Behavioral characterization of the *Fmr1*-KO mouse model of Autism Spectrum Disorder

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BACKGROUND

Fragile X syndrome is known as a monogenic cause of Autism Spectrum Disorder (ASD) and one of the most common inherited forms of intellectual disability. The *Fmr1* Knock-Out (KO) mouse model is a fundamental tool to study ASD and to assess the efficacy of pharmacological compounds ameliorating ASD-like behavior. The aim of the current study was to behaviorally characterize and compare the *Fmr1*-KO mouse model with C57BL/6JRj (control) animals. Additionally, the effect of a serotonergic compound (LP-211) and the widely used GABAergic drug R-Baclofen was tested in *Fmr1*-KO mice.

MATERIAL & METHODS

Male B6.129P2-*Fmr1*^{tm1Cgr}/J (*Fmr1*-KO) mice were allocated to three different groups (n=15 per group) 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 the age of 7 and 10 weeks.

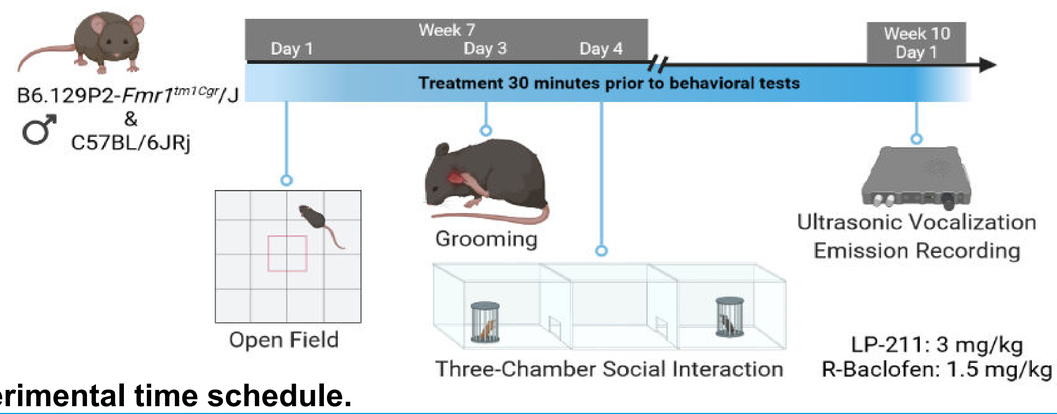

Figure 1: Experimental time schedule.

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RESULTS

The **Open Field** test revealed significantly higher activity, hyperactivity and distance moved in *Fmr1*-KO-vehicle-treated mice compared to C57BL/6JRj animals. However, the LP-211 and R-Baclofen treatment did not significantly affect those parameters.

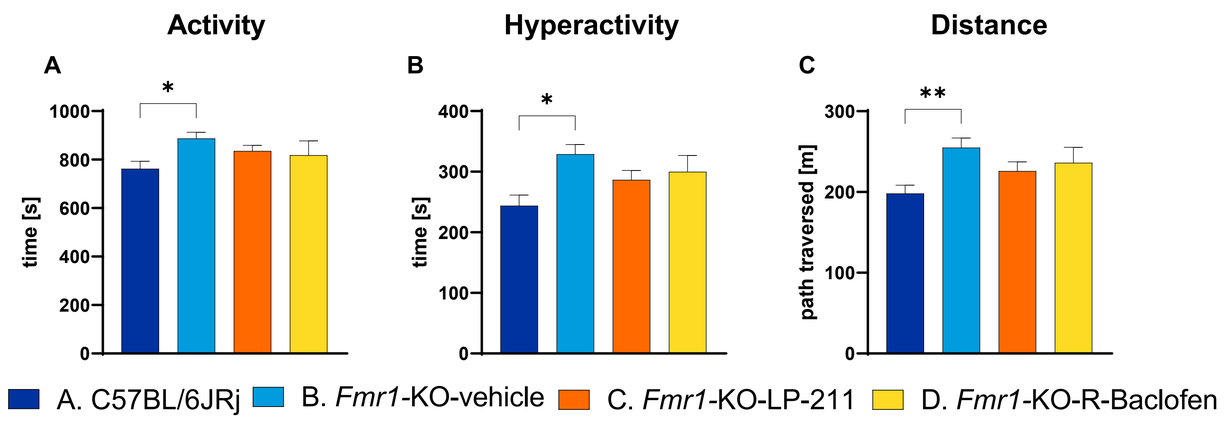

Figure 2: Open Field test. Activity (**A**), hyperactivity (**B**), distance moved (**C**). Animals were recorded for 30 minutes. Data is displayed as group mean (n=15) + SEM. One-way ANOVA followed by Bonferroni's (B) or Kruskal-Wallis (A and C) *post hoc* test; all versus B; *p<0.05, **p<0.01.

The **Grooming** test showed significantly higher grooming episodes and duration in *Fmr1*-KO-vehicle-treated mice compared to C57BL/6JRj animals. R-Baclofen treatment resulted in a significant reduction of these parameters in *Fmr1*-KO mice.

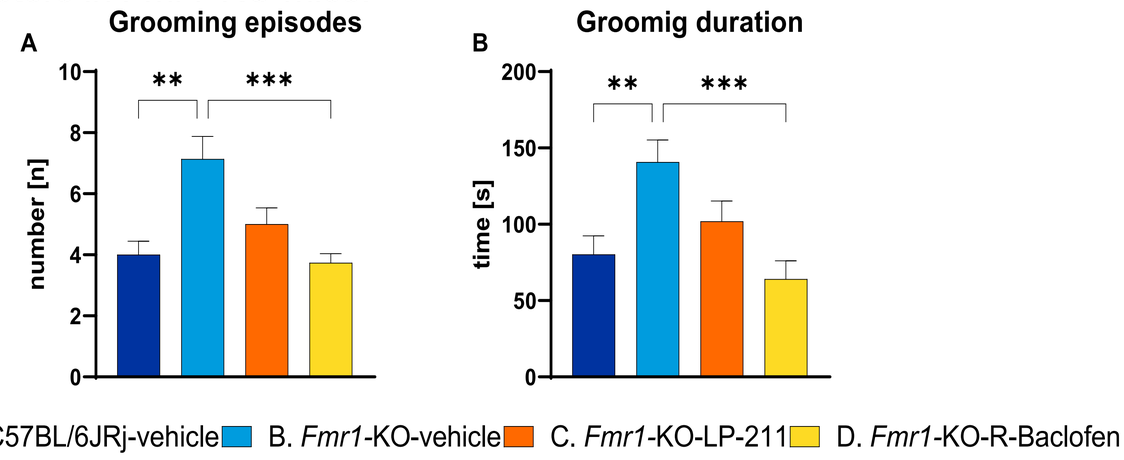

Figure 3: Grooming test. Grooming episodes (A), grooming duration (B). Animals were recorded for 10 minutes. Data is displayed as group mean (n=15) + SEM. One-way ANOVA followed by Kruskal-Wallis (A) or Bonferroni's (B) *post hoc* test; all versus B; **p<0.01, ***p<0.001.

Three Chamber Social Interaction test was performed in three phases of habituation, approach, and novelty (data from approach and novelty phases are presented here). In the approach phase, impaired social interaction and interest of *Fmr1*-KO-vehicle-treated animals compared to the control C57BL/6JRj group were detected (Figure 4 A-B). However, in *Fmr1*-KO mice, the LP-211 treated-*Fmr1*-KO mice showed a higher interest to a novel mouse in the novelty phase (Figure 4 C-D).

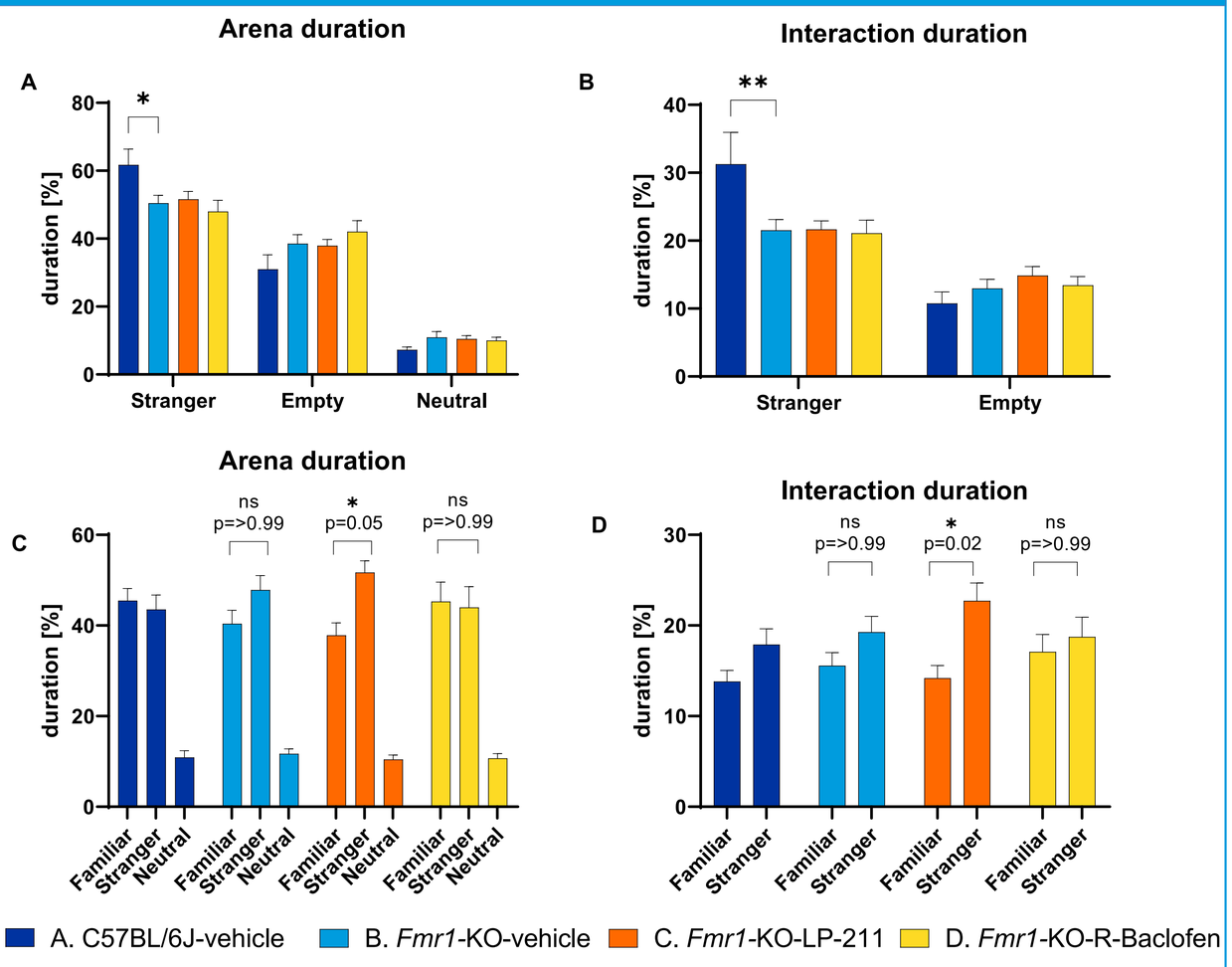

Figure 4: Three Chamber Social Interaction test: Arena duration (A) and interaction duration (B) in approach phase, arena duration (C) and interaction duration (D) in novelty phase. Animals were recorded for 10 minutes in each of the three phases. Data is displayed as group mean (n=15) + SEM. Two-way ANOVA followed by Bonferroni's *post hoc* test (A, B). Three-way ANOVA followed by Bonferroni's *post hoc* test (C, D); all versus B; *p<0.05, **p<0.01.

Ultrasonic Vocalization Emission Recording test was performed while each mouse was exposed to fresh urine of an estrous female mouse for 5 minutes. Data from these recordings showed a reduced number of vocalizations in *Fmr1*-KO-vehicle-treated mice compared to C57BL/6JRj animals, suggesting the impaired social communication and interest of these animals. LP-211 and R-Baclofen treatment did not affect those parameters.

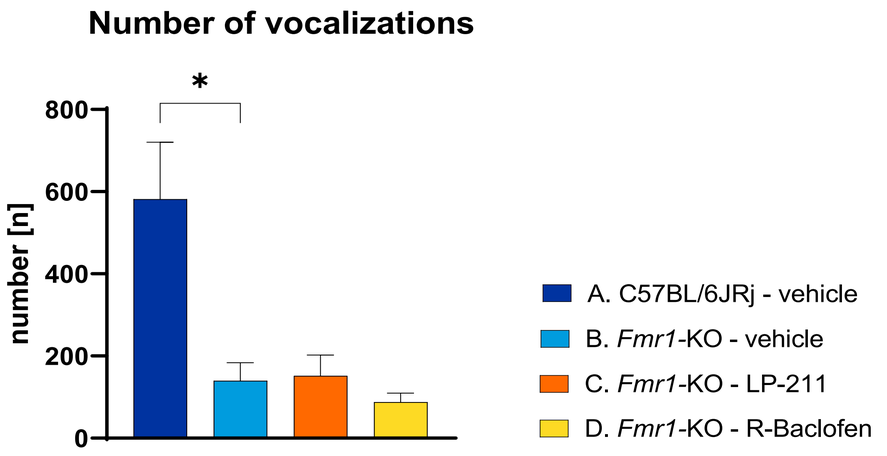

Figure 5: Ultrasonic Vocalization Emission Recording test: Number of vocalization. Recording was performed for 5 minutes. Data is displayed as group mean (n=15) + SEM. One-way ANOVA followed by Kruskal-Wallis *post hoc* test; all versus B; *p<0.05.

CONCLUSION

Increased activity, hyperactivity, and repetitive behavior, as well as decreased social interaction and impaired social communication, were observed in *Fmr1*-KO-vehicle-treated animals. R-Baclofen treatment could alleviate repetitive behavior of grooming in *Fmr1*-KO mice. In conclusion, these data propose that the *Fmr1*-KO mouse model can serve as a useful tool to investigate some core and secondary symptoms of ASD. Therefore, the *Fmr1*-KO strain could be used to design novel therapeutic approaches as an ultimate goal.

Contact for more information about the models:

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