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Short Chain Fatty Acid Supplementation After Traumatic Brain Injury Attenuates Neurologic Injury Via the Gut-Brain-Microglia Axis

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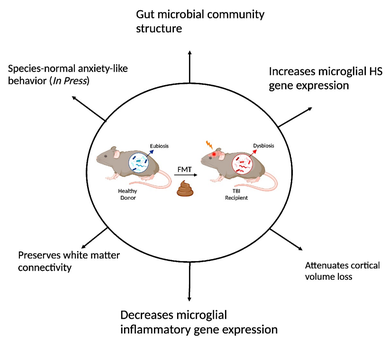
Background



Traumatic brain injury (TBI) is an underrecognized public health threat. There are limited therapeutic options for TBI, and supportive care remains the mainstay of treatment. Our previously published data demonstrate that post severe TBI fecal microbiome transplantation (FMT) can reverse TBI-induced depletion of commensal bacteria, preserve white matter connectivity, protects cognition, and decrease brain lesion size in mice after TBI. The mechanisms that underly gut bacterial modulation remain unclear.

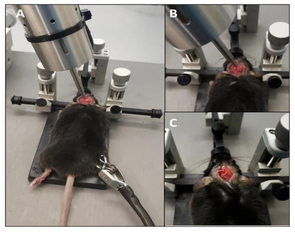
Research Objectives

We hypothesized that post-injury treatment with Short Chain Fatty Acids (SCFA), metabolites of commensal gut bacteria, would attenuate neurocognitive deficits after TBI in mice.

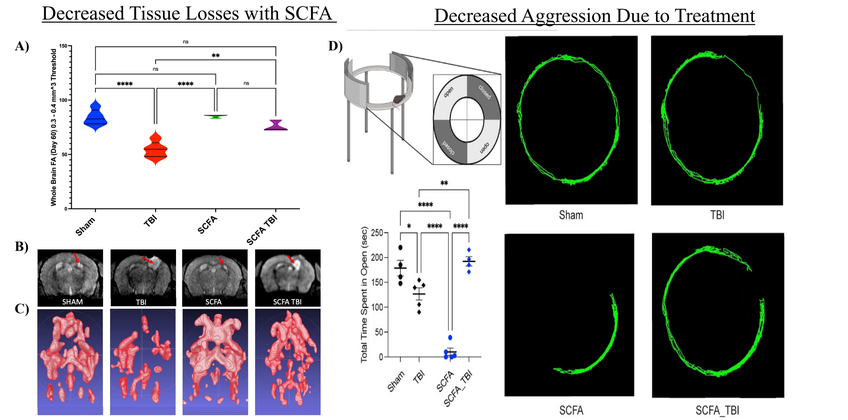
Methods

14-week-old (n=52) male C57BL/6 underwent TBI via controlled cortical impact vs. sham injury.
Post-TBI, each group was treated with **acetate, butyrate,** and **propionate** vs. salt vehicle via free access to drinking water for four weeks post-TBI.

- **Magnetic Resonance Imaging** White matter and Cortical volume loss measured at 60 days post-injury (DPI).
- **Behavioral Analysis** Open field testing to measure anxiety levels (50 DPI).
- **Neuroinflammation Measured** Brain microglia extracted to assess signs of neuroinflammation at (30 DPI).
- **Microbiome Analysis** Animal stool collected to assess microbial community structure via 16S RNA gene amplicon sequencing at (59 DPI)

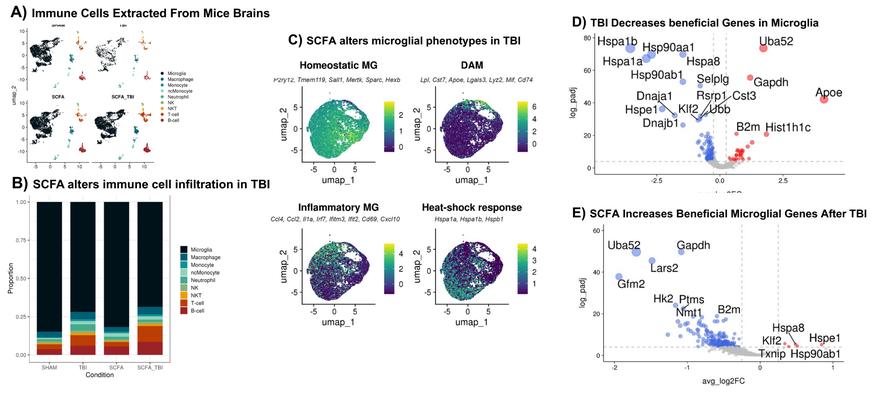


SCFA Treatment Decreases White Matter Loss and Behavioral Deficits



A) TBI is known to impede Fractional Anisotropy (white matter connectivity) in the brain. We used MRI scans of animals to measure FA through water diffusivity (60DPI). SCFA treated TBI mice were comparable sham (NS) and showed significant difference from untreated injured animals (p-value <.001)(N=3). B) Representative 3D contrast T2 weighted MRI images. C). Representative renderings of white matter connectivity. D) Open Field test used to measure Levels of Anxiety/Seeking behavior. SCFA White matter connectivity protections correlated well with amelioration of behavioral deficits in TBI. While untreated animals showed an increase in aggressive/seeking behaviors compared to Sham (p<.0001), SCFA treatment decreased TBI induced aggression by nearly 1/3rd compared to the TBI groups (p<0.001) (Two-way ANOVA)

Microglial Gene Expression Altered by Injury and Treatment

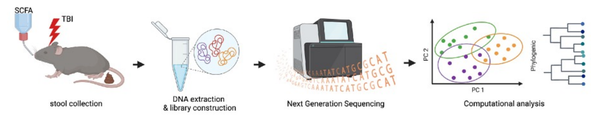


(A) Immune cells extracted & sorted. B) Immune cell proportions were revealed to be altered by injury and treatment. B) Cd45+/dim cells (microglia) were grouped by gene expression according to function. C) Plot demonstrates clear clustering of different microglia phenotypes due to treatment/injury (10X genomics) D) Volcano plot reveal functional gene expression decreases neuroprotective heat-shock genes and neurodegeneration-linked Uba52 and Gapdh in TBI mice compared to sham. E) Volcano plot reveal functional gene expression increased neuroprotective heat-shock genes and decreased neurodegeneration-linked Uba52 and Gapdh in treated TBI mice compared to untreated.

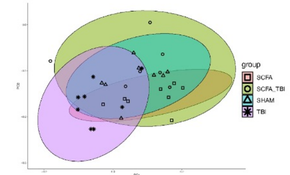
Short Chain Fatty Acids Support Gut Microbes

A) We collected stool samples and analyzed them for microbial species. B) We assessed samples for taxa similarity the unexpected protection of specific microbial species with SCFA treatment. C) DESEQ analysis found 11 differentially abundant bacteria, some associated with: Inflammation – (Clostridia1, Bacteroides3), Cognition & Neurodevelopment – (Oscillobacter1, Lachnospiracae2), SCFA production – (Blautia1, Lachnospiraceae3 & Bacteroides3)

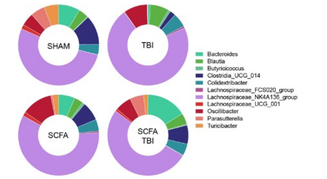
A) Stool samples processed and analyzed for microbial taxa via 16S RNA analysis



B) SCFA treated TBI α diversity similar to sham



C) SCFA treatment stabilizes microbial after severe TBI



Limitations

- Limitations to CCI include the need for craniotomy and the expense of acquiring the impactor and actuating device.
- There is a dearth of consistent foundational literature highlighting TBI in females. As sex is a confounding factor in TBI, female mice are being assessed in a separate study.

Conclusions

- SCFA treatment improved white matter connectivity, protected normal behavior patterns, and maintained healthy gut microbial species, while reducing microglial inflammatory gene expression and increasing protective heat shock protein gene expression.
- These data suggest SCFA could mimic benefits of FMT for TBI treatment, offering a novel therapeutic approach for an injury with limited treatment options

Funding

NIH Grants - R01 GM130662, R01 GM130662-01S, R21 NS116638, L70 GM151741-01, 3K99NS130277, 1K99NS130277

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