BTBR *T+ Itpr3tf/J* mice, a suitable animal model to study anxiety and repetitive behaviors in Autism Spectrum Disorder

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BACKGROUND

Research on Autism Spectrum Disorders (ASDs) lags behind other neuronal diseases due to multiple causal factors. Animal models are valuable tools to understand the physiology of ASDs and are needed to develop novel therapeutic strategies. The current study aimed to behaviorally characterize and compare the BTBR *T+ Itpr3tf/J* (BTBR) mouse model of ASD to C57BL/6JRj (control) animals and evaluate deficits while treating mice with a serotonergic compound (LP-211) or the widely used GABAergic drug R-Baclofen.

MATERIAL & METHODS

Male BTBR mice were allocated to three different groups (n=15) and treated either with LP-211, R-Baclofen or vehicle. Additionally, 15 male C57BL/6JRj control mice received vehicle only. Behavioral tests were conducted at six different ages.

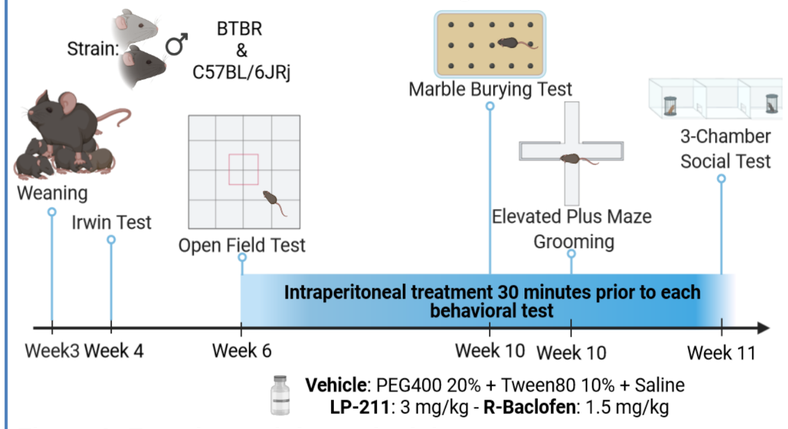

Figure 1: Experimental time schedule. Created with BioRende.com

CONCLUSION

Increased anxiety as well as stress-related and repetitive behaviors were detected in BTBR vehicle-treated mice. LP-211 and R-Baclofen treatment resulted in a trend to alleviate these behaviors in BTBR animals. **In conclusion**, these findings propose BTBR animals as a suitable animal model to study anxiety and repetitve behaviors in ASD research.

RESULTS

The **Open Field** test revealed higher thigmotaxis and lower rearing activity as well as reduced time spent in the center of the open field box (Fig. 2A-D) in BTBR vehicle-treated mice compared to C57BL/6JRj controls. These data indicate higher anxiety and compromised non-selective attention/fear-like behavior in BTBR animals. LP-211 and R-Baclofen treatment did not significantly affect those parameters.

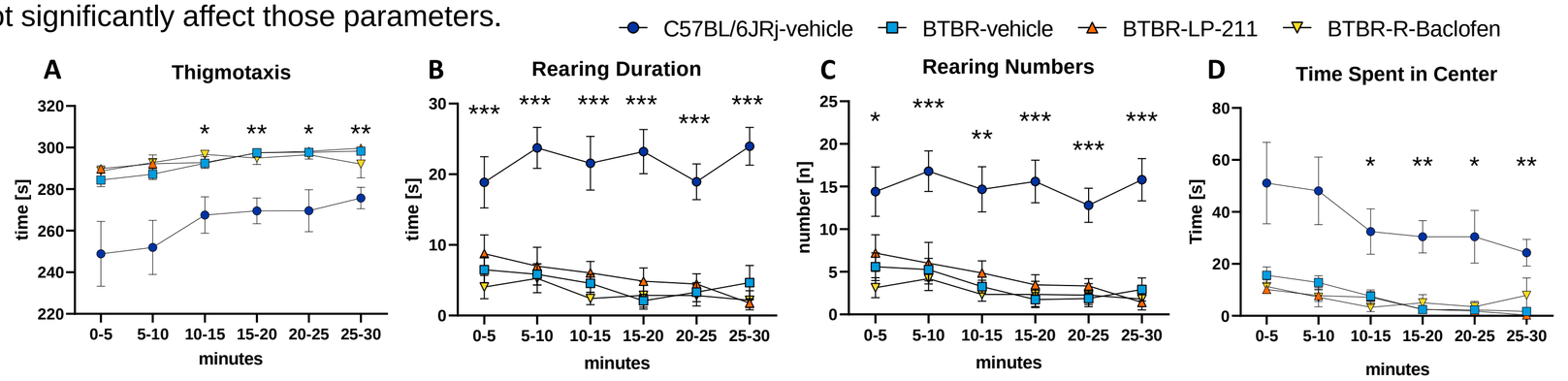

Figure 2: Open Field Test. Thigmotaxis (**A**), Rearing duration (**B**), Rearing numbers (**C**), Time spent in the center (**D**). Animals were recorded for 30 minutes and evaluated in 5-minutes intervals. Two-way ANOVA followed by Bonferroni's *post hoc* test all versus B; *p<0.05, **p<0.01, ***p<0.001.

BTBR vehicle-treated animals have a significantly higher **Grooming** duration compared to controls. Also, a trend towards a reduced grooming duration was observed after LP-211 and R-Baclofen treatment. However, number of grooming events was similar between all groups. The **Marble burying** test revealed a trend of increased digging behavior in BTBR-vehicle treated mice. Whereas LP-211 and R-Baclofen treatment of BTBR animals resulted in a trend of decreased digging behavior (Fig. 3C).

In the **Elevated Plus Maze** test a higher velocity and an increased number of entries to the closed arms suggest a higher anxiety and stress-related behavior in BTBR vehicle-treated mice compared to C57BL/6JRj animals (Fig. 4A and B). The increased frequency to enter the central zone (Fig. 4C) could further indicate repetitive behavior in BTBR mice as already shown in the Grooming and Marble burying test (Fig. 3).

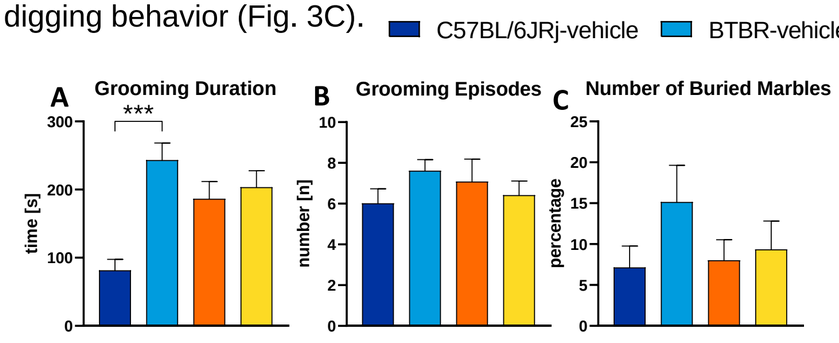

Figure 3: Grooming and Marble burying Test. Duration of Grooming (**A**), Grooming episodes (**B**), Number of buried marbles (**C**). One-way ANOVA followed by Kruskal-Wallis *post hoc* test; all versus B; ***p<0.001.

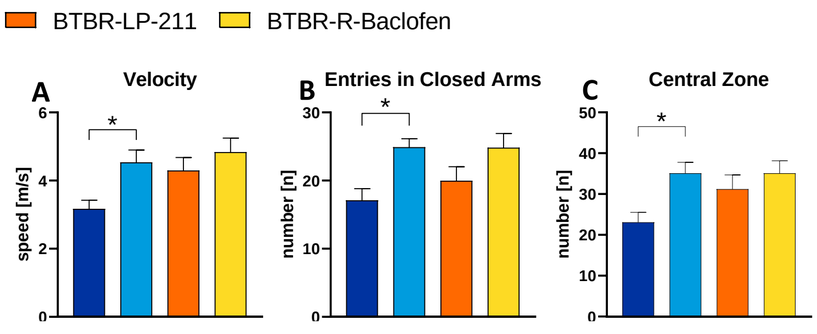

Figure 4: Elevated plus maze Test. Velocity (**A**), Number of entries in closed arms (**B**), Frequency to the central zone (**C**). One-way ANOVA followed by Kruskal-Wallis (A) and Bonferroni's (B and C) *post hoc* test; all versus B; *p<0.01.

The **Three-Chamber Social Test**, evaluating the social interaction index, frequency and time spent with stranger and novel mouse did not reveal any significant differences between experimental groups (data not shown).