INTRODUCTION TO THE CGG KI MOUSE

To investigate the pathological and behavioral consequences of the fragile X premutation, a transgenic CGG knock-in (KI) mouse was developed in which the 5′ UTR containing 8 CGG repeats in the endogenous murine *Fmr1* gene was replaced, via homologous recombination, with a human Nhel—Xhol fragment containing 98 CGG repeats (Hunsaker et al., 2011; Willemsen et al., 2003). Similar to what has been shown in human premutation carriers, the CGG KI mouse shows a moderate level of instability across generations in the length of the CGG trinucleotide repeats with a bias toward expansions, particularly upon paternal transmission (Brouwer et al., 2008; Kim et al., 2010; Willemsen et al., 2003). The CGG KI mouse also shows elevated levels of *Fmr1* mRNA as well as moderate reductions in Fmrp protein levels that exacerbate as CGG repeats approach the full mutation range.

Importantly for the development of a mouse model for a genetic disease, the CGG KI mouse provides a compelling model for the molecular consequences of the premutation, including mitochondrial dysfunction and specific alterations to miRNA processing (Sellier et al., 2010). Additionally, it has been clearly shown in both male and female CGG KI mice that there is the presence of intranuclear masses or inclusions, the hallmark pathological feature of FXTAS, present in neurons and astroglia

in the brain, and some evidence for microglial pathology (*i.e.*, amorphic cytoplasmic masses) as has been observed in FXTAS in rare cases as well as in CGG KI mice (Brouwer et al., 2008; Hunsaker et al., 2009; Schluter et al., 2012; Tassone et al., 2012; Wenzel et al., 2010; Willemsen et al., 2003; Greco et al., personal communication). Additional pathological features of FXTAS, including periventricular white matter disease, nodular heterotopia, and white matter hyper intensities on T2 weighted or FLAIR magnetic resonance images (MRI) in the middle cerebellar peduncle and pons are absent in the CGG KI mouse model, but that is likely due to differences among species or else to limitations of histological techniques rather than any limitations with the murine model.

CGG KI MOUSE BEHAVIORAL ANALYSES

CGG KI MOUSE SPATIOTEMPORAL PROCESSING

So far as neurobehavioral deficits in the CGG KI mouse are concerned, the CGG KI mouse shows a number of basic processing deficits for spatial and temporal information. The CGG KI mouse model of the fragile X premutation shows spatial memory deficits on the Morris water maze when they are older than 52 weeks of age (Van Dam et al., 2005). These deficits, however, appear to be very mild and are not as profound as the general memory

deficits demonstrated in FXTAS patients (*cf.*, Leehey, 2009). Since the Van Dam et al. (2005) study, a new approach has been developed to evaluate memory function in CGG KI mice, called behavioral endophenotyping (*cf.*, Hunsaker, 2012a,b). This approach emphasizes behavioral tasks designed to test specific hypotheses concerning the cognitive strengths and weaknesses reported in fragile X premutation carriers asymptomatic for FXTAS, spatiotemporal function was specifically assayed in the CGG KI mice (*cf.*, Goodrich-Hunsaker et al., 2011a,b). These domains were chosen as it has been suggested that a number of neurodevelopmental disorders share a fundamental spatiotemporal processing deficit called a spatiotemporal hypergranularity. This hypergranularity results in impaired spatiotemporal memory resolution, such that a greater difference among elements in spatial or temporal separation is required before they can be discriminated (*cf.*, Hunsaker, 2012a,b; Simon, 2007, 2008, 2011,).

Using a pair of behavioral tasks to evaluate spatial resolution of spatial memory function in CGG KI mice, it was demonstrated that CGG KI mice show deficits for processing the specific distances that separate two objects in space using a metric change detection task (also called a coordinate change--*cf.*, Figure 1A). These deficits were present as early as 3

months of age, but in a cross sectional study did not appear to be more profound at 6, 9, or 12 months of age (Hunsaker et al., 2009, 2012). However, performance on a task that required the mice to remember which side was occupied by an object after the objects were transposed (called a topological or categorical change) did not show deficits at early ages. In fact, CGG KI mice did not show deficits for this topological change detection task until they were 9 and 12 months of age, not differing from wild type littermate controls at 3 and 6 months of age (Hunsaker et al., 2009, 2012). What can be learned from these data are twofold: (1) that the resolution of spatial processing in CGG KI mice is reduced from a very young age, presumably as early as development, and this resolution appears to be fixed across time, such that the resolution does not progressively worsen as a function of time. (2) general spatial memory as measured by the topological change detection task does show a progressive worsening across age, with deficits emerging in middle life and worsening at advanced ages in CGG KI mice, a pattern similar to those seen with the water maze (Hunsaker et al., 2009, 2012, Van Dam et al., 2005).

To evaluate temporal memory in CGG KI mice, a temporal ordering for visual objects task was used (Hunsaker et al., 2010, 2012; Figure 1B). For this task, in a clear box mice were

presented with two copies of an object for 5 min, then removed from the box for 5 min. The mice were then presented with two copes of a second object in the box for 5 min. After another 5 min break, they were exposed to two copies of a third object for 5 min. After the mice were removed after this third object exposure for a 5 min break, they received one of two tests. The first test is a temporal ordering test during which the mouse is presented with a copy of the first and a copy of the third object and allowed to explore. Typically, mice will preferentially explore the first over the third object.

On another day after a different set of object presentation, the mice receive a second test, a novelty detection task. In this task, the first object they were presented that day as well as a never before sen novel object were presented. In general, mice will preferentially explore the novel object over the familiar one. Intact performance during this novelty task suggest that any deficits on the temporal ordering task are not due to general memory deficits or forgetting the first object before the test session, as they can discriminate a familiar object from a novel object, suggesting intact visual object memory. On this task, the CGG KI mice show intact performance on the novelty detection task, but impairments during performance on the temporal ordering task,

with the CGG KI mice not showing as strong a preference for exploring the first over the third object (Hunsaker et al., 2010, 2012).

As a follow up to these experiments, an explicit spatiotemporal processing task was performed. In this task, mice were presented with a large object in a first spatial location for 5 min in a large box with clear visual cues present. After a 5 min break, the mice explored the same object in a second location. This was repeated for a third location. In this way, the mouse explored the same object in three locations, which we will call exploration of a location. After these presentations, one of three tests were given (over three days with new object-location pairings each day--Figure 1C). The first is a temporal ordering for spatial locations test wherein the first and third locations were marked with identical objects identical to that used to present the locations. Importantly, these locations were always 180º from the mouse's starting location, thus minimizing spatial interference. Preferential exploration the first over the third location was used to index spatiotemporal memory.

The second test was a pure spatial memory control during which the first location and a novel fourth location were marked by identical objects, which were 180º from the mouse's starting point. Preferential exploration of the novel location suggests

intact general spatial memory processing. The final test was a spatial resolution test during which the first and novel object were only separated by 45-90º from the mouse's starting position, increasing the spatial interference to isolate spatial processing in the mice. On this task, the CGG KI mice showed no deficit for spatiotemporal novelty detection wherein the locations were separated by 180º and thus interference was minimized. However, the CGG KI mice did show impairments when the interference was maximized, as well as during the temporal ordering test--strongly suggesting spatial and temporal processing impairments (Borthwell et al., 2012).

An important element to these behavioral results is that both male and female CGG KI mice showed deficits. This is not a minor point as female premutation carriers show reduced disease severity due to the protective effect of a second, non mutated *FMR1* gene on the second X chromosome, which males lack (Berry-Kravis et al., 2005; Berry-Kravis et al., 2007; Schluter et al., 2012; Tassone et al., 2012). The presence of these deficits in both male and female CGG KI mice suggests impairments to these processes are fundamental consequences of the premutation, since deficits are present and identifiable even in the least affected of the population. Similar effects are currently being identified and characterized in human premutation carriers both

symptomatic and asymptomatic for FXTAS symptomatology (Aguilar et al., 2008; Allen et al., 2008; Hagerman et al., 2001; Narcisa et al., 2011).

CGG KI MOUSE VISUOMOTOR FUNCTION

The identification and characterization of spatial and temporal processing anomalies in the fragile X premutation has important consequences for motor function in premutation carriers and the CGG KI mouse model. It has been demonstrated that visuomotor function depends on the integration of spatial and temporal information (Aghdaee and Cavanagh, 2007; Alvarez and Franconeri, 2007; Benson and Haith, 2009; Bertenthal and Von Hofsten, 1998; Bertenthal et al., 1997; Casasanto and Boroditsky, 2008; Chaston and Kingstone, 2004; Jeannerod, 1997; Ladavas and Serino, 2008; Marshall and Fink, 2001; Verstraten et al., 2000; Warren, 2006; Weiss et al., 2000; Yeshurun and Levy, 2003). This means that for an agent to act in space, they must be able to (1) identify the goal of action and (2) plan the movements, (3) initiate the movements, (4) modify the movement online using updated spatiotemporal information, and (5) properly terminate the movement as intended. Intact spatiotemporal updating is what prevents the agent from reaching for a cup and knocking it off the table because they either did

not or were unable to stop or slow the progress of their hand. This is increasingly important if reaching or acting in space extends to processing angular relationships among stimuli or targets that change over time. This updating process is what may be deficient in premutation carriers and FXTAS patients if they show similar spatiotemporal processing deficits as the CGG KI mouse, and thus show reduced spatial and temporal resolution. At present, these assertions are only supported by anecdotal reports of subclinical apraxia and general clumsiness among premutation carriers (R Hagerman & D Hall personal communication).

Van Dam et al. (2005) have identified motor deficits in the CGG KI mouse model at advanced ages, showing a decreased ability of these mice to stay upon an accelerating rotarod. Importantly, these results have been independently replicated in our laboratory (G Arque, unpublished observations). These are important data as FXTAS results in postural and gait instability, both somewhat important for performance on an accelerating rotarod, but the analogy between a gait ataxia and impairments on an accelerating rotarod is strained at best. To more efficiently characterize motor deficits in the CGG KI mouse, more sensitive measures of motor function analogous to

the behaviors measures in humans are required (*i.e.*, CATSYS; Aguilar et al., 2008; Allen et al., 2008; Narcisa et al., 2011).

To specifically evaluate visuomotor functioning in CGG KI mice, a skilled forelimb reaching task was developed by modifying existing protocols to emphasize the acquisition of the skilled reaching rather than performance (as the CGG KI mice are born with the mutation and it cannot be simply turned off until after the training). In this task, the mouse was required to reach through a narrow window to obtain a reward pellet just out of reach of the tongue at a 30º angle off the edge of the window (to require the mouse to reach with the non preferred paw). The number of pellets the mouse was able to obtain without dropping or knocking away the pellet was recorded, as were the number of errors. The CGG KI mice showed different learning curves than wild type mice, with CGG KI mice learning the task on average 1-2 days later than wildtype littermates and never quite learned the task to the same level of asymptotic performance (Diep et al., 2012). Importantly, these deficits were subtle, only becoming apparent when the mice were forced to perform a rather difficult task. There were no observed differences in these mice for grip strength measures or other measures of gross limb usage (unpublished observations). We interpret these data as suggesting there is a fundamental impairment in one of two

neural systems: (1) the parietal cortex and it's interactions with the superior colliculus and cerebellum were unable to provide adequate spatiotemporal updating to allow the CGG KI mice to reach the same level of success as the wild type mice. This interpretation is supported by recent experiments demonstrating superior colliculus activation during reaching tasks in humans using functional MRI (fMRI; Linzenbold and Himmelbach, 2012). (2) the pontocerebellar system shows disruptions (as has been suggested in FXTAS) in a way we could not identify histologically and the deficits arise from an inability of the cerebellum to control the fine motor skills required to skillfully reach, grasp, and consume the reward.

The qualitative data suggests that the CGG KI mice reached with more of a circular or radial motion rather than a directed vector toward the reward pellet, and that mice with longer CGG repeat lengths showed less directed / more radial trajectories than wildtype littermate mice (Figure 2; Supplemental Movie 1). This resulted in the CGG KI mice knocking the reward away or having difficulty in grasping the reward. Once the CGG KI mice grasped the reward pellet, however, they were able to consume it, not showing any difference in the ability to hold onto the pellet and consume it.

To evaluate potential subclinical gait ataxia or general clumsiness in the CGG KI mice, two variants of a skilled ladder walking task were employed. These tasks evaluated the ability of mice to walk across a series of very thin rungs perpendicular to the direction of travel (similar to walking across a ladder set on the ground). The number of times that the mouse made an error in foot placement was recorded as a dependent variable and will be referred to as a foot slip. To perform these tasks, the mouse was placed at one end of the apparatus and allowed to cross from one end to the other into a darkened box.

The first apparatus developed to perform these experiments was a manual ladder rung task (Hunsaker et al., 2011). The apparatus wasted clear plexiglass walls separated by approximately 5 cm with 2 mm diameter steel rungs making up the floor of the apparatus. For this initial study, the mice were placed at one end of the apparatus and were allowed to walk back and forth for 2 minutes. The number of foot slips we recorded for the duration of the 2 min, except for when the mouse was turning around. The number of times the mouse went from one end of the apparatus to the other was also recorded as a general locomotor measure. On this task, mice as young as 2 months of age already showed an increased number of foot slips than wild type litter mate controls. Importantly, the mice showed both

forelimb and hindlimb slips, something that suggests both visuospatial and basic motor deficits. These data suggest visuospatial processing deficits in that there were a high number of forelimb slips in the CGG KI mice, suggesting a difficulty in planning where in space to place the forepaw as well as a difficulty for updating the movement as the step progressed (*i.e.*, as the mouse moved forward the initial planned step has to be modified subtly and an inability to do so results in a foot slip). Hind foot slips however, do not have a visuospatial planning component, but rather reflect a subtle motor effect. This is because stepping with the hind limbs has been shown to be more efficient and easier for the mouse to perform. An inability, or at least increased difficulty with, to step with the hind limb may reflect some form of ataxia that has not been picked up using other apparatus and methods. Additionally, as a model of FXTAS, during performance of this task, the CGG KI. Mice showed a shaking behavior visually similar to an intention tremor in human FXTAS. This is important as this task was rather difficult, and may have required a high degree of effort from the CGG KI mice that was not required from the wild type mice, that in no cases ever presented similar tremoring or shaking behaviors.

Independently, and in parallel with the development of the ladder rung task, a group at Erasmus MC in Rotterdam developed a version of a skilled walking task that was very similar in form, but more elegant in function than the manual ladder rung task. This apparatus, called the Erasmus ladder (Saab et al., 2012), automated the data collection and performance of this task. The apparatus was designed to use light puffs of air to induce the mouse to move from one start box to another across a set of rungs that were attached to electronics capable of capturing step times, foot slips, misplacement of limbs, etc. Additionally, this apparatus also provided a motor associative learning paradigm wherein a tone was played and after 250 ms the next rung was lifted, requiring a motor movement from the mouse to avoid being tripped. (Cupido, 2009) in his dissertation work, evaluated CGG KI mice as well as *Fmr1* KO mice for motor function on the Erasmus ladder apparatus.

Though unpublished, Cupido's (2009) data indicate that the CGG KI mice did not show differences in step time, suggesting intact motor function, but showed a significantly increased number of foot slips and missteps (Figure 3). Additionally, the CGG KI mice were able to nominally perform the motor associative learning task as well as wild type littermate control animals, suggesting intact basal ganglia function. Interestingly,

however, the *Fmr1* KO mice showed the same number of footslips and missteps as the control group and the same step times, suggesting intact visuospatial, visuomotor, and intact basic motor function. The *Fmr1* KO mice did, however, show a deficit for the associative learning task, never performing as well as CGG KI and wild type control animals. These data suggest that the *Fmr1* KO, but not the CGG KI mice, show deficient motor associative processing. In parallel, the CGG KI, but not the *Fmr1* KO mice, show visuospatial processing deficits measures by increased missteps. These data, and this apparatus, doubly dissociated the CGG KI and *Fmr1* KO mice for motor performance. These data have clear implications for the premutation and full mutation underlying fragile X syndrome in humans as brain functions appear to be impaired by both mutations, but the nature of the impairments can be behaviorally doubly dissociated.

CGG KI MOTOR NEUROCOGNITIVE DEFICITS, COMPARISONS WITH HUMANS

One important aspect of modeling a genetic disorder in a mouse model is the comparison of the clinical manifestations of disease with the phenotype of the mouse model. Due to the nature of the disparate research protocols between human and mouse behavioral research, we will focusing the core similarities and

differences between premutation carriers with and without FXTAS and the CGG KI mouse with an emphasis on the degree of analogy/homology in the paradigms or behaviors.

As stated above, premutation carriers with FXTAS demonstrate a range of motor and cognitive symptoms that are recapitulated or phenocopied by the CGG KI mouse in some way. FXTAS patients often present in the clinic with an intention tremor and/or a cerebellar gait ataxia. Importantly, the tremor and ataxia seem to present with an oscillatory component, such that the gait ataxia becomes more profound as the individual walks until they lose balance. They appear relatively normal for the first few steps, but then a postural away emerges that grows in amplitude with each step until the patient either braces against a wall or falls over. For the intention tremor, FXTAS patients appear to show normal motor function at first, but as the trial continues (*i.e.*, spiral or Archimedes or drawing a third line in the space separating two lines), a minute oscillation emerges which increases in amplitude until the patient stops. Gait ataxia shows a similar tendency with the amplitude of the postural sway increasing until the patient braces themselves with a cane or against a wall, after which the pattern of increasing instability repeats (RJ Hagerman & DA Hall, unpublished observations). These data suggest there may be

some sort of abnormal feedback among cortical and cerebellar systems that prevents the fine online correction of movements so errors accumulate and exacerbate out of control. In other words, it is possible the cerebellum never receives the vestibular/kinesthetic feedback that signals the accumulating error present during each movement, so the amplitude of the error term builds exponentially with each subsequent movement until the patient completely loses control and has to abruptly stop the movement to reset. The implication for these data is that tasks requiring temporally extended performance of motor movements (*e.g.*, long trials) and/or be sufficiently difficult to induce stress are required to induce an intention tremor or ataxic gait in any mouse model for the fragile X premutation and FXTAS.

Data that might just directly model for these oscillatory behaviors mentioned above was observed during the skilled forelimb reaching task. When the CGG KI mice were reaching for the reward pellets, they often reached with a slightly circuitous path, not a direct vector to the pellet but not too far off. If they missed the pellet, the subsequent reaches took an increasingly wide and uncontrolled pattern that continued across 3-5 reaches until the mouse knocked the pellet off the platform (*cf.*, Figure 2; Supplemental Movie 1 with particular focus on the CGG KI mouse with 143 repeats). In these cases,

they never retrieved the reward pellet. Although these data can be similarly interpreted as a frustration response, the absence of this behavior in wild type litter mates suggests otherwise. When the wild type mice missed the pellet, they continued reaching at a clear vector toward the pellet until they retrieved it, which they usually were able to. Unfortunately, during this task only limited data were available to support these assertions (data from 4 CGG KI (Low CGG = 85, 92; High CGG = 143, and 198 repeats) and 2 wild type litter mate mice are provided in Figure 2 as vector sketches traced from recorded trials). Future work is needed to develop methods to either capture trials at super high temporal resolution or else automate the collection of vectors taken by the paw to obtain the reward pellet to further flesh out these effects and extend them into potential behavioral markers for disease progression in the CGG KI mouse.

FUTURE DIRECTIONS

Despite the ability to identify and characterize visuomotor deficits in the CGG KI mice, it has been to date impossible to determine whether these motor deficits derive from cerebral or cerebellar dysfunction. Both the skilled reaching as well as the skilled walking deficits can just as easily be explained by parietal or superior colliculus dysfunction as dysfunction in pontocerebellar or cerebellar dysfunction itself. What is most likely based upon the converging data from the CGG KI mouse, the cortico-cerebellar circuits described by Diep et al. (2012) may underlie these deficits. Although elegant and straightforward at first glance, this distributed network explanation actually raises more issues than it answers. For one, an important question that remains unanswered is whether disruptions to one node of the network (*i.e.*, the inferior parietal lobe) result in qualitatively and quantitatively similar deficits as cerebellar or superior collicular disruptions? To date these questions remain unanswered.

One way to evaluate the differential contributions of these different networks is to apply transgenic mouse technologies to the problem. Hashem et al. (2009) demonstrated that a premutation length CGG repeat expressed specifically into Purkinje cell populations via a L7/pcp2 promoter resulted in

profound, progressive impairments on the accelerated rotarod. These data were interpreted as ataxia like gait disturbances, although no explicit analysis of fine motor function were attempted. An analysis of these mice on skilled reaching as well as skilled ladder rung walking or on the Erasmus ladder would provide data sufficient to determine the precise locus of dysfunction in the CGG KI mice and perhaps the fragile X premutation and FXTAS. Stated more simply, comparing a mouse with cerebellum specific expression of the premutation would give a cerebellum only pattern of behavioral pathology, isolating the role of the cerebellum in the cortico-cerebellar networks proposed by Diep et al. (2012) to underly motor deficits in CGG KI mice.

Similarly, the application of mice with premutation length CGG repeats expressed in neurons of the forebrain, but not cerebellum or subcortex would potentially isolate the role of the cortex for motor function, allowing independent analysis of this portion of the cortico-cerebellar network. These experiments would potentially allow for functional dissociations between the cerebellar and cortical foci that may affect motor function. A comparison of the behavioral data from these models with the CGG KI mouse would allow a more sophisticated analysis of motor function as affected by the premutation with higher

precision than possible in clinical and imaging studies of FXTAS. Additional studies using cell type specific promotors may also be able to isolate the contribution of different cell types and populations in the CGG KI mice. Such data would be critical to inform and target potential treatments to relevant cell classes.

CONCLUSIONS

Figure Captions

Figure 1: Diagrammatic representation of spatial and temporal behavioral paradigms used in studies of CGG KI mouse neurocognitive function.

Figure 2: Traces for reaching trajectories over 10 consecutive reaches for 2 wildtype mice (WT), 2 Low CGG mice (CGG repeats = 85, 92) and 2 High CGG mice (CGG repeats = 143, 198). Note the increasingly radial trajectories in mice with expanded CGG repeats compared to the WT mice.

Figure 3: Data redrawn from (^Cupido, 2009, #74895) demonstrating the dissociation between motor performance and motor associative learning in CGG KI mice and the *Fmr1* KO mouse model of fragile S syndrome.

Supplemental Movie 1: Example trials for a WT and High CGG (CGG repeat = 143) mouse demonstrating the difference in reaching trajectories for these populations.

References

Aghdaee, S. M., & Cavanagh, P. (2007). Temporal limits of long-range phase discrimination across the visual field. Vision Research, 47(16), 2156-2163. doi:10.1016/j.visres.2007.04.016

Aguilar, D., Sigford, K. E., Soontarapornchai, K., Nguyen, D. V., Adams, P. E., Yuhas, J. M., … Hagerman, R. J. (2008). A quantitative assessment of tremor and ataxia in FMR1 premutation carriers using CATSYS. American Journal of Medical Genetics. Part A, 146A(5), 629-635. doi:10.1002/ajmg.a.32211

Allen, E. G., Juncos, J., Letz, R., Rusin, M., Hamilton, D., Novak, G., … Sherman, S. L. (2008). Detection of early FXTAS motor symptoms using the CATSYS computerised neuromotor test battery. Journal of Medical Genetics, 45(5), 290-297. doi:10.1136/jmg.2007.054676

Alvarez, G. A., & Franconeri, S. L. (2007). How many objects can you track? Evidence for a resource-limited attentive tracking mechanism. Journal of Vision, 7(13), 14.1-1410. doi:10.1167/7.13.14

Benson, J. B., & Haith, M. M. (2009). Language, Memory, and Cognition in Infancy and Early Childhood. London: Academic Press.

Berry-Kravis, E., Abrams, L., Coffey, S. M., Hall, D. A., Greco, C., Gane, L. W., … Leehey, M. A. (2007). Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. Movement Disorders, 22(14), 2018-30, quiz 2140. doi:10.1002/mds.21493

Berry-Kravis, E., Potanos, K., Weinberg, D., Zhou, L., & Goetz, C. G. (2005). Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. Annals of Neurology, 57(1), 144-147. doi:10.1002/ana.20360

Bertenthal, B., & Von Hofsten, C. (1998). Eye, head and trunk control: the foundation for manual development. Neuroscience and Biobehavioral Reviews, 22(4), 515-520.

Bertenthal, B. I., Rose, J. L., & Bai, D. L. (1997). Perception-action coupling in the development of visual control of posture. Journal of Experimental Psychology: Human Perception and Performance, 23(6), 1631-1643.

Borthwell, R. M., Hunsaker, M. R., Willemsen, R., & Berman, R. F. (2012). Spatiotemporal processing deficits in female CGG KI mice modeling the fragile X premutation. Behavioural Brain Research, 233(1), 29-34.

Brouwer, J. R., Huizer, K., Severijnen, L. A., Hukema, R. K., Berman, R. F., Oostra, B. A., & Willemsen, R. (2008). CGG-

repeat length and neuropathological and molecular correlates in a mouse model for fragile X-associated tremor/ataxia syndrome. Journal of Neurochemistry, 107(6), 1671-1682. doi: 10.1111/j.1471-4159.2008.05747.x

Casasanto, D., & Boroditsky, L. (2008). Time in the mind: using space to think about time. Cognition, 106(2), 579-593. doi: 10.1016/j.cognition.2007.03.004

Chaston, A., & Kingstone, A. (2004). Time estimation: the effect of cortically mediated attention. Brain and Cognition, 55(2), 286-289. doi:10.1016/j.bandc.2004.02.013

Cupido, A. (2009). Detecting Cerebellar Phenotypes with the Erasmus Ladder. Ph.D. Erasmus MC, Rotterdam.

Diep, A. A., Hunsaker, M. R., Kwock, R., Kim, K., Willemsen, R., & Berman, R. F. (2012). Female CGG knock-in mice modeling the fragile X premutation are impaired on a skilled forelimb reaching task. Neurobiology of Learning and Memory, 97(2), 229-234. doi:10.1016/j.nlm.2011.12.006

Goodrich-Hunsaker, N. J., Wong, L. M., McLennan, Y., Srivastava, S., Tassone, F., Harvey, D., … Simon, T. J. (2011). Young adult female fragile X premutation carriers show age- and genetically-modulated cognitive impairments. Brain and Cognition, 75(3), 255-260. doi:10.1016/j.bandc.2011.01.001

Goodrich-Hunsaker, N. J., Wong, L. M., McLennan, Y., Tassone, F., Harvey, D., Rivera, S. M., & Simon, T. J. (2011). Adult Female Fragile X Premutation Carriers Exhibit Age- and CGG Repeat Length-Related Impairments on an Attentionally Based Enumeration Task. Frontiers in Human Neuroscience, 5, 63. doi:10.3389/fnhum.2011.00063

Hagerman, R. J., Leehey, M., Heinrichs, W., Tassone, F., Wilson, R., Hills, J., … Hagerman, P. J. (2001). Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology, 57(1), 127-130.

Hashem, V., Galloway, J. N., Mori, M., Willemsen, R., Oostra, B. A., Paylor, R., & Nelson, D. L. (2009). Ectopic expression of CGG containing mRNA is neurotoxic in mammals. Human Molecular Genetics, 18(13), 2443-2451. doi:10.1093/hmg/ddp182

Hunsaker, M. R. (2012a). Comprehensive neurocognitive endophenotyping strategies for mouse models of genetic disorders. Progress in Neurobiology, 96(2), 220-241. doi: 10.1016/j.pneurobio.2011.12.001

Hunsaker, M. R. (2012b). The importance of considering all attributes of memory in behavioral endophenotyping of mouse models of genetic disease. Behavioral Neuroscience, 126(3), 371-380. doi:10.1037/a0028453

Hunsaker, M. R., Goodrich-Hunsaker, N. J., Willemsen, R., & Berman, R. F. (2010). Temporal ordering deficits in female

CGG KI mice heterozygous for the fragile X premutation. Behavioural Brain Research, 213(2), 263-268. doi:10.1016/j.bbr.2010.05.010

Hunsaker, M. R., Kim, K., Willemsen, R., & Berman, R. F. (2012). CGG trinucleotide repeat length modulates neural plasticity and spatiotemporal processing in a mouse model of the fragile X premutation. Hippocampus,. doi:10.1002/hipo.22043

Hunsaker, M. R., von Leden, R. E., Ta, B. T., Goodrich-Hunsaker, N. J., Arque, G., Kim, K., … Berman, R. F. (2011). Motor deficits on a ladder rung task in male and female adolescent and adult CGG knock-in mice. Behavioural Brain Research, 222(1), 117-121. doi:10.1016/j.bbr.2011.03.039

Hunsaker, M. R., Wenzel, H. J., Willemsen, R., & Berman, R. F. (2009). Progressive spatial processing deficits in a mouse model of the fragile X premutation. Behavioral Neuroscience, 123(6), 1315-1324. doi:10.1037/a0017616

Jeannerod, M. (1997). The cognitive Neuroscience of Action. Cambridge, MA: Blackwell.

Kim, K., Taylor, S., Ta, B. T., Hagerman, P. J., & Berman, R. F. (2010). Expansion bias for transmission of expanded CGG-repeat murine Fmr1 alleles. Proceedings from 60th Annual Meeting of The American Society of Human Genetics, Washington, D. C.

Ladavas, E., & Serino, A. (2008). Action-dependent plasticity in peripersonal space representations. Cognitive Neuropsychology, 25(7-8), 1099-1113.

Leehey, M. A. (2009). Fragile X-associated tremor/ataxia syndrome: clinical phenotype, diagnosis, and treatment. Journal of Investigative Medicine, 57(8), 830-836. doi: 10.231/JIM.0b013e3181af59c4

Marshall, J. C., & Fink, G. R. (2001). Spatial cognition: where we were and where we are. Neuroimage, 14(1 Pt 2), S2-7. doi: 10.1006/nimg.2001.0834

Narcisa, V., Aguilar, D., Nguyen, D. V., Campos, L., Brodovsky, J., White, S., … Hagerman, R. J. (2011). A Quantitative Assessment of Tremor and Ataxia in Female FMR1 Premutation Carriers Using CATSYS. Current Gerontology and Geriatrics Research, 2011.

Saab, A. S., Neumeyer, A., Jahn, H. M., Cupido, A., Simek, A. A. M., Boele, H. J., … Monyer, H. (2012). Bergmann Glial AMPA Receptors Are Required for Fine Motor Coordination. Science Signalling, 337(6095), 749.

Schluter, E. W., Hunsaker, M. R., Greco, C. M., Willemsen, R., & Berman, R. F. (2012). Distribution and frequency of intranuclear inclusions in female CGG KI mice modeling the

fragile X premutation. Brain Research, 1472, 124-137. doi: 10.1016/j.brainres.2012.06.052

Sellier, C., Rau, F., Liu, Y., Tassone, F., Hukema, R. K., Gattoni, R., … Charlet-Berguerand, N. (2010). Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. EMBO Journal, 29(7), 1248-1261. doi:10.1038/emboj.2010.21

Simon, T. J. (2007). Cognitive characteristics of children with genetic syndromes. Child and Adolescent Psychiatric Clinics of North America, 16(3), 599-616. doi:10.1016/j.chc. 2007.03.002

Simon, T. J. (2008). A new account of the neurocognitive foundations of impairments in space, time and number processing in children with chromosome 22q11.2 deletion syndrome. Developmental Disabilities Research Reviews, 14(1), 52-58.

Simon, T. J. (2011). Clues to the foundations of numerical cognitive impairments: evidence from genetic disorders. Dev Neuropsychol, 36(6), 788-805. doi: 10.1080/87565641.2010.549879

Tassone, F., Greco, C. M., Hunsaker, M. R., Berman, R. F., Seritan, A. L., Gane, L. W., … RJ, H. (2012). Neuropathological, Clinical, and Molecular Pathology in Female Fragile X Premutation Carriers with and without FXTAS. Genes, Brain and Behavior, 11(5).

Van Dam, D., Errijgers, V., Kooy, R. F., Willemsen, R., Mientjes, E., Oostra, B. A., & De Deyn, P. P. (2005). Cognitive decline, neuromotor and behavioural disturbances in a mouse model for fragile-X-associated tremor/ataxia syndrome (FXTAS). Behavioural Brain Research, 162(2), 233-239. doi: 10.1016/j.bbr.2005.03.007

Verstraten, F. A., Cavanagh, P., & Labianca, A. T. (2000). Limits of attentive tracking reveal temporal properties of attention. Vision Research, 40(26), 3651-3664.

Warren, W. H. (2006). The dynamics of perception and action. Psychological Review, 113(2), 358-389. doi:10.1037/0033-295X. 113.2.358

Weiss, P. H., Marshall, J. C., Wunderlich, G., Tellmann, L., Halligan, P. W., Freund, H. J., … Fink, G. R. (2000). Neural consequences of acting in near versus far space: a physiological basis for clinical dissociations. Brain, 123 Pt 12, 2531-2541.

Wenzel, H. J., Hunsaker, M. R., Greco, C. M., Willemsen, R., & Berman, R. F. (2010). Ubiquitin-positive intranuclear inclusions in neuronal and glial cells in a mouse model of

the fragile X premutation. Brain Research, 1318, 155-166. doi:10.1016/j.brainres.2009.12.077

Willemsen, R., Hoogeveen-Westerveld, M., Reis, S., Holstege, J., Severijnen, L. A., Nieuwenhuizen, I. M., … Oostra, B. A. (2003). The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. Human Molecular Genetics, 12(9), 949-959.

Yeshurun, Y., & Levy, L. (2003). Transient spatial attention degrades temporal resolution. Psychological Science, 14(3), 225-231.