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Fecal Microbiota Transplant with Aged Gut Microbiota Increase Species-Normal Anxiety as Compared to Young Gut Microbiota and Increases Tc17 Population in the Brain in Germ-Free Mice.

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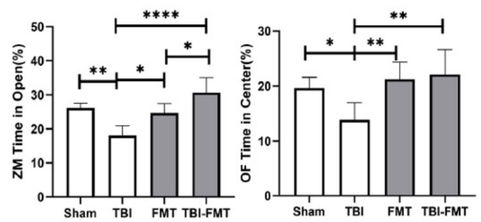
Traumatic Brain Injury (TBI)

Traumatic brain injury affects approximately 3 million Americans each year. Furthermore, aged patients (>65 years) account for an increasing proportion of TBI patients and aged patients suffer a higher rate of long-term neurocognitive morbidity as compared to their younger counterparts. **In fact, TBI is the leading non-genetic risk factor for the development of Alzheimer's disease (AD) and related dementias (ADRD).**

Brain-Gut-Microbiome Axis: Treatment for TBI

The brain-gut-microbiota connection has emerged as a potential mechanism in treating neurological diseases. Indeed, our lab has shown that *gut dysbiosis occurs in response to TBI.* Moreover, we have published that **fecal microbiota transfer (FMT) following TBI markedly reduced neurocognitive decline after injury [1]**. Interestingly, we have also shown that aging alone led to an increase of disease-associated microbial taxa and that this dysbiosis worsened after TBI. FMT also attenuates white matter loss ultimately leading to better neurocognitive outcomes in young TBI mice. **However, the potential benefits of FMT in aged TBI subjects has yet to be determined.**

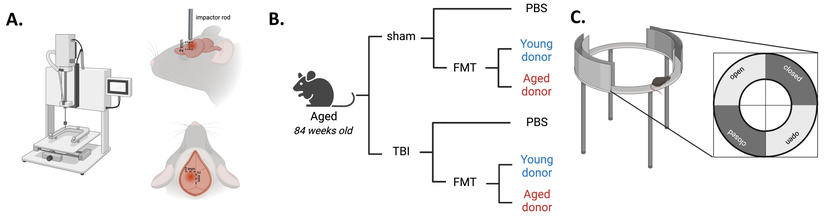


In previous studies, FMT attenuates post-traumatic anxiety and preserves exploratory behavior in mice after TBI. *FMT treated TBI mice spent a greater percentage of time in the center region of the Open Field (OF) and in the open region of the Zero Maze (ZM) as compared to vehicle treated TBI mice 45 days post injury.* ***Data from*** **Davis et al. Shock (2022) [1]**

We hypothesized that *restoring a youthful gut microbial community* structure will **improve neurocognitive outcomes in aged mice after TBI.**

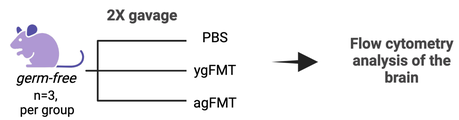
Experimental Design:

Effect of youthful gut microbiome in aged mice:



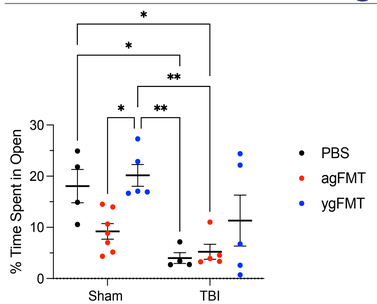
A. Controlled Cortical Impact. A consistent and clinically applicable neurologic injury of TBI for rodent models. **B. Experimental groups.** Animals either received vehicle (PBS), FMT from healthy young or aged donors following sham injury or TBI. **C. Elevated Zero Maze.** Two weeks following initial injury and respective treatments, animals were subjected to elevated zero maze – a neurocognitive test measuring species-normal anxiety. The time spent in closed and open spaces were measured to calculate percent of time spent in the open region of the maze.

Effect of Healthy Young vs Aged Gut Microbiota in Germ-Free Mice



Total 9 germ-free mice were treated twice with vehicle (PBS), or FMT from healthy young (ygFMT) or aged B6 stool (agFMT) ***in the absence of injury*** over the course of 3 days**.** 3 weeks following initial treatment, animals were euthanized for flow cytometry analysis to assess various changes that may arise from FMT in the brain.

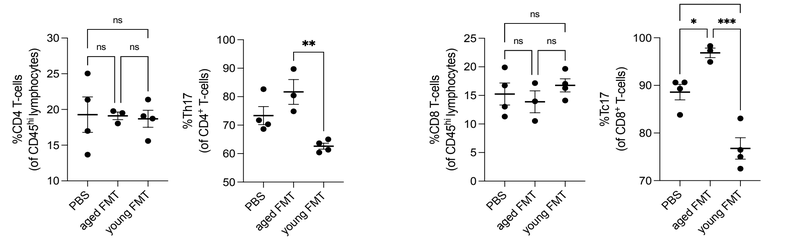
Elevated Zero Maze of aged Mice:



- Contrary to our hypothesis, FMT from young donors following TBI did not attenuate alterations in species-normal anxiety in aged mice.
- **Interestingly, FMT with stool from aged mice significantly altered species normal anxiety in sham injured mice.**

agFMT = FMT from healthy aged donors, ygFMT = FMT from healthy young donors. Two-way ANOVA with Tukey's multiple comparisons testing used for statistical analysis. Data are reported as the mean +/- standard error of the mean.

Flow Cytometry of Germ-Free Brains:



4C.A2 BD FACSymphony A5-Laser Anaylzer was used. Two-way ANOVA with Tukey's multiple comparisons testing used for statistical analysis. Data are reported as the mean +/- standard error of the mean.

- While we saw no difference in total $CD4^+$ and $CD8^+$ T-cell population in the brain across groups
  - **healthy, young B6 stool (young FMT): ↓** in Th17 (compared to aged-FMT) & **↓** in Tc17 population
  - **healthy, aged B6 stool (aged FMT): ↑** in Tc17 population

Conclusions

- FMT from youthful gut microbiota did not improve neurocognitive deficits in aged mice following TBI
- However, with sham injury alone, aged gut microbiota alone caused increased anxiety compared to those treated with young stool.
- Flow cytometry analysis of germ-free mice showed a decrease in inflammatory T-cell population (Th17 & Tc17) from young B6 gut microbiota while aged gut microbiome caused an increase in Tc17 population.
- This is consistent with previously published data regarding the role of Th17 with gut microbiome [2].

Limitations & Future Directions

- Low N's for behavior analysis → increase the number of TBI-young FMT animals
- Coinciding T-cell population responding to gut microbiome unknown → expand flow cytometry panel
- Complement flow cytometry data with cytokine analysis

References

[1] Davis, B. T. *et al.* Fecal Microbiota Transfer Attenuates Gut Dysbiosis and Functional Deficits After Traumatic Brain Injury. *Shock* **57**, 251–259 (2022).

[2] Kedmi, R. et al. A RORγt+ cell instructs gut microbiota-specific T-reg cell differentiation. *Nature*, **610**, 737-743 (2022).

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