The Interaction of Striatal Dopamine on Glutamate-Mediated Repetitive Self-Grooming Behavior in the BTBR T+tf/J Mouse Model for Autism.

Olubusola O. Olukoya '15 and Hewlet G. McFarlane Ph.D
Department of Neuroscience, Kenyon College, Gambier OH

Introduction

Background

- Repetitive behavior is a diagnostic symptom of the autism spectrum disorder (ASD). [2]
- The BTBR T+tf/J (BTBR) mouse model for autism has been used to evaluate potential behavioral treatments for ASD. [2]
- Methyl-6-phenylethynyl-pyridine (MPEP), a selective mGluR5 antagonist, has been shown to significantly reduce repetitive self-grooming in BTBR and to up regulate dopamine (DA) release. [3] [1]
- Dopamine D1 and D2 receptors may modulate repetitive behaviors. [1]

Hypothesis

- MPEP reduction of repetitive self-grooming behavior is mediated through increased striatal dopamine (DA) acting on dopamine D1 and D2 receptors.
- Treatment of adult BTBR mice with MPEP and either SCH 23390 (SCH) or Haloperidol (HAL), dopamine D1 and D2 receptor antagonists respectively, would attenuate the effect of MPEP.

Methods

Subjects

Animals were reared from existing stock at the Kenyon College Research Laboratory (Gambier, Ohio). Standard housing weaning and feeding procedures were carried out as stipulated by Silverman et al (2012) with male and female BTBR used in relatively equal proportions. Testing was usually conducted between 0830 and 1730h and animals allowed 1h to acclimate to testing environment.

Drug Administration

- MPEP (1.0 mg/kg) was dissolved in 0.85% NaCl solution and administered 30 min before testing
- SCH (0.0075 mg/kg) was dissolved in distilled water
- HAL (0.06 mg/kg) was dissolved in tartaric acid, diluted with DI water, and brought to a neutral pH with NaOH
- Animals were administered SCH and HAL treatments 45 min before testing to allow for maximum effectiveness
- All injections were administered i.p. at vol. 3.6μL/g body weight.

Experiment 1. Locomotor Activity

Locomotor activity was assessed as total distance traveled in 30 min using VersaMax Animal Activity Monitor Cages (AccuScan Instruments, Columbus, OH, USA) and Fusion v4 software. Animals were allowed 30 min to acclimate to testing cages. Data was analyzed with a repeated measures two way analysis of variance on Prism software with α=0.05.

Experiment 1A

Locomotor activity of 28 BTBR was assayed in groups administered SAL (n=8), HAL (n=10) and SCH (n=10) treatments.

Experiment 1B

We assayed locomotor activity of 28 BTBR mice in response to MPEP double and single injection protocols (n=10 per group) with saline used both as a pre-treatment for the double-injection and as a control group (n=8).

Experiment 2. Self-grooming Assay

The behavior of animals were recorded for 10 min by placing in plastic chambers affixed with aerial Noldus video recorder. A thin layer of bedding was provided to reduce both neophobia and digging behavior, and animals allowed 5 min to acclimate to testing chambers. Both frequency (2A) and duration (2B) of repetitive self-grooming behavior were scored using Noldus Observer XT 7 software. Data analysis carried out using a one-way non-parametric analysis of variance on Prism software with α=0.05

Results

Experiment 1A

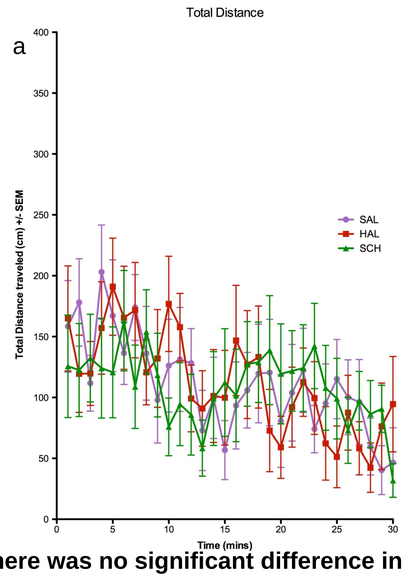

Fig 1. (a) Locomotor activity of BTBR mice in response to SAL, HAL and SCH treatments (b) Total distance moved by BTBR mice in each 5 min interval over a 30 min testing session. **Locomotor activity reduced over time** for all treatments F(29)=2.745, p<0.0001

There was no significant difference in locomotor activity in response to drug treatment F(2)=0.009676, p=0.9904, and with the interaction between drug treatment and time F(58)=0.7915, p=0.8672.

Experiment 1B

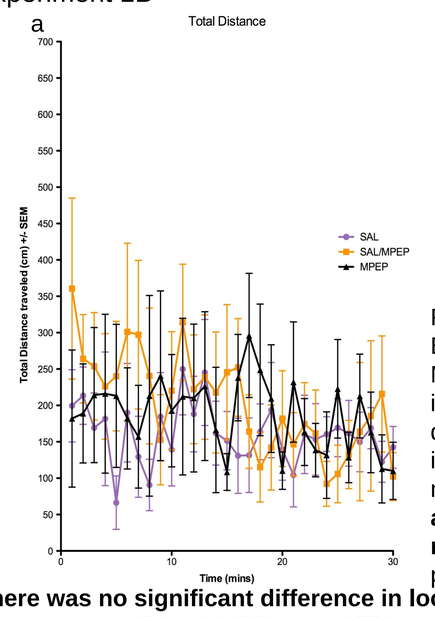

Fig 2. (a) Locomotor activity of BTBR mice in response to SAL, MPEP single and MPEP double injection treatments (b) Total distance moved by the BTBR mice in each 5 min interval over a 30 min testing session. **Locomotor activity did not significantly reduce** over time F(29)= 1.440, p=0.0642

There was no significant difference in locomotor activity in response to drug treatment F(2)=0.01574, p=0.8552, and with the interaction between drug treatment and time F(58)=1.077, p=0.3290.

Experiment 2A

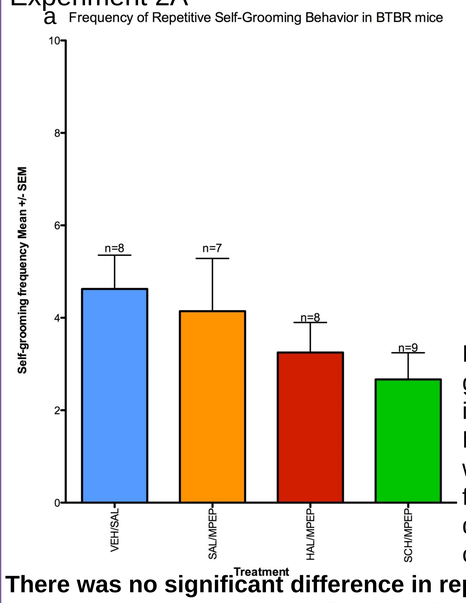

Fig 3. (a) Frequency of self-grooming behavior of BTBR mice in response to drug treatments Mean+/-SEM (b) Box-and-whiskers plot of self-grooming frequency showing the range of distribution per group with 5-95% confidence intervals.

There was no significant difference in repetitive self-grooming behavior in response to drug treatment F(3)=1.360, p=0.2753. However, there is a trend in data that pre-treatment with D1 and D2 antagonists may cause an even greater reduction of repetitive self-grooming behavior in BTBR.

Experiment 2B

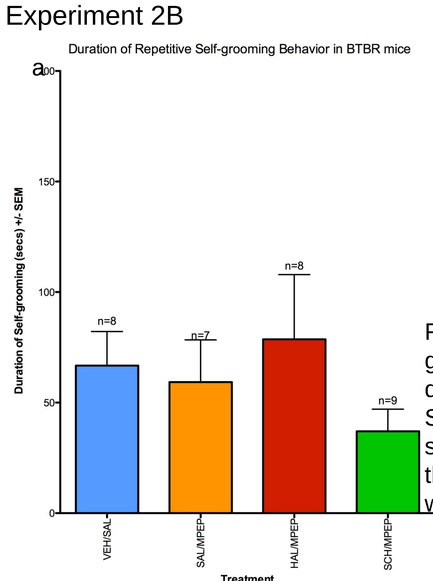

Fig 4. (a) Duration of self-grooming behavior in response to drug treatments Duration (secs)+/- SEM (b) Box-and-whiskers plot of self-grooming duration showing the range of distribution per group with 5-95% confidence intervals.

There was no significant difference in repetitive self-grooming behavior in response to drug treatment F(3)=0.8800, p=0.4633. However, data suggests a trend of greater reduction of the duration of self-grooming behavior in BTBR pre-treated with SCH than when treated with MPEP alone. HAL pre-treatment did not seem to show a similar effect.

Discussion

- Locomotor activity was not significantly affected by any of the treatments used.
- There was a significant reduction of locomotor activity over time in response in experiment 1A which may have been due to injection stress
- Results were not significant in experiment 2 possibly due to the small sample size per group
- Data trend suggests possible difference in the effects of treatments that is not in line with hypothesis.
- D1 and D2 receptor antagonists may play a role in the reduction of repetitive self-grooming behavior
- Future research should investigate same effect on greater sample size as well as the influence of D1 antagonism on the duration of repetitive self-grooming behavior.

Acknowledgements

Many thanks to Dr. Hewlet McFarlane for his patience and guidance, Becky Gallagher for her assistance with animal care, Shanon Hashman for the many (necessary) hugs, Dudley Thomas for his time, the Neuroscience Department at Kenyon and the Kenyon College Summer Science Program for the funding and support provided for the duration of the study, and to my mom for the countless hours spent going over last minute details such as the color of this poster.

References

1. Chau, P., Soderpalm, B., Ericson, M. (2011). The mGluR5 antagonist MPEP elevates accumbal dopamine and glycine levels; interaction with strychnine-sensitive glycine receptors. Addiction Biology 16, 591-599.
2. McFarlane, H.G., Kusek, G.K., Yang, M., Pheonix, J.L., Bolivar, V.J., & Crawley, J.N. (2008). Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes, Brain and Behavior 7, 152-163.
3. Silverman, Jill L., Tolu S. S., Barkan, C. L., and Crawley, J. N., (2009). Repetitive Self-Grooming Behavior in the BTBR Mouse Model of Autism Is Blocked by the MGluR5 Antagonist MPEP. Neuropsychopharmacology 35(4), 976-89.