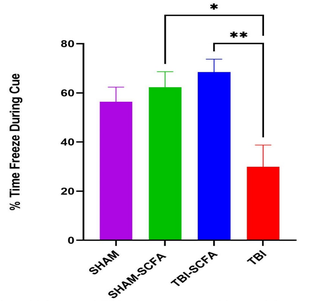
Figure 4. SCFA treated TBI Show Preservation Of Anxiety-Like



(A) Open Field tracing of mice based on regions. The blue boxes indicate center regions (B) TBI mice supplemented with SCFA post-injury displayed preservation of normal anxiety-like behavior than vehicle-treated TBI mice as measured by time spent in the center region of the open field (19.75 ± 6.1% time vs. 29.9 ± 4.9% time, p=0.004 **). With the greatest difference between Sham treated mice and TBI (16.08 ± 2.3% vs. . 29.9 ± 4.9% , p <0.0001 ****). and aged TBI (16.08 ± 2.3% vs. 5.85 ± 1.34% time, p =0.0008). Multiple comparison test shown no significant interactions between the Sham and TBI-SCFA.

RESULTS

Figure 5. SCFA Treated TBI mice Show Preservation Of Connectivity Between Amygdala, Hippocampus, and Prefrontal Cortex



SCFA treated TBI mice as compared to the vehicle-treated TBI mice as measured by cued fear conditioning (68.44s ± 12.99% time freezing vs. 29.90 ± 17.77 % time freezing, p=0.0053 **). This demonstrates preserved connectivity in the SCFA treated brain as compared to the young brain after TBI. Non-treatment group has a greater % change in learning and memory loss in compare to aged animals

CONCLUSION

Dietary supplementation with short-chain fatty acids markedly improved memory, learning and anxiety measures as compared to vehicle-treated TBI post injury.

- These data suggest preservation of the connectivity between the hippocampus and prefrontal cortex in SCFA treated TBI mice.
- Vehicle-treated TBI mice showed marked disinhibition of normal anxiety-like behavior suggesting a greater loss of connectivity between the amygdala and hippocampus as compared to SCFA treated TBI mice.
- These data suggest a therapeutic benefit of SCFA supplementation after TBI.

ACKNOWLEDGMENTS

This research was funded by NIH Grant 1R01GM130662