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# Genetic reduction of MMP-9 in the *Fmr1* KO mouse partially rescues prepulse inhibition of acoustic startle response

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## Abstract

Sensory processing abnormalities are consistently associated with autism, but the underlying mechanisms and treatment options are unclear. Fragile X Syndrome (FXS) is the leading known genetic cause of intellectual disabilities and autism. One debilitating symptom of FXS is hypersensitivity to sensory stimuli. Sensory hypersensitivity is seen in both humans with FXS and FXS mouse model, the *Fmr1* knock out (*Fmr1* KO) mouse. Abnormal sensorimotor gating may play a role in the hypersensitivity to sensory stimuli. Humans with FXS and *Fmr1* KO mice show abnormalities in acoustic startle response (ASR) and prepulse inhibition (PPI) of startle, responses commonly used to quantify sensorimotor gating. Recent studies have suggested abnormally high levels of matrix metalloproteinase-9 (MMP-9) as a potential mechanism of sensory abnormalities in FXS. Here we tested the hypothesis that genetic reduction of MMP-9 in *Fmr1* KO mice rescues ASR and PPI phenotypes in adult *Fmr1* KO mice. We measured MMP-9 levels in the inferior colliculus (IC), an integral region of the PPI circuit, of WT and *Fmr1* KO mice at P7, P12, P18, and P40. MMP-9 levels were higher in the IC of *Fmr1* KO mice during early development (P7, P12), but not in adults. We compared ASR and PPI responses in young (P23–25) and adult (P50–80) *Fmr1* KO mice to their age-matched wildtype (WT) controls. We found that both ASR and PPI were reduced in the young *Fmr1* KO mice compared to age-matched WT mice. There was no genotype difference for ASR in the adult mice, but PPI was significantly reduced in the adult *Fmr1* KO mice. The adult mouse data are similar to those observed in humans with FXS. Genetic reduction of MMP-9 in the *Fmr1* KO mice resulted in a rescue of adult PPI responses to WT levels. Taken together, these results show sensorimotor gating abnormalities in *Fmr1* KO mice, and

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suggest the potential for MMP-9 regulation as a therapeutic target to reduce sensory hypersensitivity.

**Keywords**

Fragile X Syndrome; acoustic startle response; prepulse inhibition; sensorimotor gating; matrix metalloproteinase-9; autism

## 1. Introduction

Fragile X syndrome (FXS) is a leading genetic cause of intellectual disability and autism. In conjunction with learning, anxiety, communication, and social deficits (Hatton et al., 2006), individuals with FXS are often hypersensitive to sensory stimuli. FXS is linked to a genetic mutation in the X chromosome with varying degrees of severity, due to the expansion of CGG repeats in the Fragile X mental retardation-1 (*Fmr1*) *gene* (Snow et al., 1993). If the repeated sequence exceeds ~200 repeats, this can lead to a full mutation and methylation of the *Fmr1* gene which in turn causes transcriptional suppression of the *Fmr1 gene* and the loss of Fragile X mental retardation protein (FMRP). Loss of FMRP has been associated with abnormal protein synthesis, particularly those involved with synaptic plasticity and maturation (Huber et al., 2002; Sidorov et al., 2013).

Debilitating sensory hypersensitivity is a common symptom in humans with FXS (Tsiouris and Brown, 2004), manifesting as intolerance to even mild sensory inputs. An animal model of FXS, the *Fmr1* knock out (*Fmr1* KO) mouse, shows many of the core abnormalities of FXS including sensory hypersensitivity (Rais et al., 2018; Sinclair et al., 2017). Sensorimotor gating is a low-level (pre-attentive) sensory filtering mechanism to reduce sensory overload reaching cortical areas. Abnormal sensorimotor gating may contribute to hypersensitivity in autism spectrum disorders (Scott et al., 2018), including FXS (Sinclair et al., 2017). Acoustic startle response (ASR) and prepulse inhibition (PPI) of ASR are behavioral outcomes used to test sensorimotor gating in humans and rodents. The circuits underlying these behaviors are present in the brainstem and midbrain of the auditory system. FMRP, the protein product of *Fmr1* gene, is expressed across all levels of the central auditory system, including strong expression in the cochlear nucleus and other regions of the brainstem (Zorio et al., 2017). Abnormal functions of these regions in *Fmr1* KO mice have been reported (Garcia-Pino et al., 2017; Mott and Wei, 2014; Wang et al., 2018) and both humans with FXS and *Fmr1* KO mice show altered ASR and PPI responses (human: Frankland et al., 2004; Yuhas et al., 2011; Hessl et al., 2009; mouse: Chen and Toth, 2001; Nielsen et al., 2002; Renoux et al., 2014; Yun et al., 2006).

One of FMRP's translational targets is matrix metalloproteinase-9 (MMP-9), an endopeptidase important in CNS development through extracellular matrix remodeling and synaptic plasticity (Reinhard et al., 2015). Increased MMP-9 levels are seen in the *Fmr1* KO mouse and FXS human brains (Bilousova et al., 2009; Dziembowska et al., 2013; Sidhu et al., 2014). Genetic reduction of MMP-9 levels in the *Fmr1* KO mouse restored auditory cortex responses to WT levels (Lovelace et al., 2016; Wen et al., 2018), rescued dendritic spine abnormalities in the hippocampus and reduced anxiety-like behaviors (Sidhu et al.,

2014). Beneficial effect of MMP reduction on synaptic arborization is seen in the drosophila model of FXS (Siller and Broadie, 2011). Inhibition of MMP-9 with minocycline reduces multiple symptoms in the *Fmr1* KO mice and humans with FXS (Bilousova et al., 2009; Dziembowska et al., 2013; Schneider et al., 2013). However, it is not known if MMP-9 levels are high in circuits involved in sensorimotor gating (Li et al., 1998) and if reduction of MMP-9 in FXS reduces sensorimotor gating abnormalities. In this study, we focused on the inferior colliculus (IC) because this region of the midbrain is a main source of inhibition that causes PPI (Fendt et al., 2001). We found increased MMP-9 levels in the IC during development, but not in adults. The second aim was to determine if ASR and PPI responses in *Fmr1* KO mice were different from WT mice from a young age. We found reduced PPI in the *Fmr1* KO mice compared to WT mice in both age groups tested: young (P23–25) and adult. A genotype difference in ASR was found only in the young age group. Finally, we tested the hypothesis that genetic reduction of MMP-9 in *Fmr1* KO mice would alleviate PPI abnormalities in the *Fmr1* KO mice. For this purpose, we generated *Fmr1* KO mice, which were heterozygous for MMP-9 and found that these mice show ASR and PPI responses that are comparable to the WT mice.

## 2. Results

### 2.1. Gelatin Zymography for MMP-9 levels in the inferior colliculus

Gelatin zymography was used to measure MMP-9 levels in the IC of WT and *Fmr1* KO mice at four different ages: postnatal day (P) 7, 12, 18, and 40. At P7 ($t(4)=-2.47$, p=0.0345) and P12($t(7)=-2.054$, p=0.0395), *Fmr1* KO mice showed greater MMP-9 levels compared to WT (Figure 1). There was no genotype difference in MMP-9 levels at P18 ($t(8)=-0.267$, p=0.398) and P40 ($t(8)=-0.299$, p=0.772). These results show that as in the forebrain (Lovelace et al., 2016), loss of FMRP in the midbrain also causes an increase in MMP-9 levels.

### 2.2. Acoustic startle response and prepulse inhibition

The ASR was measured in young and adult mice. Young (P23–25) *Fmr1* KO mice showed a significant reduction in ASR compared to age-matched WT mice (one-way ANOVA, $F(1,16)=6.190$, p=0.024) (Figure 2A). To examine PPI, two-way ANOVA was used with prepulse intensity and genotype as factors. PPI was significantly reduced in young *Fmr1* KO mice compared to WT mice (Genotype: $F(1,48)=9.226$, p=0.004) (Figure 2B). While prepulse intensity affected PPI (Intensity: $F(2,48)=3.267$, p=0.047; Tukey HSD, 75dB vs 85dB, p=0.596; 75dB vs 95dB, p=0.038; 85dB vs 95dB, p=0.273), there were no significant interactions between genotype and prepulse intensity (Genotype*Intensity: $F(2,48)=0.499$, p=0.610). This suggests that the genotype difference in PPI is not due to change in any specific intensity tested.

The ASR was not different between adult (P50–80) WT, *Fmr1* KO, and MMP-$9^{+/-}$/*Fmr1* KO mice (one-way ANOVA, $F(2,24)=1.019$, p=0.376) (Figure 3A). A two-way ANOVA with genotype and prepulse intensity as factors showed that there was a main effect of genotype ($F(2,72)=5.583$, p=0.006), and prepulse intensity ($F(2,72)=8.156$, p=0.001), but no significant interactions ($F(4,72)=0.401$, p=0.807) (Figure 3B). Post hoc Tukey HSD analyses

revealed that *Fmr1* KO PPI was significantly reduced compared to WT (WT vs. *Fmr1* KO, p=0.004) as seen in the young mice. There was no difference in PPI responses between WT and MMP-9+/−/*Fmr1* KO (WT vs. MMP-9$^{+/-}$/*Fmr1* KO, p=0.427) or between MMP-9$^{+/-}$/*Fmr1* KO and *Fmr1* KO mice (*Fmr1* KO vs. MMP-9$^{+/-}$/*Fmr1* KO, p=0.106). Additionally, prepulse intensity post hoc analysis revealed that as the sound intensity increased, the PPI increased as well (75dB vs 85dB, p=0.044; 75dB vs 95dB, p=0.000431, 85dB vs 95dB, p=0.267).

One potential methodological concern is that the mice show startle response to the pre-pulse, particularly at 85 and 95dB SPL (Valsamis and Schmid, 2011). However, because the ASR itself was not different at this age, it is unlikely that the genotype PPI differences were due to startle to the pre-pulse. In addition, the genotype effects are present even if only the 75 dB SPL prepulse is considered, which is unlikely to cause startle by itself (one-way ANOVA analyses of PPI for the 75dB prepulse ($F$(2, 24)=3.446, p=0.04). Taken together, these data indicate that genetic reduction of MMP-9 in the *Fmr1* KO mice, provides a rescue of the PPI deficit, without impacting the baseline ASR.

### 2.3. Habituation

Habituation of electrophysiological response to repeated stimulation is reduced in both humans with FXS and the *Fmr1* KO mice (Ethridge et al., 2016; Lovelace et al., 2016; Schneider et al., 2013). Genotype differences in ASR may arise due to differences in habituation to startle stimuli. Therefore, we examined whether ASR habituation is altered in the *Fmr1* KO mice. Habituation was calculated by dividing the average of the final five ASR values by the average first five ASR values (see Methods). There was no genotype difference in habituation in young ($F$(1,16)=0.316, $p$=0.582) (Figure 4A) or adult ($F$(2,24)=0.368, $p$=0.696) (Figure 4B) mice. This suggests that in the young mouse, genotype differences in ASR do not occur because of altered habituation.

## 3 Discussion

We found that in young, but not in adult, *Fmr1* KO mice, there was a significant reduction in ASR amplitude compared to age matched WT mice. In both age groups, PPI was significantly reduced in the *Fmr1* KO mice compared to WT mice, suggesting that sensorimotor gating abnormalities present in young *Fmr1* KO mice are maintained into adulthood. We also found increased MMP-9 levels in the IC of the developing, but not adult, *Fmr1* KO mice. This suggests that abnormal MMP-9 levels in the IC may be associated with reduced PPI. While WT and *Fmr1* KO mice were significantly different from each other in terms of PPI, the MMP-9$^{+/-}$/*Fmr1* KO mice were not different from either WT or *Fmr1* KO mice. We interpret this to mean that there was a partial rescue of the PPI phenotype when MMP-9 was reduced in the *Fmr1* KO mice. The partial correction implies other mechanisms in the ASR/PPI pathway such as abnormal ion channel function may be involved in sensorimotor gating dysfunction in FXS (Deng et al., 2013; Zaman et al., 2017). The adult mouse data are consistent with data from humans with FXS who show no differences in ASR and reduced PPI suggesting that these sensorimotor gating measures can be used as potential biomarkers (Frankland et al., 2004; Hessl et al., 2009). Together these results

strongly suggest that increased MMP-9 in FXS underlies abnormal sensorimotor gating and may cause sensory hypersensitivity. This conclusion is consistent with the findings of Gkokas et al., (2014), who over-expressed MMP-9 in mice and found FXS-like symptoms.

ASR in *Fmr1* KO mice has been examined by a number of groups, but the results are mixed in a manner indicating sensitivity of measures to variations in stimulus parameters, age, and genetic background. Chen and Toth (2001) showed that ASR is significantly reduced in 7–10 weeks old *Fmr1* KO mice compared to WT mice on the FVB background. Yun et al. (2006) showed reduced ASR in *Fmr1* KO mice compared to WT in FVB strain mice older than 4 weeks, but ASR was not different in mice younger than 3 weeks. In the C57B6/J genetic background, ASR was shown to be higher in the *Fmr1* KO mice compared to WT for stimuli in the 70–80 dB range (Nielson et al., 2002). However, when a 120dB startle stimulus was used, the WT mice showed stronger ASR than the *Fmr1* KO mice. Frankland et al. (2004) tested C57B6/J mice and found no difference in ASR for stimuli <90dB SPL. However, for >95dB SPL, ASR was stronger in the WT mice compared to *Fmr1* KO mice. Ding et al. (2014) and Veeraragavan et al. (2011) showed no genotype difference in ASR. Together the preponderance of evidence suggest that ASR is either not different between the genotypes, or decreased in *Fmr1* KO mice compared to WT. Our data are consistent with this trend, with young *Fmr1* KO mice showing reduced ASR and adults showing no genotype difference, although the mechanisms for age-dependent genotype differences in ASR are unclear. There were no significant differences in average weight of mice between the two genotypes in either young or adult ages, suggesting that this is not a factor in the age effect on genotype difference in ASR. It is possible that the muscle tone is different in young mice. Largo and Schinzel (1985) suggested a developmental delay in motor function, with boys with FXS showing reduced muscle tone. But, very little is known about development of muscle function in the *Fmr1* KO mice. Future studies should examine electromyographic responses in these mice to determine potential delays in muscle function. The difference between young and adult ASR responses should, however, not be surprising because developmentally transient genotype differences appear to a prominent feature in FXS (see Meredith et al., 2012 for a review).

Several studies have examined PPI in *Fmr1* KO mice, also with mixed results. Chen and Toth (2001) showed PPI was increased in *Fmr1* KO mice compared to WT (FVB background) at both 75dB and 85dB SPL prepulse intensities. The differences with our PPI result may arise from different protocols used in the two studies. We included all prepulse intensities in one session, whereas, Chen and Toth (2001) tested the lower intensity a month prior to testing the higher intensity. Nielson et al. (2002), Frankland et al. (2004), and Ding et al. (2014) also reported increased PPI in *Fmr1* KO mice on the C57B6/J background. Veeraragavan et al. (2011) reported no difference and De Vrij et al. (2008) used eye-blink response and reported decreased PPI in the *Fmr1* KO mice. Despite this evidence suggesting increased PPI in the *Fmr1* KO mouse, humans with FXS show significantly reduced PPI (Frankland et al., 2004 and Hessl et al., 2009), which is consistent with our results.

A novel finding of this paper is that abnormal MMP-9 activity may underlie sensorimotor gating abnormalities in FXS. MMP-9 is one of the targets of FMRP, so with the absence of FMRP, there is an upregulation of MMP-9 (Dziembowska et al., 2013) in several regions of

the brain in FXS. The ASR pathway consists of the auditory nerve input to the ventral cochlear nucleus, which connects to the caudal pontine reticular nucleus, from there synapsing onto the motor neurons of the spinal cord whose activity elicits the startle response (Koch and Schnitzler, 1997). We show here that MMP-9 levels are increased in the IC of *Fmr1* KO mice as well. The IC is a major hub of both the ascending and descending auditory pathway, and is critically involved in PPI (Fendt et al., 2001). The activation of the IC by the prepulse sound is rapidly relayed as a long duration inhibition of the neurons of the pontine reticular nucleus neurons. The inhibition generated by the IC reduces ASR, leading to the classic PPI of startle response. Interestingly we found increased MMP-9 in the IC at P7 and P12, but not at P18 or P40. We found reduced PPI at ~P23 and ~P50, when MMP-9 levels in the IC are similar to WT. This suggests that MMP-9 plays a crucial role in the development of IC, and abnormalities in early development of IC are sustained into adulthood, even though MMP-9 levels are normalized. This makes the crucial prediction that any pharmacological treatment through MMP-9 manipulation to reduce sensorimotor gating abnormalities would be more effective if given during the P7-P12 window, rather than in adulthood. Future studies will test this prediction.

## Conclusions

In findings similar to those seen in humans with FXS, we provide evidence for reduced PPI in the *Fmr1* KO mice. PPI, may therefore, be developed as biomarker for pursuit of translation-relevant therapeutic avenues in FXS. Reduced PPI was seen in both adult and young *Fmr1* KO mice, suggesting early developmental origin of sensorimotor gating abnormalities in FXS and the necessity to provide treatment early in development. Finally, our data suggest a potential target for reducing sensory hypersensitivity in FXS through a reduction of MMP-9 levels using specific inhibitors and at specific developmental time points.

## 4. Methods and Materials

### 4.1. Mice

FVB.129P2−Pde6b+Tyr$^{c\text{-}ch}$/AntJ controls (WT) and FVB.129P2−*Fmr1tm1Cgr*/J (*Fmr1* KO) mice were received from Jackson Laboratories and housed on a 12:12 light/dark cycle, with standard lab chow and water given *ad libitum* in the vivarium. To generate the *Mmp9* $^{+/-/}$ *Fmr1* KO mice, FVB.Cg−*Mmp−9tm1Tvu*/J mice were backcrossed with *Fmr1* KO mice. These mice had a reduced expression of MMP-9 in the auditory cortex (Sidhu et al.., 2014 and Wen et al.., 2018). Genotypes of mice were verified by sending tail samples to Transnetyx (Cordova, TN). There were two age groups tested: young (“Y”, PND 23–25) and adult (“A”, PND 50–80). Table 1 provides information about the age and average weight of each group. Weights between genotypes were not significantly different in either the young ($t(16)=1.792$, $p=0.092$) or adult ($F(2,24)=0.71$, $p=0.502$) groups. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Riverside. Each experimental group consisted of n=9 mice. All mice tested were males.

### 4.2 Apparatus

**Acoustic Startle.—**The Coulbourn Animal Acoustic Startle System (Coulbourn Instruments, Whitehall, PA) was used to measure the ASR. The apparatus consisted of a single subject anechoic startle chamber with a ventilating fan built into the ceiling to provide background noise (68dB SPL). A weight-sensitive startle platform was centered inside of the chamber. The A10–21B Startle Controller Software was used to generate the PPI protocol and measure ASR peak magnitude. Each mouse was placed into individual ventilated holding cages.

### 4.3. Procedures

Mice habituated for 20 minutes in their home cages after being transported to the lab. Afterwards, each mouse was weighed, and allowed to habituate for 10 additional minutes in the holding cage, before being placed in the startle chamber. A five-minute delay was built into the program to allow the animal to acclimate to the chamber prior to the stimulus presentation. The built-in fan provided background noise of 68dB SPL, measured with a digital sound level meter (BK Precision, Model 732A). Table 2 provides information on the various trials used. Trial type 1 consisted of the initial six and final five trials, which presented only 115dB SPL startle stimulus. These trials were used to calculate the ASR and habituation. Between the stimulus alone trials, eight of each prepulse trials were presented in pseudorandom order, with the prepulse – 75, 85, or 95dB SPL – preceding the startle stimulus by 100ms. Duration of the prepulse sound was 20ms and duration of the startle pulse was 40ms. Additional prepulse trials were excluded from analysis, due to being of similar sound levels to the startle stimulus (105dB). The inter-trial interval ranged from 15–25s to minimize anticipation of the startle stimulus delivery.

### 4.4. Gelatin Zymography

Inferior colliculus (IC) tissue samples were taken from P7, P12, P18, and P40 mice. The mice were euthanized with isofluorane, the IC was dissected and immediately flash frozen over dry ice then stored at −80°C. The gelatin zymography protocol was performed as previously described in Wen et al., 2018. The zymography buffer used to resuspend the IC included 100 μL of 100 mM Tris-HCl (pH = 7.6), 150 mM NaCl, 5 mM $CaCl_2$, 0.05% Brij35, 0.02% $Na_3N$, 1% Triton X-100, 100 μM PMSF and protease inhibitor cocktail (Sigma, catalog# P8340). Gelatin agarose beads (Sigma, catalog# G5384) were added to the sample lysates to pull down MMP-9 on 10% Tris-Glycine gel with 0.1% gelatin as the substrate (Life Technologies). Once pulled down, the gels were placed in renaturing buffer (Life Technologies, catalog# LC2670) for 90 minutes and developing buffer (Life Technologies, catalog# LC2671) for 96 hours. Gels were then stained with Commassie blue overnight and de-stained for subsequent analysis. Total protein concentration was measured per lysate using the BCA colorimetric protein assay (Pierce, 23 235). Levels of MMP-9 protein were analyzed using Photoshop CS4. All samples were normalized to the WT values per each individual age group (n=3–7).

### 4.5. Data analysis

Weight comparisons were done by independent t-tests to compare the young groups and one-way ANOVA was used to compare the adult groups. One-tailed unpaired t-test was used to compare WT and *Fmr1* KO MMP-9 levels. ASR was calculated by averaging the peak magnitude of the startle stimulus alone responses and across the 11 Trial Type 1 responses. Percent habituation was calculated as %Habituation=100 x (average last five Trial Type 1 responses/average first five Trial Type 1 response). Percent PPI was calculated as %PPI=1-(average startle amplitude from prepulse trial/average startle amplitude from startle stimulus alone trial) × 100. To compare PPI across genotypes, a two-way ANOVA was used with prepulse intensity (75, 85, 95 dB) and genotype (KO, WT) as factors. One-way ANOVA was used for comparison of ASR across genotypes separately for each age group tested. Standard error is shown by the error bars in all figures. Age was not tested as a factor in any analysis. Post-hoc tests are as described in the Results section.

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## References

Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM, 2009 Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J. Med. Genet 46, 94–102. 10.1136/jmg.2008.061796 [PubMed: 18835858]

Chen L, Toth M, 2001 Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience 103, 1043–1050. 10.1016/S0306-4522(01)00036-7 [PubMed: 11301211]

Cho Y, Gong T-WL, Stöver T, Lomax MI, Altschuler RA, 2002 Gene Expression Profiles of the Rat Cochlea, Cochlear Nucleus, and Inferior Colliculus. JARO J. Assoc. Res. Otolaryngol 3, 54–67. 10.1007/s101620010042 [PubMed: 12083724]

de Vrij FMS, Levenga J, van der Linde HC, Koekkoek SK, De Zeeuw CI, Nelson DL, Oostra BA, Willemsen R, 2008 Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. Neurobiol. Dis 31, 127–132. 10.1016/j.nbd.2008.04.002 [PubMed: 18571098]

Deng PY, Rotman Z, Blundon JA, Cho Y, Cui J, Cavalli V, Zakharenko SS and Klyachko VA, 2013 FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. Neuron, 77(4), 696–711. [PubMed: 23439122]

Ding Q, Sethna F, Wang H, 2014 Behavioral analysis of male and female Fmr1 knockout mice on C57BL/6 background. Behav. Brain Res 0, 72–78. 10.1016/j.bbr.2014.05.046

Dziembowska M, Pretto DI, Janusz A, Kaczmarek L, Leigh MJ, Gabriel N, Durbin-Johnson B, Hagerman RJ, Tassone F, 2013 High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. Am. J. Med. Genet. A 161, 1897–1903. 10.1002/ajmg.a.36023

Ethridge LE, White SP, Mosconi MW, Wang J, Byerly MJ, Sweeney JA, 2016 Reduced habituation of auditory evoked potentials indicate cortical hyper-excitability in Fragile X Syndrome. Transl. Psychiatry 6, e787 10.1038/tp.2016.48 [PubMed: 27093069]

Fendt M, Li L and Yeomans JS, 2001 Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology, 156(2–3), pp.216–224. 10.1007/s002130100794 [PubMed: 11549224]

Frankland PW, Wang Y, Rosner B, Shimizu T, Balleine BW, Dykens EM, Ornitz EM, Silva AJ, 2004 Sensorimotor gating abnormalities in young males with fragile X syndrome and Fmr1-knockout mice. Mol. Psychiatry 9, 417–425. 10.1038/sj.mp.4001432 [PubMed: 14981523]

Garcia-Pino E, Gessele N, Koch U, 2017 Enhanced Excitatory Connectivity and Disturbed Sound Processing in the Auditory Brainstem of Fragile X Mice. J. Neurosci 37, 7403–7419. 10.1523/JNEUROSCI.2310-16.2017 [PubMed: 28674175]

Gkogkas CG, Khoutorsky A, Cao R, Jafarnejad SM, Prager-Khoutorsky M, Giannakas N, Kaminari A, Fragkouli A, Nader K, Price TJ and Konicek BW, 2014 Pharmacogenetic inhibition of eIF4E-dependent Mmp9 mRNA translation reverses fragile X syndrome-like phenotypes. Cell reports, 9(5), pp.1742–1755. https://10.1016/j.celrep.2014.10.064 [PubMed: 25466251]

Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB, Roberts J, Mirrett P, 2006 Autistic behavior in children with fragile X syndrome: Prevalence, stability, and the impact of FMRP. Am. J. Med. Genet. A 140A, 1804–1813. 10.1002/ajmg.a.31286 [PubMed: 16700053]

Hessl D, Berry-Kravis E, Cordeiro L, Yuhas J, Ornitz EM, Campbell A, Chruscinski E, Hervey C, Long JM, Hagerman RJ, 2009 Prepulse inhibition in fragile X syndrome: Feasibility, reliability, and implications for treatment. Am. J. Med. Genet. B Neuropsychiatr. Genet 150B, 545–553. 10.1002/ajmg.b.30858 [PubMed: 18785205]

Huber KM, Gallagher SM, Warren ST, Bear MF, 2002 Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc. Natl. Acad. Sci 99, 7746–7750. 10.1073/pnas.122205699 [PubMed: 12032354]

Koch M, Schnitzler H-U, 1997 The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. Behav. Brain Res 89, 35–49. 10.1016/S0166-4328(97)02296-1 [PubMed: 9475613]

Largo RH and Schinzel A, 1985 Developmental and behavioural disturbances in 13 boys with fragile X syndrome. European Journal of Pediatrics, 143(4), pp.269–275. 10.1007/BF00442299 [PubMed: 2580709]

Li L, Korngut LM, Frost BJ, Beninger RJ, 1998 Prepulse inhibition following lesions of the inferior colliculus: prepulse intensity functions. Physiol. Behav 65, 133–139. 10.1016/S0031-9384(98)00143-7 [PubMed: 9811375]

Lovelace JW, Wen TH, Reinhard S, Hsu MS, Sidhu H, Ethell IM, Binder DK, Razak KA, 2016 Matrix metalloproteinase-9 deletion rescues auditory evoked potential habituation deficit in a mouse model of Fragile X Syndrome. Neurobiol. Dis 89, 126–135. 10.1016/j.nbd.2016.02.002 [PubMed: 26850918]

Meredith RM, Dawitz J, Kramvis I, 2012 Sensitive time-windows for susceptibility in neurodevelopmental disorders. Trends Neurosci 35, 335–344. 10.1016/j.tins.2012.03.005 [PubMed: 22542246]

Mott B, Wei S, 2014 Firing Property of Inferior Colliculus Neurons Affected by FMR1 Gene Mutation. J. Otol 9, 86–90. 10.1016/S1672-2930(14)50020-7

Nielsen DM, Derber WJ, McClellan DA, Crnic LS, 2002 Alterations in the auditory startle response in Fmr1 targeted mutant mouse models of fragile X syndrome. Brain Res 927, 8–17. 10.1016/S0006-8993(01)03309-1 [PubMed: 11814427]

Rais M, Binder DK, Razak KA, Ethell IM, 2018 Sensory Processing Phenotypes in Fragile X Syndrome. ASN NEURO 10 10.1177/1759091418801092

Reinhard SM, Razak K, Ethell IM, 2015 A delicate balance: role of MMP-9 in brain development and pathophysiology of neurodevelopmental disorders. Front. Cell. Neurosci 9 10.3389/fncel.2015.00280

Renoux AJ, Sala-Hamrick KJ, Carducci NM, Frazer M, Halsey KE, Sutton MA, Dolan DF, Murphy GG, Todd PK, 2014 Impaired sensorimotor gating in Fmr1 knock out and Fragile X premutation model mice. Behav. Brain Res 267, 42–45. 10.1016/j.bbr.2014.03.013 [PubMed: 24657592]

Schneider A, Leigh MJ, Adams P, Nanakul R, Chechi T, Olichney J, Hagerman R, Hessl D, 2013 Electrocortical changes associated with minocycline treatment in fragile X syndrome. J. Psychopharmacol. (Oxf.) 27, 956–963. 10.1177/0269881113494105

Scott KE, Schormans AL, Pacoli K, De Oliveira C, Allman BL, Schmid S., 2018 Altered auditory processing, filtering, and reactivity in the Cntnap2 knockout rat model for neurodevelopmental disorders

Setz C, Brand Y, Radojevic V, Hanusek C, Mullen PJ, Levano S, Listyo A, Bodmer D, 2011 Matrix metalloproteinases 2 and 9 in the cochlea: expression and activity after aminoglycoside exposition. Neuroscience 181, 28–39. 10.1016/j.neuroscience.2011.02.043 [PubMed: 21354273]

Sidhu H, Dansie LE, Hickmott PW, Ethell DW, Ethell IM, 2014 Genetic Removal of Matrix Metalloproteinase 9 Rescues the Symptoms of Fragile X Syndrome in a Mouse Model. J. Neurosci 34, 9867–9879. 10.1523/JNEUROSCI.1162-14.2014 [PubMed: 25057190]

Sidorov MS, Auerbach BD, Bear MF, 2013 Fragile X mental retardation protein and synaptic plasticity. Mol. Brain 6, 15 10.1186/1756-6606-6-15 [PubMed: 23566911]

Siller SS, Broadie K, 2011 Neural circuit architecture defects in a Drosophila model of Fragile X syndrome are alleviated by minocycline treatment and genetic removal of matrix metalloproteinase. Dis. Model. Mech 4, 673–685. 10.1242/dmm.008045 [PubMed: 21669931]

Sinclair D, Oranje B, Razak KA, Siegel SJ, Schmid S, 2017 Sensory processing in autism spectrum disorders and Fragile X syndrome—From the clinic to animal models. Neurosci. Biobehav. Rev 76, 235–253. 10.1016/j.neubiorev.2016.05.029 [PubMed: 27235081]

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN, 1993 Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am. J. Hum. Genet 53, 1217–1228. [PubMed: 7902673]

Tsiouris JA, Brown WT, 2004 Neuropsychiatric Symptoms of Fragile X Syndrome. CNS Drugs 18, 687–703. 10.2165/00023210-200418110-00001 [PubMed: 15330685]

Valsamis B and Schmid S, 2011 Habituation and prepulse inhibition of acoustic startle in rodents. JoVE (Journal of Visualized Experiments), 55, p.e3446 10.3791/3446

Veeraragavan S, Bui N, Perkins JR, Yuva-Paylor LA, Carpenter RL, Paylor R, 2011 Modulation of behavioral phenotypes by a muscarinic M1 antagonist in a mouse model of fragile X syndrome. Psychopharmacology (Berl.) 217, 143 10.1007/s00213-011-2276-6 [PubMed: 21487657]

Wang X, Zorio DAR, Schecterson L, Lu Y, Wang Y, 2018 Postsynaptic FMRP Regulates Synaptogenesis In Vivo in the Developing Cochlear Nucleus. J. Neurosci 38, 6445–6460. 10.1523/JNEUROSCI.0665-18.2018 [PubMed: 29950504]

Wen TH, Afroz S, Reinhard SM, Palacios AR, Tapia K, Binder DK, Razak KA, Ethell IM, 2018 Genetic Reduction of Matrix Metalloproteinase-9 Promotes Formation of Perineuronal Nets Around Parvalbumin-Expressing Interneurons and Normalizes Auditory Cortex Responses in Developing Fmr1 Knock-Out Mice. Cereb. Cortex 28, 3951–3964. 10.1093/cercor/bhx258 [PubMed: 29040407]

Yuhas J, Cordeiro L, Tassone F, Ballinger E, Schneider A, Long JM, Ornitz EM, Hessl D, 2011 Brief Report: Sensorimotor Gating in Idiopathic Autism and Autism Associated with Fragile X Syndrome. J. Autism Dev. Disord 41, 248–253. 10.1007/s10803-010-1040-9 [PubMed: 20521090]

Yun S-W, Platholi J, Flaherty MS, Fu W, Kottmann AH, Toth M, 2006 Fmrp is required for the establishment of the startle response during the critical period of auditory development. Brain Res 1110, 159–165. 10.1016/j.brainres.2006.06.086 [PubMed: 16887106]

Zaman T, De Oliveira C, Smoka M, Narla C, Poulter M, Schmid S., 2017 BK channels mediate synaptic plasticity underlying habituation in rats. J. Neurosci, 37 (17): 4540–4551. [PubMed: 28348135]

Zorio DA, Jackson CM, Liu Y, Rubel EW, Wang Y. (2017) Cellular distribution of the fragile X mental retardation protein in the mouse brain. J Comp Neurol 525:818–849. 10.1002/cne.24100 [PubMed: 27539535]

## Highlights

- Adult Fmr1 KO mice show reduced pre-pulse inhibition of acoustic startle.
- Pre-pulse inhibition in Fmr1 KO mice is normal if MMP-9 is genetically reduced.
- MMP-9 is involved in sensory gating.
- MMP-9 may be a potential target for sensory hypersensitivity symptoms in FXS.

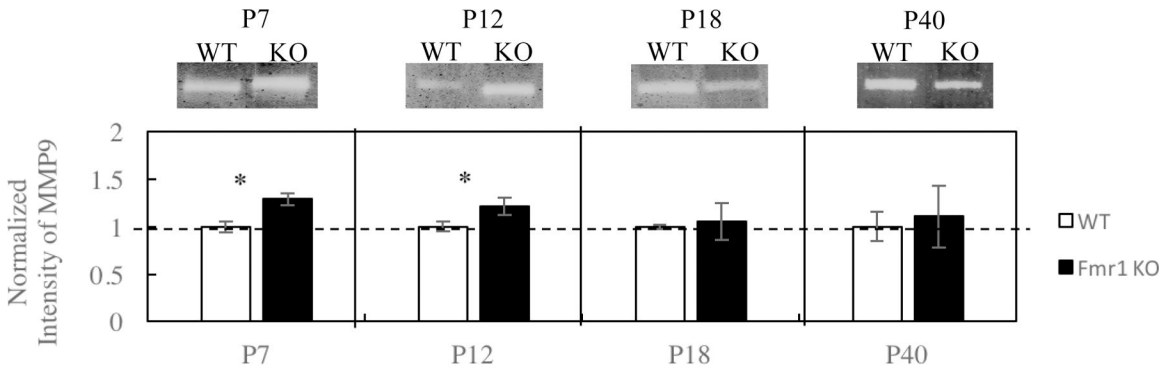


**Figure 1. MMP-9 levels in IC differ between WT and adult *Fmr1* KO mice at P7 and P12, but not P18 or P40.**

There was an increase in MMP-9 levels in *Fmr1* KO mice earlier in development (P7: $t(4)=-2.47$, p=0.0345); P12: $t(7)=-2.054$, p=0.0395). However, at later ages there was no difference between the two genotypes (P18: P18 ($t(8)=-0.267$, p=0.398); P40 ($t(8)=-0.299$, p=0.772). Standard error is shown in the bar graphs.

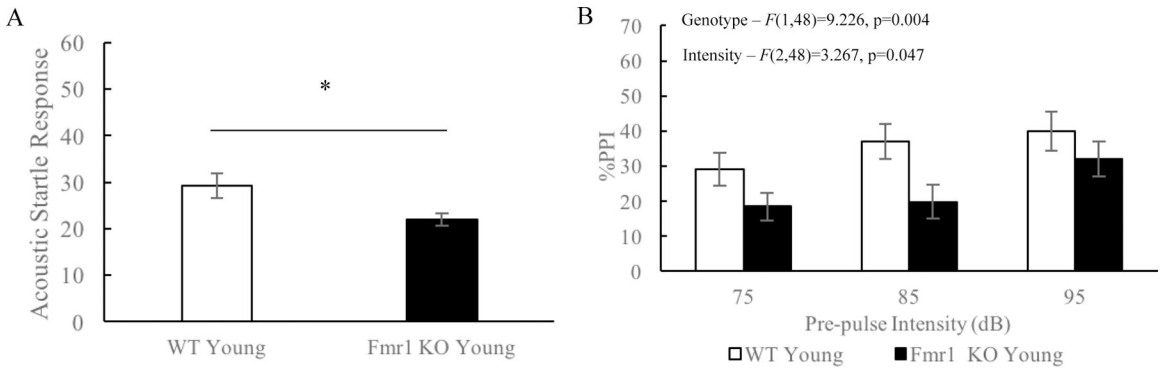


**Figure 2. Startle is reduced in *Fmr1* KO mice at young, but PPI is not different from WT.**

A) The baseline ASR was significantly different between young WT and *Fmr1* KO mice (one-way ANOVA - $F(1,16)=6.190$, $p=0.024$). B) Two-way ANOVA with prepulse intensity and genotype as factors in the young mice revealed that PPI increased with sound intensity (Intensity – $F(2,48)=3.267$, $p=0.047$; Tukey HSD - 75dB vs 85dB, $p=0.596$; 75dB vs 95dB, $p=0.038$; 85dB vs 95dB, $p=0.273$) and was reduced in *Fmr1* KO compared to WT mice (Genotype – $F(1,48)=9.226$, $p=0.004$). There were no significant interactions between genotype and prepulse intensity (Genotype*Intensity – $F(2,48)=0.499$, $p=0.610$). Standard error is shown in the bar graphs.

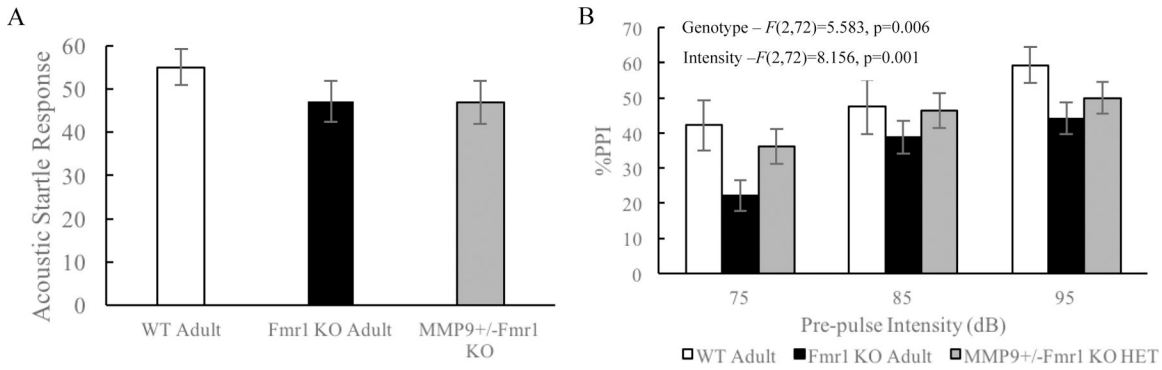


**Figure 3. Genetic reduction of MMP-9 in *Fmr1* KO mice partially rescues the PPI deficit.**

A) In adult mice, there was no statistical difference in ASR across the three genotypes (one way ANOVA - $F(2,24)=1.019$, p=0.376). B) Two way ANOVA with genotype and prepulse intensity as factors shows that in adult mice, there was a significant effect of genotype ($F(2,72)=5.583$, p=0.006; Tukey HSD – WT vs *Fmr1* KO, p=0.004; WT vs MMP9+/−*Fmr1* KO, p=0.427; *Fmr1*KO vs MMP9+/−*Fmr1* KO, p=0.106) and intensity ($F(2,72)=8.156$, p=0.001; Tukey HSD – 75dB vs 85dB, p=0.044; 75dB vs 95dB, p=0.000431; 85dB vs 95dB, p=0.267), but no interactions (Genotype*Intensity - $F(4,72)=0.401$, p=0.807). Standard error is shown in the bar graphs.

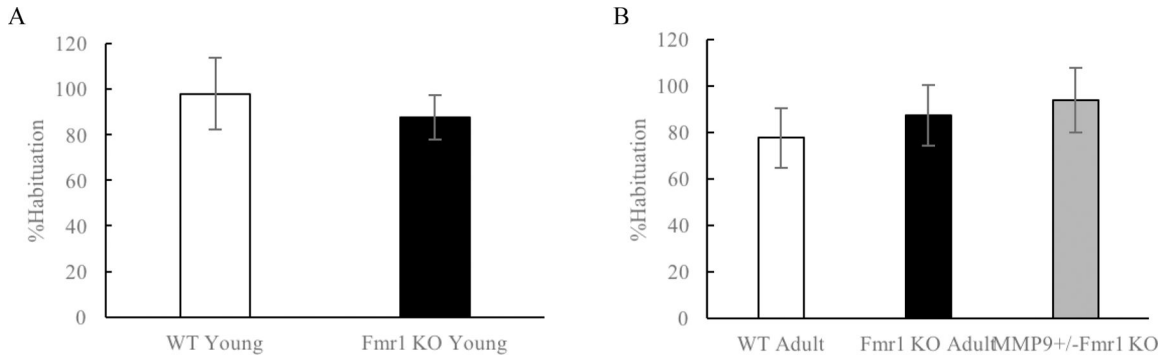


**Figure 4. Habituation to ASR stimuli is normal in both young and adult *Fmr1* KO mice.**

A) Young WT and *Fmr1* KO mice did not show any significant difference in habituation from the beginning of the protocol to the end ($F(1,16)=0.316$, $p=0.582$). B) There was no significant difference in percent habituation between any of the adult age groups ($F(2,24)=0.368$, $p=0.696$). Standard error is shown in the bar graphs.

**Table 1.**

Summary of genotype and age studied.

| Genotype | Number of mice | Age(postnatal days) | Average weight (g) | Average standard error |
|---|---|---|---|---|
| WT Adult | 9 | 53–55 | 28.5 | 0.98 |
| *Fmr1* KO Adult | 9 | 53–54 | 28.5 | 0.82 |
| MMP9 +/− *Fmr1* KO Adult | 9 | 65–67 | 27.3 | 0.715 |
| WT Young | 9 | 24–25 | 17.4 | 0.72 |
| *Fmr1* KO Young | 9 | 23–24 | 15.6 | 0.70 |

**Table 2.**

**Trial types for acoustic startle and prepulse inhibition.**

In trials looking at prepulse inhibition, the interval between the prepulse and the startle pulse was 100ms. The inter-trial interval ranged from 15–25 seconds.

| Trial Type | Prepulse Intensity (dB) | Startle Stimulus (dB) |
|---|---|---|
| 1 | 0 | 115 |
| 2 | 75 | 115 |
| 3 | 85 | 115 |
| 4 | 95 | 115 |
| 5 | 105 | 115 |
| 6 | 0 | 115 |