BEHAVIORAL PATTERNS OF AN AUTISM MOUSE MODEL WHEN EXPOSED TO AVERSIVE AUDITORY STIMULI

By

Alyssa Dominguez

A capstone project submitted for Graduation with University Honors

December 15, 2023

University Honors
University of California, Riverside

APPROVED

Dr. Khaleel A. Razak
Department of Psychology and Neuroscience

Dr. Richard Cardullo, Howard H. Hays Jr. Chair
University Honors

ABSTRACT

A leading cause of autism known as Fragile X Syndrome (FXS) is among the most prevalent causes of hereditary intellectual impairment worldwide (Stone et al., 2023). One major symptom of FXS is abnormal sensory sensitivity which may lead to elevated anxiety and behavioral problems as seen in transgenic mouse models and human patients. Studies have established that FXS model mice exhibit innate hyperactivity and hypersensitivity especially when exposed to novel environments, but the connection to abnormal behavioral patterns remains unclear (Crawley, 2007). Here, we examined adult male C57BL/6J Fmr1 KO mice and C-57 wildtype (WT) mice, in an open field test (OFT) and nest building test when challenged with aversive auditory stimuli and without such stimuli. In the OFT, we assessed velocity, distance traveled, and duration spent in each respective area to analyze anxiety-like behaviors and hyperactivity. Results revealed genotypic effects such that KO mice spent significantly less time in thigmotaxis and significantly more time in area B compared to WT mice in the presence of the auditory stimulus. In the nest building test, WT mice scored significantly higher compared to KO without the sound stimulus and introduction of the auditory stimulus resulted in a narrowing of the scores between KO and WT mice. These findings have potential implications to human patients with FXS to prevent the emergence of behavioral issues by limiting or gradually exposing patients to known environmental triggers and learning how to manage and treat such symptoms more effectively over the long term.

ACKNOWLEDGMENTS

I want to first thank my faculty mentor Dr. Khaleel Razak for giving me the opportunity to explore the field of neuroscience in-depth. He has been instrumental in the advancing and supporting of my research efforts. I want to also thank my graduate student mentor Courtney Scaramella for always being enthusiastic and available.

TABLE OF CONTENTS

INTRODUCTION

The most prevalent cause of intellectual disability worldwide and a leading cause of autism spectrum disorder (ASD) is known as Fragile X syndrome (FXS), a genetically inherited disorder. FXS is an X-linked disorder that affects approximately 1 in 4000 males, which is about two times more than in females (Niu et al., 2017). Over ninety percent of males with FXS exhibit autistic-like traits to a certain extent (Niu et al., 2017). It is brought on by the abnormal expansion, of more than two hundred copies of CGG repeats in the fragile X messenger ribonucleoprotein 1 (*FMR1*) gene, resulting in silencing of the *FMR1* gene and therefore a reduction or loss of the fragile X messenger ribonucleoprotein 1 protein (FMRP) (Niu et al., 2017). In the absence of FMRP, an RNA-binding protein that represses translation, regulation of critical synaptic plasticity and maturation is reduced leading sensory, motor, and cognitive impairments (Sidorov et al., 2013). Due to these impairments, the intelligence quotient (IQ) scores for almost all males with the full FXS mutation is below seventy (Niu et al., 2017).

The physical representations of FXS includes large ears, a long face, hyperflexible joints, and macroorchidism (Niu et al., 2017). Though we have already established that the full mutation consists of more than two hundred copies of CGG repeats, there also exists a premutation which can be subclinical. Compared to the typical of fewer than forty-five repetitions among individuals without FXS, the premutation is a greater increase of fifty-five to two hundred CGG repeats (Hagerman et al., 2009). Typically, the premutation does not result in decreased FMRP levels but rather increases the production of FMR1 mRNA and carriers may experience clinical features such as primary ovarian insufficiency and the fragile X-associated tremor/ataxia syndrome (FXTAS), which do not occur in full mutation carriers (Hagerman et al., 2009). The magnitude of intellectual disability and the physical phenotype of FXS frequently coincide with

the degree of FMRP deficiency (Hagerman et al., 2009). Similarly, the prognosis of individuals with FXS is significantly impacted by the comorbidity of FXS with ASD and the severity of ASD symptoms in these patients. Though the genetic origins of FXS are well understood, it is crucial to examine the gene-environment interactions to map out how behavioral abnormalities are expressed in FXS.

Behavioral Phenotypes

Male individuals with FXS exhibit a range of behavioral phenotypes including social anxiety, tactile defensiveness, repetitiveness, attention deficits, hyperactivity, impulsivity, and hyperarousal to sensory stimuli (Hagerman et al., 2009). The comorbidity of FXS and attention deficit/hyperactivity disorder (ADHD) is also rather prevalent even when not accounting for the severity of the autism symptoms or nonverbal cognitive abilities (Grefer, 2017). ADHD symptoms such as hyperactivity, impulsivity, and inattention may prove just as critical as cognitive abilities in predicting later socialization skills in individuals with FXS (Chromik et al., 2019). Due to these symptoms, humans with FXS are challenged with daily tasks including self-care, social interactions, and the introduction to novel environments which can worsen quality of life. Caregivers also bear significant medical and psychological challenges when taking care of FXS patients due to their often-low degrees of independence. Investigating if and how FXS patients respond atypically to a variety of stimuli allows intervention and treatment options to be explored and can promote neuroplasticity and improve well-being.

Auditory Hypersensitivity

More specific sensory dysfunction includes auditory hypersensitivity as a characteristic feature of ASD. In human patients with FXS, sensory and cognitive processing is measured by looking at event-related brain potentials (ERP), which represent the activity of populations of neurons as

they respond to specific stimuli and processes (Rotschafer & Razak, 2014). ERPs can be detected by electro-encephalograms (EEG) and magneto-encephalograms (MEG) to measure such electrical activity of the brain (Rotschafer & Razak, 2014). Previous findings indicate that auditory hypersensitivity in ASD is a response of the primary auditory cortex potentially resulting from neurological immaturity or functional abnormalities in it (Matsuzaki et al., 2012). More specifically, slower transmission and delayed response rates occur in the central auditory pathways due to decreased interneuron activity and abnormal brain connectivity and myelination processes (Matsuzaki et al., 2012). It becomes evident that hypersensitivity to auditory stimuli is significant in ASD, although difficult to investigate in humans due to its severity and ethical challenges which has given rise to another approach to analysis known as translational neuroscience.

Novel research aims to examine symptom manifestation in humans by using the *FMR1* knock-out (KO) mouse as a model for FXS as many behavioral phenotypes are conserved from human to mouse. In the prepulse inhibition paradigm, where a moderately intense prepulse tone is first introduced and suppresses the startle response to a succeeding and strong auditory stimulus, fragile X mice displayed a significantly stronger effect compared to control mice (Chen & Toth, 2001). Similarly, previous studies in our lab have shown that KO mice display a propensity for audiogenic seizures suggesting a hyper-responsiveness of auditory neurons and abnormal spectro-temporal processing (Rotschafer & Razak, 2013). This abnormality in temporal processing further indicates cortical region-specific delays where the frontal cortex fails to follow rapid changes in sounds (Croom et al., 2023). Further analysis of the developing *FMR1* KO auditory cortex showed a transient decrease in perineuronal net expression around GABAergic interneurons, which corresponds with hyperexcitability of individual neurons (Wen,

2019). Though the mechanisms and structural components underlying sensory sensitivity specific to auditory stimuli are understood, the connection between behavior changes is unclear. Studies that have assessed behaviors showed that KO mice expressed anxiety-like and hyperactivity outcomes in an open field arena compared to wild-type (WT) mice (Sorensen et al.,). Given the idea of a novel environment, the anxiety related with an OFT, and the behavioral phenotypes of the KO mice, such results seem as expected. On the contrary, another experiment demonstrated that KO mice explored the center of the open field, proportional to total distance traveled, significantly more than WT littermates, indicative of less anxiety-like behavior (Spencer et al., 2005). Perhaps the motivation of the KO mice to explore the novel environment outweighed their anxiety more so compared to WT mice. When chronic stress resembling real-life conditions was manipulated, behavioral sensitivity to stress was dramatically reduced in KO mice compared to WT mice (Lemaire-Mayo et al., 2017). This supports the behavioral differences between KO and WT mice, while most exploratory-based models show a reduction in anxiety-like behaviors in KO mice. However, findings are inconsistent, and most experiments assess several different behavioral tasks concurrently, which can result in order effects and carryover effects.

The goal of the present study was to determine if and how, in comparison to adult mice without the FXS genetic defect, adult FXS mice behave differently when challenged with intense background auditory stimuli during two distinct behavioral tasks. Importantly, much of the literature on FXS has concentrated on children and not the developmental stages later in life, including adulthood. This gap in research limits our understanding of how behavioral phenotypes may decline, improve, or remain consistent over time. The connection between auditory

hypersensitivity and its potential impact on the behaviors of an FXS mouse model has not been explored in previous studies.

METHODOLOGY

Mice

All procedures and animal care and use protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Riverside. Experiments were conducted in accordance with the NIH "Guide for the Care and Use of Laboratory Animals." Mice were male C57BL/6 wild type (WT, RRID:IMSR JAX:000,664) and male *Fmr1* knock-out mice (RRID: IMSR_JAX:003,025, The Jackson Laboratory) obtained from Jackson Laboratories. We chose this strain due to their decreased susceptibility for audiogenic seizures. The ages of both WT and KO mice ranged from p65-102 and genotypes were confirmed by PCR analysis of genomic DNA isolated from mouse tails. Mice were maintained in an AAALAC accredited facility in 12-h light/dark cycles, and food and water were available ad libitum.

Auditory Stimulus

The cacophony of sound was a continuously alternating up and down frequency modulated sweep with frequencies between 2–8 kHz, presented at a sound level between 60–95 dB SPL. The stimulus was generated using computer software (RPvdsEx, Tucker Davis Technologies, FL) and delivered using the RZ6 hardware system (Tucker Davis Technologies, FL) to an amplifier (Marantz, Integrated Amplifier PM8004) and then to the external speaker (FT17H, Fostex International). The sound level was measured with a portable sound meter (BK PRECISION 735) just before each experiment was run to maintain stable sound output across days.

Experiment 1: Open-Field Test

An open-field test (OFT) was conducted in a brightly lit behavioral room. Prior to testing, mice were habituated to the room for one hour. One mouse at a time was placed in the corner of the open-box arena made of white acrylic sheets measuring 43 x 43 x 43 cm. KO and WT (N = 10-11 mice per group) mice were placed into the arena at random order to control for confounds. The experiment lasted three consecutive days to habituate the mice to the novel arena prior to presenting the sound stimulus. Each mouse was placed in the arena at approximately the same time once (for 10 minutes) each day between 8:00 AM and 5:00 PM for three days for testing and video recording. Using SMART video tracking software from PanLab Apparatus from a web camera mounted on the ceiling above the open-field, ten minutes of subject behavior was analyzed each day. After three consecutive days of habituation, each mouse is expected to have spent a total of thirty minutes in the arena. Immediately after the ten minutes on the third day of habituation, mice remained in the arena for an additional ten minutes and were introduced to the aversive auditory stimuli. The sound stimulus was controlled by the researcher throughout the entire ten minutes. The open-field arena floor and walls were sprayed with 95% ethanol solution after each mouse, wiped with a clean paper towel, and air-dried for an additional three minutes to eliminate odor trails. The recorded arenas were then digitally overlaid to TopScan Lite Software (Clever Sys., Inc., Reston, VA 201,090, USA) which designed arenas to track and score locomotor activity. The analysis divided the open-box arena into three distinct areas: (1) Thigmotaxis, area directly adjacent to the outer wall about the width of the body of a mouse; (2) Area B, area between thigmotaxis and the center zone; (3) Center zone, area directly in the center of the arena (see appendix A). Analysis of the total ten-minute duration was split into two five-minute intervals across all three days. Three aspects of behavior were assessed in each five-minute interval: (1) Duration (s); (2) Distance (mm); (3) Velocity (mm/s). Statistical analysis

was performed with a two-way repeated measures ANOVA using GraphPad Prism 10 software. Data represents the mean ± standard error of the mean (SEM).

Experiment 2: Nest Building Test

A nest building test was conducted in a brightly lit behavioral room. Clean cages were prepared for one mouse to be placed and studied in each cage with new bedding and food. Mice were placed side by side (four at maximum) in their respective new cages in the behavioral room for an hour prior to the experiment to habituate to their new room and cage. After an hour of habituation, one square nestlet made of pulped cotton fiber was placed into each cage at the same time in the middle of the cage. Each nestlet was documented and weighed using a weigh boat and analytical balance prior to being placed in the cages. Each mouse was given a total of two hours to interact with the nestlet in their cage. To determine if there were differences in nest building before and after experimental manipulation between KO and WT mice, the nest building test was performed with and without the auditory stimulus. There were 11 WT mice and 13 KO mice in the control group that were not presented with any sound. The experimental group consisted of 13 WT and 12 KO mice that were presented with auditory stimulus for approximately 68 minutes out of the total 120 minutes, or approximately 57% of the total two-hour duration. After two hours, each nestlet or formed nest was carefully pulled apart using the researcher's thumb and index finger without forcing any pieces apart to separate the largest piece of the nestlet that remained. This piece was then weighed and divided by the weight of the original nestlet then multiplied by 100 to quantify the percent of the original nestlet that is intact. A picture of the entire nest or nestlet material in the cage was also taken after the experiment to visually evaluate the structure of any nestlet material. Adapted from the Binder lab, the combination of the structure of the nest along with the weight of the original nestlet was used to

assign each nestlet or formed nest a final number score between 1-5 including half scores. The criteria for the final scores were as follows: 1- nestlet largely untouched (>90% intact); 2 - partially torn nestlet (50-90% intact); 3 - nestlet is mostly shredded (<50% intact); 4 - identifiable nest (<10% intact); 5- near-perfect nest (<10% intact) (see appendix B). Nests that do not fall into one distinct category are assigned half scores. For example, a nest that visually appears to be partially torn yet only 46% of the original nestlet still remains will be scored 2.5. Statistical analysis was performed with a two-way ANOVA using GraphPad Prism 10 software. Data represents the mean ± standard error of the mean (SEM).

RESULTS

The main goal of this study was to determine if and how, in comparison to mice without the FXS genetic defect, FXS mice behave differently when challenged with aversive auditory stimuli during a behavioral exploratory task and nest building task. Our first hypothesis was that KO mice would exhibit innate hyperactivity upon first introduction to the open-field arena due to novelty and their range of behavioral phenotypes. Results revealed that there existed no significant differences between KO and WT mice on day 1 (see Table 1) and day 2 (see Table 2) of the open-field experiment during the entire 10 minutes with the assessment of velocity, distance, and duration in the respective areas.

Distance	Thigmotaxis	F (1, 40) = 0.3996	P=0.5309
	Area B	F (1, 40) = 0.6600	P=0.4214
	Center	F (1, 40) = 0.005696	P=0.9402
Duration	Thigmotaxis	F (1, 40) = 2.603	P=0.1145
	Area B	F (1, 40) = 4.030	P=0.0515
	Center	F (1, 40) = 0.1801	P=0.6736
Velocity	Thigmotaxis	F (1, 40) = 0.3806	P=0.5408
	Area B	F (1, 40) = 0.009851	P=0.9214
	Center	F (1, 40) = 3.247	P=0.0791

Table 1. *Genotype effects (WT vs. KO) on day 1 of OFT including distance, duration, and velocity behavioral assessments.* A two-way repeated measures ANOVA was performed indicating no significant differences across all areas and aspects of behavior (all p-values = $p > 0.05$) n = 11 KO & n = 11 WT.

Distance	Thigmotaxis	F (1, 40) = 0.004710	P=0.9456
	Area B	F (1, 40) = 0.07825	P=0.7811
	Center	F (1, 40) = 0.2660	P=0.6088
Duration	Thigmotaxis	F (1, 40) = 1.613	P=0.2114
	Area B	F (1, 40) = 2.848	P=0.0993
	Center	F (1, 40) = 0.02041	P=0.8871
Velocity	Thigmotaxis	F (1, 40) = 0.4368	P=0.5125
	Area B	F (1, 40) = 0.3805	P=0.5408
	Center	F (1, 40) = 1.446	P=0.2362

Table 2. *Genotype effects (WT vs. KO) on day 1 of OFT including distance, duration, and velocity behavioral assessments.* A two-way repeated measures ANOVA was performed indicating no significant differences across all areas and aspects of behavior (all p-values = $p > 0.05$) n = 11 KO & n = 11 WT.

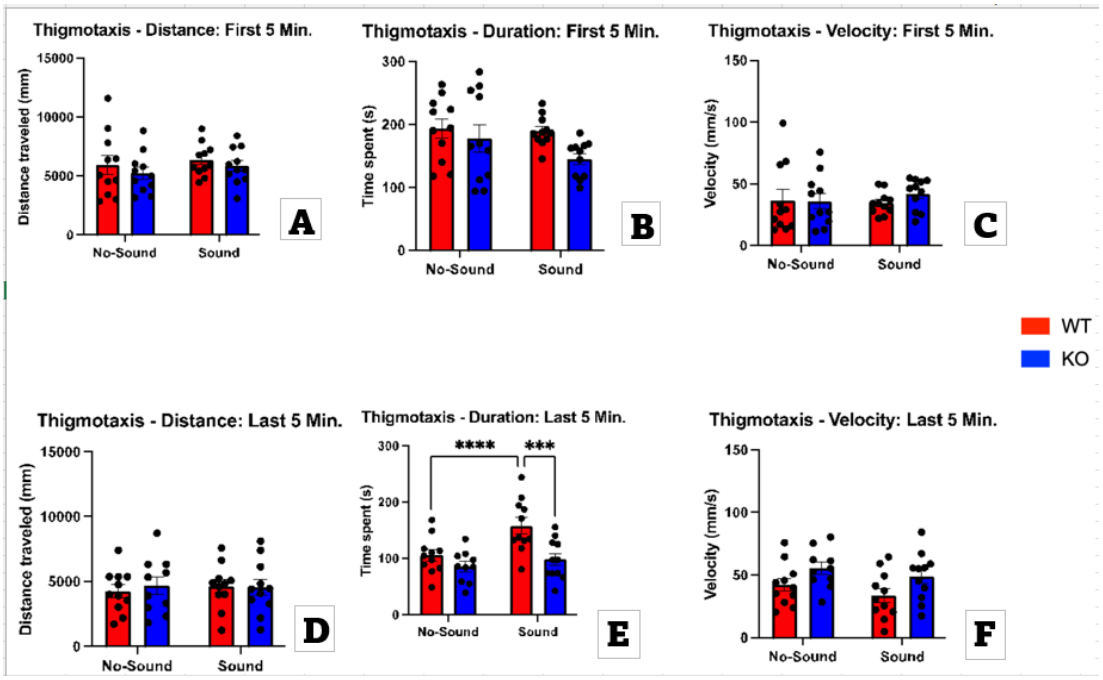


Fig. 1. OFT analysis on day 3 comparing sound vs. no-sound conditions, genotypic effects, and interaction effects in thigmotaxis split into two five-minute intervals. A two-way repeated measures ANOVA revealed significant differences in the second five-minute interval when analyzing duration in thigmotaxis. All differences in velocity and distance regarding thigmotaxis were not significant including the first five-minute interval for duration. n = 10 KO no-sound; n = 11 KO sound & n = 11 WT sound; n = 11 WT no-sound.

Our primary analysis focused on the third day of the experiment, comparing the sound versus no-sound conditions. To analyze changes across time and within trials, the ten-minute duration was split into two five-minute intervals. Regarding thigmotaxis, results revealed that WT mice spent significantly more time in thigmotaxis compared to KO mice in the second five-minute interval when the auditory stimulus was present (Fig. 1E.) [genotype effect: $F\ (1,20) = 8.305,\ p < 0.01$]. WT mice also spent significantly more time in thigmotaxis when the sound stimulus was present compared to without the stimulus (Fig. 1E.) [condition effect: $F\ (1,19) = 19.73,\ p < 0.001$], this effect is not seen in KO mice. This supports our second hypothesis that WT mice would exhibit typical anxiety-like behavior when exposed to the aversive stimuli, and that KO mice would behave abnormally (Fig. 1E.) [interaction sound x genotype: $F\ (1,19) = 7.593,\ p < 0.05$]. Specifically, in the second five-minute interval, KO mice spend a similar amount of time in thigmotaxis when the sound stimulus is present compared to when it is not.

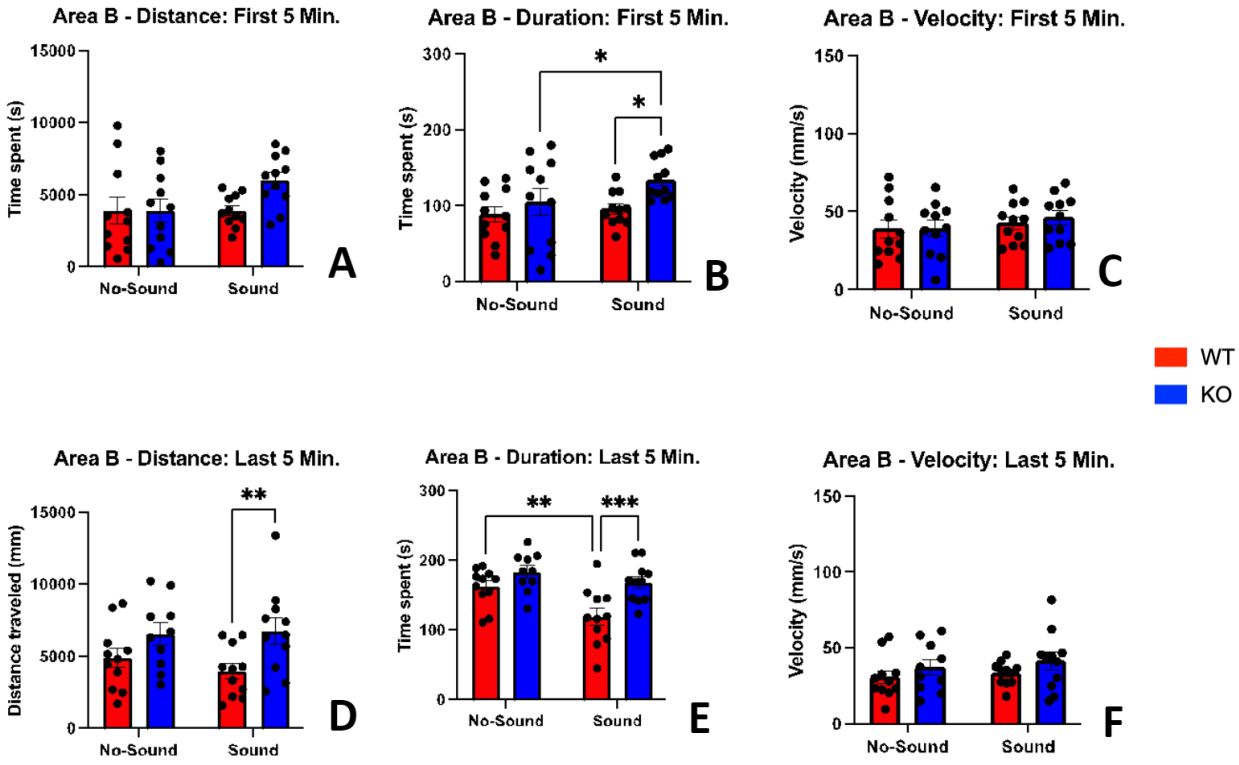


Fig. 2. OFT analysis on day 3 comparing sound vs. no-sound conditions, genotypic effects, and interaction effects in area B split into two five-minute intervals. A two-way repeated measures ANOVA was performed demonstrating significant differences in both five-minute intervals when analyzing duration spent in area B. There were significant differences regarding distance traveled in area B in the second five-minute interval. Differences in velocity were not significant. n = 10 KO no-sound; n = 11 KO sound & n = 11 WT sound; n = 11 WT no-sound.

Analysis of area B revealed that KO mice spent significantly more time in area B compared to WT mice in the first five-minute interval (Fig. 2B.) [genotype effect: $F\ (1,20) = 4.378, p < 0.05$] and in the second five-minute interval (Fig. 2E.) [genotype effect: $F\ (1,20) = 10.96, p < 0.01$], only when the sounds stimulus was present. WT mice further spent significantly less time in area B during the last five minutes in the presence of the sound compared to without, consistent with their previously established behavior of spending time in thigmotaxis (Fig. 2E.) [condition effect:

F (1,19) = 11.80, p < 0.01]. KO mice also traveled a significantly greater distance compared to WT mice in area B during the last five minutes with the presence of the sound (Fig. 2D.) [genotype effect: *F (1,20) = 5.806, p < 0.05*]. This data supports the idea of hyperactivity and atypical behavior in KO mice. They showcased increased locomotion and decreased anxiety by not remaining in thigmotaxis, particularly favoring area B in the OFT, in contrast to WT mice when exposed to the noxious sound.

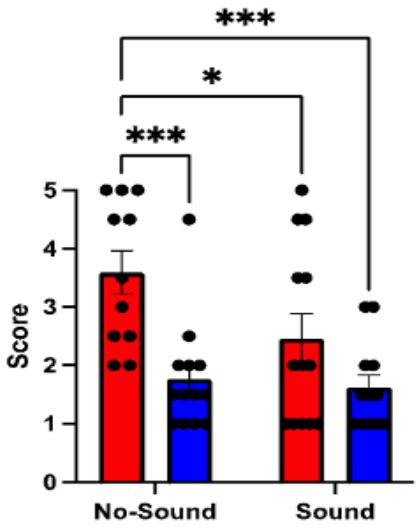


Fig. 3. Nest building test analysis comparing sound vs. no-sound conditions, genotypic effects, and interaction effects. A two-way ANOVA revealed significant differences between KO and WT mice only without the sound stimulus.

Nesting behavior analysis illustrated that WT mice scored significantly higher compared to KO mice without the aversive sound [genotype effect: *F (1,45) = 16.02, p < 0.001*]. There were no significant differences between the sound versus no-sound conditions or interactions effects. This does not support the hypothesis that KO mice would exhibit increased hypersensitivity to the intense auditory stimuli and display reduced nest-building behavior compared to WT mice. Instead, the introduction of the auditory stimulus resulted in a narrowing of the scores between KO and WT mice, compared to when the stimulus was absent.

DISCUSSION

Though much of the literature on behavior analysis through exploratory-based models, including the OFT, tends to support the presence of distinct behavioral differences between WT and KO mice, our study revealed no differences upon the initial introduction to the OFT. It is understood that rodents in particular display anxiety provoked fear or flight responses and distinct aversions to large, brightly lit, exposed and novel environments as they are assumed to be conditioned to view environments like such as dangerous (Seibenhener & Wooten, 2015). This principle is however mediated by the tendency for mice to explore novel environments and is consistent with the present study such that both KO and WT mice traveled the greatest distance and at the highest velocity on the first day of the experiment.

The notion that auditory hypersensitivity leads to hyperactivity was displayed on the third day of the experiment as KO mice traveled a greater distance than WT mice in area B in the presence of the aversive sound. Given the behavioral distinctions observed between KO and WT mice in the presence of the sound, it prompts an important question: To what extent does auditory hypersensitivity, as a phenotype, influence behavior compared to a range of other environmental stimuli? In future studies, an approach could involve introducing mice to the open-field arena and promptly introducing stimuli, such as light or sound, to observe whether the intensity of the stimulus is sufficient to elicit discernible differences between KO and WT mice.

In the nest building experiment, the introduction of the auditory stimulus resulted in a narrowing of the scores between KO and WT mice, compared to when the stimulus was absent. However, scores for the KO mice remained consistent both in the presence of the sound and without it. This suggests a robust phenotype, indicating that KO mice either struggle to construct a nest within the allotted two-hour time limit, lack sufficient motivation to do so, or both. The latter

explanation is particularly concerning, considering that nest building is thought of as an intrinsic behavior in mice that is highly linked to their survival. Considering the small size of mice, they are susceptible to heat loss, making nests crucial for thermoregulation and functioning as clothes and houses in humans (Deacon, 2006). Nesting also serves a protective role, potentially reducing the risks of predation in the wild, as mice can hide, camouflage, and shield themselves from environmental conditions (Deacon, 2006). Nest building in mice when translated to humans can serve as an indicator of well-being and self-care behavior.

In both experiments, KO mice exhibited atypical behavior compared to WT mice, raising the question of how these behavioral abnormalities in KO mice and FXS human patients might be regulated or mitigated. Potential solutions could involve strategies to limit exposure to stimuli to prevent the onset of distress, expressed through behavioral abnormalities. Alternatively, progressively introducing stimuli as a means of desensitization could prove more practical to address abnormalities as any given environment is accompanied by a variety of inputs, some of which may be unexpected. In this attempt to decrease hypersensitivity, subjects are gradually exposed to sensory stimuli over an extended period of time in an effort to alter their reaction to such stimulus which in this context could prove beneficial.

Another approach includes the introduction of specific drugs that could relieve the behavioral symptoms seen in FXS. In one study, researchers chronically injected an endogenous neurotransmitter known as agmatine to KO mice and WT mice and examined the effects of the drug on compulsion, hyperactivity, memory impairment, social deficits, and more in a series of behavioral tasks that included an OFT (Jeon et al., 2022). Given the mood-enhancing properties of agmatine, results demonstrated alleviation of anxiety or hyperactivity and reversal in KO mice of compulsion, learning and memory deficits, hyperactivity, abnormal social interaction and

communication deficiencies (Jeon et al., 2022). Similar to this, it has been demonstrated that individuals with FXS have decreased receptor availability for γ-aminobutyric-acid (GABA), the primary inhibitory neurotransmitter in the brain, which can mediate the symptoms of FXS (Cogram et al., 2019). In this study, gaboxadol, a drug that acts as a GABA receptor agonist, was introduced to KO and WT mice to analyze hyperactivity, anxiety, aggression, and repetitive behaviors in a number of behavioral tasks that included an OFT (Cogram et al., 2019). Results revealed that all aberrant behaviors were normalized in KO mice to WT levels (Cogram et al., 2019). While the introduction of drugs may improve the behavioral challenges in FXS, the threat of developmental side effects, other cost-related obstacles, or complexity in the number of variables within in experiment can exceed their advantages.

The findings of this study provoke further research to assess behavior in the context of how distressing stimuli may be presented to individuals naturally in various environments. This supports the idea that further exploring the connection between known sensitivity to stimuli and behavior in FXS is crucial before developing strategies to clinically improve daily life. Ultimately, examining the gene-environment interactions is essential to map out how behavioral abnormalities are expressed in FXS. Furthermore, understanding the mechanisms and variables that contribute deficiencies in behavioral tasks can be used to predict future outcomes of developmental processing. In line with prevention of such behavioral challenges, limiting or gradually exposing FXS patients to sensory stimuli that triggers abnormal behavior responses can lead to more effective long-term symptom management.

REFERENCES

Chen, L., & Toth, M. (2001). Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience*, *103*(4), 1043–1050. https://doi.org/10.1016/s0306-4522(01)00036-7

Chromik, L. C., Quintin, E., Lepage, J., Hustyi, K. M., Lightbody, A. A., & Reiss, A. L. (2019). The influence of hyperactivity, impulsivity, and attention problems on social functioning in adolescents and young adults with fragile X syndrome. *Journal of Attention Disorders*, *23*(2), 181-188. https://doi.org/10.1177/1087054715571739

Cogram, P., Deacon, R. M. J., Warner-Schmidt, J. L., von Schimmelmann, M. J., Abrahams, B. S., & During, M. J. (2019). Gaboxadol Normalizes Behavioral Abnormalities in a Mouse Model of Fragile X Syndrome. *Frontiers in behavioral neuroscience*, *13*, 141. https://doi.org/10.3389/fnbeh.2019.00141

Croom, K., Rumschlag, J. A., Erickson, M. A., Binder, D. K., & Razak, K. A. (2023). Developmental delays in cortical auditory temporal processing in a mouse model of Fragile X syndrome. *Journal of neurodevelopmental disorders*, *15*(1), 23. https://doi.org/10.1186/s11689-023-09496-8

Deacon, R. Assessing nest building in mice. *Nat Protoc* **1**, 1117–1119 (2006). https://doi.org/10.1038/nprot.2006.170

Grefer, M. L. (2017). *ADHD symptoms, social problems, and independence in adolescent and young adult males with FXS* (Order No. AAI10165123). Available from APA PsycInfo®. (1928259763; 2017-05719-009). Retrieved from https://www.proquest.com/dissertations-theses/adhd-symptoms-social-problems-independence/docview/1928259763/se-

Hagerman, R. J., Berry-Kravis, E., Kaufmann, W. E., Ono, M. Y., Tartaglia, N., Lachiewicz, A., Kronk, R., Delahunty, C., Hessl, D., Visootsak, J., Picker, J., Gane, L., & Tranfaglia, M.

(2009). Advances in the treatment of fragile X syndrome. *Pediatrics*, *123*(1), 378–390. https://doi.org/10.1542/peds.2008-0317

Jeon, S. J., Kwon, H., Bae, H. J., Gonzales, E. L., Kim, J., Chung, H. J., Kim, D. H., Ryu, J. H., & Shin, C. Y. (2022). Agmatine relieves behavioral impairments in Fragile X mice model. *Neuropharmacology*, *219*,1-10. https://doi.org/10.1016/j.neuropharm.2022.109234

Lemaire-Mayo, V., Subashi, E., Henkous, N., Beracochea, D., & Pietropaolo, S. (2017). Behavioral effects of chronic stress in the Fmr1 mouse model for fragile X syndrome. *Behavioural brain research*, *320*, 128–135. https://doi.org/10.1016/j.bbr.2016.11.051

Matsuzaki, J., Kagitani-Shimono, K., Goto, T., Sanefuji, W., Yamamoto, T., Sakai, S., Uchida, H., Hirata, M., Mohri, I., Yorifuji, S., & Taniike, M. (2012). Differential responses of primary auditory cortex in autistic spectrum disorder with auditory hypersensitivity. *Neuroreport*, *23*(2), 113–118. https://doi.org/10.1097/WNR.0b013e32834ebf44

Niu M, Han Y, Dy ABC, et al. Autism Symptoms in Fragile X Syndrome. *Journal of Child Neurology*. 2017;32(10):903-909. doi:10.1177/0883073817712875

Rotschafer, S. E., & Razak, K. A. (2014). Auditory processing in fragile x syndrome. *Frontiers in cellular neuroscience*, *8*, 19. https://doi.org/10.3389/fncel.2014.00019

Rotschafer, S., & Razak, K. (2013). Altered auditory processing in a mouse model of fragile X syndrome. *Brain research*, *1506*, 12–24. https://doi.org/10.1016/j.brainres.2013.02.038

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of visualized experiments : JoVE*, (96), e52434. https://doi.org/10.3791/52434

Sidorov, M. S., Auerbach, B. D., & Bear, M. F. (2013). Fragile X mental retardation protein and synaptic plasticity. *Molecular Brain*, *6*, 11. https://doi.org/10.1186/1756-6606-6-15

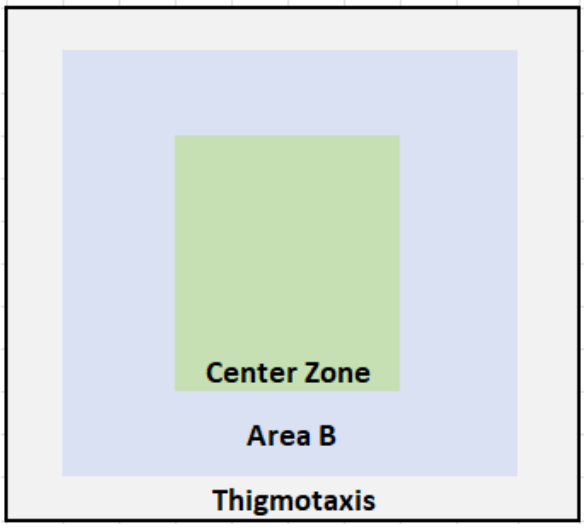
Sørensen, E. M., Bertelsen, F., Weikop, P., Skovborg, M. M., Banke, T., Drasbek, K. R., & Scheel-Krüger, J. (2015). Hyperactivity and lack of social discrimination in the adolescent Fmr1 knockout mouse. *Behavioural pharmacology*, *26*(8 Spec No), 733–740. https://doi.org/10.1097/FBP.0000000000000152

Spencer, C. M., Alekseyenko, O., Serysheva, E., Yuva-Paylor, L. A., & Paylor, R. (2005). Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. *Genes, brain, and behavior*, *4*(7), 420–430. https://doi.org/10.1111/j.1601-183X.2005.00123.x

Wen, T. H. (2019). *Development of structural and functional abnormalities underlying auditory hypersensitivity in a mouse model of Fragile X Syndrome* (Order No. AAI10787952). Available from APA PsycInfo®. (2154837383; 2018-58620-097). https://www.proquest.com/dissertations-theses/development-structural-functional-abnormalities/docview/2154837383/se-2

APPENDIX

Appendix A



Appendix B