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 littermates.
- *Fmr1* KO and wildtype (WT) littermates 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) innate 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.

Figure 1A

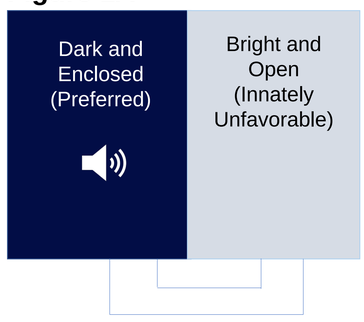
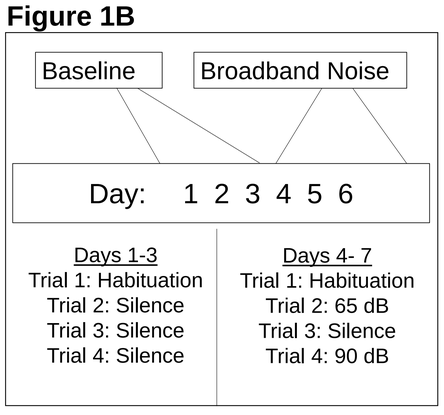
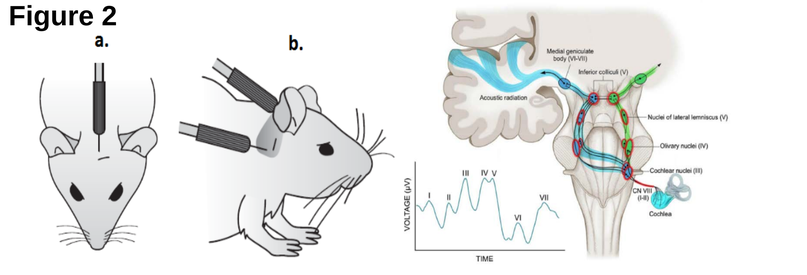


Figure 1B



- For ABR recordings animals were anesthetized using a Ketamine/ Xylazine mix (75/7.5 mg/kg)
- ABRs were collected and analyzed using the IHS (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 2

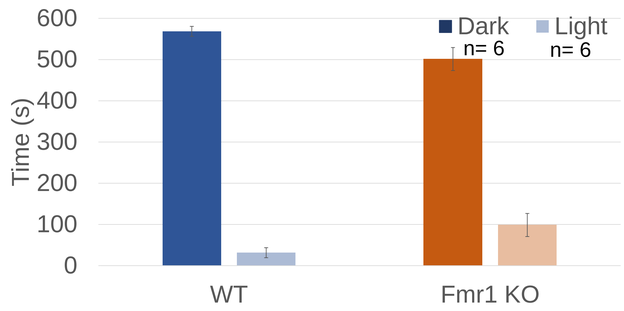


https://www.mousephenotype.org/impress/protocol/149/7

http://www.hearingfoundation.in/Images/BERA1.jpg

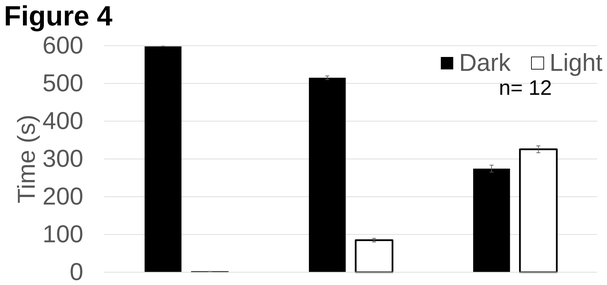
Results

Figure 3



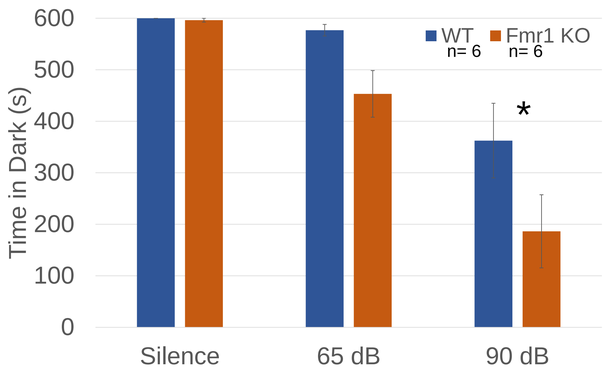
During baseline testing, both *Fmr1* KO and WT rats show an innate behavioral preference to the dark.

Figure 4



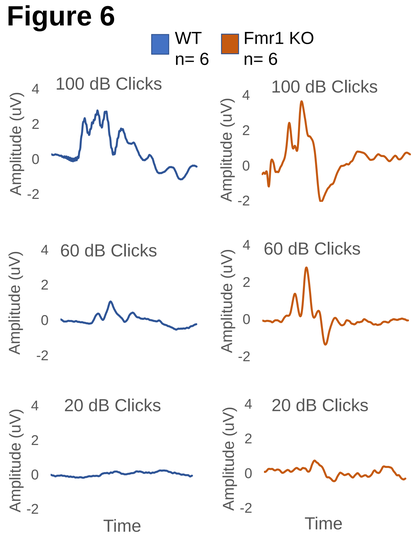
As all animals (*Fmr1* KO and WT) are exposed to increasingly loud sounds they spend less time in the dark and more in the light. This indicates a sound avoidance response and is a way to quantify the aversiveness of a sound.

Figure 5



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 2-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 ($F= 1.876$, $P= .1708$).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

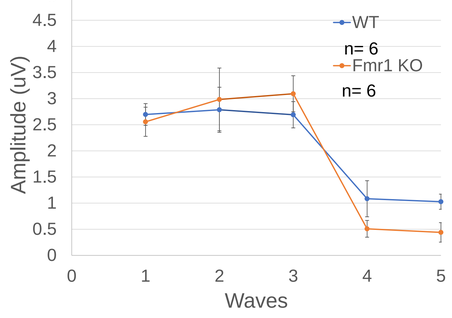


Example ABR waveforms from WT and *Fmr1* KO rats

Figure 7

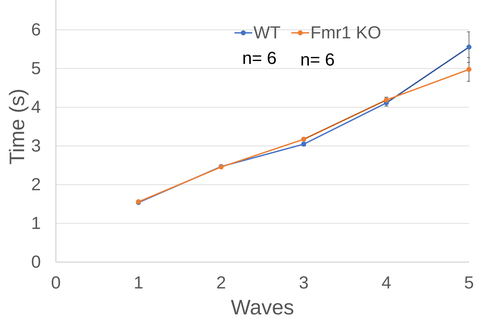
No difference in ABR threshold between WT and *Fmr1* KO animals

Figure 8



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

Figure 9



No difference in 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 aversive quality 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 hypersensitivity.
- There is not a 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 hypersensitivity 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.
- These results support future studies which examine novel treatments of FX and ASD.

Acknowledgements

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References

1- Rotschafer, S. E. & Razak K. A. (2014). Auditory Processing in Fragile X Syndrome. Frontiers in Cellular Neuroscience, 8 (19).