Acquisition of Avoidance Responding in the *Fmr*1 Knockout Mouse

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Fragile X Syndrome (FXS) is the most common inherited cause of mental retardation. Much work has been done characterizing the behavioral phenotype of the animal model of FXS, the *Fmr*1 knockout mouse. However, very little literature exists on knockout performance in the active avoidance task. This study evaluated if *Fmr*1 knockouts differed from wild type littermates in avoidance acquisition. Data revealed no difference in acquisition between knockouts and wild types.

Keywords: Fragile X Syndrome, Fmr1 Knockout, Active Avoidance

In fragile X syndrome (FXS), the most common inherited cause of intellectual disability, a repeat of the trinucleotide sequence CGG (> 200) in the promoter region of the FMR1 gene (located on the X chromosome) leads to a silencing of the gene. FMR1 silencing results in a lack of fragile X mental retardation protein (FMRP) production (Brown, 2002) and, in the absence of FMRP, abnormal dendritic development occurs involving an overgrowth of immature spines (Beckel-Mitchener & Greenough, 2004; Irwin, Galvez, & Greenough, 2000). The mechanism by which this occurs is unknown, but is hypothesized to be attributed to enhanced mGluR activity (Bear, Huber, & Warren, 2004). Since FXS is an X-linked disorder, it is more common in males than females (Sherman, 2002). Males with FXS present with specific physical and behavioral phenotypes. Physically, males with FXS have long narrow faces, prominent ears, macroorchidism, ophthalmologic problems, and unusual growth patterns (initial rapid growth followed by a decline in adolescence) (Hagerman, 2002). Behaviorally, males with FXS present with hyperarousal and hyperactivity which is sometimes manifested in tantrums (Hagerman, 2002). Additionally, individuals with FXS are also commonly diagnosed with autism (Dölen & Bear, 2009).

Research has also demonstrated that individuals with FXS engage in a high degree of avoidance behavior. One proposed explanation of this behavior has been the high degree of social anxiety experienced by those with FXS which appears to manifests itself in the form of social avoidance (e.g., withdrawal, eye-gaze avoidance, self-injurious behavior (SIB), and aggression) (Kau, Reider, Payne, Meyer, & Freund, 2000). Indeed, in a survey of psychotropic medication use among those diagnosed with FXS, Valdovinos and colleagues (2009) found that approximately 36% of their sample were reported to engage in aggressive behavior, 42% in refusals or opposition, 43% in SIB, and 25% in withdrawal. These findings are significant as research has demonstrated that in a majority of cases problem behaviors such as aggression and SIB are maintained by negative reinforcement or escape from some noxious stimulus (e.g., demands, social interactions) (Iwata et al., 1994). Case in point, in a survey of families with boys diagnosed with FXS, Symons and colleagues (2003) found that a majority of their sample was reported to engage or have engaged in self-injurious behavior and that SIB was more likely to have occurred after the presentation of a difficult demand suggesting an escape function. Another possible explanation for avoidance behavior in those diagnosed with FXS is that perhaps those with FXS experience hyperarousal of the sympathetic nervous system in response to stimuli (auditory, visual, and tactile) (Hagerman, 2002) as evidence by increased cortisol reactivity (Hessl, Glaser, Dyer-Friedman, & Reiss, 2006) and increased magnitude of electrodermal activity (Miller et al., 1999).

The *Fmr1* knockout mouse has been demonstrated to be an appropriate model for the human condition as similarities have been observed in both physical and behavioral characteristics (The Dutch Belgium Consortium, 1994). In assessments of avoidance behavior in the knockout, data published have been on the knockout's performance on the passive avoidance test, an assessment of learning and memory. Results of have not revealed any differences between wild type and knockout performance (The Dutch Belgium Consortium, 1994; Qin, Kang, & Smith, 2005). However, limited data on the performance of the *Fmr1* knockout on the active avoidance test exist. The difference between these two tasks is that in one test, the ability to associate one side of a cage (*i.e.*, the dark side as opposed to bright side) with shock is measured whereas with the other test, the ability to associate a cue with the shock and subsequent avoidance of the shock is measured.

Thus, with the human condition in mind, we assessed negatively reinforced behavior in *Fmr1* knockout (KO) and wild type (WT) littermates using an active avoidance test. Given the research on avoidance behavior in individuals with FXS, we

wanted to determine if the *Fmr1* KO would show accelerated acquisition of avoidance compared to their WT littermates.

Subjects for these experiments were the offspring ($N = 10$ KO; $N = 13$ WT) of five breeding pairs obtained from Dr. James Malter's lab at the University of Wisconsin Waisman Center. The *Fmr1* KO mice were originally developed by William Greenough (University of Illinois, Urbana, Illinois) and backcrossed > 6 times to C57BL/6 mice. Breeding pairs consisted of a female heterozygous for a null mutation in *Fmr1* (McKinney, Grossman, Elisseou, & Greenough, 2005) and a male wild type. Breeding in this manner produced male offspring that were either wild type (WT) or hemizygous (*Fmr1* KO) for the null mutation. All breeding was conducted at Drake University and genotyping was performed by polymerase chain reaction at the Waisman Center (Madison, WI). Mice were weaned at 3 weeks of age, housed individually, maintained on a light to dark schedule of 12/12 hours, and had ad lib access to food and water. Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed.

Mice were trained to actively avoid a mild footshock (0.5 mA, 2 sec) by moving between chambers in an automated two-chambered shuttle box (San Diego Instruments, San Diego, CA). An avoidance trial commenced with the onset of a cue lamp. If the mouse failed to move into the other chamber during cue lamp onset (maximum of 10 sec), footshock was administered. If the mouse moved into the other chamber during cue lamp onset, avoidance was recorded by the computer. Following a 30-min acclimation period, mice were placed into a clean activity monitor. Sessions consisted of 15 avoidance trials and were conducted once a day for 10 days (Monday through Friday).

As shown in Figure 1 (left panel), avoidances increased with training (two-way repeated measures ANOVA; main effect of day, ($F(9,21) = 34.05$, $p < 0.001$). However, there was no significant effect of genotype, ($F(1, 21) = 0.19$, $p = 0.67$) or interaction between day and genotype ($F(9, 189) = 0.76$, $p = 0.66$). The right panel of Figure 1 illustrates data for the number of escape responses, which is the number of times mice crossed to the dark side of the chamber once shock was delivered. Again, there was a main effect of day ($F(9, 21) = 27.76$, $p < 0.001$) but

there was no effect of genotype ($F(1, 21) = 2.14$, $p = 0.16$) or interaction between day and genotype ($F(9, 189) = 1.28$, $p = 0.25$). Figure 2 (left panel) shows the number of inter-trial interval crosses for both *Fmr1* KO and WT. As with the other measures, there was a main effect of day ($F(9,21) = 13.78$, $p < 0.001$) but no effect of genotype ($F(1, 21) = 0.72$, $p = 0.41$) or interaction between day and genotype ($F(9, 189) = 1.39$, $p = 0.20$). Finally, the right panel of Figure 2 depicts data for the average latency of crossing after presentation of the cue light. For each mouse on each day a latency score was calculated by taking the mean latency of the 15 trials. Trials in which there were no avoidances were assigned a latency of 10 sec. The data points on the graph are group means of these latency scores. Two-way repeated measures ANOVA revealed main effect of day ($F(9,21) = 28.88$, $p < 0.001$) but not genotype ($F(1, 21) = 0.32$, $p = 0.58$) or interaction between day and genotype ($F(9, 189) = 0.74$, $p = 0.68$).

Based on the high degree of avoidance behavior observed in individuals with FXS, we had hypothesized that the *Fmr1* KO mice would demonstrate a more rapid acquisition of avoidance responding than their WT counterparts. These data, however, demonstrate that *Fmr1* KO do not have enhanced avoidance learning which is similar to what was observed in the passive avoidance test (The Dutch Belgium Consortium, 1994; Qin et al., 2005). Performance on negatively reinforced behavior (lever press) has also been evaluated in the *Fmr1* KO (Brennan, Albeck, & Paylor, 2006). Researchers had found that although WT mice were able to avoid shock delivery as the study progressed, the *Fmr1* KO mice were not. It appeared that an operant avoidance task may not be the most appropriate paradigm to study avoidance responding in the *Fmr1* KO as the *Fmr1* KO never acquired the lever-pressing response. Our data suggest that although the *Fmr1* KO mouse is a valid model for many of the behaviors often observed in those with FXS, with regards to aversion of noxious stimuli, this mouse might not demonstrate the heightened aversion often observed in individuals diagnosed with FXS. Further research should be conducted with other behavioral tests to determine if heightened avoidance of noxious stimuli is present in the *Fmr1* KO model and thus congruent with what is observed in humans with FXS.
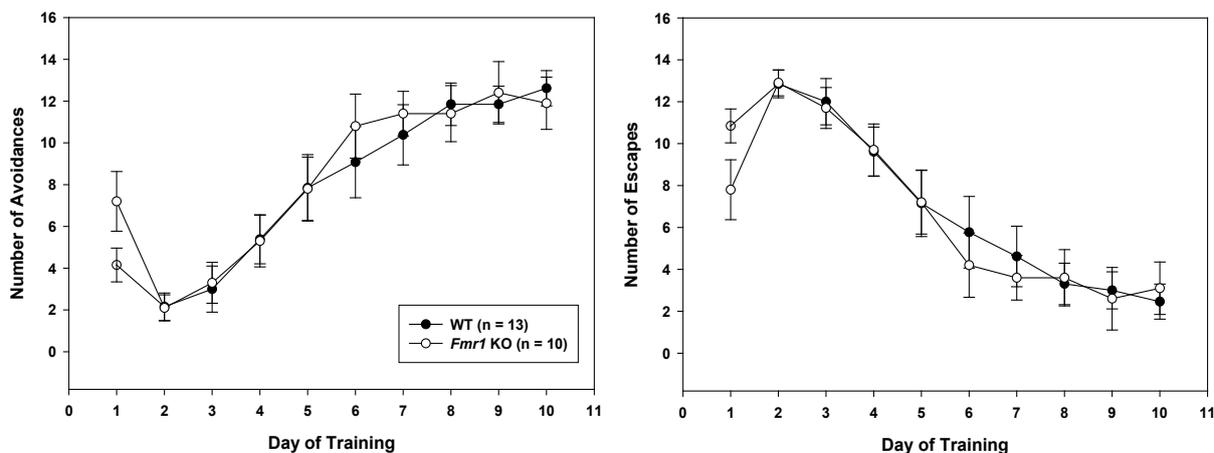

Figure 1.
Left Panel: Number of avoidance crossings for wild type (WT) (N = 13) versus Fmr1 knockout (KO) (N = 10) mice per day of active avoidance training. Right Panel: Number of shock escape crossings for WT versus Fmr1 KO mice per day of active avoidance training.
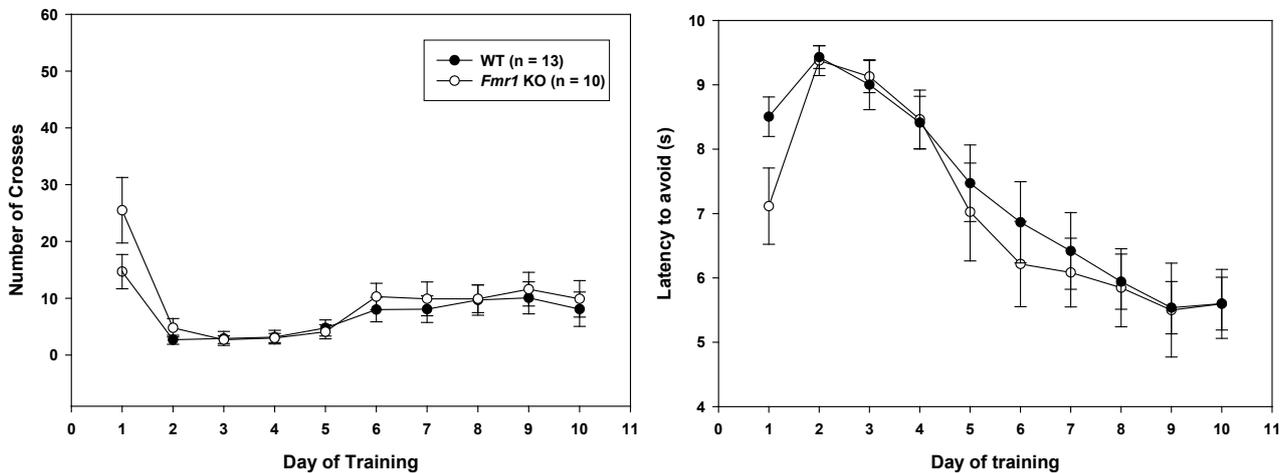
Figure 2.

Left Panel: Number of inter-trial interval (ITI) crossings for WT versus Fmr1 KO mice per day of active avoidance training. Right Panel: Mean latency to cross into dark chamber after cue light presentation for WT versus Fmr1 KO mice per day of active avoidance training.

Acknowledgements

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