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Spatiotemporal Processing Deficits in Female CGG KI Mice Modeling the Fragile X Premutation

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Abstract

The fragile X Premutation is a tandem CGG trinucleotide repeat expansion in the fragile X mental retardation 1 (*FMR1*) gene between 55 and 200 repeats in length. A CGG knock-in (CGG KI) mouse has been developed that models the neuropathology and cognitive deficits reported in fragile X Premutation carriers. It has been suggested that carriers of the premutation demonstrate a spatiotemporal hypergranularity, or reduced resolution of spatial and temporal processing. A temporal ordering of spatial locations task was used to evaluate the ability of CGG KI mice to process temporal and spatial information with either high or low levels of spatial interference. The results indicate that CGG KI mice showed difficulty performing a spatial novelty detection task when there were high levels of spatial interference, but were able to perform the novelty detection task when there was low spatial interference. These data suggest that CGG KI mice show reduced spatial and temporal resolution that are modulated by the dosage of the *Fmr1* gene mutation, such that when behavioral tasks require mice to overcome high levels of either spatial or temporal interference, the CGG KI mice perform increasingly poorly as the CGG repeat length increases.

Keywords

Fragile X Premutation; CGG KI Mouse; Pattern Separation; Temporal Order; Spatiotemporal Processing

INTRODUCTION

Fragile-X Associated Tremor/Ataxia Syndrome (FXTAS) is a late onset neurodegenerative disease resulting from a tandem CGG trinucleotide repeat expansion between 55 and 200 in the 5' untranslated region of the fragile X mental retardation 1 gene (*FMR1*) called the fragile X Premutation [38]. Though not all carriers of the fragile X Premutation develop FXTAS, it is estimated that 40% of male and 16% of female premutation carriers develop FXTAS [39]. Clinical manifestations of FXTAS include intention tremor, gait ataxia, and

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Parkinsonism, along with post-mortem presence of eosinophilic, ubiquitin positive intranuclear inclusions in neurons and astrocytes throughout the brain [38,39].

Where it was previously thought that fragile X Premutation carriers were cognitively unaffected by the mutation, a growing body of evidence now demonstrates a spectrum of neurocognitive impairment [15,17–18,31–34, 37]. Despite this debate, studies into cognitive effects of the fragile X Premutation reveal an association between the length of CGG repeat expansion with genetic dosage or CGG repeat length. Goodrich-Hunsaker et al. [12–14] reports genetically modulated cognitive performance on spatial and numerical magnitude comparison tasks using asymptomatic female fragile X Premutation carriers between 21–42 years of age. Similarly, results from an attentionally based enumeration task also demonstrated some slight, but significant impairments in numerical cognition related to genetic dosage [12]. These studies along with previous results in male fragile X Premutation carriers implicate cognitive deficits across several domains: including working memory, executive function memory, and arithmetic processing [*cf.*, 6,13–18]. In order to better understand the extent and nature of neurocognitive impairments in the fragile X Premutation, the behavior of a CGG knock-in (CGG KI) mouse model of the fragile X Premutation has been evaluated. In accordance with human carriers of the fragile X Premutation, the CGG KI mice develop intranuclear inclusions throughout the brain [32,46]. Recent studies report both temporal ordering deficits and visuomotor impairments in female CGG KI mice modeling the fragile X Premutation, with the latter displaying a correlation with CGG repeat length [10,22,24]. These results are consistent with reports of genetic dosage modulating neurocognition among premutation carriers [12–14, 36].

In a review of neurocognitive impairments in space, time, and number processing in children with neurodevelopmental disorders such as fragile X-associated disorders (*i.e.*, fragile X syndrome and the fragile X Premutation) and the 22q11.2 deletion syndrome, Simon [42–44] proposed a *spatiotemporal hypergranularity* underlies deficits present across the spatial and temporal domains. Recently, Hunsaker [19] has extended this theory into research into mouse models of neurodevelopmental genetic disease and showed it was consistent with the spatial processing and temporal ordering deficits observed in male and female CGG KI mice.

More specifically, the spatiotemporal hypergranularity proposed to underlie spatiotemporal dysfunction in CGG KI mice stems from abnormal development of neural networks that underlie spatial and temporal attention; namely the hippocampus, parietal cortex, and rostral cortices. As the premutation is present at development, these mice have abnormal neural circuitry that process spatial and temporal relationships among stimuli in a suboptimal manner. What this theory proposes is that for a CGG KI mouse to discriminate among spatial stimuli they need to be separated in space more than a wildtype mice needs to make the same discrimination. Similarly, temporal relationships among stimuli need to have a greater separation than wildtype mice require to make the temporal judgment. This can be conceptualized as a reduction of resolution in spatial and temporal processing capabilities. These spatial and temporal judgments have been proposed to be similar in nature to the spatial and temporal pattern separation processes that act to minimize spatial and temporal interference to allow for efficient encoding of stimuli [40,42–44].

In order to specifically evaluate spatiotemporal processing deficits in CGG KI Mice, female CGG KI mice heterozygous for the fragile X Premutation were tested for their ability to process the temporal order in which objects occupying specific spatial locations were presented [20–23]. The paradigm chosen has been shown to evaluate the temporal processing of spatial information--which has been shown to be subserved by different neural networks than temporal processing of simple object information [20–21]. We hypothesize

that a spatial hypergranularity resulting from suboptimal spatial pattern separation accounts for any inability to perform a spatial novelty detection tasks when very high levels of spatial interference must be overcome; and a temporal hypergranularity resulting from suboptimal temporal pattern separation accounts for impaired temporal ordering for spatial locations in cases of very low spatial interference. Taken together, these deficits compound to result in a spatiotemporal hypergranularity, impairing the ability of the CGG KI mice to efficiently process a temporal order for spatial locations. We further hypothesize that CGG KI mice will be able to perform spatial novelty detection tasks when the levels of spatial interference are minimal, suggesting intact spatial memory function.

The results for the present study support our initial hypotheses: female CGG KI mice heterozygous for the fragile X Premutation showed hypergranular spatial and temporal processing (*i.e.*, reduced memory resolution) that resulted in inefficient encoding or retrieval of spatiotemporal relationships required for task performance, but showed intact spatial memory.

MATERIALS AND METHODS

Mice

Sixteen female CGG KI mice heterozygous for the fragile X Premutation at 6 months of age (n=8 Low CGG repeat (CGG 77–110); n=8 High CGG repeat (CGG 145–194)) and eight female wildtype mice (CGG 8–12) of the same age were used as subjects for this task. All wildtype mice were littermates with CGG KI mice included in the study. All CGG KI mice were bred onto a congenic C57BL/6J background over >12 generations from founder mice on a mixed FVB/N × C57BL/6J background [45]. Mice were housed in same sex, mixed genotype groups with three to four mice per cage in a temperature and humidity controlled vivarium on a 12 h light-dark cycle. Mice had ad libitum access to food and water throughout experimentation. Mouse weights did not differ among genotypes throughout experimentation. All experiments were conducted during the light phase of the diurnal cycle. The experiment were conducted under University of California, Davis approved IACUC protocols.

Genotyping

Genotyping was carried out upon tail snips. DNA was extracted from mouse tails by incubating overnight at 55°C with 10 mg/mL Proteinase K (Roche Diagnostics; Mannheim, Germany) in 300 μL lysis buffer containing 50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 150 mM NaCl, 1% SDS. One hundred μL saturated NaCl was then added and the suspension was centrifuged. One volume of 100% ethanol was added, gently mixed, and the DNA was pelleted by centrifugation and the supernatant discarded. The DNA was washed and centrifuged in 500 μL 70% ethanol. The DNA was then dissolved in 100 μL milliQ-H2O. CGG repeat lengths were determined by PCR using the Expanded High Fidelity Plus PCR System (Roche Diagnostics). Briefly, approximately 500–700 ng of DNA was added to 50 μL of PCR mixture containing 2.0 μM/L of each primer, 250 μM/L of each dNTP (Invitrogen; Tigard, OR), 2% dimethyl sulfoxide (Sigma-Aldrich; St. Louis, MO), 2.5 M Betaine (Sigma-Aldrich), 5 U Expand HF buffer with mg (7.5 μM/L). The forward primer was 5'-GCT CAG CTC CGT TTC GGT TTC ACT TCC GGT-3' and the reverse primer was 5'-AGC CCC GCA CTT CCA CCA CCA GCT CCT CCA-3'. PCR steps were 10 min denaturation at 95°C, followed by 34 cycles of 1 min denaturation at 95°C, annealing for 1 min at 65°C, and elongation for 5 min at 75°C to end each cycle. PCR ends with a final elongation step of 10 min at 75 °C. DNA CGG repeat band sizes were determined by running DNA samples on a 2.5% agarose gel and staining DNA with ethidium bromide [7,10]. For female CGG KI mice heterozygous for the fragile X premutation there were two

bands present, one corresponding to the wildtype allele (CGG repeat length 8–12), and another corresponding to the expanded premutation allele (CGG repeat length 70–200). For wildtype mice, only the wildtype allele was present. Genotyping was performed twice on each mouse, once using tail snips taken at weaning and again on tail snips collected at sacrifice. In all cases the genotypes matched.

Behavioral Apparatus

For this experiment, a white square Plexiglas box was used measuring 70 cm on all sides, and 40 cm in height. A digital camera was positioned over the box capturing an overhead view of the experimental set-up. Different, distinctive geometric shapes were placed on the walls as spatial cues to help spatially orient the mice. These cues were placed slightly off center on each of the walls. Two identical cylindrical or conical objects measuring 4–7 cm in diameter and 11–15 cm in height were chosen and set in distinct spatial locations during experimentation for each task. Different pairs of identical objects were used for each task. Objects placed in different spatial locations were placed approximately 5 cm from each wall or corner of the box. This distinct placement of the object allowed for complete circumnavigation of the object by the mouse. The apparatus and experimental parameters were modified from a task developed for use in rats [20–21]. At the start of each experiment, the box was thoroughly wiped down using 70% ethanol to dilute and spread out any unwanted odors.

Experimental Methods

The order of experiments was pseudorandomized for all mice to control for any effects of task order influencing behavioral performance. Two experimenters independently scored the data blinded to mouse genotype from the digital recordings. A computerized tracking system (ANY-Maze v4.3; Stoelting Co.; Wood Dale, IL) collected locomotor activity from the videos.

Temporal Order for Spatial Locations (Figure 1A)

For this experiment a mouse was then placed in the lower left hand corner of the box, as viewed from above, and allowed to explore a cylindrical or conical object placed in the first spatial location (location 1). At the end of 5 min, a blue plastic rectangular container measuring 7.5 cm in length, 7.5 cm in height, and 7.5 cm in width was used to cover the mouse and slowly moved until positioned in the original starting point. This method was used rather than removing the mouse between each session to reduce stress and/or anxiety responses in the mouse that may mask behavioral performance. A 5 min intersession period followed. The blue container was then removed and the mouse was allowed to explore the same object in the second spatial location (location 2) for another 5 min. The mouse was covered once more and moved to the original starting point for a 5 min intersession interval before being allowed to explore the object in the last spatial location for 5 min (location 3). Once the mouse had explored the object in all three spatial locations, the mouse was covered with the blue plastic rectangular container for 10 min [20,21]. For the test phase, the mouse was presented with two identical objects, one placed in the first spatial location (location 1) and the other in the third spatial location (location 3) along opposite walls of the apparatus for 5 min. The distance between the objects measured 80 cm. The start location of each mouse was determined such that the starting location for each mouse was equidistant from the first and third locations during the test session. Mouse performance was video recorded and was later assessed using the videos acquired during each trial. For each mouse, time spent in active exploration (*i.e.*, sniffing, touching) with the object was recorded, and total locomotor activity was collected. It was determined that when mice climbed, stood, or sat upon an object was not considered exploratory activity.

Novelty Detection for Spatial Location with High Spatial Interference (Figure 1B)

The three locations were presented the same way as in the temporal ordering for spatial locations task, but with a different object pair that the mice had never previously encountered. However, during this high interference novelty test, the familiar spatial location (location 1) and the novel spatial location (location 4) were located very near each other (along adjacent walls of the apparatus). The distance between the objects measured 35 cm, less than half the distance apart as the temporal ordering test. Conceptually, this testing procedure results in increased spatial interference between the two spatial locations occupied by the identical objects. For this task, the start location for each mouse was adjusted so the start location for all mice during the last session was equidistant from the familiar and novel spatial location.

Novelty Detection for Spatial Location with Low Spatial Interference (Figure 1C)

The location presentations were presented similarly to the temporal ordering for spatial locations task, but using a different object pair the mice had never previously encountered with the following modification: The locations were chosen so that the familiar and novel spatial locations used during the test session were along opposite walls of the apparatus. This larger spatial separation was the same separation as the temporal ordering test, and a greater separation than the high spatial interference test (*cf.*, Figure 1B). This modification was used to reduce spatial interference among the locations. The distance between the objects measured 80 cm, and was the same as during the temporal ordering for spatial locations test. The start location of each mouse was determined such that the starting location for each mouse was equidistant from the familiar and novel spatial locations during the test session.

Dependent measures

For the temporal ordering and novelty detection for spatial locations tasks, spatial location exploration was defined as active physical contact with the object in the spatial location using the forepaws, whiskers or nose. With this definition, standing near an object without interacting with it would not be counted as exploration, nor would standing or sitting upon an object. 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 was calculated as follows: (exploration in location 1 − exploration in location 3)/(exploration in location 1 + exploration in location 3). Exploration during the novelty detection test sessions was similarly converted into a ratio score, using exploration of the object in location 1 and an object in the novel location 4 in the calculation: (exploration in novel location 4 − exploration in location 1)/(exploration in location 1 + exploration in novel location 4).

A ratio value near 1 means that the mouse showed more exploration of the first location presented in the temporal ordering task. A score near −1 suggests the mouse preferentially explored the last location presented. A score near 0 reflects equal exploration of spatial locations indicating a failure to detect or retrieve the temporal order of spatial locations [21].

In the novel location tests, a score approaching 1 would indicate a preferential exploration for the novel location, and therefore intact spatial novelty detection, a score approaching −1 indicated preferential exploration for the object in location 1, also indicating memory for the first spatial location. A score near 0 would indicate equal exploration for the familiar and novel locations, suggesting that either an excess of interference at retrieval or forgetting had occurred as reflected in a failure to discriminate between the locations [21]. As a measure of general activity levels, locomotor activity was collected by an overhead tracking system and

confirmed by experimenters recording the number of crossings of a 3 × 3 grid overlaid on the video [*cf.*, 20–21].

Statistical methods

Locomotor activity was analyzed using a 3 (binned CGG repeat group: wildtype, Low CGG repeat (CGG 77–110), High CGG repeat (CGG 145–194)) × 4 (session) repeated measures ANOVA. Object/Location exploration data from each session were analyzed with 3 (CGG repeat group) × 3 (session) repeated measures ANOVA to verify that mice explored all the locations similarly during the study sessions to verify that unequal exploration would not confound measures of temporal ordering. Prior to comparing CGG KI mice and wildtype mice for the ratio scores, it was verified that the ratio score for the wildtype mice was ≠ 0 via a one-tailed t-test against the null hypothesis of a ratio score = 0 to verify preferential exploration of the first location during the temporal order test and novel location during the novelty tests.

Exploration data that were converted to ratio values were analyzed by one-way ANOVA with experiment order as a covariate. To more fully characterize any differences among groups, Tukey's HSD post hoc pairwise comparisons test was performed when the overall group comparison was significant. To verify that locomotor behavior and location exploration during earlier sessions did not contribute to temporal ordering and/or novelty detection measures recorded during the test sessions, ANOVA were performed with both locomotor behavior and location exploration during all sessions as well as experimental order as covariates. To elucidate a role for CGG repeat length modulation of any effects within the CGG KI mice, Pearson's correlation coefficients were calculated to assess the relationship of the ratio values with CGG repeat length in only the CGG KI mice as the range of CGG repeat values were too limited in wildtype mice to perform such analyses (range 8–12 in wildtype compared with 77–194 in CGG KI mice). Furthermore, it was determined that, owing to the large gap in CGG repeat values between the wildtype and any CGG KI mice (gap in the CGG repeat lengths = 55), including wildtype mice in any correlation was inappropriate given the structure of the dataset. All p values were adjusted to control for false discovery rate (FDR) and were considered significant at $p_{(adj)}<0.05$ when power was maintained at 1-ß>.80.

For plotted data, Low CGG and High CGG mice were plotted separately as boxplots to demonstrate group differences. These groups were treated separately for ANOVA as there is a 35 CGG repeat between the groups, suggesting the potential for two separate groups. Below the boxplots are scatterplots of the combined CGG KI mouse data with the correlation analyses that were performed. As the y axis of these plots have been adjusted to best visualize the relationship between performance and CGG repeat in the CGG KI mice, the wildtype mouse data were not included in these plots. This decision was made to better characterize the association between increasing CGG repeat lengths and neurocognitive function in the CGG KI mice, and not to directly characterize differences between CGG KI mouse groups and wildtype mice [*cf.*, similar presentation and statistical analyses presented in 10,24].

RESULTS

Temporal ordering for spatial locations

For the temporal ordering for spatial locations task, there was a significant main effect of CGG repeat group ($F(2,21)=121.52$, $p_{(adj)}<.0001$). Tukey's HSD post hoc pairwise comparisons demonstrated that the High CGG repeat group performed significantly more poorly than the Low CGG repeat group and wildtype group (both $p_{(adj)}<.0001$), and the Low

CGG group performed more poorly than the wildtype group ($p_{(adj)}<.001$). There were no significant differences among groups for either locomotor behavior or spatial location exploration during the three, 5 min location presentation sessions (all $p_{(adj)}>.30$), and experimental order did not contribute to task performance ($p_{(adj)}=.71$).

To characterize any possible relationship between CGG repeat length and temporal ordering for spatial locations in CGG KI mice with expanded CGG trinucleotide repeats, a Pearson's correlation coefficient was calculated across Low CGG and High CGG groups of CGG KI mice. A negative association was observed between the CGG trinucleotide repeat length and the ratio value during performance of the temporal ordering for spatial locations task (Figure 2A; corr $\rho = -.85$; $p_{(adj)}<.0001$, $R^2_{(adj)}=.73$).

Novelty detection for spatial locations with high spatial interference

Similar to the temporal ordering for spatial locations task, there was a significant main effect of CGG repeat group for the spatial locations with high spatial interference task ($F(2,21)= 89.92$, $p_{(adj)}<.0001$). Tukey's HSD post hoc pairwise comparisons demonstrated that the High CGG repeat group performed significantly worse than the Low CGG repeat group and wildtype group (both $p_{(adj)}<.0001$), and the Low CGG group performed worse than the wildtype group ($p_{(adj)}<.001$). There were no significant differences among groups for either locomotor behavior or spatial location exploration during the three, 5 min location presentation sessions (all $p_{(adj)}>.40$), and experimental order did not contribute to task performance ($p_{(adj)}=.71$).

To characterize any possible relationship between CGG repeat length and spatial location novelty detection with high interference in CGG mice with expanded CGG trinucleotide repeats, a Pearson's correlation coefficient was calculated across Low CGG and High CGG groups of CGG KI mice. A negative association was observed between the CGG trinucleotide repeat length and the ratio value during performance of the spatial location novelty detection with high spatial interference task (Figure 2B; corr $\rho = -.88$; $p_{(adj)}<.0001$, $R^2_{(adj)}=.77$).

Novelty detection for spatial locations with low spatial interference

For the novelty detection for spatial locations with low spatial interference task there was no significant effect for CGG repeat group ($F(2,21)=.12$, $p_{(adj)}=.88$), nor were there significant differences among groups for either locomotor behavior or spatial location exploration during the three, 5 min object presentation sessions (all $p_{(adj)}>.20$), and experimental order did not contribute to task performance ($p_{(adj)}=.71$). Furthermore, no association was observed between the CGG trinucleotide repeat length and the ratio value during performance of the spatial location novelty detection with low spatial interference (Figure 2C; corr $\rho = -.03$; $p_{(adj)} =.92$, $R^2_{(adj)}=.0007$).

DISCUSSION

Female CGG KI mice heterozygous for the fragile X premutation were impaired on a temporal order for spatial locations task, a task shown previously to require intact spatial and temporal processing [*cf.*, 20,21]. These mice also showed impairments for a novelty detection for spatial locations task requiring the mice to overcome high levels of spatial interference to make the discrimination between the novel and familiar spatial location. However these mice were unimpaired on a novelty detection for spatial locations task wherein the spatial interference was minimized, suggesting these mice showed intact spatial memory.

In a review of neurocognitive impairments in children with genetic disorders, Simon proposed the theory of a spatiotemporal hypergranularity to describe deficits spanning the spatial and temporal domains [19,42–44]. He proposed a coarser or "grainier" resolution of mental representations with respect to space and time accounts for deficits observed in these disorders. In the present study, whereas wildtype mice are able to recall and separate events in time and space, with increasing CGG repeat length, both spatial and temporal processing become more coarse or grainier, resulting in impaired discrimination of spatial and temporal relationships among stimuli [42–44; *cf.*, 19,40].

The present temporal ordering for spatial locations data can be interpreted as a deficit in spatiotemporal processing because the CGG KI mice were unable to determine which spatial location presented during a test came earlier in a sequence. These impairments cannot be attributed to global deficits for spatial processing because the same CGG KI mice selectively explored a novel spatial location, suggesting the mice showed intact spatial memory. Based on the spatiotemporal hypergranularity model, it is likely the resolution of temporal processing in CGG KI mice is coarser than that of wildtype mice--such that larger temporal distances are required for the CGG KI mice to correctly process the temporal order for spatial locations.

To explore the resolution of spatial processing in CGG KI mice, mice were tested on a spatial location novelty task with high levels of spatial interference among the familiar and novel spatial location. The CGG KI mice were unable to discriminate these spatial locations as well as wildtype mice, evidenced by reduced preferential exploration of the novel spatial location over the familiar location. Based on the spatiotemporal hypergranularity model, it is likely the resolution of spatial processing in CGG KI mice is coarser than that of wildtype mice, as has been previously reported [23].

Critical to the interpretation of the spatial novelty with high interference task as being related to reduced resolution of spatial processing is the finding that CGG KI mice were able to perform a spatial novelty detection task when the spatial interference was minimal (*i.e.*, 80 cm separation of locations). As mentioned above, the same CGG KI mice were overcome by the levels of spatial interference in the high interference condition (*i.e.*, 35 cm separation). In other words, a larger spatial separation is necessary for CGG KI mice to discriminate the familiar and novel spatial location than wildtype mice require to make a similar discrimination.

An nontrivial point in this analysis is the fact that wildtype mice performed similarly well for both spatial novelty irrespective the level of spatial interference. One potential hypothesis would be that the high levels of spatial novelty would result in impaired discrimination in the wildtype mice. If the levels of spatial interference were increased sufficiently, this would surely be the case. In the present experiment, the high spatial interference condition was defined as the two spatial locations to be discriminated being located within 35 cm of each other, whereas the low spatial interference condition was defined as a separation of the two spatial locations of approximately 80 cm. We propose the lack of impairment on the part of the wildtype mice was due to the degree of interference being too low to result in behavioral deficits. This idea is supported by previous work with male CGG KI mice that showed intact spatial processing at distances as low as 30 cm [23] and rats have been shown to have intact spatial discrimination as low as 15 cm [11]. Also, in the original version of the present task used in rats, there was no statistical difference for the high and low interference conditions in control, CA3, or CA1 lesioned rats--only the dentate gyrus lesioned rats [21]. Despite the fact that we did not see an effect of spatial interference in the wildtype mice, it remains a clear possibility that increasing spatial interference sufficiently would indeed impair spatial discrimination in wildtype mice.

Critically, the effects of this spatiotemporal hypergranularity on task performance scaled with the dosage or the fragile X premutation. In other words, the performance of the female CGG KI mice worsened as the number of CGG repeats on the *Fmr1* gene increased for both the temporal ordering and spatial novelty task with high interference. These data support the assertion that increasing CGG repeats are associated with increasingly coarse spatial and temporal mental representations as posited by Simon [42–44]. This negative association between gene dosage and behavioral performance supports previous findings in the CGG KI mice, namely that visuomotor function as measured by skillful walking and skilled reaching tasks similarly deteriorates as a function of increasing CGG repeat length [11,22]. This provides evidence for impaired spatiotemporal processing as a potential neurocognitive endophenotype for the fragile X premutation, as the degree of impairment scales with dosage of the genetic mutation [19, 42–44].

As has been described previously, CGG KI mice show neuropathological features, including intranuclear inclusion bodies, throughout neural networks known to subserve spatial and temporal processing including: the hippocampus and limbic cortices (*i.e.*, anterior cingulate), the parietal lobe, and the murine analog of the medial prefrontal cortex (*i.e.*, infralimbic/prelimbic cortices) [22,23,46]. There are also evidence for reduced Fmrp levels and concomitant elevation in *Fmr1* mRNA levels in these networks, which may negatively affect spatiotemporal processing in CGG KI mice. We propose that molecular and anatomic pathology in neural networks involving these structures underly the present results. Furthermore, these are the same networks proposed by Simon [42–44] to subserve the attentional processes that underly the spatiotemporal hypergranularity observed in many neurodevelopmental disorders.

What the present experiment was unable to address was the exact nature of the spatiotemporal hypergranularity observed in CGG KI mice. Simon's [42–44] postulation for the spatiotemporal hypergranularity was that it results from an alteration or abnormal development of the neural circuits underlying spatial and temporal attention, not directly from impairments to the temporal and spatial pattern separation processes commonly tested in rodent lesion models [*cf.*, 2,9,10,11,19,35,40,41,47]. More recently, however, it has been suggested that pattern separation occurs not only at the most basic level of information processing, but also at a mnemonic level analogous to the attentionally-modulated description of the spatiotemporal hypergranularity (*i.e.*, pattern separation among memory representations rather than among stimuli) [*cf.*, 2]. We propose these impairments in attentional or mnemonic pattern separation processes are analogous to the spatiotemporal hypergranularity and thus underlie the reduced or course temporal spatial resolution observed in CGG KI mice.

In summary, female CGG KI mice heterozygous for the fragile X premutation showed impaired spatiotemporal processing that are consistent with a spatiotemporal hypergranularity. These data demonstrate the fragile X premutation alters the manner by which CGG KI mice process spatial and temporal relationships among stimuli in the environment in a dose-dependent manner. These data support models postulating abnormal development negatively influences spatiotemporal processing in neurodevelopmental disorders [19,42–44], and suggests spatiotemporal processing serves as a valid outcome measure that can be used in studies evaluating neurocognitive sequelae in these disorders--particularly in studies evaluating potential treatment options.

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- Female CGG KI mice modeling the fragile X premutation show spatiotemporal processing deficits
- Female CGG KI mice modeling the fragile X premutation show decreased spatial and temporal resolution
- Female CGG KI mice modeling the fragile X premutation show a spatiotemporal hypergranularity that results in coarse mental representations involving time and space

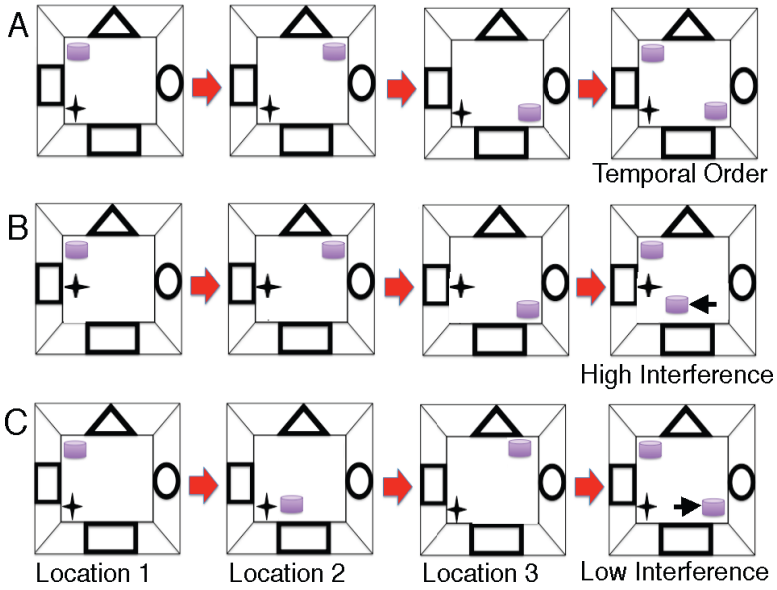

Figure 1.

Experimental Apparatus and Task Design. A. Temporal Ordering for Spatial Locations. 80 cm separates location 1 from location 3. B. Novelty Detection for Spatial Locations with High Spatial Interference. 35 cm separates location 1 from the novel spatial location. C. Novelty Detection for Spatial Locations with Low Spatial Interference. 80 cm separates location 1 from the novel location. The arrows in B and C denote the novel spatial location. The start location of the mouse is marked in each plate. In all cases during the test session the mosue was started equidistant from the two locations being presented.

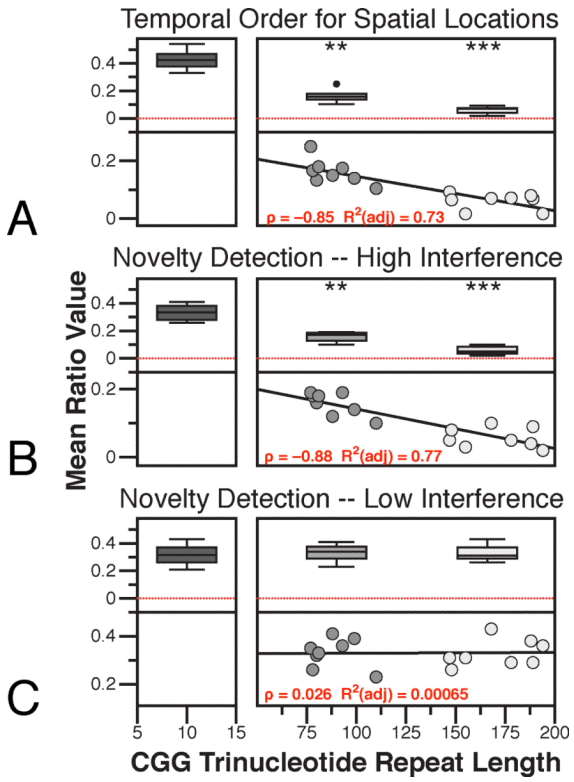

Figure 2.

Experimental Data. A. Temporal Ordering for Spatial Locations. B. Novelty Detection for Spatial Locations with High Spatial Interference. C. Novelty Detection for Spatial Locations with Low Spatial Interference. Boxplots represent the following groups: wildtype mice, Low CGG repeat mice with CGG repeats from 77–110 repeats, and High CGG repeat mice with 145–194 repeats. The scatterplots below the boxplots have the axes adjusted to emphasize the relationship between CGG repeat length and behavioral performance. As such, the wildtype mice are not shown. ** p<0.001; *** p<0.001