Auditory Hypersensitivity in a Rat Model of Fragile X Syndrome

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Abstract

Autism spectrum disorder (ASD) is a neurological disorder characterized by impaired social skills and sensory deficits. Fragile X syndrome (FX) is the leading known inherited cause of ASD and symptoms associated with FX are similar to those of ASD, particularly auditory hypersensitivity and impaired communication [1]. The known genetic cause of FX allows for the creation of animal models and supports the investigation of the fundamental neurological impairments underlying ASD. We utilized a sound avoidance paradigm to assess loudness sensitivity in a rat model of FX (Fmr1 KO rat). In this paradigm, rats were free to move between a preferred (dark and enclosed space) and an innately unfavorable (bright and open) environment. Both Fmr1 KO and WT littermates remained in the preferred environment until an aversive sound was played. However, in comparison with WT animals, the Fmr1 KO rats left the preferred environment at lower loudness levels, indicating loudness intolerance in Fmr1 KO animals. We assessed hearing function by measuring auditory brainstem responses (ABRs) and found no significant difference between genotypes. Future studies will examine the neural mechanisms underlying loudness intolerance in these animals.

Materials and Methods

- We bred heterozygous Fmr1 females (SAGE Labs) with wildtype (WT) males (Charles River Labs) to produce male WT and Fmr1 KO littersmates.
- Fmr1 KO and wildtype (WT) littersmates were subjected to a Sound Avoidance Paradigm which consisted of an innately preferred (dark) and unfavorable (light) environment that the animals could freely move between (Fig. 1A).
- Following habituation to the apparatus, animals were subjected to three 10 minute (600 seconds) trials per test day (Fig 1B).
- On Baseline days (1-3) rats were housed in a neutral light- dark preference (assessed by time spent in dark) was established for each animal.
- After baseline testing, animals were subjected to 3 conditions (silence, 65 dB, and 90 dB) to measure light- dark preference.
- For ABR recordings animals were anesthetized using a Ketamine/ Xylazine mix (75/7.5 mg/kg).
- ABRs were collected, and analyzed using the HYS (Intelligent Hearing System) in response to clicks, 4kHz, 8kHz, 12 kHz, 16kHz, 24 kHz, and 32 kHz from 0 to 100 dB SPL (Fig 2).

Figure 1A

![Figure 1A](https://www.mousephenotype.org/impress/protocol/149/7)

**Dark and Enclosed (Preferred)**

**Bright and Open (Innately Unfavorable)**

![Figure 1B](https://www.mousephenotype.org/impress/protocol/149/7)

**Baseline**

**Broadband Noise**

**Day:** 1 2 3 4 5 6

![Figure 2](https://www.mousephenotype.org/impress/protocol/149/7)

Figure 2

- **Figure 2a.** Rat BrainstemEATURE
- **Figure 2b.** Example ABR waveforms from WT and Fmr1 KO rats. No difference in ABR thresholds between WT and Fmr1 KO animals.

Figure 3

![Figure 3](https://www.mousephenotype.org/impress/protocol/149/7)

**During baseline testing, both Fmr1 KO and WT rats show an innate behavioral preference to the dark.**

Figure 4

![Figure 4](https://www.mousephenotype.org/impress/protocol/149/7)

**As all animals (Fmr1 KO and WT) are exposed to increasingly loud sounds they spend less time in the dark and more in the light. This indicates a sound avoidance response and is a way to quantify the aversiveness of a sound.**

Figure 5

![Figure 5](https://www.mousephenotype.org/impress/protocol/149/7)

**The Fmr1 KO rats show a greater sound avoidance behavior than WT rats. The Fmr1 KO rats spend less time in the dark as intensity increases compared to WT rats. A two-way ANOVA demonstrates a significant main effect of Genotype (F = 7.374, P = .0109) and Sound Intensity (F = 27.17, P < .0001) but no interaction (F = 1.876, P = .178). Post-hoc analysis found a significant difference in time spent in dark between WT and KO rats in presence of 90 dB sound (p = <.05).**

Figure 6

![Figure 6](https://www.mousephenotype.org/impress/protocol/149/7)

**No difference in ABR amplitude of each wave (I – V) in response to click stimuli between genotypes.**

Figure 7

![Figure 7](https://www.mousephenotype.org/impress/protocol/149/7)

**No difference in ABR latency for each ABR wave (I – V) in response to click stimuli between genotypes.**

Conclusions and Future Works

**Conclusions**

- There is no difference in light- dark preference between genotypes.
- Sound avoidance behavior is a way to quantify the aversiveness of a sound.
- Fmr1 KO rats spend less time in the dark than WT rats when exposed to aversive noise. This indicates that Fmr1 KO rats exhibit loudness hyperresponsivity.
- There is no significant difference in threshold, amplitude, or latency of auditory brainstem responses (ABRs) between Fmr1 KO and WT rats. This indicates lower level auditory function is not affected in Fmr1 KO rats and suggests that the auditory hyperresponsivity in Fmr1 KO rats originates elsewhere.

**Future Works**

- Further assessment of peripheral auditory function through distortion product otoacoustic emissions (DPOAE) and compound action potential (CAP) measurements.
- Assessment of central auditory function, (ex: recording responses from the inferior colliculus) will indicate if there is more central gain in FX models in higher regions of the auditory pathway.
- Determine if loudness sensitivity involves an emotional response, by examining anxiety tests (open field or elevated plus maze) and/or amygdala function in Fmr1 KO rats.
- These results support future studies which examine novel treatments of FX and ASD.

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References