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Short Communication

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

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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.

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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 this 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.

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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

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[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 soundproof cabinet.

On day one, basal startle response was measured with a startle input–output program run in succession with a 15 min break between trials. Noise was played for 40 ms 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 100 dB SPL acoustic startle and 50 ms pre-pulses of narrow-band or broad band noise at 40, 50, or 65 dB SPL played 100 ms before the startle with 5–9 s 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 25 dB.

%PPI was calculated as follows: 100 − [(response to pre-pulse plus startle stimulus/startle response alone) × 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 ± 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 (12 kHz, 24 kHz, 48 kHz), 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: 12 kHz = 26.3 ± 0.92 24 kHz = 35 ± 2.39 48 kHz = 60.1 ± 3.79; Fmr1 KO: 12 kHz = 25 ± 2.19 24 kHz = 34.2 ± 4.06 48 kHz = 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: 12 kHz = 23.14 ± 2.14, 24 kHz = 24.71 ± 4.16, 48 kHz = 46 ± 13.39, $n = 7$; CGG KI: 12 kHz = 27 ± 1.74, 24 kHz = 40.22 ± 5.27, 48 kHz = 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 (60–120 dB SPL) demonstrated no significant difference between Fmr1 KO animals compared to WT (Fig. 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 (Fig. 1B; $F(1, 48) = 6.864$, $P < 0.05$; post hoc Fisher's LSD at 65 dB $t = 2.887$, $P < 0.05$, $n = 8$). 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 (Fig. 2A; $F(1, 112) = 9.916$, $P < 0.05$, $n = 10$ KI, 6 WT; $t = 2.164$, $P < 0.05$ for 105 dB point by Fisher's LSD). However, we observed no significant difference in PPI between premutation and control animals (Fig. 2B; $F(1, 42) = 0.2025$, NS, $n = 10$ KI, 6 WT).