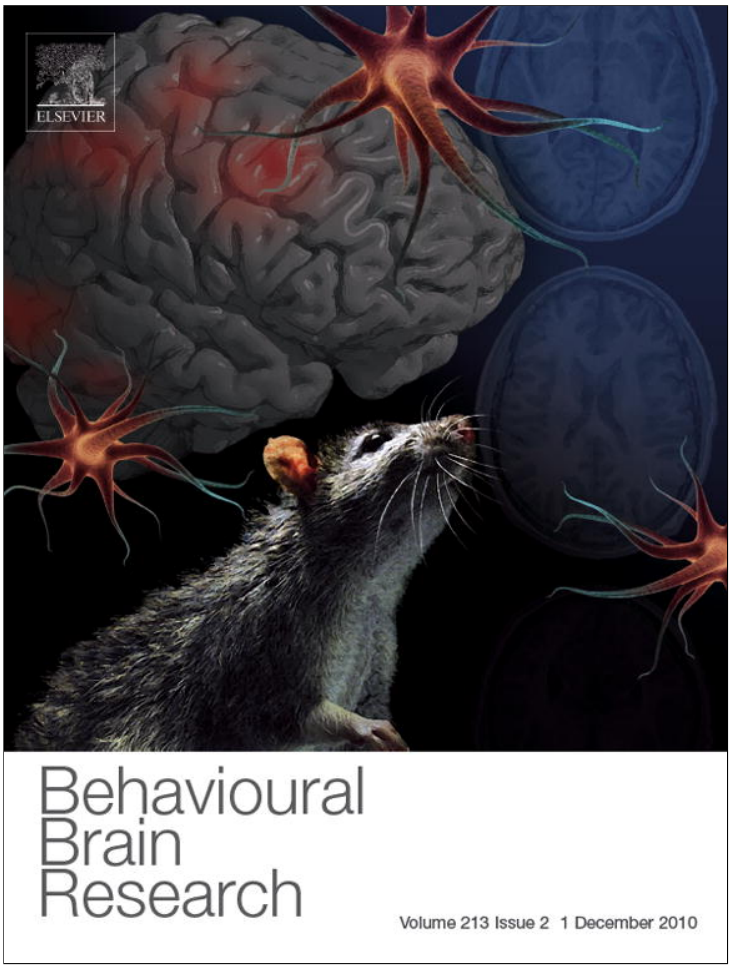

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Research report

Temporal ordering deficits in female CGG KI mice heterozygous for the fragile X premutation

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ABSTRACT

The fragile X premutation is a tandem CGG trinucleotide repeat expansion on the *FMR1* gene between 55 and 200 repeats in length. A CGG knock-in (CGG KI) mouse with CGG repeat lengths between 70 and 350 has been developed and used to characterize the histopathology and cognitive deficits reported in carriers of the fragile X premutation. Previous studies have shown that CGG KI mice show progressive deficits in processing spatial information. To further characterize cognitive deficits in the fragile X premutation, temporal ordering in CGG knock-in (CGG KI) mice was evaluated. Female CGG KI mice were tested for their ability to remember the temporal order in which two objects were presented. The results demonstrate that at 48 weeks of age, female CGG KI mice with CGG repeat expansions between 150 and 200 CGG repeats performed more poorly on tests of temporal order than wildtype mice, whereas female CGG KI mice with between 80 and 100 CGG repeats performed similarly to wildtype mice. No mice had any difficulty in detecting the presence of a novel object. These data suggest female CGG KI mice show a CGG repeat length-sensitive deficit for temporal ordering.

1. Introduction

The fragile X premutation is defined as a CGG trinucleotide repeat expansion between ~55 and 200 repeats in length in the 5′ untranslated region (5′ UTR) of the fragile X mental retardation 1 (*FMR1*) gene. The fragile X premutation results in a 3–8 fold increase in *FMR1* mRNA levels in leukocytes and, paradoxically, decreased *FMR1* protein (FMRP) levels due to translational inefficiency of the mutant *FMR1* mRNA [63]. Some carriers of the fragile X premutation develop a late onset neurodegenerative disorder: fragile X-associated tremor/ataxia syndrome (FXTAS). This is in contrast to the full mutation in fragile X syndrome (FXS), which results in intellectual disability [21]. In FXS the CGG repeat expansion is longer than 200, and *FMR1* mRNA and FMRP levels are too low to be detected due to hypermethylation of the *FMR1* promoter region and subsequent transcriptional silencing [61,62].

Until recently, cognitive function in fragile X premutation carriers was presumed to be largely unaffected by the mutation. However, studies into potential cognitive effects of the fragile X premutation are demonstrating neurocognitive impairments related to the length of the CGG repeat expansions, *FMR1* mRNA levels, and FMRP levels [2,41,60,63]. Fragile X premutation carriers have reduced hippocampal volumes relative to the general population and this volume reduction correlates with poor performance on memory tests [32,47]. Using functional magnetic resonance imaging (fMRI), Koldewyn et al. [41] reported that fragile X premutation carriers have reduced hippocampal activation during episodic retrieval compared to the general population. These studies suggest that cognitive processing in fragile X premutation carriers is fundamentally altered. These findings, however, are difficult to interpret as there are not always differences in performance between fragile X premutation carriers and the general population for behavioral performance; making comparisons between neural activation as recorded by fMRI and cognitive processing difficult.

To evaluate the nature of neurocognitive deficits in carriers of the fragile X premutation, a CGG knock-in (KI) mouse model has been studied [4,8,30,65,67,68]. This CGG KI mouse has been shown to model much of the neuropathology seen in fragile X premutation carriers with FXTAS, including intranuclear inclusions in neurons and astrocytes, the neuropathological hallmark of FXTAS [4,8,30,65,67,68].

In a recent review, Simon [59] showed that many neurogenetic disorders including FXS, Turner syndrome, Williams syndrome,

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and chromosome 22q11.2 deletion have overlapping cognitive impairments across the spatial and temporal domains. Simon [58,59] proposed that these deficits in spatiotemporal cognition may result from "reduced resolution, or clarity, of mental representations" referred to as 'spatiotemporal hypergranularity'. In other words, processing spatial distances or temporal separations between objects becomes increasingly difficult as the spatial or temporal differences become smaller due to cognitive interference. Thus, compared to the general population, individuals with neurodevelopmental disorders may have coarser mental representations, so that identification of one spatial location or time point from another requires a larger between-item difference before they are perceived as distinct. Although the spatiotemporal hypergranularity model was developed and subsequently validated in 22q11.2 deletion syndrome [58,59], it can be extended to include other neurodevelopmental disorders that show spatial and/or temporal processing deficits, which may include FXS [33,36].

Previous research indicates that the hippocampus has a role in processing spatial and temporal relationships between stimuli, and moreover receives inputs from all sensory modalities; suggesting that one function of the hippocampus may be to encode and separate events in time and space by a process called 'pattern separation' [16,17,25,38,40,44,50–52,54–56,64]. Pattern separation ensures that incoming sensory information is orthogonalized to minimize interference. One hypothesis is that spatiotemporal hypergranularity may result from impaired spatial and temporal pattern separation processes. Hunsaker et al. [30] demonstrated that male CGG KI mice have progressive spatial processing deficits using a task specifically designed to evaluate spatial pattern separation [18,19]. They further demonstrated that development of spatial processing deficits coincided with the appearance of hippocampal pathology (e.g., intranuclear inclusions).

The spatial processing deficits in CGG KI mice can be interpreted as resulting in or from a hypergranularity in spatial information processing. The present study was designed to evaluate temporal pattern separation in CGG KI mice by evaluating their ability to process temporal relationships between stimuli. This study also serves as an additional test of the spatiotemporal hypergranularity hypothesis in the CGG KI mouse model of the fragile X premutation by directly evaluating temporal pattern separation in CGG KI mice [25,38].

In the present study, heterozygous female CGG KI mice at 48 weeks of age were tested on a temporal ordering paradigm used previously in rats [23,24,28]. This paradigm exploits the tendency of rats and mice to explore the earlier item in a sequence of items presented over time if given a choice between two [48,66]. Performance on this task depends either on temporal sequencing of presented stimuli to guide performance or on judgments of the relative memory strengths among previously presented stimuli [3,15]. An impairment in either of these memory processes results in similar temporal ordering deficits—namely the lack of preferential exploration of an object presented earlier in a sequence over one presented later [28,29,38,39,43].

Female mice were used in this study as the frequency of the fragile X premutation is higher in females than males (1:250–813 in males and 1:113–259 in females) [20], and there are increasing reports of neurocognitive and psychiatric abnormalities in female fragile X premutation carriers [1,8,11,21,22,31,34,35,42,53,70]. While most research into FXS and the fragile X premutation has been carried out in males because the mutation is X-linked, it is also important to characterize pathology resulting from X-linked mutations in females [10,66]. The results of the present study suggest that 48-week-old female CGG KI mice have difficulty in temporal ordering, but only when the CGG trinucleotide repeat expansion is beyond a certain threshold. These same female CGG KI mice responded normally to a novel visual stimulus. The results of this study suggest that female CGG KI show impaired temporal ordering, supporting the hypothesis that a spatiotemporal hypergranularity may underlie cognitive deficits seen in the fragile X premutation.

2. Materials and methods

2.1. Mice

The generation of a CGG knock-in (CGG KI) mouse model of the fragile X premutation has been described in detail [5,68]. The CGG KI mice were developed on a on a mixed FVB/N × C57BL/6J background, then backcrossed with C57BL/6J mice from Jackson Labs (Bar Harbor, ME) until congenic.

CGG KI mice used in the present study were housed in same sex, mixed genotype groups of up to four littermates per cage with food and water ad libitum, constant temperature, and a 12 h light–dark cycle. Temporal ordering was evaluated in 43 female mice at 48 weeks of age. Fourteen mice were wildtype, with both X alleles having CGG trinucleotide repeats of between 9 and 11 repeats. The remaining 29 mice were heterozygous for the CGG repeat expansion (i.e., one wildtype and one mutant X allele), with 14 carrying a large CGG repeat expansion between 150 and 190, and 15 carrying a smaller CGG repeat expansion between 80 and 100 on the mutant X allele. The high and low CGG repeat expansion mouse lines were bred from CGG KI mice with spontaneous expansions or contractions of the trinucleotide repeat and then maintained as separate lines in order to examine the relationship between CGG repeat length and pathological features. All CGG KI mice used in this study were from separate litters and wildtype mice were littermate controls for the CGG KI mice. All experimental procedures and protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC).

2.2. Genotyping

DNA was extracted from mouse tails and the number of CGG trinucleotide repeats was quantified by PCR following published protocols [6,30,67,68]. Genotypes were determined twice, once from tail snips taken at weaning and again from tail snips taken at the time of sacrifice to verify genotype. No discrepancies were observed between the two measured genotypes.

2.3. Experimental apparatus

To evaluate temporal ordering in CGG KI mice as a function of CGG trinucleotide repeat length, the three groups of mice (i.e., wildtype, low and high CGG repeats) were tested on a temporal ordering for visual objects task [23,24,28]. The task was run in a transparent Plexiglas box 26 cm long × 20 cm wide × 16 cm tall. Eight objects in triplicate were used as stimuli for this study. These objects ranged in size from 6 cm diameter × 6 cm tall to 4 cm × 2 cm. All objects and the apparatus were wiped down with 70% ethanol between sessions in order to reduce unwanted odor cues. Behavioral data were scored by two experimenters blind to the genotype of the mouse [25,28].

2.4. Experimental protocols

2.4.1. Temporal ordering for visual objects

During session 1, two identical copies of a first object (object 1) were placed at the ends of the box 2.5 cm from the end walls and centered between the long walls (Fig. 1). The mouse was placed in the center of the box facing away from both objects. The mouse was given 5 min to freely explore the objects. After 5 min, the mouse was removed to a small holding cup for 5 min. During this time, the first objects were replaced with two duplicates of a second object (object 2). For session 2, the mouse was again placed in the apparatus and allowed to explore. After 5 min, the mouse was removed to the holding cup for 5 min and the objects were replaced with two duplicates of a third object (object 3). For session 3, the mouse was given 5 min to explore. After 5 min, the mouse was removed into a small cup for 5 min and an unused copy of the first and an unused copy of the third object were placed into the box. The mouse was again placed into the box and allowed to explore the two objects (e.g., object 1 and object 3) during a 5 min test session. Mice typically show increased exploration of the first object compared to the third object, and this was used as an index of memory of the temporal order of the object presentation. A lack of preferential exploration of one object over the other indicates temporal ordering impairments [25]. An alternative explanation based on relative memory strengths for the visual objects is possible and is considered in Section 4.

2.4.2. Visual object novelty detection

In addition to reflecting impaired temporal ordering, increased exploration of the first object over the third could also be interpreted as being due to difficulty in remembering the first object prior to the test session. In order to minimize and control for such general memory deficits, a novelty detection of visual objects task was performed. Briefly, on a different day mice received a three sessions during which they were allowed to explore three novel sets of objects (objects 1, 2, 3) similar to the temporal ordering tasks. During the test session, the first object and a

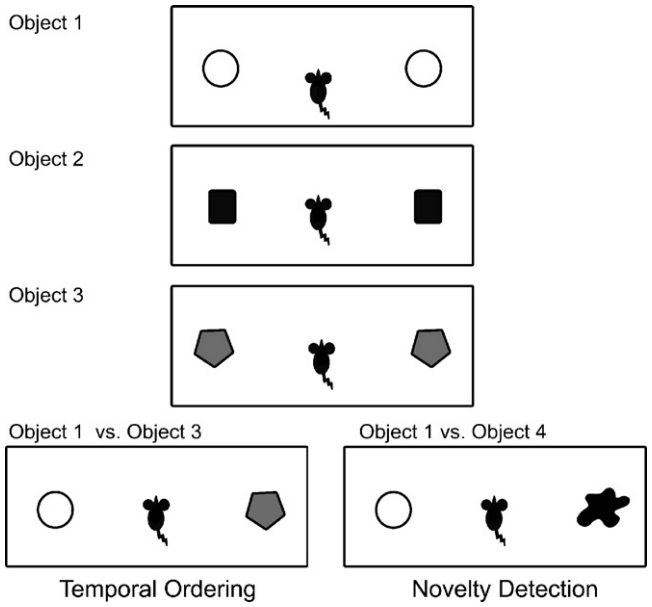

Fig. 1. *Temporal ordering for visual objects paradigm.* Mice are presented with two copies of object 1 for 5 min followed by a 3 min intersession interval. This is repeated for objects 2 and 3. After the presentation of all three objects, mice are given a preference test wherein object 1 and object 3 are presented to the mouse and they are allowed to explore. Preferential exploration of object 1 over object 3 reflects intact temporal ordering. *Visual object novelty detection paradigm.* The novelty detection for visual objects paradigm has identical sessions 1–3, but the test session consists of object 1 and a never before seen novel object 4. Preferential exploration of a novel object 4 over object 1 reflects intact memory of object 1 as well as intact novelty detection.

novel fourth object (object 4) were presented and the mice were allowed 5 min to explore. Preferential exploration of the novel object 4 over object 1 would indicate that the mouse remembered having previously explored object 1, while equal levels of exploration of the two objects would indicate that forgetting had occurred [48].

2.4.3. Dependent measures

For the temporal ordering task, object exploration was defined as active physical contact with the object with the forepaws, whiskers, or nose. With this definition, an mouse standing near an object without interacting with it would not be counted as exploration. Object exploration was recorded in .5 s increments (e.g., >.25 s was recorded as .5 s and >.75 s was recorded as 1 s). This conservative definition slightly underestimates the amount of exploration and reduces confounds/experimenter bias if a more liberal criterion for exploration such as if the mouse were within 1 cm of the object was to be employed. These data were collected during each initial object exploration session as well as during the test session by experimenters blind to the mouse genotype. To control for differences in exploration levels between mice, exploration during the temporal ordering test sessions was converted into a ratio score to constrain the values between −1 and 1. The ratio calculated as follows:

$$\frac{(\text{exploration of object 1} - \text{exploration of object 3})}{(\text{exploration of object 1} + \text{exploration of object 3})}$$

Exploration during the novelty detection test sessions was similarly converted into a ratio score, using exploration of objects 1 and 4 in the calculation.

A ratio value near 1 means that the mouse showed more exploration of the first item presented in the temporal ordering task. A score near −1 suggests the mouse preferentially explored the last object presented. A score near 0 reflects equal exploration of objects indicating a failure to detect the temporal order of visual object presentation. In the novel object test a score near either 1 (i.e., preference for the novel object) or −1 (preference for object 1) would indicate intact memory of object 1, while a score near 0 would suggest that forgetting had occurred. As a measure of general activity levels, locomotor activity was determined by recording the number of times the mouse crossed the midline of the box with all four paws during each session.

2.4.4. Statistical analysis

Prior to running an analysis of variance (ANOVA), the data were tested for normalcy (Shapiro–Wilk test) and homoscedacity (Browne–Forsythe test). Locomotor activity was analyzed using a 3 (group; wildtype, low CGG repeat, high CGG repeat) × 4 (session; session 1, session 2, session 3, test session) repeated measures ANOVA. Any differences in locomotor activity were more fully characterized using Tukey's HSD post hoc paired comparisons test. Object exploration data from each session were analyzed with 3 (group) × 4 (session) repeated measures ANOVA to verify that mice explored all the objects similarly during the study sessions to verify that unequal exploration would not confound measures of temporal ordering [48]. Furthermore, side preferences during object sessions 1–3 were tested with individual paired *t*-tests against the null hypothesis of 50% exploration for the object on each side. Exploration data that were converted to ratio values were analyzed by one-way ANOVA. To more fully characterize any differences among groups, Tukey's HSD post hoc paired comparisons test was performed. To verify that locomotor behavior and object exploration during earlier sessions did not contribute to temporal ordering and/or novelty detection measures recorded during the test sessions, analyses of covariance (ANCOVA) were run with both locomotor behavior and object exploration during session 1, both locomotor behavior and object exploration during session 3, as well as locomotor behavior during the test session as covariates. All results were considered significant at $\alpha \leq .05$ and $1 - \beta \geq .8$.

3. Results

3.1. Temporal ordering of visual objects

3.1.1. Locomotor activity

Statistical analysis showed no difference in locomotor activity between groups $F(2, 39) = 1.49$, $p = .22$. There was a significant effect of session $F(3, 167) = 13.93$, $p < .001$, but the group × session interaction was not significant, $F(6, 167) = .06$, $p = .81$. Individual comparisons between sessions revealed that locomotor activity during session 1 was higher than activity during the other three sessions ($p < .05$), but the other three sessions did not differ (all $p > .1$). The lack of a significant interaction is important, because it suggests that there were no differences in the slope of habituation across groups.

3.1.2. Object exploration

A two-way repeated measures ANOVA with group (wildtype, low CGG repeat, high CGG repeat) and session (session 1, session 2, session 3, test session) as factors was performed on the object exploration data. The ANOVA showed no difference in exploration between groups, $F(2, 39) = 2.64$, $p = .11$, no effect of session, $F(3, 167) = 1.51$, $p = .21$, and no interaction, $F(6, 167) = 1.86$, $p = .14$. These data suggest that all mice similarly explored objects across sessions 1–3 and did not differ in total object exploration during the test session. Furthermore, no mouse showed side biases during exploration sessions 1–3 (all two-tailed *t*-tests $p > .1$).

3.1.3. Temporal ordering (ratio values)

To determine group differences in temporal ordering, a one-way ANOVA was performed on the ratio values computed from the object exploration during the test session (Fig. 2). Since previous research has implicated differences in object exploration during exploration sessions and locomotor activity in exploration measures during preference tests [48], locomotor activity and object exploration during sessions 1 and 3 were included as covariates in the analysis, as was locomotor activity during the test session. The one-way analysis of covariance (ANCOVA) revealed that there were group differences for the ratio score, $F(2, 34) = 13.63$, $p < .001$. The locomotor activity or object exploration covariates during sessions 1 or 3 were not statistically significant (all $p > .1$), nor locomotor activity during the test session ($p > .1$). To further characterize the main effect of group, Tukey's HSD post hoc paired comparisons test revealed that the high CGG repeat group showed temporal ordering impairments relative to the wildtype and low CGG repeat groups ($p < .001$, $p < .01$, respectively). The low CGG repeat group showed similar temporal ordering as the wildtype group ($p > .1$).

3.1.4. Visual object novelty detection

The same analyses performed for the temporal ordering for visual objects were performed for the visual object novelty detection task. The only significant effect was the main effect of session

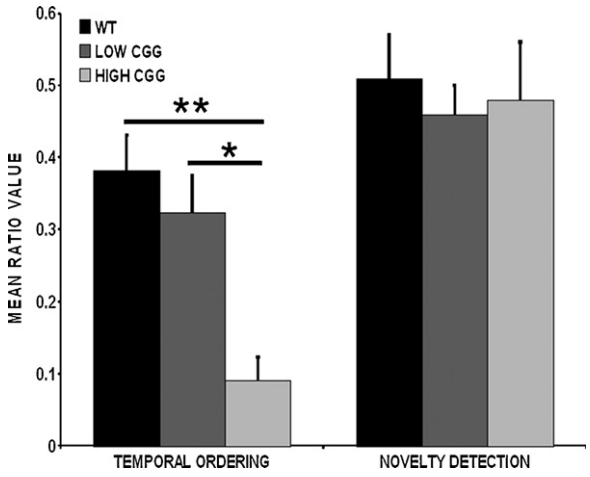

Fig. 2. *Temporal ordering and novelty detection for visual objects in female CGG KI mice*. Wildtype mice and CGG KI mice from the low CGG repeat group (70–100 CGG repeats) do not significantly differ, whereas the high CGG repeat group (150–200 CGG repeats) shows a significantly lower ratio value than the other two groups (plotted are means ± SEM; $^{*}p > .01$; $^{**}p < .001$). There were no differences for novel object detection.

for locomotor behavior, $F(3, 167) = 8.56$, $p < .001$. Again, just as in the temporal ordering for visual objects task, locomotor activity during session 1 was higher than the other three sessions ($p < .05$). Session 2, session 3, and the test session did not differ (all $p > .1$). There were no differences in how the different groups reacted to visual object novelty (ANCOVA $p > .2$). These results suggest CGG KI mice reacted similarly to wildtype mice to visual object novelty (Fig. 2).

4. Discussion

The present results show that CGG KI mice with trinucleotide repeat expansions between 150 and 200 CGG repeats did not preferentially explore the earlier presented object in a sequence of objects, indicating either a deficit in processing sequential relationships among remembered stimuli, or a deficit in judging the relative memory strength among the stimuli [26,27,37,43,45]. While the deficit could reflect impaired learning or impaired recall of the order of exposure to the objects, it is not possible to make this distinction based on the present data. This is because the experimental design of the temporal order for visual objects task was not explicitly designed to test for strength of memory for sequences, and thus it is not possible to discriminate between the use of sequential processing per se and judgments of relative memory strengths among stimuli [3,15].

We assume that the present task could be solved either by explicit sequential processing of the visual object information or by weighting the relative strengths of the memory traces of experienced visual objects [28,43]. Since the present task was designed to evaluate temporal ordering in CGG KI mice, not to elucidate the component processes underlying the temporal ordering in CGG KI mice, we shall refer to both processes collectively as "temporal ordering" (cf. [28,38]).

Interestingly, CGG KI mice with CGG trinucleotide repeat expansions >150 repeats (high CGG repeat) showed temporal ordering deficits compared to wildtype mice, whereas those with CGG trinucleotide repeat expansions <100 repeats (low CGG repeat) showed no evidence for temporal ordering deficits and performed similarly to wildtype mice. Both groups of female CGG KI mice were able to identify and selectively explore a novel visual object, suggesting that the temporal ordering deficits reflected impaired temporal processing/judging of relative memory strength and not global memory deficits or altered visual perception [25,48]. This is the first report of impaired temporal ordering in the female CGG KI mouse as previous studies have been focused on the spatial domain [30,65].

The temporal ordering or visual objects task presented to CGG KI mice has been described as an episodic-like memory task, as episodic memory relies on the separation of experienced behavioral episodes in time in order to recall specific episodes, either by explicitly recalling time-stamped memories or discriminating the differential strengths of memory traces [12,38,39,43]. In this way, the temporal ordering deficits in CGG KI mice with >150 CGG trinucleotide repeats supports previous studies that have suggested abnormal hippocampal activity during episodic recall in fragile X premutation carriers [41]. The present experiment also extends previous work by Van Dam et al. [65] and Hunsaker et al. [30] who were able to demonstrate spatial processing deficits in male CGG KI mice. The Hunsaker et al. [30] report suggested that male CGG KI mice showed deficits for fine spatial processing that contribute to the memory deficits observed by Van Dam et al. [65] using the Morris water maze test of spatial learning and memory. A deficit in temporal ordering as revealed in the present study suggests that deficits in the fragile X premutation may involve larger networks of structures that interact in temporal and/or episodic memory [13,23,24,45]. Furthermore, the present data in combination with those reported by Hunsaker et al. [30] support the hypothesis that a spatiotemporal hypergranularity may underlie some cognitive deficits seen in carriers of the fragile X premutation.

In rat lesion studies, diverse brain regions such as the anterior thalamus [69], rostral infralimbic and prelimbic cortices (referred to as medial prefrontal cortex by [24,46]), perirhinal cortex [23], and the hippocampus [28,39] have been shown to be involved in temporal ordering. Lesions of any one of these structures lead to qualitatively similar deficits for temporal ordering. In humans, the parietal cortex has further been shown to be involved in processing temporal relationships [9,45]. Due to the apparently distributed nature of the processes underlying temporal ordering within the brain [13], it is difficult to assign abnormalities within any specific anatomical loci as underlying the observed deficits in temporal ordering in CGG KI mice.

Brain anomalies in fragile X premutation carriers have been reported, and include grey matter volume decreases in the hippocampus [32,47], the left thalamus [47,49], the insula, inferior temporal cortex, pre and post central gyrus, and the inferior parietal cortex, as well as white matter abnormalities in the cingulum and frontal–temporal white matter tracts [47,49]. All of these areas have been implicated in temporal and/or episodic memory [13]. Unfortunately, studies evaluating similar neuropathological features have not been performed in the CGG KI mouse [4,6,7,67,68], so it is not yet possible to make comparisons between the human and mouse. Longitudinal studies of large groups of CGG KI mice using noninvasive imaging technologies (e.g., high field MRI) that bypass fixation and histological artifacts are underway to characterize regional volumetric differences in the CGG KI mouse well enough to make a direct comparison between the CGG KI mouse and the fragile X premutation carrier.

Willemsen et al. [68] showed the presence and relative abundance of intranuclear inclusions in male CGG KI mice (on a mixed FVB/N × C57BL/6J background). These inclusions appeared similar to those reported in FXTAS cases. They found that at 48–52 weeks of age, CGG KI mice showed intranuclear inclusions in the thalamus, hypothalamus, cingulate cortex (anterior cingulate), periamygdaloid cortex, rostral cortex, and in the hippocampus at slightly lower levels—all regions implicated in temporal ordering. Hunsaker et al. [30] and Wenzel et al. [67] undertook limited analyses of intranuclear inclusions in male CGG KI mice (on a congenic C57BL/6J background) and found a similar pattern of inclusions, though quantitative analyses were not undertaken. What remains

absent from the literature is the distribution and relative abundance of intranuclear inclusions in heterozygous female CGG KI mice. Wenzel et al. [67] evaluated a pair of aged female CGG KI mice that would be in the High CGG repeat group in the present study, and found a similar distribution of inclusions as in the male CGG KI mice, but analyses of female mice in the Low CGG repeat group have yet to be undertaken, although these female mice do have intranuclear inclusions (unpublished observations).

Because levels of *Fmr1* mRNA and Fmrp levels have not yet been quantified in female CGG KI mice it is not yet possible to relate these molecular measure to the present temporal order deficits. To date, these measurements have only been carried out in male mice [6,14], and unequal X inactivation in females renders any *Fmr1* and Fmrp measurements in males and females impossible to directly compare (cf. [57]). Studies are currently underway to quantify *Fmr1* and Fmrp levels in female CGG KI mice.

The present data demonstrate impaired temporal ordering in the CGG KI mouse. Furthermore, this study provided insights into potential differences between CGG KI mice with repeat lengths below 100 CGG trinucleotide repeats and those with repeat lengths above 150 CGG trinucleotide repeats that bear further study using both the murine model as well as with fragile X premutation carriers. These data also suggest some additional parallels in the cognitive deficits seen in fragile X premutation carriers in the CGG KI mouse model of the fragile X premutation, although additional studies will be necessary in order to determine how strong such parallels might be. The present results also point to the need to develop behavioral and neurocognitive tasks for CGG KI mice that more closely model the neurocognitive phenotype of fragile X premutation carriers so that the underlying neural circuitry that is impaired in fragile X premutation carriers (both with and without FXTAS) can be more directly explored. Behavioral tests in CGG KI mice that more closely map onto the neurocognitive deficits reported in fragile X premutation carriers would also make such mice valuable tools for the development and evaluation of treatment for the disorder [33].

The present data show that female CGG KI mice developed to model the fragile X premutation show deficits in temporal ordering, but only when the repeat expansions approach the upper end of the premutation range. What remains to be studied are the implications of this finding for human fragile X premutation carriers and whether such deficits can be attributed to spatiotemporal hypergranularities as has been suggested [58,59].

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