Potential Correlation between Microglia Polarization & Synaptic Hyperconnectivity in Local Circuits in ASD

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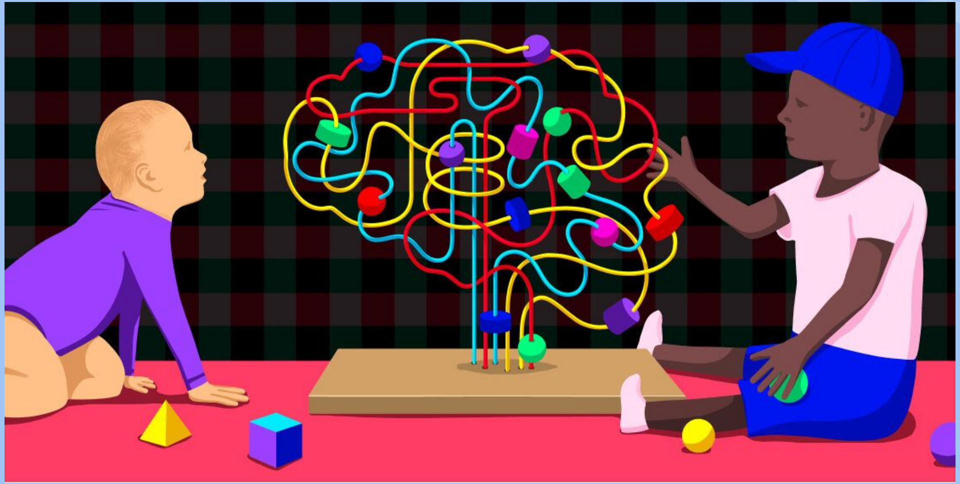
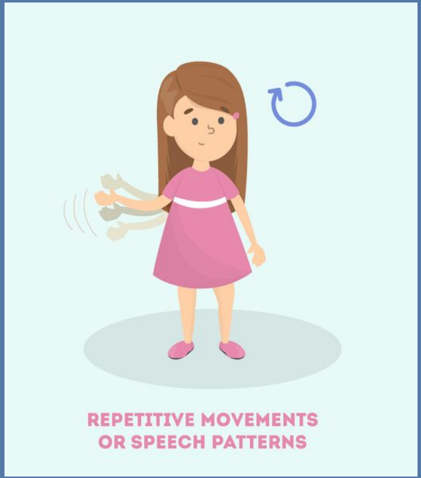


BACKGROUND INFORMATION

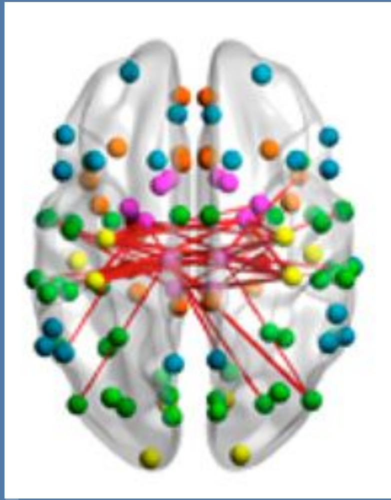
What is Autism Spectrum Disorder?

- ASD is a **neurodevelopmental** condition that affects how individuals interact with others, communicate, learn, and behave (NIMH, 2014).

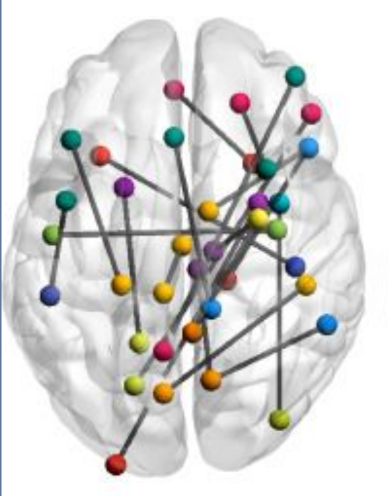


The Brain in Autism

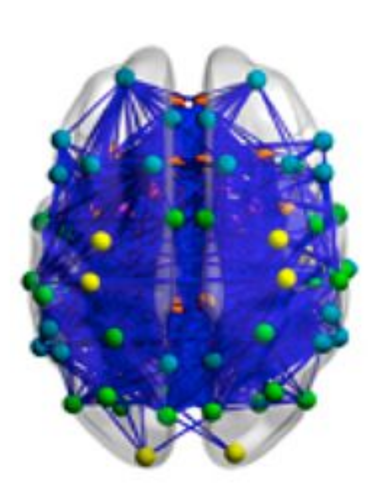
- There is **differing functional connectivity** in the ASD brain
- Long distance connections show ***hypo***connectivity while short distance connections show ***hyper***connectivity (Supekar et al. 2014),



Hypo-connectivity



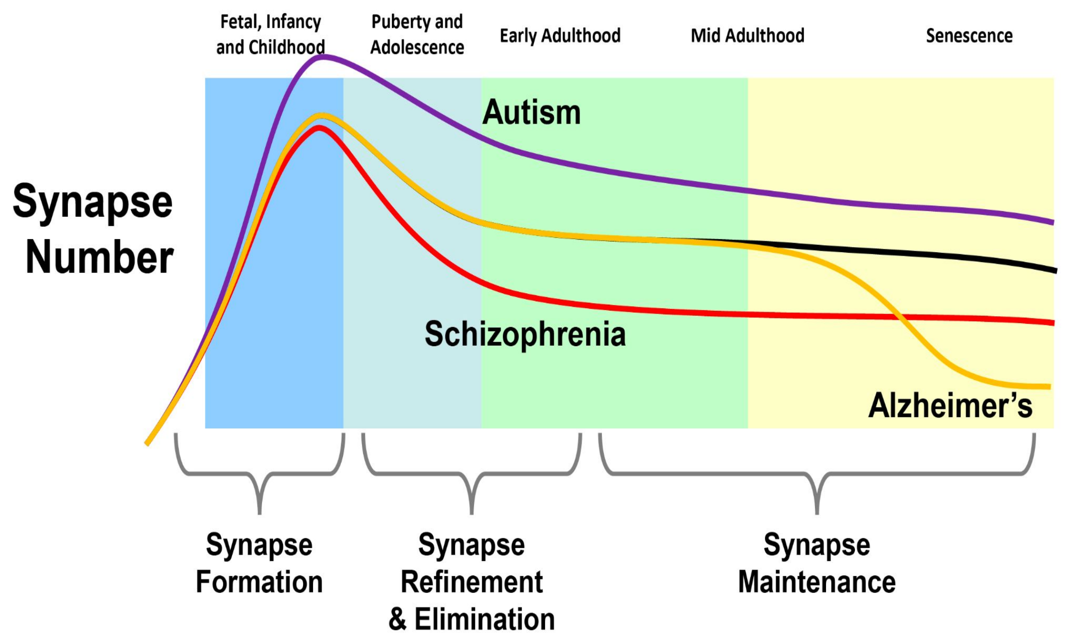
Typical connectivity



Hyper-connectivity

- **These differences** may underlie ASD behavior.
- But their cellular mechanisms are not well-understood.

Regulation of connectivity: Synaptic Pruning



Process that occurs in the brain during development in which **extra synapses are eliminated** for more efficiency (Santos, 2011)

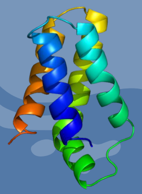
One mechanism of synaptic pruning is **phagocytosis**

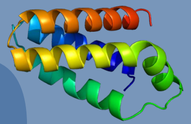
Microglia Controls Phagocytosis

- Immune cells of the brain
- Can exist in two phenotypic states

M1 Microglia: Pro-Inflammatory	M2 Microglia: Anti-Inflammatory
• Neuroinflammation • Phagocytosis of pathogens	• Restores homeostasis • **Phagocytosis of unwanted synapses**

Cambridge University Press, 2012.; Paolicelli et al., 2011





Microglia & mTOR

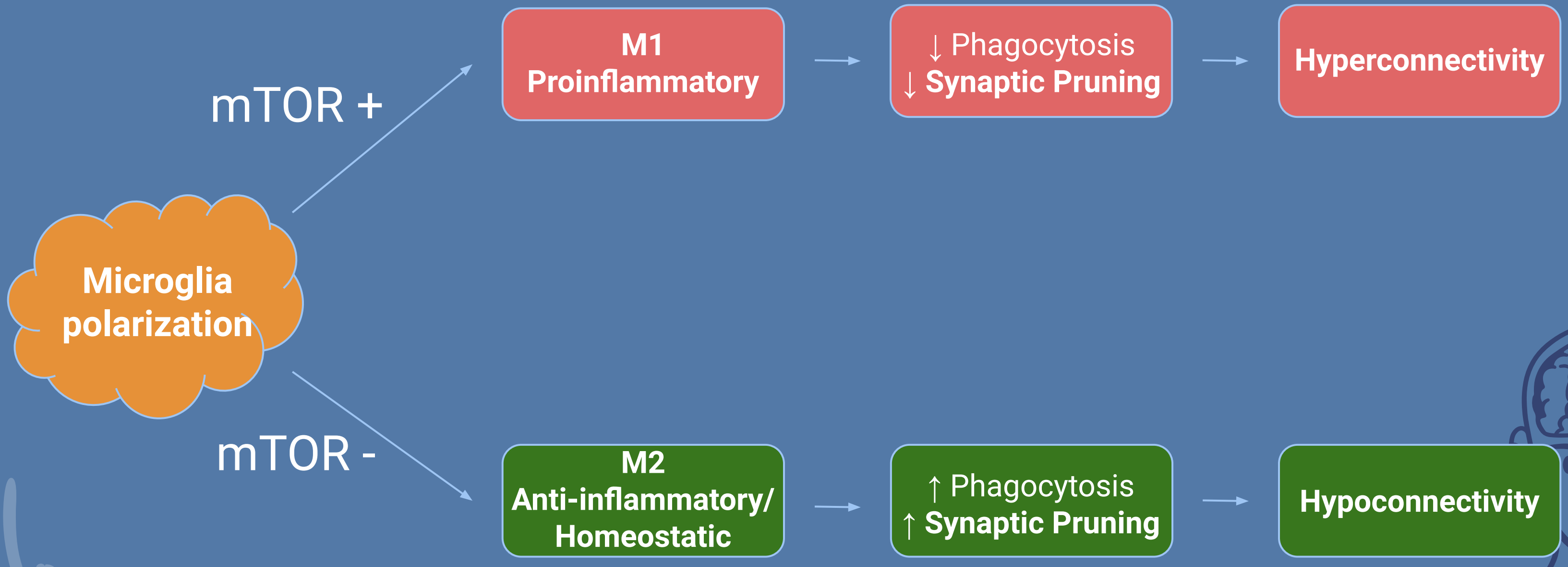
- Mammalian Target of Rapamycin (mTOR) is an enzyme that regulates cell growth, cell survival, protein synthesis, and autophagy
- ↑ mTOR results in an increase in pro-inflammatory microglia (Wang, et al., 2022)

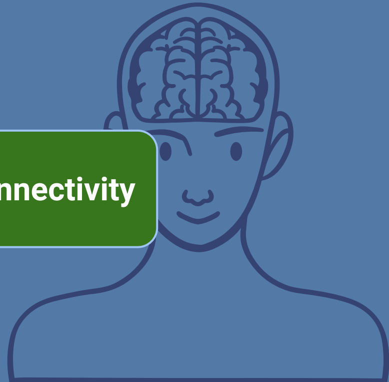
ASD & mTOR

- ↑ mTOR regulated protein synthesis can result in **hyperconnectivity** in short distance networks (Pagani et al., 2021)
- Rapamycin, an mTOR inhibitor, alleviated ASD behavioral phenotype in BTBR mouse model (Burket et al., 2014)

Background: What We Don't Know

- What is the link between **microglia polarization** and **synaptic connectivity**?
- What is the link between **mTOR activity** and **synaptic connectivity**?





GOALS & AIMS

Goals of Research

To understand the mechanisms that lead to hyperconnectivity in ASD.

General Hypothesis

In the ASD brain, mTOR activity alters microglia polarization, which disrupts circuit connectivity.

Specific Aims

- **Specific Aim 1**: To decrease mTOR activity to reduce hyperconnectivity in an autistic mouse model.
- **Specific Aim 2**: To decrease mTOR activity to promote M2 microglia polarization in an autistic mouse model.

METHODS & EXPERIMENTAL DESIGN

Animal Model of Autism: BTBR Mouse

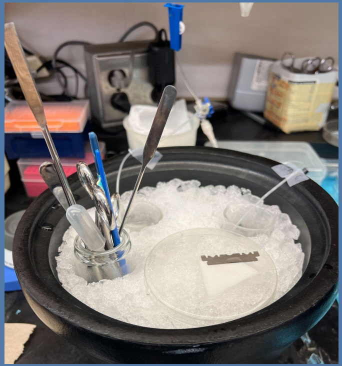
- Inbred mouse strain that demonstrates the **3 core behavioral features** of ASD
  - Deficits in social interactions
  - Unusual vocalizations
  - Repetitive behaviors

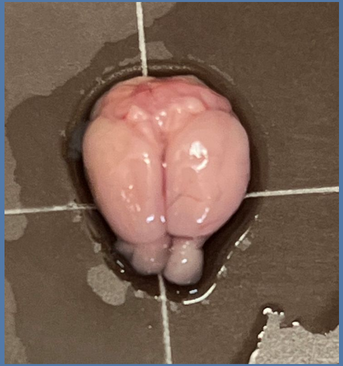
- Physical characteristics:
  - Missing corpus callosum
  - Smaller hippocampal commissure

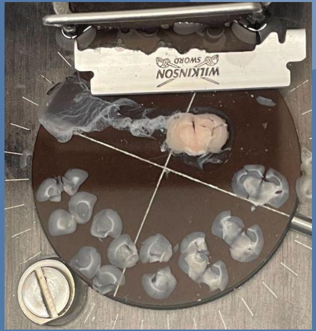
C57BL/6J

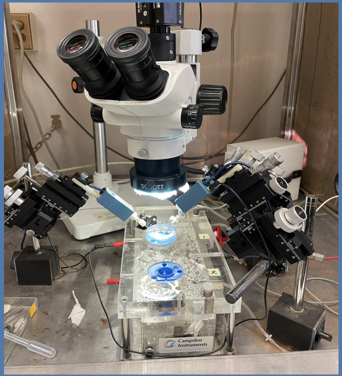
BTBR

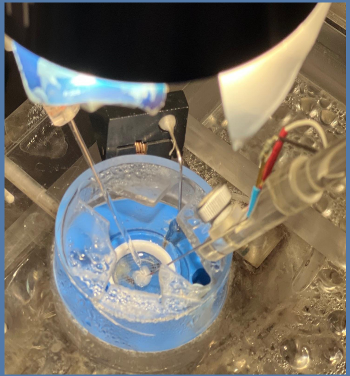
Specific Aim 1: Methods



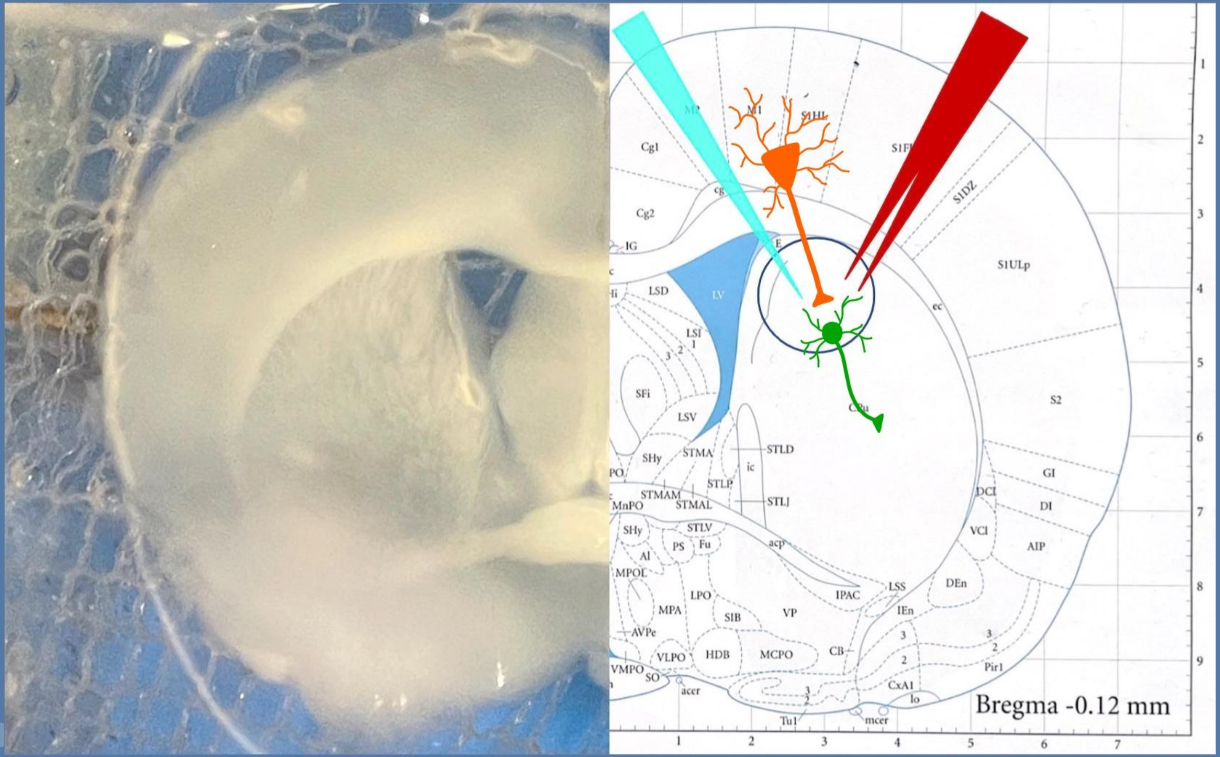








Specific Aim 1: Dorsal Striatum Recordings

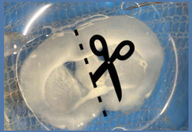


Target: corticostriatal pathway at the dorsal striatum

- Dorsal striatum supports motor behaviors frequently observed in ASD

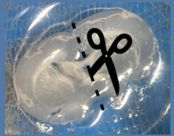
Specific Aim 1: Experimental Design

4 ♀ C57BL/6J



aCSF + DMSO ✚ aCSF + 300 nM rapamycin

4 ♀ BTBR

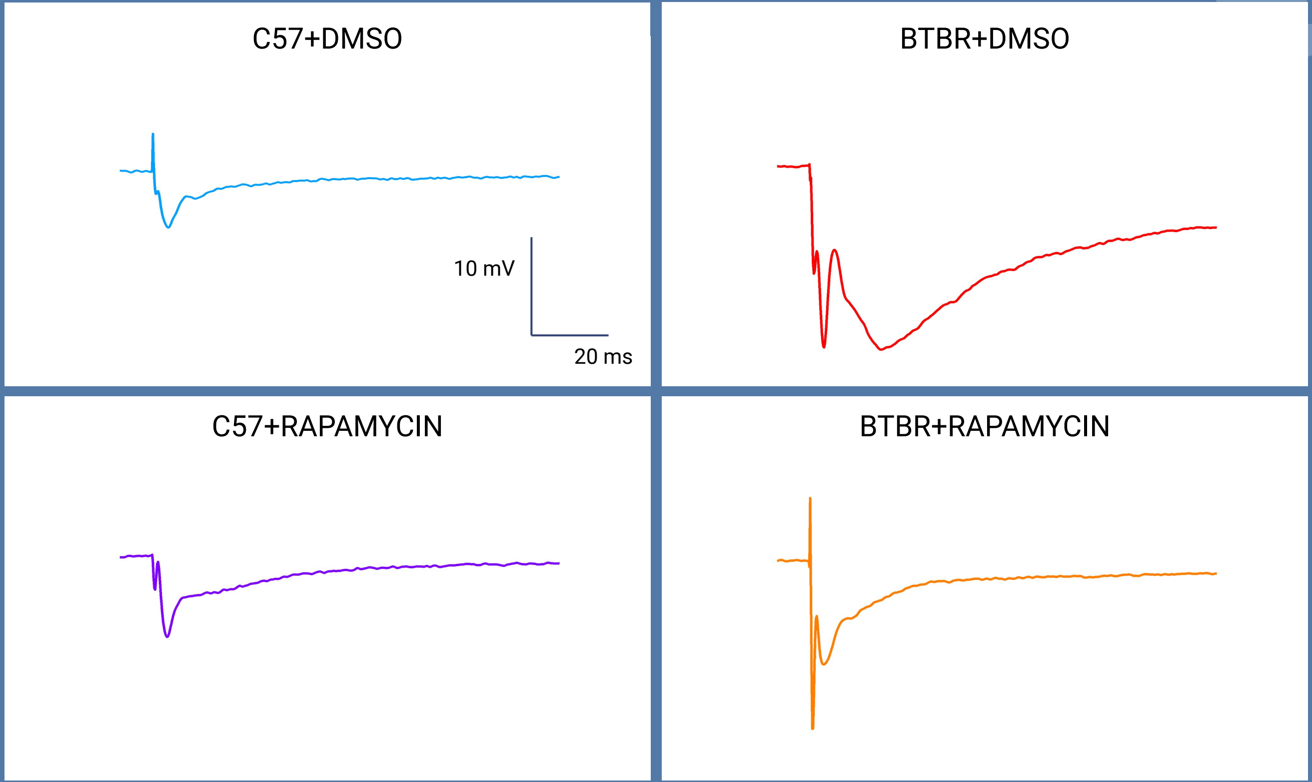


aCSF + DMSO ✚ aCSF + 300 nM rapamycin

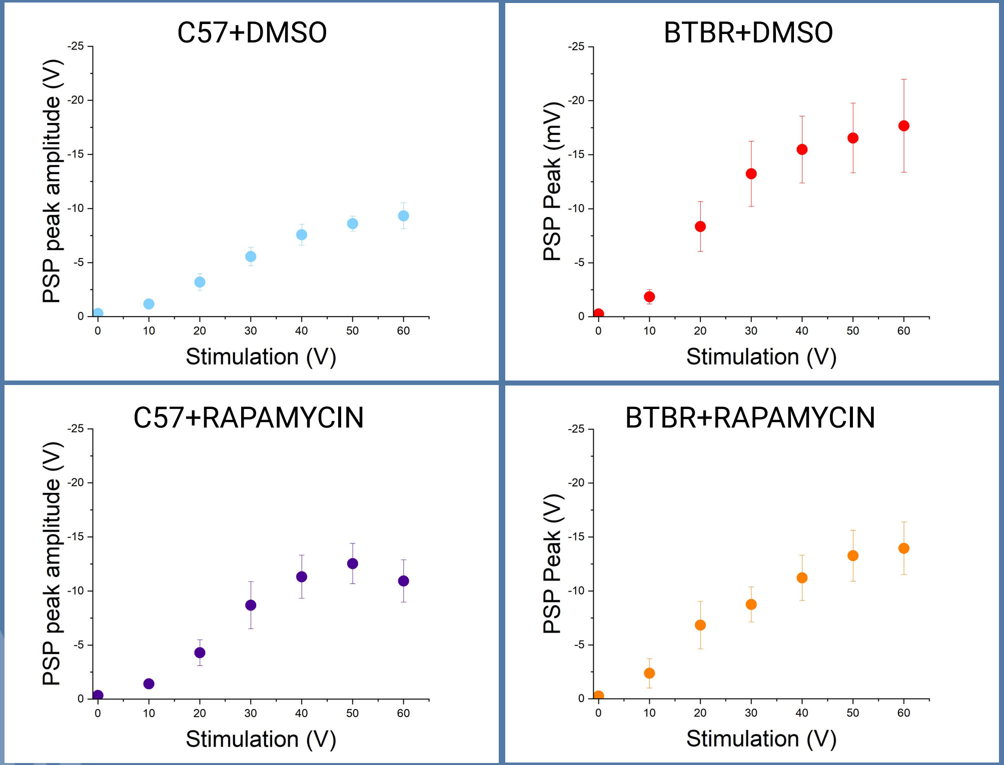
1.5 hr. incubation

RESULTS AND PREDICTIONS

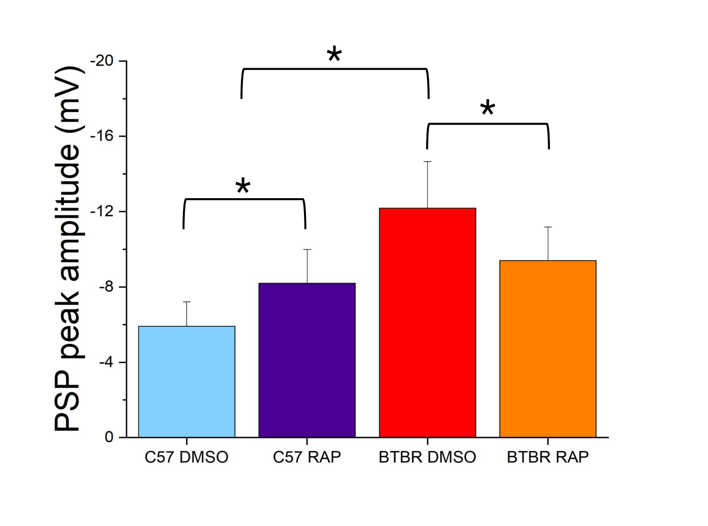
Specific Aim 1: Post-Synaptic Potentials From Dorsal Striatum



Rapamycin decreases peak of synaptic depolarization in the BTBR mouse

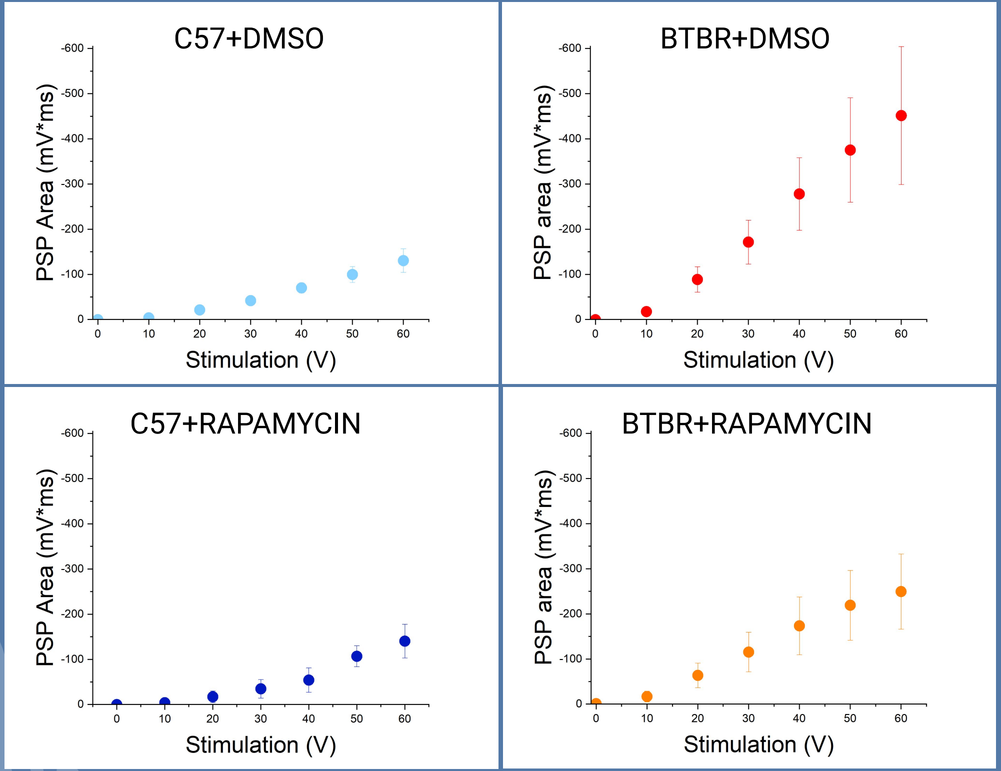


COMPARISON BETWEEN GROUPS

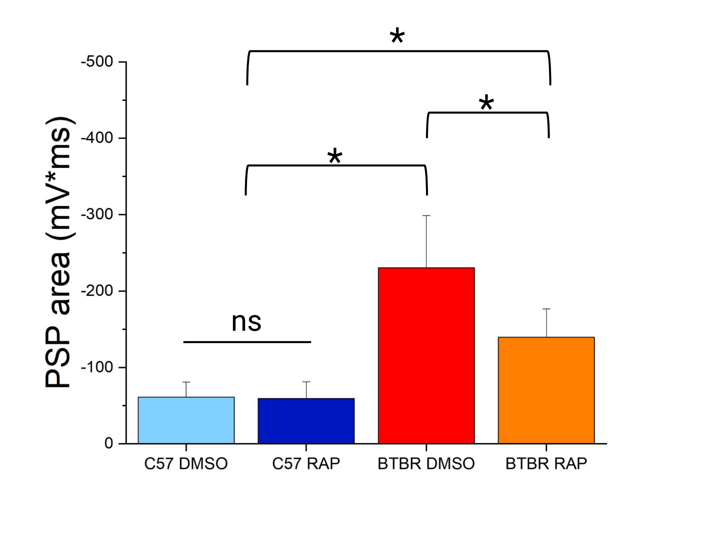


One-way ANOVA, Tukey post-hoc. * = p<0.05

Rapamycin decreases envelope of synaptic depolarization in BTBR mice

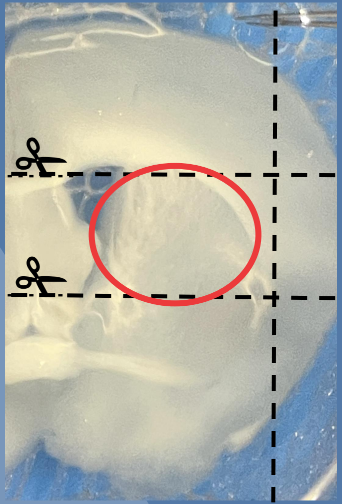


COMPARISON BETWEEN GROUPS

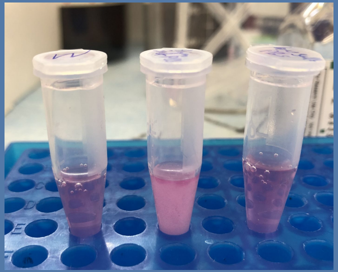


One-way ANOVA, Tukey post-hoc. * = $p<0.05$

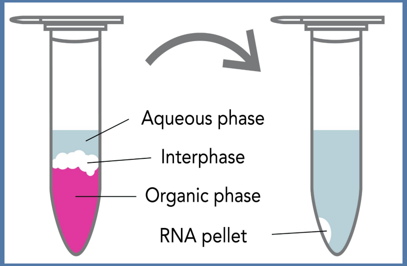
Specific Aim 2: Methods



Dorsal Striatum microdissection



Tissue collection + homogenization

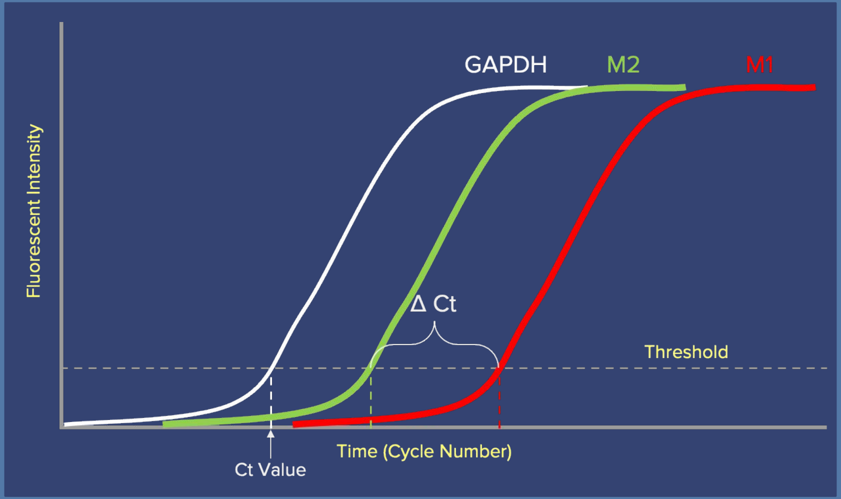


RNA Isolation

qPCR of genes of interest:
CD86 (M1 Microglia)
CD206 (M2 Microglia)

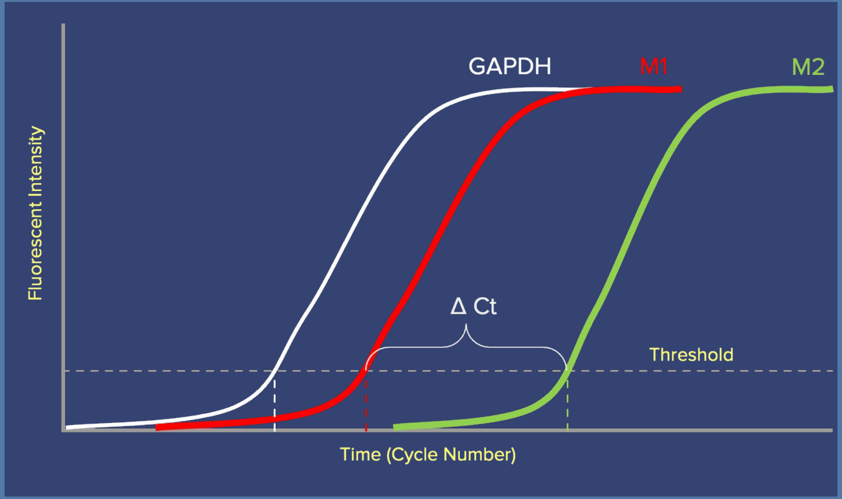
Specific Aim 2: Gene Expression Expected Results

C57BL/6J



C57: More anti-inflammatory (M2) microglia.

BTBR



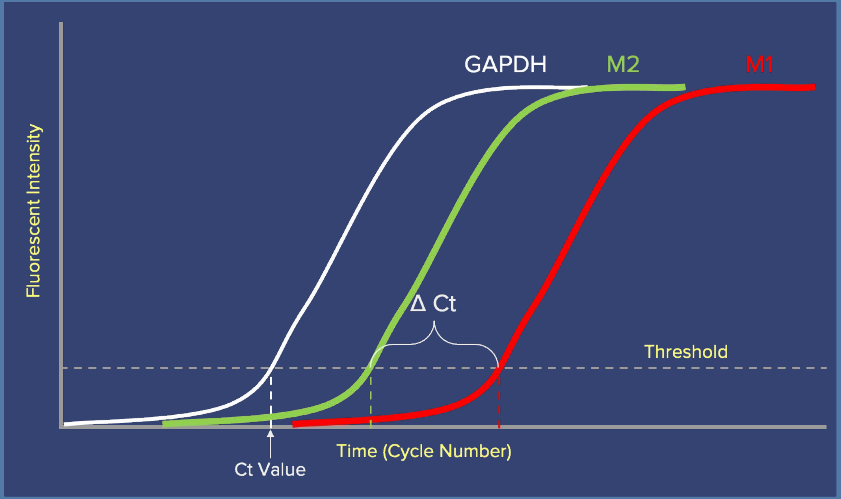
BTBR: More pro-inflammatory (M1) microglia.

CD86 (M1 Microglia)

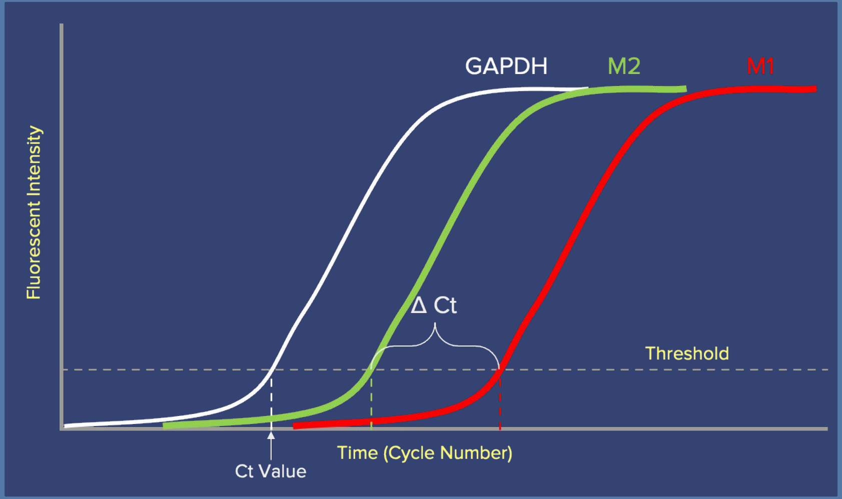
CD206 (M2 Microglia)

Specific Aim 2: Gene Expression Expected Results

C57BL/6J



BTBR + Rapamycin



C57: More anti-inflammatory (M2) microglia.

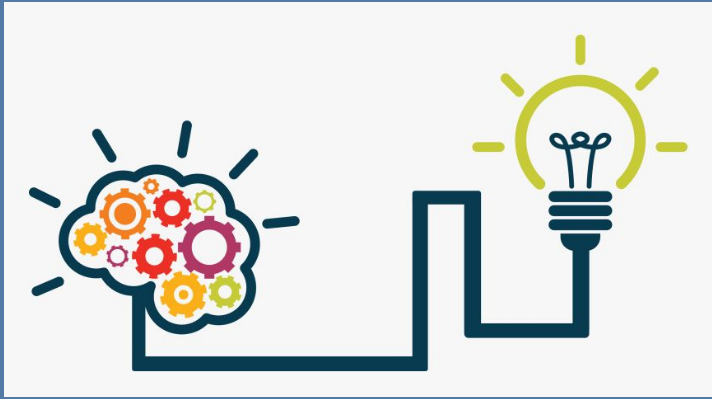
BTBR: More anti-inflammatory (M2) microglia.

CD86 (M1 Microglia)
CD206 (M2 Microglia)

CONCLUSIONS

Conclusions

- Exacerbated synaptic depolarization of the BTBR mice is decreased with rapamycin incubation.
- The level of synaptic depolarization of the BTBR mice with Rapamycin is similar to the C57 mice.



Pitfalls & Future Directions

Pitfalls

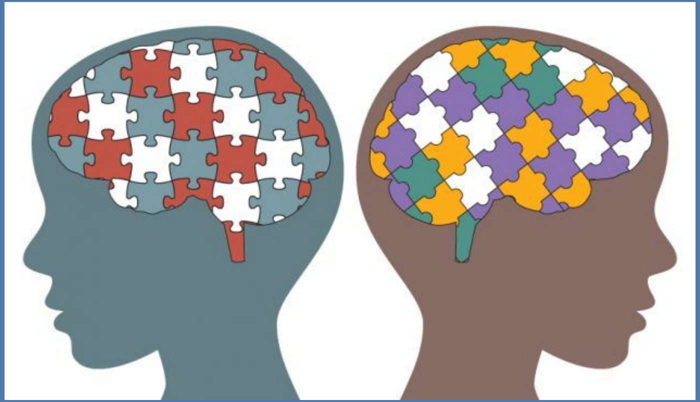
- Small sample size
- Female only population*
- Not blind to the experimental groups
- The dorsal striatum is lateralized
- Brain handling and slicing could trigger neuroinflammation
- Rapamycin may be producing other effects to reduce synaptic efficacy (i.e. down regulation of synaptic receptors)

Future Directions

- To measure dendritic spines
- To carry out qPCR for M1 and M2 microglia expression in the collected samples
- Carry out these experiments in vivo

Impact

- Add to a better understanding on the mechanisms behind hyperconnectivity in the autistic brain.
- Understanding how hyperconnectivity occurs may provide a way to address the behavioral patterns in ASD
- Pave future research on glia cell differentiation and activation in ASD.



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THANK YOU!
ANY QUESTIONS?