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Impaired sensorimotor gating in Fmr1 knock out and Fragile X premutation model mice

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Abstract

Fragile X Syndrome (FXS) is a common inherited cause of intellectual disability that results from a CGG repeat expansion in the *FMR1* gene. Large repeat expansions trigger both transcriptional and translational suppression of Fragile X protein (FMRP) production. Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is an allelic neurodegenerative disease caused by smaller “pre-mutation” CGG repeat expansions that enhance *FMR1* transcription but lead to translational inefficiency and reduced FMRP expression in animal models. Sensorimotor gating as measured by pre-pulse inhibition (PPI) is altered in both FXS patients and Fmr1 knock out (KO) mice. Similarly, FXTAS patients have demonstrated PPI deficits. Recent work suggests there may be overlapping synaptic defects between Fmr1 KO and CGG knock-in premutation mouse models (CGG KI). We therefore sought to interrogate PPI in CGG KI mice. Using a quiet PPI protocol more akin to human testing conditions, we find that Fmr1 KO animals have significantly impaired PPI. Using the same protocol, we find CGG KI mice demonstrate an age-dependent impairment in PPI compared to wild type (WT) controls. This study describes a novel phenotype in CGG KI mice that can be used in future therapeutic development targeting premutation associated symptoms.

Keywords

Fragile X; premutation carriers; prepulse inhibition; sensorimotor gating

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Fragile X Syndrome (FXS) is the most common known inherited cause of intellectual disability [1]. FXS patients exhibit motor developmental delays, executive dysfunction, and 30–50% of patients qualify for a DSM-IV diagnosis of autism [1]. FXS is caused by expansion of a CGG trinucleotide repeat in the 5' untranslated region (UTR) of the *FMR1* gene on the X chromosome to more than 200 repeats. This large expansion inhibits production of the FMR1 protein product, FMRP, by triggering hypermethylation of the repeat and *FMR1* promoter region, resulting in transcriptional silencing of *FMR1*. When *FMR1* methylation is incomplete and FMR1 mRNA is transcribed, the CGG repeat expansion inhibits FMRP translation, presumably by impairing ribosomal scanning through the repeat in the 5'UTR [2].

Associated with FXS is the allelic age-related neurodegenerative condition, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS). FXTAS patients display cognitive and neurological signs with age but are typically asymptomatic until age 50. The main features of FXTAS include action tremor and ataxia, with cognitive decline in a significant fraction of patients. FXTAS patients have an intermediate “premutation” repeat expansion between 55 and 200 CGGs [3]. In premutation carriers, the CGG repeat expansion triggers enhanced transcription, resulting in an increase of FMR1 mRNA [4]. However, as in unmethylated FXS patients, the repeat RNA forms a hairpin loop that impairs FMRP translation [5, 6]. This effect is repeat length dependent, such that larger CGG repeats elicit greater impairments in translational efficiency and lower FMRP levels [3, 7, 8]. Consistent with reduced FMRP levels, younger premutation carriers can develop symptoms more commonly associated with FXS, such as higher rates of autistic and attention deficit hyperactivity disorder (ADHD)-like symptoms [5, 9].

One phenotype observed in both FXS patients and mouse models is sensorimotor gating abnormalities. This is the process by which an acoustic startle response (ASR) is modulated, as measured by pre-pulse inhibition (PPI) [10]. FXS patients show impaired PPI, suggesting a basal failure in sensorimotor gating [11]. Similarly, mice lacking FMRP (*Fmr1* KO) also show disruptions in PPI, although the direction of change is variable [12–18].

Our group has recently demonstrated that premutation model mice exhibit deficits in a form of synaptic plasticity (mGluR-LTD) that is also implicated in *Fmr1* KO mice [6]. Additionally, symptomatic FXTAS patients exhibit altered ASR and PPI [19]. We therefore sought to define whether premutation model mice, which have 120 CGG repeats knocked in to the murine *Fmr1* 5'UTR (CGG KI; [6]), exhibit alterations in their ASR or PPI. Establishing a phenotypic readout in premutation model mice that has direct correlation with patient findings would greatly aid in preclinical testing and development of novel therapeutics for FXTAS. Furthermore, identifying areas of phenotypic overlap between CGG KI and *Fmr1* KO mice may allow cross-application of therapeutic strategies developed for FXS patients to be applied to premutation carriers.

Here we show that *Fmr1* KO mice exhibit reduced PPI. In older CGG KI mice, we see a similar impact on sensorimotor gating, with altered ASR and reduced PPI compared to controls. However this phenotype in premutation model mice is age-dependent, as younger

CGG KI mice show normal PPI. This study establishes altered PPI as a shared phenotype in CGG KI and Fmr1 KO mice that may be amenable to pharmacologic intervention.

Mouse handling regulations were followed in accordance to NIMH/NIH Guidelines on the Care and Use of Animals and the University Committee and the Use and Care of Animals at the University of Michigan (UCUCA).

Mice were maintained on a C57BL/6 background. CGG KI mice were obtained from the Usdin laboratory at the NIH (Bethesda, MD) [6]. CGG KI and littermates were genotyped as described previously [6]. Fmr1 KO mice were received from Cara Westmark (University of Wisconsin) and Jim Malter (UT Southwestern). Genotyping was performed as described previously [20].

Kinder Scientific Startle Monitor equipment and software was used to measure acoustic startle reflexes (<http://www.kinderscientific.com>). The testing chamber was fixed on a movement sensor within a small padded chamber (to mute reverberation) housed within a larger sound proof cabinet.

On day one, basal startle response was measured with a startle input-output program run in succession with a 15 minute break between trials. Noise was played for 40ms four times each in a pseudo-random order with no pre-pulse between stimuli. Fmr1 KO cohort n=7 KO, 7 WT; young CGG KI cohort n=10 KI, 6 WT; old CGG KI n=8 KI, 4 WT.

PPI trials began on day two and continued for four testing days. The quiet PPI protocol consisted of two sessions using 100dB SPL acoustic startle and 50ms pre-pulses of narrow-band or broad band noise at 40, 50, or 65dB SPL played 100ms before the startle with 5–9 seconds inter-trial intervals played in a pseudo-random order. Fmr1 KO cohort n=8 KO, 10 WT; young CGG KI n=10 KI, 6 WT; old CGG KI n=14 KI, 7 WT. Ambient sound for the quiet protocol was measured at 49.3 dBA SPL, with the majority of spectral energy falling below 1.8 kHz (Quest model 2200 sound level meter). Ambient background sound levels above 1.8 kHz (murine auditory range is 2.5–70 kHz [21]) did not exceed 25dB.

%PPI was calculated as follows: $100 - [(response\ to\ pre-pulse\ plus\ startle\ stimulus / startle\ response\ alone) \times 100]$. The average %PPI value of each session per mouse was combined for final values compared between genotypes.

Following all testing, the hearing ability of the animals was verified using Auditory Brainstem Response Testing (ABR) as described previously [22].

Values are reported as mean $\pm$ SEM. Data were compared by two-way ANOVA comparing genotype and stimulation intensity. A significant effect of stimulation intensity was expected in all experiments. Interactions are noted in the results where applicable; * indicates an overall difference in genotype of $P < 0.05$. Significant effects of genotype were followed with a Fisher's LSD for post-hoc analysis on individual points (# indicates $P < 0.05$).

To ensure that hearing function was not affecting our experiments, we evaluated ABRs in the mice we tested. Using three tonal frequencies (12kHz, 24kHz, 48kHz), there was a significant effect of frequency across all genotypes, consistent with published studies in

C57BL/6 mice [23]. In the younger (3–5 month old) Fmr1 KO and WT littermate controls, we found no difference in thresholds between genotypes (all ABR thresholds are in dB SPL; WT: 12kHz=26.3±0.92 24kHz=35±2.39 48kHz=60.1±3.79; Fmr1 KO: 12kHz=25±2.19 24kHz=34.2±4.06 48kHz=48.1±8.07; $F(1, 54) = 2.060$, not significant (NS), $n=10$ for each group; data not shown). Similarly, we found no difference in younger CGG KI mice compared to WT controls (WT: 12 kHz= 26±2.83 24 kHz=28±3.62 48 kHz=68±8.87; CGG KI: 12 kHz=37.25±13.59 24 kHz=39.75±14.33 48 kHz=76±17.30; $F(1, 18) = 1.624$, NS, $n=4$ for each group; data not shown). In older (7–8 months old) CGG KI and WT littermates, we observed a significant elevation in ABR thresholds for the CGG KI mice compared to WTs at higher frequencies (WT: 12kHz=23.14±2.14, 24kHz=24.71±4.16, 48kHz=46±13.39, $n=7$; CGG KI: 12kHz=27±1.74, 24kHz=40.22±5.27, 48kHz=77.78±2.54; $F(1, 42) = 14.99$, $P < 0.05$, $n=9$; interaction between genotype and frequency, $F(2, 42) = 3.380$, $P < 0.05$; data not shown). As ABR profiles were similar across all genotypes at the 12 kHz frequency, we restricted our analysis to either BBN or 12 kHz stimulation.

We first evaluated the ASR and PPI in Fmr1 KO mice and littermate controls. Basal ASR in animals over a range of intensities (60–120dB SPL) demonstrated no significant difference between Fmr1 KO animals compared to WT (Figure 1A; $F(1, 108) = 0.088$, NS, $n=7$ for each group).

In an effort to better mimic the PPI testing conditions used in patient studies [11, 19], we recorded PPI using an established protocol with minimal background noise [24]. Using this protocol we found a significant decrease in the %PPI of Fmr1 KO animals compared to WT controls (Figure 1B; $F(1, 48) = 6.864$, $P < 0.05$; post-hoc Fisher's LSD at 65dB $t = 2.887$, $P < 0.05$, $n=8$). While these results are consistent with studies in FXS patients and some trials in Fmr1 KO mice [11, 12, 17], other studies have observed an opposite effect, with enhanced PPI in Fmr1 KO mice [13–18]. It is notable that we observed some habituation in both WT and Fmr1 KO animals tested. However, this effect was not different between genotypes, therefore did not affect the overall results.

We next evaluated PPI and ASR in premutation CGG KI mice. We measured basal ASR in younger (2–5 month old) CGG KI and WT littermate controls, and observed a significant reduction in startle amplitude in CGG KI mice (Figure 2A; $F(1, 112) = 9.916$, $P < 0.05$, $n=10$ KI, 6 WT; $t = 2.164$, $P < 0.05$ for 105dB point by Fisher's LSD). However, we observed no significant difference in PPI between premutation and control animals (Figure 2B; $F(1, 42) = 0.2025$, NS, $n=10$ KI, 6 WT).

In patients, symptomatic male premutation carriers have altered PPI [19]. We therefore chose to evaluate older male CGG KI and littermate controls (7–8 months old), hypothesizing that they may have age-related alterations in sensorimotor gating. We saw altered basal ASR in aged CGG KI animals compared to WTs (Figure 2C; $F(1, 90) = 6.958$, $P < 0.05$, $n=8$ KI; no individual point was significant by Fisher's LSD). In contrast to our findings, symptomatic human premutation carriers are reported to have reduced basal startle response [19]. Our observation may be a hyperacusis-elicited response to age-related hearing impairment in mice as previously described [25]. We found a significant decrease in

CGG KI %PPI compared to littermate controls (Figure 2D; $F(1, 57) = 4.312$, $P < 0.05$, $n = 14$ KI, 7 WT; $t = 2.285$, $P < 0.05$ for 65dB pre-pulse, post-hoc Fisher's LSD).

In this study we demonstrate a new behavioral phenotype in premutation model mice. Both CGG KI and Fmr1 KO mice exhibit impaired PPI, a measure of sensorimotor gating, which mimics what is seen in Fragile X spectrum disorder patients [11, 17, 19]. While similar to studies in human FXS patients, this finding differs from previous reports where Fmr1 KO mice exhibit enhanced PPI [13–18]. The reason for these differences is unclear, although minor modifications in testing protocols, environmental variance or genetic drift in the Fmr1 KO background may be contributing factors. One previous study in Fmr1 KO mice, using eye blink to measure startle similar to human tests, also demonstrated impaired PPI, which is typically observed in patients [12].

The impaired PPI observed in Fmr1 KO and older CGG KI mice indicates an additional behavioral phenotype in common between these models. Recent work demonstrates that these CGG KI mice exhibit a number of features originally thought to be specific to Fmr1 KO animals. Specifically, hyperactivity, decreased anxiety in open field and elevated zero maze assays, and social behavior alterations [26]. However, not all mouse models of FXTAS exhibit these phenotypes [3], so the generalizability for these findings to other models remains unknown.

The mechanism for altered PPI in FXTAS patients and CGG KI mice is unclear. These mice do not exhibit significant neurodegeneration within the ages used here, but there could be an age-dependent decline in neuronal function which impacts sensorimotor gating. Of note, the age-dependent hearing loss in C57BL/6 mice is unlikely to be a major contributor to the change in PPI, as age-related deafness typically enhances rather than suppresses PPI at lower frequencies, opposite of what we observe [27].

An alternative explanation for the observed PPI alterations is the reduced FMRP expression in CGG KI mice. Work by our group and others demonstrate that CGG premutation model mice express reduced levels of basal FMRP and impaired activity-dependent FMRP translation [6, 7, 26]. This defect is coupled to enhanced mGluR-dependent synaptic weakening [6], a phenotype also seen in Fmr1 KO mice [28]. Interestingly, in both Fmr1 KO mice and in FXS patients, alterations in PPI are reversible with mGluR 1/5 antagonists [12]. Whether CGG KI mice or premutation patients will benefit similarly from mGluR antagonists will be an important area of future study.

These data, along with behavioral and electrophysiological assessments in premutation model mice, suggest that there are significant areas of pathophysiologic overlap in these models. The progress in treating FXS may also be applicable to premutation carriers and may have important implications for therapeutic development across the Fragile X disorder spectrum.

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Highlights

FMR1 KO Fragile X Syndrome model mice show impaired prepulse inhibition (PPI).

CGG repeat knockin FXTAS model mice show impaired prepulse inhibition.

These prepulse inhibition abnormalities in CGG knockin mice are age-dependent.

Fmr1 KO and CGG knockin animals share an impaired PPI phenotype.

Figure 1. Auditory startle response and reduced pre-pulse inhibition in Fmr1 KO animals
 (A) Startle response curves reveal that Fmr1 KO mice (n=7) ages 2–3 months have normal responses compared to littermate WT mice (n=7). (B) WT mice (n=10) show a significantly higher %PPI than Fmr1 KO mice (n=8) when tested with a 12kHz, 100dB startle and 45–65 dB pre-pulses. * $P < 0.05$ main effect of genotype by two-way ANOVA; # $P < 0.05$ post-hoc Fisher's LSD.

Figure 2. Auditory startle response and age-dependent pre-pulse inhibition in CGG KI mice
 (A) Startle response curves show that 2–5 month old CGG KI mice (n=10) have altered ASR compared to littermate controls (n=6). (B) PPI in younger CGG KI mice revealed no difference. (C) Startle response curves in older 7–8 month old CGG KI mice (n=8) have altered ASR compared to WT (n=4). (D) Older CGG KI mice (n=14) show significantly impaired PPI compared to littermate controls (n=7). *P<0.05 main effect of genotype by two-way ANOVA; #P<0.05 post-hoc Fisher's LSD.