

Visual Spatial Learning and Memory in Fragile X Syndrome and *fmr1* Knockout Mice

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Abstract

This dissertation describes separate but related studies that explore visual spatial learning and memory in Fragile X Syndrome. Across all studies, either the performance of individuals affected by FXS and/or *fmr1* KO mice was compared to comparison controls on seven H-W mazes of increasing difficulty levels. Study one employed the traditional configuration of the H-W mazes to evaluate performance variables that include latency to complete the maze and number of the errors. The results of study 1 revealed significant differences in performance for both FXS groups as compared to mental age-matched comparison individuals and wild type mice, respectively. In contrast to the FXS group, performance of the comparison group improved as indicated by significantly fewer errors across trials. A similar pattern of results was observed when latency across trials was analyzed. Taken together, the results of study one support the hypothesis that a selective deficit in spatial learning and memory characteristic of the FXS phenotype can be observed in the murine model of FXS, if equivalent tasks are employed in testing humans and mice.

Study two expanded on these findings by adding landmarks to the maze environment to evaluate how these may impact spatial learning and memory in *fmr1* KO mice. Contrary to our hypotheses, landmarks significantly impaired wild type control performance. In addition, results revealed that the performance of the *fmr1* KO mice generally did not differ between landmark and non-landmark tasks, indicating that the presence of landmarks neither enhanced nor hindered mouse performance.

Lastly, study three entailed a more in-depth behavior analysis of maze navigation performance for FXS individuals from study 1. Consistent with the hypotheses and findings from study 1, results revealed significant differences in performance variables between individuals, with FXS participants generally performing worse than the comparison group participants. Taken together, the results of study 3 generally supported the hypothesis that there was greater impairment in performance for individuals affected by FXS as compared to controls. This impairment was evident in the pattern of pathways taken to solve H-W mazes, consistent with the notion that affected individuals employed different behavioral strategies.

Contributions of Authors

Three separate manuscripts comprise the empirical portion of this thesis. At the time this dissertation was submitted, one study had been published, one was in the process of review and the other was in preparation for submission. The following is a statement regarding the specific contributions provided by the author and co-authors of the work described.

Lindsey MacLeod was the first author on all three manuscripts in the thesis. She contributed to the conceptualization and design of the experiments described, as well as tested participants/subjects, planned statistical analyses and analyzed their data. Further, she interpreted these data and wrote the manuscripts presented.

All manuscripts were co-authored by Dr. C. Kogan, who was acting in his capacity as dissertation supervisor and mentor. Dr. Kogan provided guidance and assistance in all aspects of the process, especially in the reviewing and revising of the manuscripts.

Dr. C. Collin contributed to the conceptualization and methodological design for the human Hebb-Williams Mazes in Study 1. He was available for consultation and reviewed the manuscript.

Dr. C. Messier contributed to the conceptualization, methodological design and data collection for the animal experiments in Study 1 and Study 2 and reviewed the first manuscript. He also provided the necessary laboratory space to conduct experiments 1 & 2.

Dr. S. Chartier provided programming consultation for Study 3 and wrote the MATLAB code to extract data.

Dr. E. Berry-Kravis through her affiliations with RUSH University, provided patients for the experimental groups in Study 1.

Dr. M. Holahan provided guidance on the conceptualization of study 2 and reviewed the manuscript.

Reno Gandhi contributed to the behavioral testing of mice in Study 1 & Study 2.

General Preface

This thesis is presented in a manuscript style and is composed of two research articles and a brief report that are related by a common theme - visual spatial learning and memory in Fragile X syndrome (FXS). The first manuscript reports an experiment testing affected individuals on Hebb-Williams Mazes and a second experiment testing *fmr1* Knockout mice on similar versions of the maze. The second manuscript builds on the first by exploring the impact of landmarks on maze performance in *fmr1* KO and wild-type mice. The final brief report explores navigational strategies and additional performance variables in FXS patients.

Although each manuscript includes its own introduction and discussion section, a General Introduction is included to provide a comprehensive review of the relevant background information with the goal of establishing a rationale for the experiments. A General Discussion section is also provided to establish links between the results of the manuscripts as well as to provide a more general integration of the findings in the context of research on FXS.

Table of Contents

Acknowledgements	ii
Abstract	iv
Contribution of Authors	v
General Preface	vi
List of Figures	ix
List of Tables/Appendices	x
List Of Abbreviations	x
Chapter One: General Introduction	1
Introduction	2
Background	2
Fragile X Syndrome	2
Visual Processing	6
Fragile X and Visual-Spatial Function	8
Hippocampal Functioning	11
Fragile X Knockout Mice	13
Navigation	16
Synaptic Plasticity and Pharmacological Treatment	19
Hebb-Williams maze	19
The proposed study	21
Hypotheses	23
Chapter Two: Manuscript I	24
Abstract	26
Introduction	27
Methods	30
Experiment 1: Comparing <i>fmr1</i> KO and control mouse performance	30
Subjects	30
Apparatus	31
Procedure	32
Experiment 2: Comparing FXS human and comparison performance	33
Participants	33
Measures	34
Apparatus	35
Procedure	36
Statistical Analyses	37
Results Human Participants	38
Results Mice	41

Discussion	45
Acknowledgements	52
References	52
Chapter Three: Manuscript II	58
Abstract	60
Introduction	61
Method	64
Subjects	64
Apparatus	65
Procedure	66
Statistical Analyses	67
Results	68
Discussion	77
References	85
Chapter Four: Brief Report Study III	93
Abstract	95
Introduction	96
Method & Statistical Analyses	90
Results	100
Discussion	106
References	111
Chapter Five: General Discussion	116
Complete Reference List	134
Appendices	159

List Of Figures

Figure 1. H-W Practice and Test Maze Configurations	33
Figure 2. Virtual H-W Maze. Interior view of the maze #12	37
Figure 3. FXS and Comparison individuals (a) Latency (b) Error	40
Figure 4. <i>Fmr1</i> KO mice and wild-type control Latency	42
Figure 5. <i>Fmr1</i> KO mice and wild-type control	43
Figure 6. Maze Difficulty for human and mouse performance	44
Figure 7. H-W Maze Configurations with landmarks	67
Figure 8. Performance of <i>fmr1</i> KO landmark and <i>fmr1</i> KO non-landmark mice	72
Figure 9. Performance of <i>fmr1</i> KO and wild-type control on landmark tasks	74
Figure 10. Performance of wild-type mice on landmark and non-landmark task	76
Figure 11. Distance travelled on H-W Mazes for comparison and FXS individuals	101
Figure 12. Frequency of Pauses on H-W Mazes for comparison and FXS individuals	103
Figure 13. Average Pause Duration on H-W Mazes for comparison and FXS individuals	104
Figure 14. Superimposed maze pathways for comparison and FXS participants	105

List of Tables

Table 1. Average Distance Travelled for FXS and Control Participants	102
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Appendices

Appendix A. Consent Form for participants affected by Fragile X Syndrome	159
Appendix B. Consent Form for control participants	161
Appendix C. Assent form for Participants	163
Appendix D. History Questionnaire	164
Appendix E. Maze 2 Superimposed maze pathways for controls and FXS	167
Appendix F. Maze 4 Superimposed maze pathways for controls and FXS	168
Appendix G. Maze 5 Superimposed maze pathways for controls and FXS	169
Appendix H. Maze 8 Superimposed maze pathways for controls and FXS	170
Appendix I. Maze 9 Superimposed maze pathways for controls and FXS	171
Appendix J. Maze 11 Superimposed maze pathways for controls and FXS	172
Appendix K. Maze 12 Superimposed maze pathways for controls and FXS	173

List of Abbreviations

H-W: Hebb-Williams Maze
KO: Knock out
FXS: Fragile X Syndrome
<i>FMRI</i> : Fragile X Mental Retardation 1 gene
FMRP: Fragile X Mental Retardation protein
M-pathway: Magnocellular Pathway
P-pathway: Parvocellular Pathway
LGN: Lateral Geniculate Nuclei
DS: Dorsal Stream
PPC: Posterior Parietal Cortex
MWM: Morris Water Maze
mRNA: Messenger RNA
MRI: Magnetic Resonance Imaging
IQ: Intelligence Quotient
ANOVA: Analysis Of Variance

Chapter One: General Introduction

Introduction:

Fragile X Syndrome is the most prevalent form of heritable mental retardation. It arises from a mutation in the *FMRI* gene on the X chromosome that interferes with expression of Fragile X Mental Retardation protein and leads to a wide range of behavioral and cognitive deficits. Previous studies have demonstrated a deficit in basic visual perceptual processing as well as spatial abilities in Fragile X. How such a deficit may impact spatial navigation remains unknown. The current studies will extend previous research by evaluating spatial learning and memory using both virtual and physical versions of the Hebb-Williams mazes, which allows for testing of humans and animals under comparable testing conditions. For the proposed studies, either the performance of individuals affected by FXS will be compared to typically-developing mental age-matched comparison participants or the performance of *fmr1* KO mice will be compared to wild-type control mice on seven H-W mazes of increasing difficulty levels. Across studies, it is hypothesized that individuals affected by FXS and KO mice as compared with their respective control groups would exhibit poorer performance on mazes deemed more difficult.

Background:

Fragile X Syndrome

Fragile X Syndrome (FXS) is the most prevalent form of heritable mental retardation (Turner, Webb, Wake, & Robinson, 1996), with recent estimates indicating that 1 in 2,500 individuals are affected (Hagerman, 2008). FXS arises from an intergenerational trinucleotide expansion (cytosine, guanine and guanine) of the Fragile X Mental Retardation 1 gene (*FMRI*) (Online Mendelian Inheritance in Man® [OMIM] 309550; Verkerk et al., 1991). In typically developing individuals, the number of trinucleotide (CGG) repeats varies between 6 and 53 with an average expression of 30 trinucleotide units (Fu et al., 1991; Snow et al., 1993; Patsalis et al., 1999). However, individuals affected with FXS typically have in excess of 200 CGG repeats (Chiurazzi, Neri, & Oostra, 2003). The expansion results in inactivation of the *FMRI* gene on the X chromosome (Sherman, 2002) through methylation of the promoter, which in turn prevents expression of Fragile X Mental Retardation protein (FMRP) (Verkerk et al., 1991). The single gene etiology of FXS enables a direct link to be established between the *FMRI* gene mutation and corresponding FXS phenotype. In typically developing individuals, FMRP is expressed in most cells with high

levels of FMRP in both fetal and adult brains (Abitbol et al., 1993; Devys et al., 1993). In individuals with FXS, the lack of expression of *FMRI* in somatic cells (Pieretti et al., 1991) leads to a well-defined phenotype that is characterized by a range of physical, behavioral, neuroanatomical and cognitive deficits.

Individuals affected with FXS display many distinct physical and behavioral features. Affected males, being hemizygous for the X chromosome, express a more severe phenotype of FXS, whereas, individuals with a pre-mutation (50-200 CGG repeats) generally have near normal levels of FMRP expression (Devys, Lutz, Rouyer, Bellocq, & Mandel, 1993). Individuals with pre-mutations often display average levels of intellectual functioning (Cornish et al., 2005) with subtle neuropsychological impairments (e.g., Kogan & Cornish, 2010), including a selective spatial deficit (Hocking, Kogan & Cornish, 2012), that may relate to increased production of *FMRI* mRNA (Tassone et al., 2000). Females being homozygous for the X chromosome, typically possess one unaffected X chromosome, which enables some FMRP expression (Dobkin et al., 2000). In general, this results in a less severe FXS symptomatology with the most common symptoms in the female population being deficits in executive function skills and social anxiety (Bennetto et al., 2001).

Physical features of FXS include: macroorchidism (enlarged testicles), elongated faces, large ears (Dobkin et al., 2000), and hyper-extensible joints (Beckel-Mitchener & Greenough, 2004). In addition, approximately 20 percent of individuals with FXS experience seizure activity (Hagerman, 2002; Beckel-Mitchener & Greenough, 2004; El Idrissi et al., 2005). Symptoms of Attention Deficit Hyperactivity Disorder (ADHD) and maladaptive social behaviours including impulsivity, hyper-arousal, anxiety increased sensitivity to sensory stimuli and aggression are also commonly observed in individuals affected by FXS (Turk & Cornish, 1998; Chen & Toth, 2001). Many of these social deficits share similar features with those exhibited by individuals with Autism (Beckel-Mitchener & Greenough, 2004; Dissanayake et al., 2009; Hernandez *et al.*, 2009) with comorbidity between the disorders ranging from 15-25% in affected males (Reiss & Freund, 1990; Dykens, Volkmar, & Glick, 1991; Turk, 1997; Bailey et al., 1998). In contrast to full mutation FXS, individuals with the pre-mutation are at an increased risk of developing a recently described condition called Fragile-X Associated Tremor and Ataxia Syndrome later in life (Berry-Kravis, et al., 2003; Jacquemont et al., 2005). Female carriers are at risk of

developing Premature Ovarian Failure (Murray et al., 1998; Allingham-Hawkins et al., 1999; Hundscheid et al., 2000).

There are neuroanatomical abnormalities in FXS. Areas of the brain characterized by high levels of FMRP expression in typically developing individuals may be particularly reliant on and therefore sensitive to changes in FMRP expression as occur in FXS. It has been well established that FMRP plays a critical role in synaptic development and plasticity (Feng et al., 1997; Antar et al., 2004), including pruning and maturation of dendritic spines (Irwin et al., 2000; 2001; Greenough, 2001). Individuals with FXS tend to have an abnormal increase in the density and length of dendritic spines in several cortical regions (Irwin et al., 2000; 2001; Greenough 2001). This suggests that the observed abnormal dendritic morphology may be caused by inadequate synaptic pruning (Jin & Warren, 2000), which in turn may lead to a greater number of excitatory synapses and increased cortical excitability. The increased cortical excitability may explain some of the underlying impairments observed in individuals affected by FXS such as epilepsy and anxiety (Berry-Kravis, 2002; Bear, Huber & Warren 2004; Chiurazzi, 2003).

Given the abundance of FMRP in the brain of typically developing individuals and the critical role it plays in synaptic development and functioning, it is not surprising that the brains of individuals with FXS possess several abnormalities. In general, results of *post mortem* studies evaluating gross pathological brain abnormalities in individuals with FXS have been inconsistent (Reyniers et al., 1999). There is some evidence suggesting cortical atrophy and ventricular enlargement (Wisniewski, Segan, Mizejeski, Sergen, & Rudelli, 1991). In addition, neuroanatomical imaging studies have shown a decrease in the posterior vermis of the cerebellum (Greco et al., 2011; Joshi, & Bryan, 1991; Reiss, Alyward, Freund, 1994; Mostofsky et al., 1998;) an area of the brain involved in modulating complex motor activity and fine control of automated skills (Thach, 1996; Courchesne & Allen, 1997). Brain imaging studies have shown similar abnormalities in *fmr1* KO mice (Ellegood, Pacey, Hampson, Lerch, & Henkelman, 2010). Other areas of the brain exhibiting abnormalities in FXS include enlarged caudate nuclei (Hallahan et al., 2011; Eliez, Blasey, Freund, Hastie, & Reiss, 2001), and increased thalamic and ventricular cerebral spinal fluid volumes (Eliez, Blasey, Freund, Hastie, & Reiss, 2001). Interestingly, evidence suggests that the caudate nucleus may be involved in regulating key attentional and emotional processes such as

response inhibition (Mataro, Garcia-Sanchez, Junque, Estevez-Gonzalez & Pujol, 1997; Semrud-Clikema et al., 2000). Therefore, the underlying structural abnormalities observed in the caudate nucleus may translate to the corresponding behavioral difficulties observed in individuals with FXS.

In both typically developing humans and wild type mice, high levels of *FMRI* mRNAs are expressed in the hippocampus (Abitbol et al., 1993; Hinds et al., 1993), suggesting that this is an additional area of the brain that is particularly reliant on and therefore sensitive to changes in FMRP expression as occur in FXS. Furthermore, structural MRI studies suggest that individuals affected by FXS have enlarged hippocampi (Reiss, Lee & Freund, 1994; Kates, Abrams, Kaufmann, Breiter & Reiss, 1997) and a recent *post mortem* study demonstrated hippocampal CA1 abnormalities (Greco et al., 2011). The hippocampus is known to play a critical role in aspects of learning, memory, attention and the regulation of affect (see reviews Warburton & Brown, 2010; Winocur, Moscovitch, & Bontempi, 2010; Ojemann, Cretzfeldt, Lettich & Haglund, 1988; Creutzfeldt, Ojemann & Lettich, 1989; Murray, Gaffan & Mishkin, 1993; Incisa della Rocchetta et al., 1995). Therefore, abnormalities observed in the hippocampus may produce deficits within several of these domains. Lastly, typically developing individuals have been shown to express high levels of FMRP in one of the lateral geniculate nuclei's (LGN) visual pathways (Kogan et al., 2004), suggesting that an area of the brain involved in processing visual information may be vulnerable to changes in FMRP expression. Supporting this notion, Kogan and colleagues (2004) demonstrated abnormal cytoarchitectural changes in the LGN of individuals with FXS. In summary, high levels of FMRP expression have been found in several areas of the human brain. Therefore, it is likely that changes in FMRP expression that occur in FXS result in abnormalities in several distinct cortical structures. Importantly, ongoing research continues to provide evidence demonstrating correlations between the neuroanatomical abnormalities observed and varying degrees of functional impairment in individuals with FXS (Reiss et al., 1995; Mostofsky et al., 1998; Mazzocco et al., 1997; Hessler et al., 2007; reviewed by Hessler, Rivera & Reiss, 2004).

Extensive research on individuals with FXS has focused on the cognitive domain and a well-defined cognitive profile has been established. Males with FXS typically have mild to moderate learning disabilities with their full scale intelligence quotient (IQ) ranging from the

mild to moderate retardation range (Cornish, Munir, & Cross, 1997; Bennetto & Pennington, 2002). Additionally, a mild verbal-non-verbal discrepancy has been observed with individuals with FXS typically exhibiting higher verbal than non-verbal scores (Theobald, Hay & Judge, 1987; Veenema, Veenema, & Geraedts, 1987). In general, there is a consensus that FXS is best characterized by a constellation of strengths and weaknesses within the cognitive domain that differentiate the FXS profile from that of other disorders of mental retardation (Van der Molen et al., 2010; Cornish et al., 2005). Areas of relative strength include vocabulary (Dykens, Hodapp & Leckman, 1987), verbal working memory (Schapiro et al., 1995; Jakala et al., 1997) long term memory for meaningful information (Freund & Reiss, 1991) and face emotion perception (Cornish, Munir & Cross, 1998; Turk & Cornish, 1998). Areas of weakness include deficits in attention (Baumgardner, Reiss, Freund, & Abrams, 1995; Backes, Genc, Schreck, Doerfler, & Lehmkuhl, 2002), executive functioning (Estevez-Gonzalez, Roig, Piles, Pineda & Garcia-Sanchez, 1997; Cornish, Munir, & Cross, 2001), linguistic processing (Ferrier, Bashir, Meryash, Johnson, & Wolf, 1991) visual-spatial cognition (Crowe & Hay, 1990; Cornish, Munir & Cross, 1998; 1999) and visual perceptual processing (Kogan et al., 2004).

In summary, individuals with FXS exhibit reduced expression of FMRP in somatic cells that leads to a wide range of neuroanatomical, behavioural and cognitive deficits. Areas of the brain such as the hippocampus and LGN that typically express high levels of FMRP appear to be particularly vulnerable. A well-defined cognitive profile has been established for FXS and is characterized by a profile of strengths and weaknesses that differentiate FXS from that of other disorders. Ongoing research continues to provide evidence demonstrating correlations between the neuroanatomical abnormalities observed and varying degrees of functional impairment in individuals with FXS. One such area of research has focused on the visual system and visual spatial abnormalities characteristic of individuals with FXS.

Visual Processing:

The visual system is one of the mostly widely studied and best understood of the sensory systems. Much of our understanding has been based on animal models, which share very similar features with the human system. In both humans and primates, visual processing begins at the retina where axons from ganglion cells first exit the retina to form two optic nerves. The optic nerves then join at the optic chiasm and continue to form the

optic tract. The latter projection synapses on the lateral geniculate nucleus (LGN) of the thalamus; a structure that plays a critical role in visual processing.

The LGN consists of six well-defined layers with layers 2, 3, and 5 receiving input from the ipsilateral eye and layers 1, 4, and 6 receiving input from the contralateral eye (Hubel & Wiesel, 1962; Hubel & Wiesel, & Stryker 1977). In addition, the top four layers of the LGN (6, 5, 4, 3) receive projections from smaller ganglion cells and form the parvocellular layers (P) and the bottom two layers (1, 2) receive projections from larger ganglion cells and form the magnocellular layers (M) (Hubel & Wiesel, 1962; Dreher et al., 1976; Hubel & Wiesel, & Stryker 1977; Leventhal, et al., 1981). A third sub-pathway, the koniocellular stream, has cells that are distributed in between each of the 6-layers (Casagrande, Yazar, Jones, & Ding, 2007). All three types of cells exhibit distinct morphological and functional properties. For example, P cells have smaller receptive fields (Wiesel & Hubel, 1966; Dowling, 1977; Livingstone & Hubel, 1988), demonstrate a prolonged response to visual stimuli and are significantly more sensitive to colour than low contrasts (Kaplan & Shapely, 1986; Livingstone & Hubel, 1988; Merigan, Katz, & Maunsell, 1991). Conversely, M cells have larger receptive fields (Wiesel & Hubel, 1966; Dowling, 1977; Livingstone & Hubel, 1988), demonstrate a more transient response to visual stimuli and are more sensitive to low contrasts than to color stimuli (Kaplan & Shapely, 1986; Livingstone & Hubel, 1988; Merigan, Katz, & Maunsell, 1991). In addition, there are approximately 1,000,000 P cells in the retina and parvocellular neurons in the LGN and only 100,000 M cells and magnocellular neurons (Hubel & Wiesel, 1962; Hubel & Wiesel, & Stryker 1977). K cells are estimated to represent 5-10 percent of the retinal ganglion cell population (Dacey, & Petersen, 1992)

In primates, visual information is first processed by early visual pathways comprising the dorsal and ventral visual streams (Ungerleider & Mishkin, 1982; Milner & Goodale, 1995). These streams depend on afferent input from the sub-cortical magnocellular (M) and parvocellular (P) parallel pathways. The dorsal stream is involved in the visual control of action and receives input primarily from the M-pathway (Merigan, Katz, & Maunsell, 1991; Goodale & Milner, 1992). The ventral stream is implicated in pattern identification and object distinctiveness, specifically related to chromatic stimuli, and receives input from the P-pathway (Merigan, Katz, & Maunsell, 1991). The posterior parietal cortex (PPC) is an

area that receives primary input from the dorsal visual pathway and is important for spatial processing and directing action to guide behavior (Milner & Goodale, 1995.) Single-cell recordings from primates have demonstrated that the PPC contains a large number of cells that respond preferentially during spatial navigational tasks (Hyvarinen & Poranen, 1974; Scott, Sergio, & Kalaska, 1997). In addition, research supports the involvement of the PPC in processing navigational information (reviewed by Andersen & Buneo, 2002; Spiers & Maguire, 2007), with researchers proposing the PPC may be responsible for relating spatial orientation information processed by the limbic system with information regarding navigational goals and action plans for attainment (reviewed by (Calton & Taube, 2009)

Traditional characterization of the visual pathways has focused on a distinction between pathway functions. This distinction is reflected in the terminology used to reference the “what” and “where” visual pathways, such that the responsibility of the dorsal stream is generally thought to determine object location (where pathway) and the ventral stream is thought to be involved in the recognition of object quality (what pathway) (Mishkin, Ungerleider, & Macko, 1983; Livingstone & Hubel, 1988). Goodale and Milner (1992) challenge this notion and suggest that this distinction may not be entirely accurate. They propose that that there is greater intermingling or cross-talk between systems than was originally proposed and that instead, each system may perform functions based on the output requirements of each stream (Milner & Goodale, 1995; Merigan, & Maunsell, 1993; Braddick, O’Brien, Wattam-Bell, Atkinson, & Turner, 2000; Tanskanen, Saarinen, & Parkkonen, 2008). Accordingly, the dorsal stream may be responsible for pre-conscious vision for action that is utilized to interact with objects and move through the environment (Goodale & Milner 1992). The ventral stream would then be implicated in conscious vision that allows for the recognition of objects (Goodale, & Milner, 1992).

Fragile X and Visual-Spatial Function:

Individuals affected with FXS have reliably been shown to experience difficulties on visual spatial tasks including those requiring participants to manipulate objects in space. These include tasks that rely on a range of skills, such as drawing (Crowe & Hay, 1990; Freund & Reiss, 1991) manipulating blocks to generate designs (Theobald et al., 1987; Loesch et al., 1993; Cornish et al., 1999) and tasks dependent on psycho-motor coordination (Cornish et al., 1999). Research suggests that different aspects of spatial processing may be

dependent on different areas of the brain, such that cognitive tasks tapping visual-perceptual processing abilities may be differentiated from visual-spatial processing tasks (Newcombe & Russell, 1962; Mehta, Newcombe, & Damasio, 1987) and visual-memory tasks (De Renzi, 1982). Indeed, Cornish and colleagues (1999) provided evidence suggesting that FXS males are impaired on visuo-motor and visuo-constructive tasks but perform similar to control groups on visuo-perceptual tasks. The implications of these results suggest that individuals with FXS experience difficulty manipulating objects in space and constructing concrete and abstract designs (Koukoui & Chaudhuri, 2007).

Given the deficits in visual-motor function observed in FXS, Kogan and colleagues (2004) investigated if these deficits might be an expression of underlying neuroanatomical and functional abnormalities specific to subcortical visual pathways. Kogan and colleagues (2004 a, b) provided neurobiological and behavioural evidence suggesting that individuals with FXS may have an early visual processing deficit that impacts dorsal stream functioning. First, using immunohistochemical staining techniques, they demonstrated that the LGN of a typically developing human male contained higher levels of FMRP expression within the M layers, a finding that was recently replicated in Old World monkeys (Zangenehpoura, Cornish, & Chaudhuri, 2009). Next, Kogan and colleagues (2004 a, b) demonstrated that corresponding layers in an FXS male patient displayed cytoarchitectural abnormalities. It can be inferred from these results that the lack of production of FMRP that results from the silencing of the *FMR1* gene contributes to the observed M-Pathway pathology in individuals with FXS. This abnormality would likely have downstream effects because the M-Pathway sends information to the dorsal stream, which is primarily responsible for the visual control of action (Milner & Goodale, 1992).

To investigate this notion behaviorally, Kogan and colleagues (2004) employed psychophysical tasks that selectively probe either the M or P pathway. Using contrast sensitivity measures, participants were shown both black/white low spatial frequency gratings to probe the M pathway or chromatic high spatial frequency gratings of which the P pathway is known to be more sensitive. They found that male patients with FXS were less sensitive to stimuli that probe the M pathways than the P pathway. In addition, they found deficits for a global motion task, which is thought to tap dorsal stream (DS) function. These deficits were not observed on a form perception task, which is thought to probe VS function.

Kogan and colleagues (2004) suggest that these impairments were likely due to the atypical development of the M-pathway neurons in the LGN. Similar to these results, a selective impairment on contrast sensitivity tests of the M pathway has been found in FXS premutation carriers (Kéri & Benedek, 2009). Consistent with this, Farzin, Whitney, Hagerman and Rivera (2008) investigated visual processing in infants with FXS and showed that they had higher detection thresholds for second order motion stimuli. Lastly, researchers demonstrated that infants with FXS could maintain the identity of static (VS processing), but not dynamic (DS processing), object information during occlusion events (Farzin & Rivera, 2010). Furthermore, Keri and Benedek (2011) showed that healthy individuals with fewer FMRP positive markers performed worse on tasks selectively targeting M-pathway and dorsal stream processing. This relationship was not observed on tasks targeting P-pathway and ventral stream processing (Keri & Benedek, 2011). Similar patterns of results have been demonstrated in FXS permutation carriers (Keri & Benedek, 2012). Taken together, these findings support the role of abnormal DS functioning in FXS and suggest that DS dysfunction is present during early stages of development.

Further evidence of a M-pathway deficit in FXS has been provided by Mazzocco, Singh Bhatia, and Lesniak-Karpiak (2006) who examined dorsal stream dysfunction in individuals with FXS and Turner Syndrome. Previous research has shown that visuospatial deficits exist in Turner Syndrome (Alexander et al., 1966; Cornoldi et al., 2001) as well as FXS (Cornish et al., 1998; 1999; Kogan et al., 2004). A battery of visual perception tasks administered to each group, consisting of “what” versus “where” tasks, and global versus spatial visuo-spatial problems (Mazzocco, Singh Bhatia, & Lesniak-Karpiak, 2006). In comparison to the Turners’ Syndrome group, individuals with FXS displayed greater difficulty locating the target objects; an ability dependent on the “where” or dorsal pathway of visuospatial processing. As well, the FXS group had greater difficulty focusing on the general spatial properties of the target item, or the global aspect of visual perception; a similar ability dependent on dorsal stream function. Similar results were found in study by Woodcock, Humphreys and Oliver (2009), who demonstrated that individuals with FXS exhibited greater impairment on a task tapping dorsal stream functioning (object location) than a task dependent on ventral stream functioning (shape identity). Finally, FXS premutation carriers display impairment on motion perception tasks, and in particular,

showed a reduction in sensitivity to biological motion as compared to mechanical motion (Kéri & Benedek, 2010). Taken together, these findings provide compelling evidence for the role of abnormal DS functioning in FXS and suggest that the M-pathway deficit may disrupt visuospatial perception in individuals with FXS, and may therefore be responsible for the visuospatial deficits characteristic of FXS.

Hippocampal Functioning:

In addition to intact basic visual processing (Tees, Midgley, & Nesbit, 1981), spatial learning, and memory are also dependent on intact hippocampal functioning (O'Keefe, & Dostrovsky, 1971; Morris, Garrud, Rawlins & O'Keefe, 1982; Ghaem et al., 1997, Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Ekstrom et al., 2003; Rogers & Kesner, 2006; Hunsaker, Tran & Kesner, 2008). The hippocampus is part of the limbic system and plays a critical role in mediating memory and spatial navigation. O-Keefe and Nadel (1978) first proposed a cognitive-mapping theory that was reliant on hippocampal functioning. This theory proposed that as an animal navigates through its environment it creates an internal representation of the environment in the form of a cognitive map that is located in the hippocampus. Supporting this theory is research conducted by Sherry, Jacobs and Gaulin (1992) who demonstrated that the hippocampus in birds that cache or store food is significantly larger than bird that do not. Food storing birds often store items in diverse locations and must remember the location of the sites in order to retrieve them. Interestingly, if the hippocampus of the food-storing birds is damaged, they continue to cache but are unable to retrieve their food (Sherry, Jacobs & Gaulin, 1992). Similarly, lesion studies in mice have demonstrated that successful performance on paradigms thought to measure visual spatial abilities such as the radial arm maze, T-maze and water maze rely on intact hippocampal processing (Mitchell, Rawlins, Steward & Olton, 1982; Morris, Garrud, Rawlins & O'Keefe, 1982; Hock & Bunsey, 1998; Okada & Okaichi, 2009). In addition, several classes of cells that preferentially respond to spatial behavior have been located within different areas of the hippocampal formation (Anderson, Morris, Amaral, Bliss & O'Keefe, 2007). These include cells that respond when an animal is in a specific location (place cells) (Best, White & Minai, 2001; Ekstrom et al., 2003;), cells that respond when an animal faces a particular direction (head-direction cells) (Taube, 2007), and cells that fire at regular spaced intervals and appear to divide the environment into a grid (grid cells)

(Hafting, Fyhn, Molden, Moser, & Moser, 2005).

Similar results have been demonstrated in humans. For example, London taxi cab drivers have been shown to have significantly elevated grey-matter volume in their hippocampus compared to controls such as bus drivers who follow a standardized navigational route (Maguire, Burgess, & O'Keefe, 1999). In addition, the right posterior hippocampus was found to increase in size as a function of number of years driving a taxi (Maguire, Burgess, & O'Keefe, 1999). Further evidence for hippocampal involvement in spatial navigation is provided by Astur and colleagues (2002). Using a virtual water maze paradigm they demonstrated that participants with hippocampal damage were impaired in locating the platform to solve the spatial navigational tasks. They demonstrated that subjects with damage to both hippocampi were impaired in locating the platform (Astur et al., 2002). Similarly, in the famous case of H.M, an individual who had bilateral temporal cortex damage including damage to the hippocampus, it was found that he could learn simple spatial relationships, such as the location of one stimuli but was impaired if he was required to find the location of two objects or when required to learn his way through a new neighborhood (Corkin, et al 1997). Similar results have been found in animals with hippocampal damage, where it has been shown that they can learn simple spatial discrimination tasks but have impaired performance when certain parameters of the procedure are changed. In the Morris water maze rats are able to find the hidden platform from one release point but demonstrate an impaired performance if the release point is changed (Morris, Garrud, Rawlings & O'Keefe, 1982). Taken together, these findings suggest that the hippocampus may be involved in more complex spatial behavior such as organizing spatial information and expressing spatial memory flexibility (as discussed by Eichenbaum, 2003).

In both humans and animals, another role of the hippocampus seems to be its involvement in transitive inference (Heckers, Zalesak, Weiss, Ditman, & Titone, 2004). The latter is a form of inferential reasoning and can be understood as a way of organizing information to support generalizations and inferences from acquired knowledge (Bunsey, & Eichenbaum, 1996). In solving the H-W mazes, this type of reasoning may be involved in applying strategies learned from one maze to the next and implicitly learning the utility of landmarks for navigation. In addition to transitive inference, evidence suggests that the

hippocampus also plays a key role in remembering the sequences of experiences or the temporal order of events (Lee, Jerman & Kesner, 2005; Fortin, Agster, & Eichenbaum, 2002). Interestingly, the hippocampus does not appear to be particularly involved or crucial to representing single items in isolation, instead, it seems to be involved in representing items in the context in which they were experienced, or linking experiences together to form a network of memories (Fortin, Agster, and Eichenbaum, 2002; Gilbert, Kesner, and Lee, 2001). Further support for this role comes from electrophysiological research that has demonstrated that the sequences of places an animal travels to while awake, are replayed in the hippocampus during slow-wave sleep (Lee & Wilson, 2002). Similar results have been shown in humans using MRI technology (Peigneux et al., 2004). In the H-W mazes, an individual or animal must remember, and be able to predict, which alleys to avoid or travel down, as well as learn the order of turns to make to find the goal box. Therefore, it is reasonable to hypothesize that the type of sequence learning subserved by the hippocampus is likely important in learning the mazes.

Fragile X Knockout Mice

Studies of *fmr1* knockout mice, a murine model of FXS, have also demonstrated deficits that at times mirror those observed in individuals affected by FXS. The murine FMRP homologue shares 97 percent similarity with the human form which suggests that it may perform a similar role in both species (Mineur, Sluyter, De Wit, Oostra, & Crusio, 2002). Due to this similarity, researchers have developed several murine models of FXS (The Dutch-Belgian Fragile X Consortium, 1994).

In contrast to FXS, in the fragile X murine model (*fmr1* KO) the mutation does not disrupt the CGG expansion, but instead replaces the *fmr1* gene with a non-functional *fmr1* substitute which results in a loss of gene function and FMRP production (Kooy, 1996). When evaluated, *fmr1* KO were found to be viable, fertile and display many similar characteristics with the human form of FXS (The Dutch-Belgian Fragile X Consortium, 1994). These characteristics include macroorchidism, learning deficits, hyperactivity (The Dutch-Belgian Fragile X Consortium, 1994; Kooy et al., 1996) and age-dependent audiogenic seizures (Chen & Toth, 2001; Musumeci et al., 2007). In addition, *fmr1* KO mice also exhibit hippocampal abnormalities during both development and adulthood that resemble those observed in FXS humans and are found to have longer dendritic spines in

pyramidal cells in subfield CA1 (Grossman et al., 2006), smaller intra-infra pyramidal mossy fibre terminal fields (Mineur, Sluyter, De Wit, Oostra & Crusio, 2002), as well as shorter dendrites, fewer dendritic spines and functional synaptic connections (Braun & Segal, 2000). Abnormalities in dendritic spine formation have also been observed in layer V pyramidal neurons of the visual cortex (Gimenez & Montoliu, 2001) and layers II/III and in a KO mouse model of the FXS permutation (Berman, Murray, Arque, Hunsaker, & Wenzel, 2012).

As discussed previously, spatial navigation is largely dependent on intact hippocampal functioning (O'Keefe, & Dostrovsky, 1971; Morris, Garrud, Rawlins & O'Keefe, 1982; Ghaem et al., 1997; Ekstrom et al., 2003; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). Lesion studies in mice have demonstrated that successful performance on paradigms such as the radial arm maze, T-maze and water maze are thought to rely on intact hippocampal processing (Mitchell, Rawlins, Steward & Olton, 1982; Morris, Garrud, Rawlins & O'Keefe, 1982; Hock & Bunsey, 1998; Okada & Okaichi, 2009). In both typically developing humans and wild-type mice, high levels of *FMRI/fmr1* mRNAs are expressed in the hippocampus (Abitbol et al., 1993; Hinds et al., 1993), suggesting that this brain area is reliant on and therefore sensitive to changes in FMRP expression as occur in FXS.

Impairments have been described for a range of behavioural tasks including Conditioned Fear Response (Paradee *et al.*, 1999), Prepulse Inhibition (Chen & Toth, 2001; Baker et al., 2010), Open Field Activity (Mineur, Sluyter, De Wit, Oostra & Crusio, 2002), and Social Interaction tasks (Spencer, Alekseyenko, Serysheva, Yuva-Paylor & Paylor, 2005; Mineur, Huynh & Crusio, 2006; Baker, et al., 2010). Despite some concordance, results from tests of the murine model of FXS focusing on replicating the spatial processing deficit observed in affected humans have been mixed (The Dutch-Belgian Fragile-X Consortium 1994; Kooy et al., 1996; D'Hooge et al., 1997; Paradee et al., 1999; Dobkin, et al., 2000; Peier et al., 2000; Mineur et al., 2002). One of the most commonly used paradigms to assess visual spatial learning in animals is the Morris Water Maze (MWM). In this task, animals must swim through a circular pool of opaque water and learn how to locate a hidden platform. Once the animals have learned how to find the platform, a reversal phase is often executed whereby the location of the platform is changed and animals must adapt and changed a learned spatial navigation strategy. In *fmr1* KO mild learning deficits have been

shown on the MWM; particularity during the reversal phase (The Dutch-Belgian Fragile X Consortium, 1994; Kooy et al., 1996; D'Hooge et al., 1997; Dobkin, 2000; Van Dam et al., 2000). A recent study by Baker and colleagues (2010) found that *fmr1* KO animals traveled a longer distance prior to finding the escape platform in the MWM and exhibited increased time spent near the walls of the tank. In addition, during the reversal phase of the test *fmr1* KO mice also exhibited impaired performance (Baker, 2010). Additional spatial impairments have also been observed on several other paradigms including the radial arm maze task (Mineur, Sluyter, De Wit, Oostra & Crusio, 2002; Yan, Asafo-Adjei, Arnold, Brown, & Bauchwitz, 2004) and Barnes Maze (Yan, Asafo-Adjei, Arnold, Brown, & Bauchwitz, 2004).

The inconsistencies that have been found between studies may be due to several factors including; differences in background strain used (Spencer et al., 2011; 2006), number of generations of back crossing (Gu et al., 2002), pre/post natal environmental factors (Spencer et al., 2011), maternal variables (Francis, Szegda, Campbell, Martin, & Insel, 2003) and the choice of the spatial task employed as well as the equivalency of such tasks to those of human spatial cognition. Since the first *fmr1* KO model was created, many other models have been generated on different genetic backgrounds with the most commonly utilized being the C57BL/6 and FVB (Kooy, 2003). Early research with *fmr1* KO may have produced conflicting results partially due to the strains not being fully congenic and the presence of retinal degradation in some lines, specifically *fmr1* KO mice on an FVB background (Kooy, 2003; Errijgers, et al., 2007). Although, a sighted version of the FVB strain is available as well as congenic lines of other background strains, controversy as to the implications of the results produced by the model remains. For example, Paradee and colleagues (1999) demonstrated that the C57BL/6 and FVB-129 background strains produced different results on the Morris water maze task. Furthermore, Dobkin and colleagues (2000) demonstrated reduced learning on the Morris water maze in the FVB-129 strain but not in C57BL/6 strain, consistent with the excellent spatial ability of C57BL/6 mice. In the present studies, a FVB background will be used because of its more modest spatial abilities may be a better murine model to investigate the visual phenotype of FXS. Use of this strain may also avoid the possibility that the C57BL/6 strain, with its superior spatial abilities can overcome the spatial deficits associated with absence of *fmr1* expression.

It therefore remains controversial as to whether the murine model of FXS exhibits a similar spatial deficit as has been documented for affected individuals. In an effort to resolve the ambiguity in the literature, in the present study we employ Hebb-Williams (H-W) mazes, which are well-established measures of spatial cognition (Hebb & Williams, 1946; Rabinovitch & Rosvold, 1951) and are reported to be sensitive to detecting alterations of hippocampal dependent spatial abilities (Winocur & Moscovitch, 1990) better than radial or water maze tasks (Pereira, Cosquer, Schimchowitsch, & Cassel, 2005).

Navigation:

The capacity to use information to adaptively move through the environment is a complex skill that is used on a daily basis and is critical for species survival. Navigation, or wayfinding, refers to how one uses information to move through the environment to reach specific locations or goals (Spiers & Maguire, 2007). Navigation has been described as being comprised of three processes that include; spatial orientation; manipulation of spatial representations to compute planned route; and execution of the plan (Calton & Taube, 2009). In addition, navigation has both a motor and cognitive component, meaning it involves both movement in space and the identification of relevant environmental information and appropriate strategies (Livingstone, & Skeleton, 2007).

Successful navigation is thought to depend on the ability to adopt different navigational strategies based on frame of reference (Bohbot, Lerch, Thorndyraft, Iaria, & Zijdenbos, 2007). Allocentric and egocentric frame of reference are generally the primary navigational strategies investigated. They can be differentiated by cues used to initiate their use and anatomical structures involved in processing (Nadel & Hardt, 2004). These two frame of reference were first described by Bisiatch and Luzzatti (1978) in their Piazza del Duomo experiment. In this study participants with damage to their parietal cortex were asked to recall the layout of the Piazza del Duomo in Italy. Researchers demonstrated that allocentric recall was intact (e.g. buildings and layout of the Piazza) but egocentric recall was impaired, where depending on the viewpoint of the observer, participants' experienced spatial neglect (Bisiatch & Luzzatti, 1978). This finding supports the notion that navigational strategies appear to rely on different areas of the brain (Iaria et al., 2003) and for humans and many other species, the visual system plays an essential role in the navigational processes.

Since the classic Piazza del Duomo study Allocentric strategies have been extensively studied and are related to salient features in the environment and code spatial stimuli such as landmarks and object interrelations in the environment (Milner & Goodale, 1995). For example, successful completion of versions of the Morris Water Maze task is thought to be largely dependent on the use of allocentric strategies (Morris, 1981; Maaswinkel & Whishaw, 1999) and requires an organism to move around the pool and locate the platform relative to other salient features in the environment, such as landmarks (Milner & Goodale, 1995). In contrast, egocentric (ideothetic) strategies are related to the body (O'Keefe & Nadel, 1978) and code location in relation to the body's trunk and moveable parts (i.e., retinocentric, head centered, arm centered, etc.) (Woodin & Allport, 1998; Burgess, Jeffery, & O'Keefe, 1999; Berthoz, 2000). Understanding the involvement of egocentric strategies in navigation is less clear. They are thought to be employed on the Morris water maze task when an organism does not have access to extra-maze cues but is still able to locate the hidden escape platform based on self-movement information (Morris, 1981; Kealy et al., 2008).

There has been a general consensus that allocentric strategies are largely dependent on the hippocampus, whereas egocentric strategies are thought to rely on the posterior parietal cortex (Milner & Goodale, 1995; Save & Poucet, 2000a & 2000b; Rogers & Kesner, 2006). In animals, lesions to the hippocampus have consistently been shown to disrupt navigation that is based on distal landmarks (e.g. Morris, Garrud, Rawlins & O'Keefe, 1982; Jarrard et al., 1993; Save & Poucet 2000b; Schmitt et al., 2003; Jarrard et al., 2004; Jenkins et al., 2004; Parron, Poucet, & Save, 2004). Similar results have been found in humans with hippocampal damage (Maguire et al., 1998; Astur et al., 2002; Livingstone, & Skelton, 2007) and single unit recording from the hippocampus have demonstrated allocentric coding in spatial-view cells and place cells (Rolls & O'Mara, 1995; Georges-Francois, Rolls, & Robertson, 1999).

Egocentric frame of reference plays an important role in visual guided action and is thought to rely on dorsal stream functioning and the parietal cortex (Creem, & Proffitt, 2001; Milner & Goodale, 2004). A study by Roger and Kesner (1996) evaluated spatial strategies used by rats to solve two versions of the Hebb-Williams maze. They demonstrated that rats with hippocampal lesions exhibited greater deficits on the allocentric version of the maze

that included translucent walls to allow for the use of landmarks. However, on the egocentric version of the maze that included opaque walls to exclude the use of landmarks, the rats with parietal lobe lesions exhibited poorer performance (Roger & Kesner, 1996). This double dissociation has also been demonstrated in other studies using the Morris water maze (Save & Poucet 2000) with research clearly demonstrating that the hippocampus and parietal cortex both process spatial information (as reviewed by Rogers & Kesner, 2006) Humans with parietal damage often experience unilateral neglect and experience difficulty reaching for objects (Critchley, 1953), or as in Balint's syndrome, are unable to generate accurate limb movement towards a target location (Balint, 1909; Holmes, 1918; Damasio & Benton, 1979).

Few studies have explored egocentric strategies in humans using virtual reality paradigms. However, a recent study demonstrated that individuals with parietal lobe damage experienced deficits on egocentric spatial memory tasks (Weniger, Ruhleder, Wolf, Lange, & Irle, 2009). A similar study conducted by the same investigators (Weniger et al., 2010) had healthy participants navigate through a first-person virtual environment while being scanned by functional magnetic resonance imaging. The maze utilized in this study was thought to depend on egocentric navigation strategies because it did not include any landmarks and all intersections appeared identical. Authors found that there was no change in hippocampal activity during the completion of the task but they did observe increased parahippocampal activity (Weniger et al., 2010), an area of the brain that surrounds the hippocampus and that is strongly connected to the parietal cortex (Cavada & Goldman-Rakic, 1989; Suzuki & Amaral, 1994; Lavenex, Suzuki, & Amaral, 2002). Furthermore, previous research using the same virtual maze configuration demonstrated that individuals with parahippocampal damage were not able to learn the maze task but individuals with hippocampal damage did not exhibit an impairment in performance (Weniger, & Irle, 2006). Weniger and colleagues (2006) suggest that the parahippocampus may serve as a bridge that enables communication between both allocentric and egocentric structures which would facilitate the creation of a comprehensive representation of space.

Navigation is a complex behaviour that likely involves the coordination and interplay of several neuroanatomical structures and strategies. Lesion studies and imaging technology have provided insight into the function of these structures during navigation. Earlier

research seemed to support the notion that allocentric strategies were more dependent on the hippocampus, whereas egocentric strategies were thought to rely on the posterior parietal cortex. Recent studies suggest that there may be more intermingling between systems. More research in this area is required to distinguish between systems and function.

Synaptic Plasticity and Pharmacological Treatment

As previously discussed, the inactivation of the FMR1 gene on the X chromosome results in reduced or absent levels of FMRP, an mRNA binding protein thought to play a significant role in glutaminergic synaptic plasticity (Weiler et al., 1997; Feng et al., 1997; Antar et al., 2004). More specifically, research suggests FMRP functions as a negative modulator of mGluR-mediated dendritic protein synthesis (Huber et al., 2002; Koekkoek et al., 2005; Hessler, Rivera & Reiss, 2004), with studies demonstrating that an absence of FMRP has several negative consequences including increased spines on dendrites due to lack of synaptic pruning, extensive synaptic weakening and immature and elongated synaptic connections (Bear et al 2004; Irwin et al., 2000; Beckel-Mitchener & Greenough, 2004). Significant evidence suggests that changes in spinal properties and morphology may impact neuronal plasticity, which may underlie cognitive deficits in learning and memory that characterize FXS (Huber et al., 2002; Hayashi and Majewska 2005; Segal, 2005; Yuste and Bonhoeffer, 2004). Given the current understanding of the molecular role of FMRP, many treatment studies have been conducted using pharmacological compounds aimed at targeting receptors such as mGluR. Several studies have demonstrated the reversal of aberrant neuroanatomical characteristics as well as behavioral and cognitive impairments in animal models of FXS (Liu et al, 2011; Mines et al, 2010; Yuskaitis et al, 2010; Bilousova et al, 2009) and some of the cognitive and behavioural deficits of observed in individuals with FXS (Jacquemont et al, 2011; Berry-Kravis *et al.*, 2009; 2006; 2004). However, there have been challenges in establishing reliable outcome measures with many cognitive measures being too difficult for the majority of individuals with FXS to complete and further have produced unacceptable levels of variability (Berry-Kravis *et al.*, 2006). Therefore there is a need to identify or develop behavior assays that are sensitive to deficits observed in affected individuals and are reliable over time.

Hebb-Williams maze:

Hebb-Williams (H-W) mazes are well-established measures of spatial cognition

(Hebb & Williams, 1946; Rabinovitch & Rosvold, 1951) and have been used to test a variety of species including mice, rats, cats, goldfish and monkeys (as discussed by Shore et al., 2001). The mazes were first developed by Hebb and Williams (1946) and were designed to assess intelligence in the rat using a closed field apparatus. The original design and procedure was later refined and standardized by Rabinovich and Rosvold (1951) to include 12 maze configurations, or problems, of increasing difficulty. Since the paradigm's development, it has been utilized in a range of different applications including to evaluate the effects of aging (Winocur & Moscovitch, 1990), posterior cingulate cortical lesions (Meunier & Destrade, 1986;1988), hippocampal and prefrontal cortical lesions (Winocur & Moscovitch, 1990), differential strain abilities in mice (Standford & Brown, 2003), environmental enrichment (Hoplight, Sherman, Hyde, & Denenber, 2001), drug compounds (Paban, Soumireu-Mourat, Alescio-Lautier, 2003) and gender differences (Shore, Stanford, MacInnes, Klein, & Brown, 2001).

Although H-W mazes have traditionally been used to test spatial learning in animals (Shore, Stanford, MacInnes, Klein, & Brown, 2001), more recently, a computerized version of these mazes has been designed to allow researchers to evaluate spatial learning in humans under comparable testing conditions (Shore et. al., 2001), a criterion not met in previous studies. In both versions of the maze, subjects must learn to navigate through increasingly more complex configurations of mazes to arrive at a goal box where they are rewarded. The time to complete each of the mazes and the number of errors are the principal dependent variables measured. Shore and colleagues (2001) were the first to undertake a cross-species comparison of human and mouse performance on the H-W maze and found that the learning curves across both species were remarkably similar. Interestingly, another widely utilized paradigm, the Morris Water Maze (MWM), has also been translated into a computerized version and has been largely used to evaluate gender differences in spatial learning (Astur, Ortiz, & Sutherland, 1998; Sandstrom, Kaufman, & Huettel, 1998). A more recent study by Goodrich-Hunsaker and colleagues (2010) also evaluated spatial deficits on the MWM task in amnesic patients with hippocampal damage. However, the benefit of utilizing the H-W paradigm is that unlike the MWM that consists of one problem, the variety of H-W maze problems provides a broader profile of results (Shore et. al., 2001).

The use of virtual environments to evaluate navigational abilities in humans is not a

novel concept. In fact virtual environments have been used to study efficient route learning (Janzen, Wagensveld, & Van Turenout, 2008), navigational strategies (Bohbot, Lerch, Thorndycraft, Giuseppe, & Zijdenbos, 2007; Etchamendy, & Bohbot, 2007) deficits in wayfinding in individuals with traumatic brain injury (Livingstone, & Skelton, 2007), and to further delineate between neuroanatomical structures that may play a critical role in spatial learning (Weniger et al., 2010). Undoubtedly, these studies have greatly advanced our understanding of spatial learning and various key elements of navigation. However, they also present several challenges in terms of replication and comparison with previous literature (Shore et. al., 2001). For example, many of the studies use novel programs and environments (Moffat, Hampson, & Hatzipantelis, 1998; Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000), require specialized equipment (Gillner & Mallot, 1998) and may not publish maze specifications or programs in adequate detail (Gron et al., 2000) to allow for replication.

Therefore, the development of the novel H-W experimental paradigm has several distinct advantages. First, this paradigm allows for a comparison across species to directly evaluate performance of individuals affected by FXS to *fmr1* KO mice. Second, H-W mazes are reported to be sensitive to detecting alterations of hippocampal dependent spatial abilities (Winocur & Moscovitch, 1990) better than radial or water maze tasks (Pereira, Cosquer, Schimchowitsch, & Cassel, 2005). Third, establishing that behavioural assays, such as the H-W maze, are able to detect behavioural deficits in both human and mice is advantageous because they can be used to evaluate pharmacological and behavioural interventions to reverse or mitigate the symptoms of FXS. In addition, the H-W paradigm and protocol have been standardized and are supported by an immense literature for which to draw comparisons (Shore et. al., 2001). Finally, findings of comparable deficits among affected individuals and *fmr1* KO mice on a spatial navigation task may provide further insight into the neurobiological basis of the FXS phenotype.

The proposed study:

Fragile X Syndrome is the most prevalent form of heritable mental retardation. It arises from a mutation in the *FMRI* gene on the X chromosome that interferes with expression of Fragile X Mental Retardation protein and leads to a wide range of behavioural and cognitive deficits. Previous studies have demonstrated a deficit in basic visual

perceptual processing as well as spatial abilities in Fragile X but how such a deficit may impact spatial navigation remains unknown. Studies of *fmr1* knockout mice, a murine model of FXS, have also demonstrated deficits that at times mirror those observed in individuals affected by FXS. Despite some concordance, results from tests of the murine model of FXS focusing on replicating the spatial processing deficit observed in affected humans have been mixed. In an effort to resolve the ambiguity in the literature and extend previous research, the proposed studies will employ a novel experimental approach to evaluate spatial learning and memory.

Hebb-Williams mazes are well-established measures of spatial cognition and using both virtual and physical versions of the mazes allows for testing of humans and animals under comparable testing conditions. The proposed study will be divided into three components that will be presented as three separate studies. Study one will employ the traditional configuration of the Hebb-Williams mazes to evaluate performance variables that include latency to complete the maze and number of the errors. It is hypothesized that individuals affected by FXS and *fmr1* KO mice will exhibit poorer performance on mazes deemed more difficult as compared with their respective control groups. Study two will use the same Hebb-Williams maze configurations and performance measures as study one, but will include the addition of landmarks to the maze environment. Incorporating landmarks into the H-W mazes is hypothesized to provide additional spatial cues that will facilitate the use of alternative navigational strategies only to wild type mice. Research has demonstrated that landmarks tend to favor hippocampal processing, suggesting that their utilization in a navigational task should reveal deficits in allocentric processing. Therefore, it is hypothesized that only wild type mice will benefit from the introduction of landmarks. Due to hippocampal abnormalities in *fmr1* KO mice that result from a lack of FMRP, it is expected they will show relatively poorer performance as compared to wild type mice on landmark tasks because they will be less effective in developing a cognitive map of the mazes. Lastly, study three will entail a more in-depth behavior analysis of maze navigation performance for FXS individuals from study 1. All maze trials generated from Study 1 will be digitized allowing additional performance measures to be created and providing further insight into how individuals are solving the mazes. Additional performance measures include the total distance travelled across maze trials, and the frequency and duration of time

spent immobile (e.g. pausing/inspecting). It is hypothesized that as a result of visual-spatial memory and learning difficulties, individuals affected by FXS as compared to the typically-developing mental ages matched comparison participants will exhibit poorer results on all performance variables. In addition, affected individuals will exhibit differences in behavioural strategies employed to solve the H-W mazes, which will be evident by the pattern of pathways taken to solve the maze. Given the well-established hippocampal abnormalities found in affected individuals, it is predicted that they will display a less flexible style of visual spatial navigation.

Hypotheses

Study 1: A comparison of the performance of individuals with fragile x syndrome and *fmr1* knockout mice on Hebb-williams mazes

Hypothesis: Individuals affected by FXS and *fmr1* KO mice will exhibit poorer performance on mazes deemed more difficult as compared with their respective control groups.

Study 2: Landmarks and Hebb-Williams maze performance: Evaluating visual-spatial learning of *fmr1* knockout mice

Hypothesis 1: Incorporating landmarks into the H-W mazes will provide additional spatial cues that will facilitate the use of alternative navigational strategies.

Hypothesis 2: Providing alternative strategies for learning a navigation task that favor either hippocampal or cortical processing will reveal which of these brain regions are most greatly affected by the loss of FMRP.

Study 3: A detailed behavioral analysis of Hebb-Williams maze performance for individuals with fragile X syndrome

Hypothesis 1: Individuals affected by FXS as compared to the typically-developing mental ages matched comparison participants will exhibit poorer results on all performance variables.

Hypothesis 2: Affected individuals will exhibit differences in behavioural strategies employed to solve the H-W mazes, which will be evident by the pattern of pathways taken to solve the maze.

Chapter Two: Manuscript I

Manuscript I

A comparative study of the performance of individuals with Fragile X syndrome and *fmr1* knockout mice on Hebb-Williams mazes.

MacLeod, L., Kogan, C., Collin, C., Berry-Kravis, E., Messier, C., & Gandhi, R. (2010). *Genes, Brain and Behavior*, 9, 53-64.

Abstract

Fragile X Syndrome is the most prevalent form of heritable mental retardation. It arises from a mutation in the *FMRI* gene on the X chromosome that interferes with expression of Fragile X Mental Retardation protein and leads to a wide range of behavioural and cognitive deficits. Previous studies have demonstrated a deficit in basic visual perceptual processing as well as spatial abilities in Fragile X. How such a deficit may impact spatial navigation remains unknown. The current study extended previous research by evaluating spatial learning and memory using both virtual and physical versions of the Hebb-Williams mazes, which allows for testing of humans and animals under comparable testing conditions. Thus, we compared the performance of individuals affected by Fragile X (n = 15) to typically-developing individuals of equivalent mental age as well as the performance of *fmr1* knock-out mice (n = 11) to wild-type control mice (n = 12) on the same maze problems. In human participants, performance of the comparison group improved across trials, showing expected significant decreases in both errors and latency. In contrast, the performance of the Fragile X group remained at similar levels across trials. Although wild type control mice made significantly fewer errors than the *fmr1* knock-out mice, latencies were not statistically difference between the groups. These findings suggest that affected humans and mice demonstrate similar spatial learning deficits attributable to the lack of the Fragile X Mental Retardation protein. The implications of these data are discussed including the notion that Hebb-Williams mazes may represent a useful tool to examine the impact of pharmacological interventions on mitigating or reversing the symptoms associated with Fragile X syndrome.

A comparative study of individuals with Fragile X syndrome and *fmr1* knockout mice on
Hebb-Williams maze performance

Fragile X syndrome (FXS) is the most prevalent form of heritable mental retardation (Turner, Webb, Wake, & Robinson, 1996). It arises from a trinucleotide expansion of the Fragile X Mental Retardation 1 gene (*FMRI*) (Online Mendelian Inheritance in Man® [OMIM] 309550; Verkerk *et al.*, 1991). In FXS, *FMRI* is not expressed in somatic cells (Pieretti *et al.*, 1991), which leads to a wide range of behavioural and cognitive deficits, including deficits in attention (Backes, Genc, Schreck, Doerfler, & Lehmkuhl, 2002; Baumgardner, Reiss, Freund, & Abrams, 1995), visual-spatial cognition (Cornish, Munir & Cross, 1998; 1999; Crowe & Hay, 1990), working memory (Jakala *et al.*, 1997; Schapiro *et al.*, 1995), and visual perceptual processing (Kogan *et al.*, 2004). Studies of *fmr1* knockout mice, a murine model of FXS, have also demonstrated deficits that at times mirror those observed in individuals affected by FXS. Impairments have been described for a range of behavioural tasks including Conditioned Fear Response (Paradee *et al.*, 1999), Prepulse Inhibition (Chen & Toth, 2001), Open Field Activity (Mineur, Sluyter, De Wit, Oostra & Crusio, 2002), and Social Interaction tasks (Mineur, Huynh & Crusio, 2006; Spencer, Alekseyenko, Serysheva, Yuva-Paylor & Paylor, 2005).

Despite some concordance, results from tests of the murine model of FXS focusing on replicating the spatial processing deficit observed in affected humans have been mixed (D'Hooge *et al.*, 1997; Dobkin, *et al.*, 2000; The Dutch-Belgian Fragile-X Consortium 1994; Kooy *et al.*, 1996; Mineur *et al.*, 2002; Paradee *et al.*, 1999; Peier *et al.*, 2000). These discrepancies may arise from differences in background strain used (Spencer *et al.*, 2006), number of generations of back crossing (Gu *et al.*, 2002), and possibly the choice of the

spatial task employed as well as the equivalency of such tasks to those of human spatial cognition. It therefore remains controversial as to whether the murine model of FXS exhibits a similar spatial deficit as has been documented for affected individuals.

In an effort to resolve the ambiguity in the literature, in the present study we employ Hebb-Williams (H-W) mazes, which are well-established measures of spatial cognition (Hebb & Williams, 1946; Rabinovitch & Rosvold, 1951) and are reported to be sensitive to detecting alterations of hippocampal dependent spatial abilities (Winocur & Moscovitch, 1990) better than radial or water maze tasks (Pereira, Cosquer, Schimchowitsch, & Cassel, 2005). H-W mazes have traditionally been used to test spatial learning in animals (Shore, Stanford, MacInnes, Klein, & Brown, 2001). However, more recently, a computerized version of these mazes has been designed to allow researchers to evaluate spatial learning in humans under comparable testing conditions (Shore *et. al.*, 2001), a criterion not met in previous studies. The development of this novel experimental paradigm has two distinct advantages. First, this paradigm allows for a comparison across species to directly evaluate performance of individuals affected by FXS to *fmr1* KO mice. Second, establishing that behavioural assays, such as the H-W maze, are able to detect behavioural deficits in both human and mice is advantageous because they can be used to evaluate pharmacological and behavioural interventions to reverse or mitigate the symptoms of FXS. Finally, findings of comparable deficits among affected individuals and *fmr1* KO mice on a spatial navigation task may provide further insight into the neurobiological basis of the FXS phenotype.

Spatial navigation learning and memory in general, and performance on the H-W mazes specifically, appears to be dependent on both intact basic visual processing (Tees, Midgley, & Nesbit, 1981) as well as hippocampal and parietal cortex functioning (Rogers & Kesner, 2006; Hunsaker, Tran & Kesner, 2008).

With respect to basic visual functioning that may impact spatial abilities in FXS, Kogan and colleagues (2004 a, b) provided neurobiological and behavioural evidence that individuals with FXS have an early visual processing deficit that impact dorsal stream functioning. In primates, visual information is processed through the dorsal and ventral visual streams (Milner & Goodale, 1995; Ungerleider & Mishkin, 1982). The posterior parietal cortex is an area that receives primary input from the dorsal visual pathway and is important for spatial processing to guide behavior (Milner & Goodale, 1995) such as egocentric spatial navigational tasks (Hyvarinen & Poranen, 1974; Andersen & Buneo, 2002; Spiers & Maguire, 2007). We hypothesize that impairments in H-W performance indicative of abnormal basic visual functioning would manifest as significantly greater latencies and error rates across all maze problems for affected humans and KO mice for the first trial with persisting deficits observed on subsequent trials.

Spatial navigation is also dependent on intact hippocampal functioning (Morris, Garrud, Rawlins & O'Keefe, 1982; Ekstrom *et al.*, 2003; Ghaem *et al.*, 1997, Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; O'Keefe, & Dostrovsky, 1971). Lesion studies in mice have demonstrated that successful performance on paradigms such as the radial arm maze, T-maze and water maze are thought to rely on intact hippocampal processing (Hock & Bunsey, 1998; Mitchell, Rawlins, Steward & Olton, 1982; Morris, Garrud, Rawlins & O'Keefe, 1982; Okada & Okaichi, 2009). In both typically developing humans and wild-type mice, high levels of *FMRI/fmr1* mRNAs are expressed in the hippocampus (Abitbol *et al.*, 1993; Hinds *et al.*, 1993), suggesting that this brain area is particularly reliant on and therefore sensitive to changes in FMRP expression as occur in FXS. Furthermore, structural MRI studies suggest that individuals affected by FXS have enlarged hippocampi (Kates, Abrams, Kaufmann, Breiter & Reiss, 1997; Reiss, Lee & Freund, 1994). *Fmr1* KO mice also exhibit

hippocampal abnormalities and are found to have longer dendritic spines in pyramidal cells in subfield CA1 (Grossman *et al.*, 2006), smaller intra-infra pyramidal mossy fibre terminal fields (Mineur *et al.*, 2002), as well as shorter dendrites, fewer dendritic spines and functional synaptic connections (Braun & Segal, 2000). Therefore, we hypothesize that abnormal hippocampal functioning alongside intact basic visual functioning in individuals affected by FXS and *fmr1* KO mice will result in normal latencies and errors for the first trial of each new maze problem and significantly elevated levels of these same dependent variables on subsequent trials

In the present study we compared the performance of individuals affected by FXS to typically-developing mental age-matched comparison participants on seven H-W mazes of increasing difficulty levels. We also compared the performance of *fmr1* KO mice to wild-type control mice on the same maze problems. We hypothesized that individuals affected by FXS and KO mice as compared with their respective control groups would exhibit poorer performance on mazes deemed more difficult.

Materials and Methods

Experiment 1: Comparing *fmr1* KO and control mouse performance

Subjects

Twelve male FVB.129P2-*Pde6b*⁺*Tyr*^{*c-ch*}/AntJ mice (JAX Stock # 004828) and 11 male FVB.129P2-*Fmr1*^{*tm1Cgr*}/J mice (JAX Stock # 004624) were obtained from a colony at Jackson Laboratories (Bar Harbor, Maine, USA). Each strain had been backcrossed for 11 generations. Mice were pigmented and did not carry the *rd1* mutation, indicating that they do not suffer from blindness due to retinal degeneration. Animals were shipped at 4 weeks of age and were tested when they were approximately 12 weeks old. Eight days prior to behavioural testing, all subjects were individually housed in a climate-controlled vivarium

(20-22 ° C) that was maintained on a 12 hr light-dark cycle with lights on from 07:00 to 19:00. All testing was conducted during the light phase of the cycle. Mice were fed Harlan Global Rodent Chow and tap water. To ensure that mice were motivated during testing, they were maintained at 85-90% of their *ad lib* body weight. Mice were weighed daily and fed their individually weighed ration of food 30 minutes after completion of the session. The mice were treated in accordance with the guidelines and principles set by the Canadian Council on Animal Care and tested under the protocol approved by the University of Ottawa Animal Care Committee.

Apparatus

Mice were tested using the Hebb-Williams maze apparatus as described by Meunier and colleagues (1986). The maze was constructed using black opaque Plexiglas and was covered with a clear Plexiglas top (Plastics of Ottawa Ltd, Ottawa, Canada). It consisted of a square open field (60 cm X 60 cm X 10 cm) with start and goal box compartments (20 cm X 10 cm X 10 cm) located at diagonally opposite corners. These compartments were fitted with clear Plexiglas lids that were attached with hinges and could be blocked with removable clear Plexiglas barriers. The goal box was fitted with a ledge (8 cm X 2.5 cm) with a recessed food cup in the centre (2.5 cm diameter). The floor of the maze was divided into 36-equal squares that were clearly outlined in white. The squares were used as markers for placing the barriers in different maze configurations as described by Rabinovich and Rosvold (1951) and to define error zones. Removable barriers (10 cm high) were created using black opaque Plexiglas and each were supported by two permanent bases (2.5 cm X 2.5 cm). Extra-maze cues were minimized by conducting the study in an all-black enclosure and by having a dim light as the only source of illumination.

Procedure

The protocol consisted of three consecutive phases: habituation, acquisition and testing. Initially, mice were habituated to the maze environment for 20 minutes per day on 4 consecutive days with barriers and doors to the start and goal box removed. During the last two sessions the goal box was baited (Rodent Chow, 100 mg) and mice had *ad lib* access to the food for the duration of the session. Subsequently, mice were trained on 6 acquisition mazes (Figure. 1A.) as described by Robinovich and Rosvold (1951). On any given day, mice were tested such that they completed five trials for each of two of the 6 acquisition mazes. Mice completed all 6 acquisition mazes in sequence as many times as necessary for them to attain the criterion performance, which was defined as two consecutive sessions completed successfully in less than 30 seconds each. The acquisition phase required an average of 10 days to complete. On each acquisition trials, mice received a small reinforcer (Rodent Chow, 20 mg). Immediately following acquisition, mice were given a selection of the standard test mazes (Rabinovitch & Rosvold, 1951) according to the same training protocol used during acquisition sessions, which was conducted over 4 days. None of the acquisition or testing sessions exceeded 180 seconds. Both human participants and mice completed the same Rabinovitch and Rosvold maze configurations and in the same order (i.e., #2, #4, #5, #8, #9, #11, and #12). Latency and number of errors were recorded. Latency was recorded from the moment the barrier at the start box was removed until the animal took its first bite of food. An error was scored each time the animal's two front paws crossed into an error zone (Figure. 1B). Experimenters were blind to the animal genotypes and were never visible to the mice during the runs. The maze was thoroughly cleaned between trials.

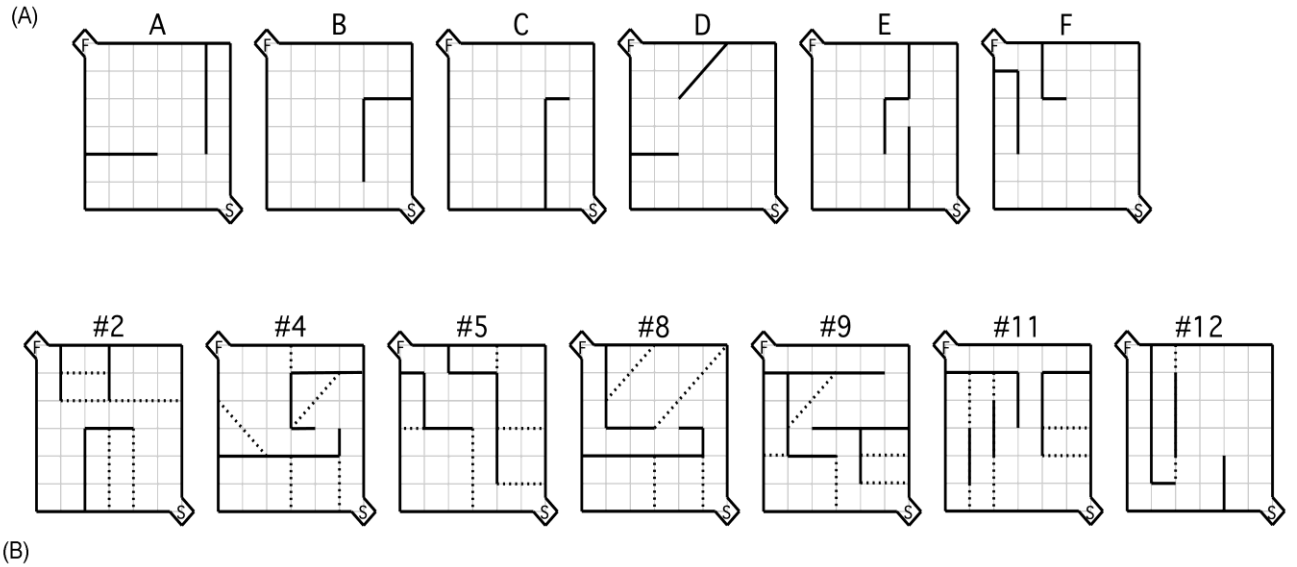


Figure 1. *Maze Configurations*. (a) Testing was conducted using the six practice mazes (A-F) and (b) the seven test mazes depicted. For each maze configuration, the (S) depicted in the bottom right hand corner represents the start box, and the (F) in the top left corner represents the goal box. Error zones are depicted by the dotted lines.

Experiment 2: Comparing FXS human and comparison performance

Participants

Fifteen male participants with FXS (mean chronological age = 24 years, SD = 4.9, mean verbal mental age = 7.57 years, SD = 1.92) were recruited from patient contact lists at Rush University Medical Center, Chicago, IL. All had a DNA-confirmed diagnosis of FXS and were full mutation carriers except for three participants who had a mosaicism of the full mutation. Seventeen male typically-developing comparison participants (mean chronological age = 5 years, SD = 2.9, mean verbal mental age = 8.07 years, SD = 2.03) were individually matched according to the verbal mental age (see Measures, Receptive Language Assessment) of the FXS participants. These individuals were recruited through a collaborator's contact list and newspaper advertising. Informed consent was obtained from caregivers of each participant (Appendix A & B). Furthermore, assent was obtained from

both control children and FXS individuals (Appendix C). All participants were paid \$25 per hour for their participation in the study and were treated in accordance to the ethical principles established by the Research Ethics Board at the University of Ottawa. Both the ethics committees of the School of Psychology, University of Ottawa, and of the Rush University Medical Center approved the study.

Measures

Receptive Language Assessment

Individual matching of FXS and comparison on verbal mental age was accomplished using the Peabody Picture Vocabulary Test Third Edition (PPVT-R, Dunn & Dunn, 1981). The PPVT is a measure of receptive vocabulary for standard English and assesses the extent of a participant's receptive verbal ability. The PPVT is a norm-referenced, individually administered test that consists of 175 vocabulary items arranged in increasing difficulty. The participant must select the image considered to best illustrate the meaning of a word presented orally by the examiner from a group of four images. Testing is terminated once a participant makes eight errors within a block of twelve trials. Raw scores were used to estimate verbal mental age according to norms provided by the test publisher (Dunn & Dunn, 1981). There were no a priori differences between the two group scores ($t = .468, p = .65$), indicating a successful matching on receptive verbal ability.

Questionnaires

A brief Medical History Questionnaire (Appendix B) was administered to all caregivers of participants to screen for any problems that would exclude them from the study. Exclusion criteria were any significant health or vision difficulties (e.g., color blindness, amblyopia, astigmatism) that would impact controlling a joystick or viewing the maze stimuli.

Apparatus

Hebb-Williams Virtual Maze

All participants were tested using a version of the virtual Hebb-Williams maze designed by Shore and colleagues (2001). Five mazes were eliminated from the original Hebb-Williams set for the purpose of this project because our pilot studies indicated that participants found these mazes too easy. In order to reduce administration time, only the most challenging mazes were used. All FXS and comparison participants were tested on the remaining subset of mazes, presented in the same order (i.e., #2, #4, #5, #8, #9, #11, and #12).

Experiments were performed on an Asus PC with a 19-inch Acer LCD monitor. Mazes were displayed at a resolution of 640 X 480 in full-screen mode. Participants navigated through the virtual environment at a constant velocity of 12 km/hr (forward, backward) and a turn rate of 50 degrees per second (left, right) using a Logitech Attack 3 joystick. Assuming a viewing height of 5 ft 6 in., the projection of the whole maze appeared to participants to measure 20m², and the diagonal straight line from start to finish was perceived as being located at a distance of 28.3 m.

Each maze was made up of a 6 X 6 room, with a 1 X 1 alcove at the entrance (start area) and exit (goal area) of the maze. Walls were created using textured rectangles that differed in colour depending on the maze configuration. A different colour was used for each maze configuration to indicate to participants that a new trial within the same maze had started (as opposed to starting a new maze). The start alcove and the floors were textured with black and grey marble effect. Each wall of the goal alcove was white and contained the image of a comic book character to provide motivation and reward for the participants. The roof was textured using beige and brown mottled square tiles (Figure. 2).

Procedure

All participants were individually tested by a research assistant, in a quiet room without their caregivers present. The tasks were administered during a 1 to 1.5 hour session and presented in a standardized order as described under apparatus above. After completing PPVT assessment, participants were trained on two types of practice mazes. An alley maze was presented first and enabled participants to establish how to adaptively maneuver through the virtual environment, while maintaining direct visual contact with the goal area. After meeting this criterion, a T-maze was presented in which participants had to choose a virtual navigational pathway in order to practice searching for the goal area of the maze. Criterion was achieved in both acquisition mazes when participants could complete three consecutive maze trials in less than 30 seconds each. At any time if a participant exceeded 120 seconds during a trial, the trial was considered finished and the participant proceeded to the next maze. For both the acquisition and testing mazes, participants received a sticker as reward after each trial, and after completing all three trials of maze they received a small piece of candy to be saved and consumed after the experiment was terminated.

After the acquisition sessions, participants completed three trials of each test maze (Figure. 1B). In between test for each maze, participants were provided with a two-minute break, at which time a children's DVD was played. After completing the fourth maze (#8) all participants were given a ten-minute break. Dependent variables were the participants' latency for solving the maze (i.e., time taken from the maze entrance to exit) and number of errors, measured as the number of times a participant crossed a predefined error line (see Figure. 1B) suggesting they were heading toward a blind alley rather than the goal box.

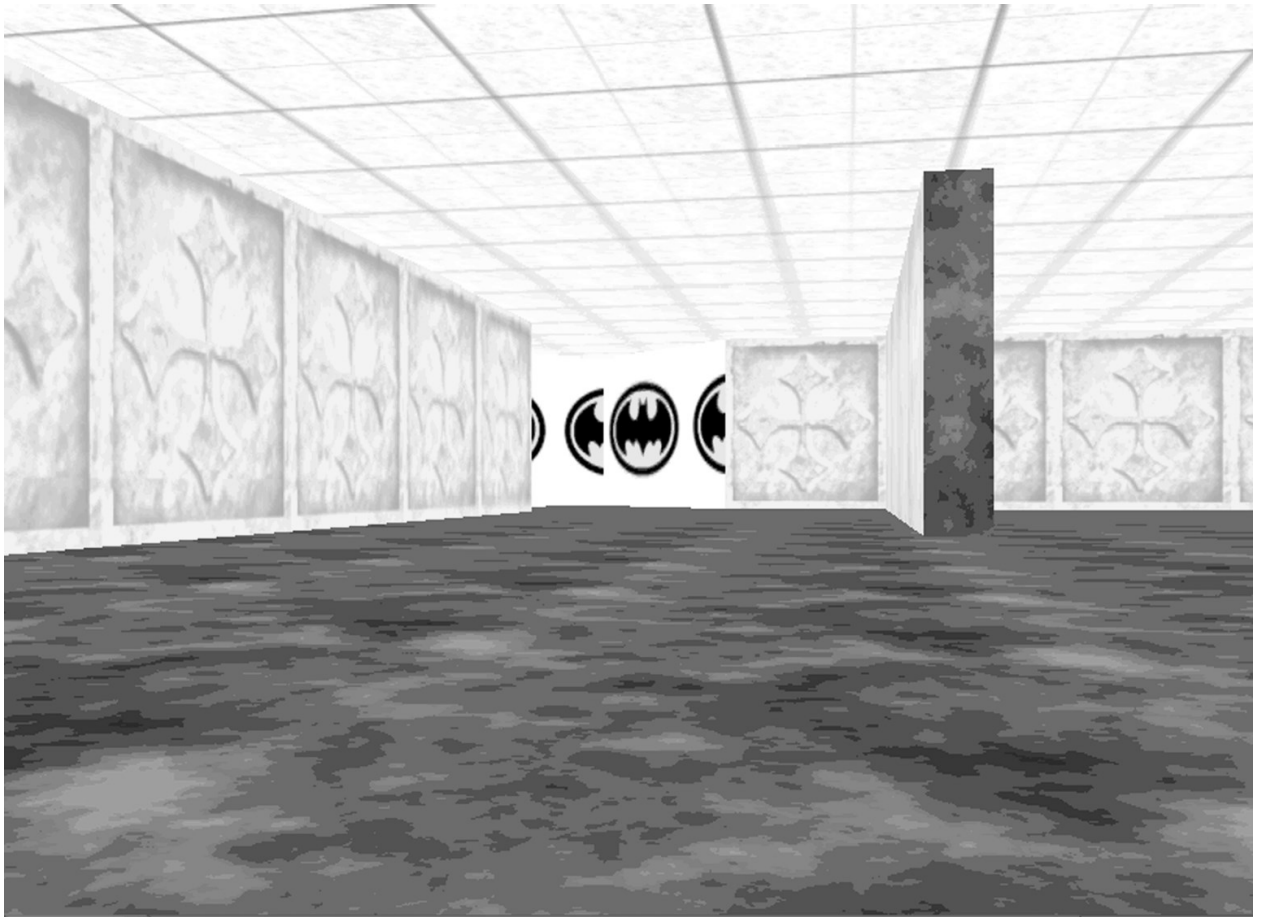


Figure 2. *Virtual Hebb-Williams maze*. Interior view of the maze #12

Statistical Analyses

The number of errors made across maze trials for human and animal studies were analyzed using a mixed-design analysis of variance (ANOVA) with Group as between-subjects variable and both Maze and Trial as within-subjects variables. Latency for completion of maze trials was analyzed in the same manner. Prior to the analyses, error and latency variables were transformed to square root scores in order to normalize the distribution of the data. Previous studies suggest that *fmr1* KO mice may exhibit increased activity levels as compared to wild type mice (e.g., Mineur et al 2002). Thus, we assessed activity levels by obtaining a count of the number of line crosses per unit of time for trial 1 of

maze 12. The latter maze was chosen because it has the least number of partitions thus allowing for the clearest observation of locomotion. We restricted our analysis to trial 1 because performance on this trial is independent of learning and memory and reflects exploratory behavior. All statistical analyses were conducted using PASW Statistics 17.0 (SPSS Inc., Chicago, USA).

Results

Human participants

Latency

A 2 X 7 X 3 ANOVA was conducted with Group (FXS, Comparison) as independent measures variable and both Maze (7 levels) and Trial (3 levels) as repeated measures variable. The latter revealed significant main effects for Group ($F_{1,30} = 12.235, p = .001$), Maze ($F_{6,30} = 12.487, p < .001$), and Trial ($F_{2,30} = 14.749, p < .001$). Furthermore, the interaction between Group and Trial was significant ($F_{2,60} = 10.933, p < .0001$) (Figure 3a). Alpha levels for *post-hoc* analyses of the interaction were adjusted using a Bonferroni correction to control for familywise error rate across trials ($\alpha = .05/9 = .006$). Thus, independent samples t-tests revealed significant differences between groups for latency for all trials (trial 1: $t_{30} = 2.78, p < .006$; trial 2: $t_{30} = -4.512, p = .001$; trial 3: $t_{30} = -7.331, p < .001$). Whereas for trial 1 the mean latency for the FXS group was significantly shorter than for the comparison participants, for subsequent trials, the comparison group completed the mazes significantly faster. We also examined differences within each group to assess for significant improvements across trials. For the comparison group, post-hoc paired samples t-tests revealed significant decreases in latency to reach the goal box between trial 1 and trial 2 ($t_{16} = 3.578, p < .001$), trial 1 and trial 3 ($t_{16} = 4.171, p < .001$). The difference in latency between groups for trial 2 and trial 3 approached significance ($t_{16} = 2.674, p = .017$). In contrast, in the FXS group there were no significant differences in latency between any of

the trials (trial 1 vs. trial 2: $t_{14} = .423, p = .679$; trial 1 vs. trial 3: ($t_{14} = .409, p = .689$) and trial 2 vs. trial 3 : $t_{14} = .086, p = .933$).

Error

A 2 X 7 X 3 ANOVA was conducted with Group (FXS, Comparison) as independent measures variable and both Maze (7 levels) and Trial (3 levels) as repeated measures variables. The latter revealed main effects for Group ($F_{1,30} = 7.398, p = 0.011$), Maze ($F_{6,30} = 23.376, p < 0.001$), and Trial ($F_{2,30} = 37.036, p < 0.001$). Furthermore, the interactions between Group and Trial ($F_{2,56} = 10.435, p < 0.001$) (Figure 3b) as well as Maze and Trial ($F_{12,336} = 2.138, p = 0.014$) were significant. Alpha levels for *post-hoc* analyses for the interaction were adjusted using a Bonferroni correction to control for familywise error rate across trials ($\alpha = .05/9 = .006$). Thus, independent samples t-tests revealed significant differences between groups for errors only for trials 2 and 3 (trial 1: $t_{28} = 1.257, p = .210$; trial 2: $t_{28} = -4.018, p < .001$; trial 3: $t_{28} = -4.556, p < .001$). We also examined differences within each group to assess for significant reductions in errors across trials. For the comparison group, *post-hoc* paired samples t-tests revealed significant decreases in the number of errors made during completion of trial 1 and trial 2 ($t_{16} = 6.059, p < .0001$), trial 1 and trial 3 ($t_{16} = 5.591, p < .0001$) but no significant difference in errors between trial 2 and trial 3 ($t_{16} = .741, p = .470$). In contrast, for the FXS group there were no significant differences in errors between any of the trials (trial 1 vs. trial 2: $t_{12} = 1.656, p = .124$; trial 1 vs. trial 3: $t_{12} = 1.492, p = .162$; trial 2 vs. trial 3: $t_{12} = .526, p = .609$).

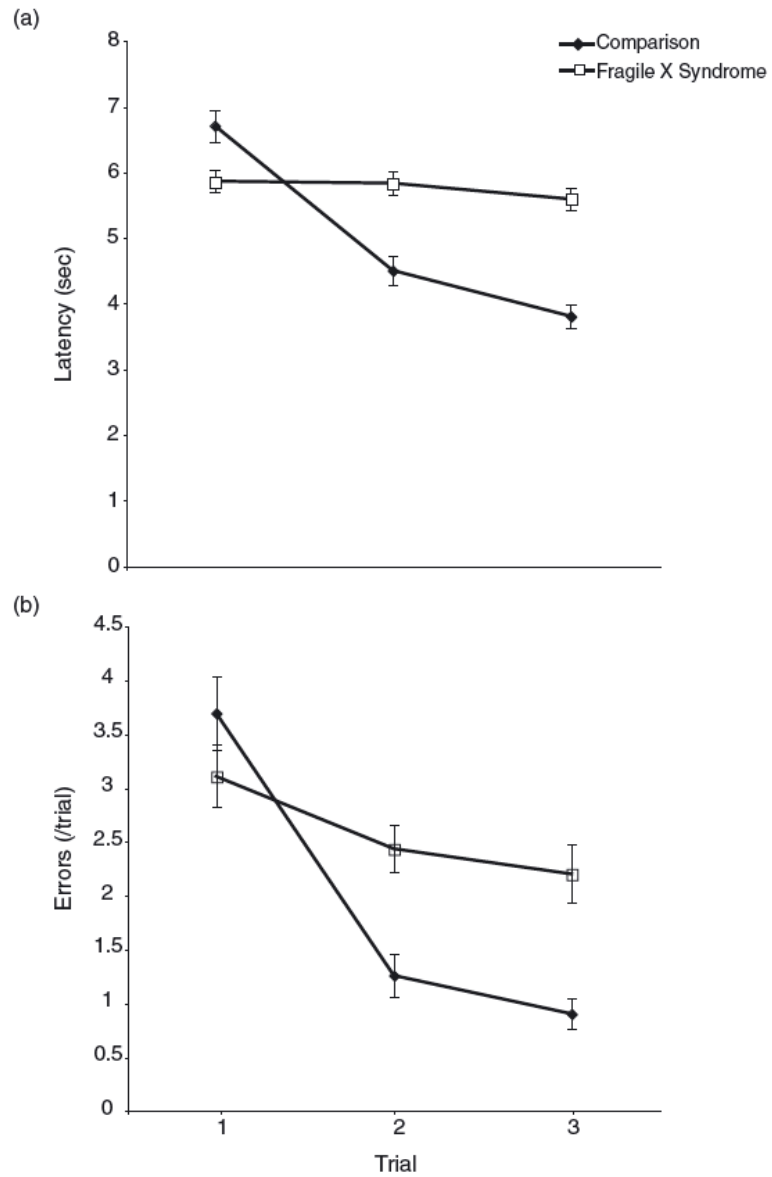


Figure 3 (a) *Human Latency*. Mean human latency measured in seconds for each of the three maze trials. Performance of comparison and FXS individuals on each of the three trials collapsed across the seven mazes. Error bars represent the standard error of the mean. (b) *Human Error*. Mean human error for each of the three maze trials. Performance of comparison and FXS individuals on each of the three trials collapsed across the seven mazes. Error bars represent the standard error of the mean.

Mice

Latency

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1* KO, control) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6,126} = 3.677$, $p = .002$) and Trial ($F_{4,84} = 26.490$, $p < .0001$) but not for Group ($F_{1,21} = .107$, $p = .746$), indicating that the latency to complete the mazes did not differ significantly between the KO and control group. However, a significant three-way interaction between Maze, Group, and Trial ($F = 1.633$, $p = .03$) was found (Figure 4). *Post-hoc* independent samples t-tests revealed only one large significant pair-wise difference attributable to longer latencies for the KO mice on trial 1 of maze 11 ($t_{21} = -2.09$, $p = 0.049$). However, when the alpha level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = .05/35 = .0014$), this difference was no longer significant.

Error

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1* KO, control) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Group ($F_{1,21} = 11.088$, $p = .003$), Maze ($F_{6,126} = 8.189$, $p < .001$), and Trial ($F_{4,84} = 16.620$, $p < .0001$). These results indicate that on average *fmr1* KO mice made more errors than control mice. Although no significant interaction between Group and Trial was found ($F_{4,84} = 1.458$, $p = .222$), a significant interaction between Maze and Trial was found ($F_{24,504} = 2.066$, $p = 0.002$). Finally, a significant three-way interaction was found for Maze, Trial, and Group ($F_{24,1008} = 2.140$, $p = .001$) (Figure 5). *Post-hoc* independent samples t-tests revealed only one

significant pair-wise difference attributable to significantly greater errors committed by the KO mice on trial 1 of maze 11 ($t_{21} = -2.33$, $p = 0.03$). However, when the alpha level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = .05/35 = .0014$), this difference was no longer significant.

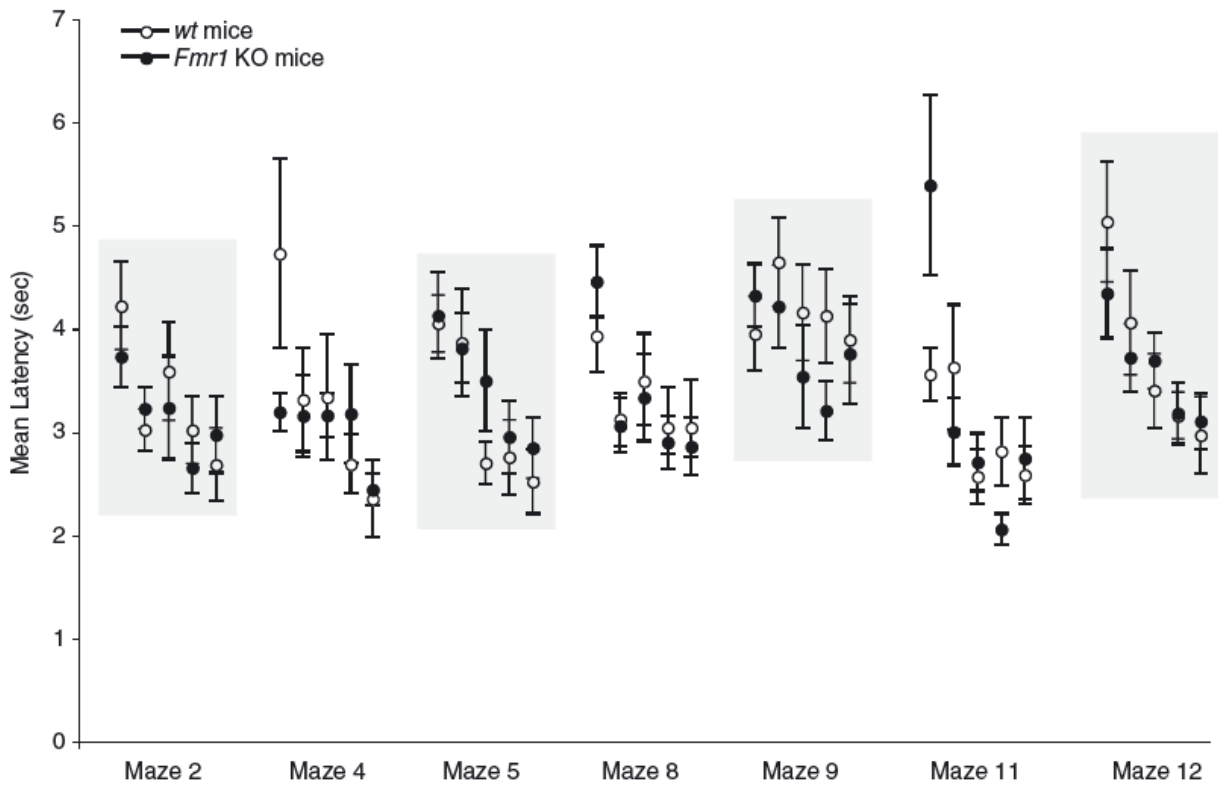


Figure 4. *Mouse latency*. Performance of wild-type control and *fmr1* KO mice for each of the seven mazes on each of the five respective trials. Error bars represent the standard error of the mean.

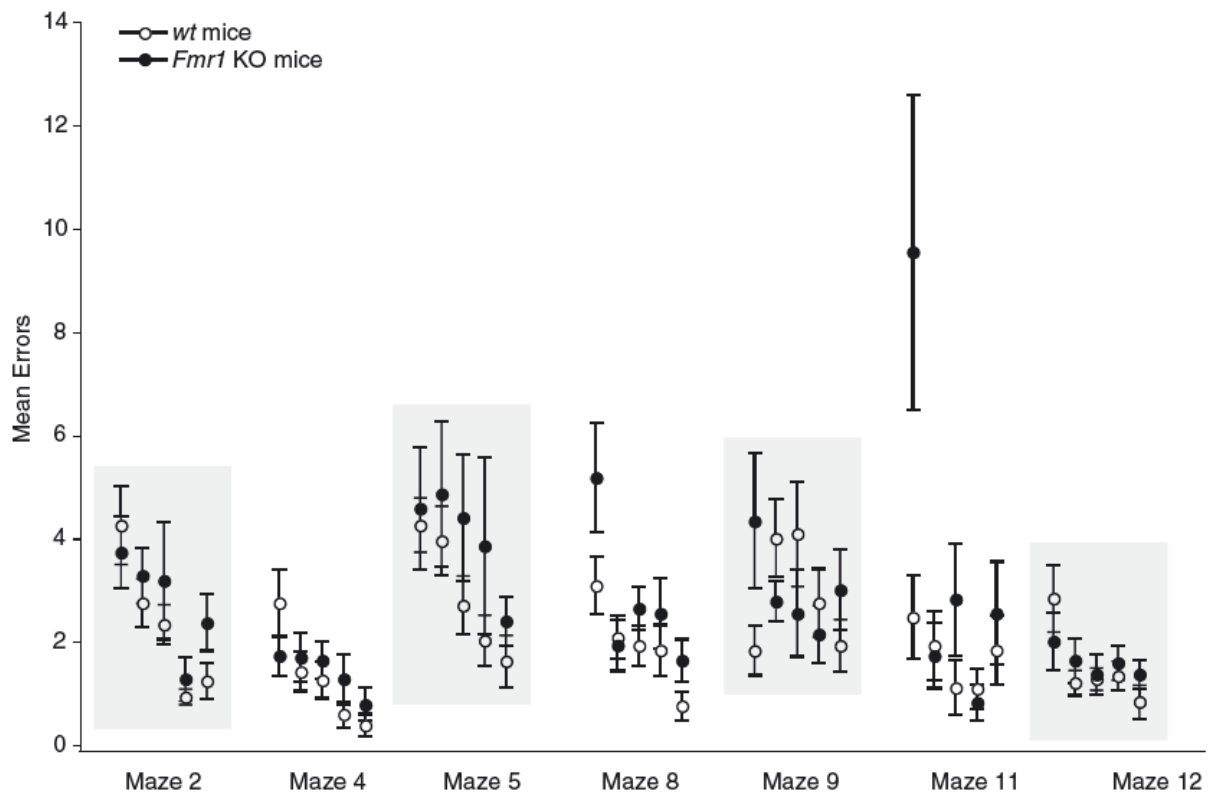


Figure 5. *Mouse error*. Performance of wild-type control and *fmr1* KO mice for each of the seven mazes on each of the five respective trials. Error bars represent the standard error of the mean.

Activity Levels

An independent samples t-test revealed no significant differences in activity levels between the *fmr1* KO and control group ($t = .661, p = .516$).

Maze Difficulty

The seven H-W mazes presented to participants and mice were selected because of reported differences in their level of difficulty (Shore et al, 2001). As described above, whereas a significant main effect of Maze was found for human and mouse latency as well as mouse error, a significant interaction between Maze and Trial was found for human error. Thus,

contrary to our hypothesis, neither the FXS group nor the *fmr1* KO group performed any worse than the respective comparison groups for mazes deemed more difficult. Although these findings confirm that mazes differed in their level of difficulty, *post hoc* analyses were not conducted because they were not relevant to the current study.

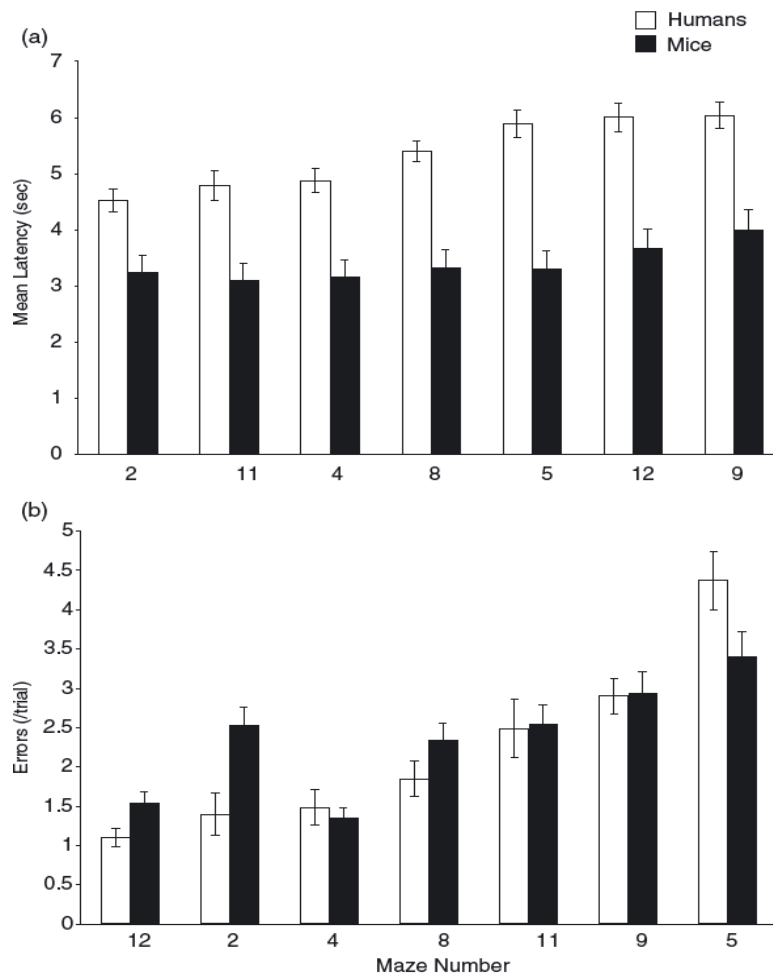


Figure 6. *Maze Difficulty*. (a) Latencies for human and mouse performance across the seven mazes collapsed across groups. (b) Number of errors for human and mouse performance across the seven mazes collapsed across groups. Note: for ease of comparison, mazes are presented according to the human data in ascending order of difficulty.

Discussion

The present study examined the spatial navigation abilities of individuals affected by FXS and a mouse model of FXS, *fmr1* KO mice, on a similar task. The results reveal significant differences in performance for both groups as compared to mental age-matched comparison individuals and wild type mice, respectively. In contrast to the FXS group, performance of the comparison group improved as indicated by significantly fewer errors across trials. A similar pattern of results was observed when latency across trials was analyzed. Although significant differences between groups were found for all trials, on trial 1 the FXS group completed mazes faster than their peers while committing a similar number of errors. That participants affected by FXS were able to successfully complete the mazes suggests that basic visual functioning necessary for solving a novel spatial task is intact in these individuals. However, with subsequent trials, the FXS group appear unable to either learn or recall the maze solution and continue to use a trial and error strategy to find the goal box.

Our findings are consistent with previous research suggesting a possible impairment of spatial processing in FXS. Individuals affected by FXS have reliably been shown to experience difficulties on visual-motor tasks including those requiring participants to manipulate objects in space, an ability similar to the one used to navigate successfully through virtual H-W mazes (Crowe *et al.*, 1990; Cornish, Munir & Cross, 1998; 1999; Freund & Reiss, 1991; Mazzocco, Singh Bahtia, & Lesniak-Karpiak, 2006). Kogan and colleagues (2004) suggest that underlying impairments in neural functioning within the thalamic magnocellular (M)-pathway contributes to visual-spatial deficits observed in individuals affected by FXS. They demonstrated that FXS individuals exhibit impairment on psychophysical tasks that tap the dorsal visual stream functioning, which is a system that is

integral to the visual control of action (Milner & Goodale, 1995). Therefore, we hypothesized that individuals affected by FXS would perform worse on the virtual H-W mazes because spatial navigation relies in part on intact vision (Tees *et al.*, 1981) and therefore efficient processing within the dorsal visual stream. However, the findings of the current study argues against a pure visual-perceptual deficit as an adequate explanation for observed differences on subsequent trials. In fact, the FXS group completed the first trial of the mazes faster than comparison participants making similar numbers of errors (Figure 3).

An alternative explanation is that our results are more suggestive of impairment in hippocampal function, which is also required for spatial navigation (Morris *et al.*, 1982; Ekstrom *et al.*, 2003; Ghaem *et al.*, 1997, Iaria *et al.*, 2003; O'Keefe, & Dostrovsky, 1971). In typically developing individuals, high levels of *FMRI* mRNAs are expressed in the hippocampus (Abitbol *et al.*, 1993; Hinds *et al.*, 1993), suggesting that this brain region is particularly reliant on and therefore sensitive to changes in FMRP expression. Indeed, functional impairments in hippocampal circuitry have been shown in *fmr1* KO mice, such as enhanced long-term depression (LTD), a form of synaptic plasticity (Huber, Kayser, & Bear, 2000; Huber, Gallagher, Warren & Bear, 2002; Zhang, Hou, Klann, & Nelson, 2009). Therefore, it is possible that these impairments could also be present in individuals affected by FXS. In support of this notion, structural MRI studies suggest that individuals affected by FXS have enlarged hippocampi (Kates, Abrams, Kaufmann, Breiter & Reiss, 1997; Reiss, Lee & Freund, 1994). Behaviorally, individuals affected by FXS exhibit impaired performance on spatial tasks (Crowe *et al.*, 1990; Cornish, Munir & Cross, 1998; 1999; Freund & Reiss, 1991; Mazzocco, Singh Bahtia, & Lesniak-Karpiak, 2006). Taken together with evidence that abnormalities in spatial learning can be attributed to hippocampal deficits (Morris *et al.*, 1982; Logue, Paylor, & Wehner, 1997), we speculate that performance of

individuals affected by FXS on the virtual H-W maze arises from such impairment.

Interestingly, *fmr1* KO mice exhibit impairments similar to those observed in the FXS group for the same H-W mazes. We found that overall wild type control mice made significantly fewer errors than the *fmr1* KO group. However, the latency to complete the mazes for both groups was not significantly different. These findings may suggest that for the *fmr1* KO but not the wild type mice there is a speed-accuracy trade-off in the navigational strategies employed by this group. Pollard and Lysons (1969) suggest that learning and memory may be best accounted for by measurements of error, whereas measurements based on time may better reflect non-problem solving behaviors, such as exploration and/or motivational factors. Similar to the human data, increased errors observed in *fmr1* KO mouse performance is more accurately a reflection of a learning or memory deficit, rather than non-specific factors or visual-perceptual ability *per se*.

An alternative interpretation for the greater number of errors committed by the *fmr1* KO mice relates to aspects of their phenotype not attributable to spatial impairments. *Fmr1* KO mice as compared to wild type mice have been reported to exhibit increased exploratory behaviour and hyperactivity (The Dutch-Belgian Fragile X Consortium, 1994; Zupan and Toth, 2008), less freezing behaviour (Paradee *et al.*, 1999), more open field entries (Yan, Asafo-Adjei, Arnold, Brown & Bauchwitz, 2004), as well as decreased anxiety (Yan *et al.*, 2004). Therefore, increases in both hyperactivity and reductions in neophobia characteristic of *fmr1* KO mice may have led to increased numbers of entries in to error zones, which in turn, resulted in poor learning or encoding of inefficient solutions to the mazes. To test this hypothesis a *post-hoc* analysis of activity levels between the murine groups was conducted for the first trial of the first maze tested. Consistent with previous reports, our results indicate that there are no significant differences in activity levels between *fmr1* KO and control mice

(The Dutch-Belgian Fragile X Consortium, 1994, Mineur *et al.*, 2002, Peier, 2000; Spencer, 2005; Zupan and Toth, 2008). However, the limited sample size of data points included in this *post hoc* analysis precludes definitive conclusion that activity level is not a factor in the observed differences in error rates between the groups. Analyses of the specific paths taken by the respective groups to reach the goal box is part of an ongoing study to establish whether increased errors committed by the KO mice is a cause or consequence of a learning deficit.

Similar to the human data, the deficits observed in the *fmr1* KO mice may relate to hippocampal dysfunction. Like typically developing humans, wild-type mice express high levels of *fmr1* mRNAs in the hippocampus (Hinds *et al.*, 1993). Cultured neurons from *fmr1* KO mouse pups harbor abnormal dendritic spines (Braun & Segal, 2000; Grossman *et al.*, 2006) and fewer functional synaptic connections (Braun & Segal, 2000) in the hippocampus. Smaller intra-infra pyramidal mossy fibre terminal fields have also been observed in the hippocampi of *fmr1* KO mice (Mineur *et al.*, 2002) Furthermore, *fmr1* KO mice show behavioral deficits on tasks that are thought to be dependent on hippocampal function, such as the radial maze (Mineur *et al.*, 2002), reversal trials of the Morris water maze (D'Hooge *et al.*, 1997; The Dutch-Belgian Fragile X Consortium, 1994; Kooy *et al.*, 1996; Van Dam *et al.*, 2000) and performance on the cross-shaped maze (Dobkin *et al.*, 2000). Therefore, we speculate that impaired performance of *fmr1* KO mice on the H-W mazes observed here is attributable to abnormal processing in the hippocampus. Future studies should examine whether spatial learning deficits in the KO mice are attributable to impairment in encoding, storage, retrieval, or some combination thereof. It is also possible that the greater number of errors committed by the KO mice relate to higher rates of perseveration for incorrect

solutions to a given maze problem. An ongoing study of the paths taken to solve the mazes will be able to address this issue.

Interestingly, although deficits in spatial memory in *fmr1* KO have been demonstrated for many tasks, findings have often been inconsistent and difficult to replicate, with most studies reporting either mild, or no differences in spatial abilities (Paradee *et al.*, 1999; Peier *et al.*, 2000; Yan *et al.*, 2004). Many possible explanations for these inconsistencies have been suggested, such as differences in background strain (Errijgers, Fransen, D'Hooge, De Deyn, & Kooy, 2008; Spencer *et al.*, 2006), the presence of a retinal degeneracy mutation in older FVB strains (Errijgers, *et al.*, 2007), and number of generations of back crossing (Gu *et al.*, 2002). For example, Paradee and colleagues (1999) demonstrated that the C57BL/6 and FVB-129 background strains produced different results on the Morris water maze task, which is consistent with the innate spatial abilities of each strain. Furthermore, Dobkin and colleagues (2000) demonstrated reduced learning on the Morris water maze in the FVB-129 strain but not in C57BL/6 strain, consistent with the excellent spatial ability of C57BL/6 mice. In the present study, a FVB background was used; its more modest spatial abilities may be a better murine model to investigate the visual phenotype of FXS. Use of this strain may avoid the possibility that the C57BL/6 strain, with its superior spatial abilities can overcome the spatial deficits associated with absence of *fmr1* expression.

One possible limitation of the present study is the differences in the composition of the comparison groups used to interpret the data from the two species. For the human study, a mental age matched comparison group was used to compare performance of the FXS group. Conversely, in the animal study the *fmr1* KO group was compared to chronologically matched controls. It is possible that the composition of the animal groups could have

increased the likelihood of observing a significant difference between the two groups tested. Studies of individuals affected by FXS primarily compare their performance to individuals who are typically-developing but score similarly on a given measure of intellectual functioning (often the PPVT). Therefore, the comparison participants are almost always chronologically younger than the affected individuals in order to avoid the confound of intelligence. As there is no established methodology for determining mental age of either KO or wild type mice, matching on this variable was not possible. A survey of the literature on *fmr1* KO mice reveals that studies either fail to inform the reader about the age of the subjects or use chronological age matching.

The present study included three participants with mosaicism of the *FMRI* gene. That is, these individuals express *FMRI* in some of their somatic cells. Given that individuals with a mosaic tend to exhibit less severe impairments across most cognitive measures (Cohen, Nolin, Sudhalter, Ding, Dobkin, & Brown, 1996; McConkie-Rosell *et al.*, 1993; Merenstein *et al.*, 1996), inclusion of these individuals in the current study could have minimized the likelihood of observing a significant difference between the two groups tested. Despite their inclusion, a significant difference in the latency of the FXS individuals was observed, as compared to mental-age matched peers. Furthermore, a review of the results obtained from individuals with mosaicism revealed that their performance was similar to that of the participants with the full-mutation.

A clear strength of the present study is its novelty. Specifically, there have been few studies comparing human and rodent performance on the same task (Shore *et al.*, 2001) and to our knowledge, none that have directly compared individuals affected by FXS to a murine model of the disorder on the same task. This is important because using such a translational approach increases the validity of the murine model as well as strengthens the notion that our

results reflect a similar underlying spatial deficit that is being targeted and expressed in both species. An additional strength of this study was the establishment of the validity of the H-W mazes as a behavioural assay able to detect behavioural deficits in both human and mice. We propose that H-W mazes could be used as a tool to evaluate therapeutic strategies both during early development in animals and later in clinical trials. Pharmacological compounds have been used to treat cognitive and behavioural deficits of FXS (Berry-Kravis *et al.*, 2006; 2004) but there have been some challenges in establishing reliable outcome measures. Many cognitive measures used previously have been too difficult for the majority of individuals with FXS to complete and further have produced unacceptable levels of variability (Berry-Kravis *et al.*, 2006). Additional studies investigating the reliability of the H-W paradigm in its ability to detect spatial deficits, as well the stability of performance measures over time would be beneficial.

In conclusion, the results of the present study support the hypothesis that a selective deficit in spatial learning and memory characteristic of the FXS phenotype can be observed in the murine model of FXS, the *fmr1* KO if equivalent tasks are employed in testing humans and mice. The present study measured the time to complete each of the mazes and the number of errors. Future research should include investigations into the types of strategies being utilized when solving the mazes. This would help clarify if perseveration or inflexibility in shifting from one learned response to another could be contributing factors in determining poorer maze performance among affected individuals and *fmr1* KO mice.

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Chapter Three: Manuscript II

Manuscript II

Landmarks and Hebb-Williams maze performance: Evaluating visual-spatial learning of
fmr1 knockout mice

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Abstract

Fragile X Syndrome is a single gene trinucleotide disorder and the most prevalent form of heritable mental retardation that is characterized by a wide range of behavioural and cognitive deficits. Previous studies have demonstrated similar visual spatial learning and memory deficits in both individuals with Fragile X syndrome and *fmr1* knock-out (KO) mice when tested on a subset of Hebb-Williams (H-W) mazes. The current study extends previous research by clarifying the nature of the spatial deficit in *fmr1* KO mice by incorporating landmarks. These additional spatial cues are hypothesized to facilitate the utilization of allocentric navigational strategies that are more reliant on hippocampal processing. Results revealed that the performance of the *fmr1* KO mice generally did not differ between landmark and non-landmark tasks, indicating that the presence of landmarks neither enhanced nor hindered mouse performance. An interesting exception to this pattern was observed on Maze 4, whereby the *fmr1* KO landmark group acquired learning faster than its non-landmark equivalent, without committing additional errors; suggesting that the presence of landmarks actually facilitated mouse performance for this specific maze. Contrary to our hypotheses, landmarks significantly impaired wild type control performance. The implications of these data are discussed, as well as possible explanations as to why landmarks, in the manner they have been implemented, do not improve allocentric processing.

Key Words: *fmr1* knockout mice, Spatial Learning and Memory, Fragile-X Syndrome, Landmarks, Behavioral Testing

Landmarks and Hebb-Williams maze performance: Evaluating visual-spatial learning of
fmr1 knockout mice

Fragile X Syndrome (FXS) is the most prevalent form of heritable mental retardation [1], with estimates indicating that 1 in 2,500 individuals are affected [2]. FXS is a single gene disorder and arises from a mutation in the Fragile X Mental Retardation 1 gene (*FMR1*) on the X chromosome [3-5] that reduces levels of Fragile X Mental Retardation protein (FMRP) [4]. The lack of expression of *FMR1* in somatic cells [6] leads to a well-defined phenotype that is characterized by a range of behavioral and cognitive deficits, including impairments in visual-spatial cognition [7-10], and visual perceptual processing [11-14].

A murine model of FXS, *fmr1* knockout mice (KO), displays similar characteristics and deficits as those observed in individuals affected by FXS, including macroorchidism, learning deficits, hyperactivity [15,16] age-dependent audiogenic seizures [17,18] and hippocampal abnormalities [19-21]. However, studies using *fmr1*KO mice, have produced mixed results on measurements of spatial processing abilities [15, 16, 22-26]. Spatial impairments in *fmr1* KO have been observed using several paradigms including the radial arm maze [26, 27] and the Barnes Maze [27], yet only mild learning deficits have been shown on the Morris Water Maze; that are generally only evident during the reversal learning [15, 16, 22, 24, 28, 29].

In an effort to clarify some of the ambiguity in the literature, Macleod and colleagues (2010) found spatial learning deficits in both affected humans and *fmr1* KO mice using virtual and physical versions of the Hebb-Williams mazes (H-W), respectively. Although significant differences were observed between groups on all trials, on the first trial, the FXS group completed the mazes faster than the comparison group, while making a similar number of errors (i.e., no speed/accuracy trade-off). The ability of the participants affected by FXS

to successfully complete the mazes suggests that the basic visual processing necessary to complete the task was intact, and that impairments observed in both affected groups appeared to be more consistent with visual spatial learning and memory deficits, in contrast to pure visual-perceptual deficits as suggested by findings of Kogan and colleagues (2004) that emphasize visual-spatial impairments at the threshold for detection of stimuli. Given the research suggesting that landmarks may promote greater flexibility in utilizing alternative navigational strategies thought to preferentially rely on different underlying neuroanatomical structures, the goal of the present study was to further clarify the nature of the spatial deficit in *fmr1* KO mice by utilizing the same H-W maze paradigm with the addition of distinct spatial cues that could be used for allocentric encoding.

Successful navigation is thought to depend on the ability to adopt different strategies based on frame of reference [31]. Allocentric and egocentric frames of reference are generally the primary navigational strategies investigated. They can be differentiated by the cues used to initiate their use as well as the anatomical structures involved in their processing [32]. Allocentric strategies are related to salient features in the environment and code spatial stimuli such as landmarks and object interrelations in the environment [33-35]. Egocentric strategies appeared to be involved in visually guided action related to body position [34] and code location in relation to the body's trunk and moveable parts (i.e., retinocentric, head centered, arm centered, etc.) [36-38].

Lesion and imaging studies have provided insight into the function of the neuroanatomical substrates implicated in spatial navigation. Although allocentric strategies have been extensively studied, the role of egocentric strategies in navigation is less well understood. Allocentric strategies are thought to be largely dependent on the hippocampus, whereas egocentric strategies are thought to rely on dorsal stream functioning and the

posterior parietal cortex [33, 35, 39, 40-43]. In animals, lesions to the hippocampus have consistently been shown to disrupt navigation that is based on distal landmarks [e.g. 40, 44-49], with similar results demonstrated in humans with hippocampal damage [50-52]. Using two versions of the H-W mazes, Roger and Kesner (1996) demonstrated that rats with hippocampal lesions exhibited greater deficits on the allocentric version of the maze that included translucent walls to facilitate the use of landmarks. However, on the egocentric version of the maze that included opaque walls to exclude the use of landmarks, the rats with parietal lobe lesions exhibited poorer performance [33]. This double dissociation has also been demonstrated in other studies using the Morris water maze [40].

Fewer studies have investigated the neuroanatomical substrates of egocentric strategies. One study demonstrated that individuals with parietal lobe damage experience deficits on egocentric spatial memory tasks [53]. In addition, a functional magnetic resonance imaging study found that healthy participants navigating through mazes thought to be dependent on egocentric navigation strategies, experienced no change in hippocampal activity but did demonstrate an increase in parahippocampal activity [54]; an area of the brain that surrounds the hippocampus and that is strongly connected to the parietal cortex [55-57]. Furthermore, individuals with parahippocampal damage were not able to learn an egocentric maze task but individuals with hippocampal damage were able to learn [58]. Weniger and colleagues (2006) suggest that the parahippocampus may serve as a bridge that enables communication between both allocentric and egocentric structures presumably facilitating the creation of a comprehensive representation of space.

Performance on H-W mazes appears to be dependent on both intact basic visual processing [59], as well as hippocampal and parietal cortex functioning [33, 60]. In both developing humans and wild type mice, high levels of *FMRI* mRNAs are expressed in the

hippocampus [61, 62]. Similarly FMRP is expressed in high levels of the magnocellular (M-pathway) layers of the lateral geniculate nuclei (LGN), a thalamic component of the visual pathways [11, 12] that projects afferents to regions of the parietal cortex, which are involved in spatial processing and directing action to guide behavior and egocentric spatial processing [35, 68].

In the present study, we evaluate the performance of *fmr1* KO compared to wild type mice on seven H-W mazes with landmarks. Incorporating landmarks into the H-W mazes was hypothesized to provide additional spatial cues that would facilitate the use of alternative navigational strategies. Research has demonstrated that landmarks tend to favor hippocampal processing, suggesting that their utilization in a navigational task should reveal deficits in allocentric processing. Hippocampal and cortical abnormalities are a prominent feature in *fmr1*KO mice. Therefore, we conjectured that providing alternative strategies for learning a navigation task that favor either hippocampal or cortical processing will reveal which of these brain regions are most greatly affected by the loss of FMRP.

Materials and Methods

Subjects. Ten male FVB.129P2-*Pde6b*⁺*Tyr*^{c-ch}/AntJ mice (JAX Stock # 004828) and 10 male FVB.129P2-*Fmr1*^{tm1Cgr}/J mice (JAX Stock # 004624) were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). Each strain had been backcrossed for 11 generations. Mice were pigmented and did not carry the *rd1* mutation, indicating that they do not suffer from blindness due to retinal degeneration. Animals were tested when they were approximately 5 months old. Eight days prior to behavioural testing, all subjects were individually housed in a climate-controlled vivarium (20-22 ° C) that was maintained on a 12 hr light-dark cycle with lights on from 0700 to 1900. All testing was conducted during the

light phase of the cycle. Mice were fed Harlan Global Rodent Chow and tap water. They were maintained at 85-90% of their *ad lib* body weight. Mice were weighed daily and fed their individually weighed ration of food 30 minutes after completion of the session. The mice were treated in accordance with the guidelines and principles set by the Canadian Council on Animal Care and tested under a protocol approved by the University of Ottawa Animal Care Committee.

Apparatus-Hebb-Williams Maze without landmarks: Mice were tested using the Hebb-Williams maze apparatus as described Meunier and colleagues (1986) [70]. The maze was constructed using black opaque Plexiglas and was covered with a clear Plexiglas top (Plastics of Ottawa Ltd, Ottawa, Canada). It consisted of a square open field (60 cm X 60 cm X 10 cm) with start and goal box compartments (20 cm X 10 cm X 10 cm) located at diagonally opposite corners. These compartments were fitted with clear Plexiglas lids that were attached with hinges and could be blocked with removable clear Plexiglas barriers. The goal box was fitted with a ledge (8 cm X 2.5 cm) with a recessed food cup in the centre (2.5 cm diameter). The floor of the maze was divided into 36-equal squares that were clearly outlined in white. The squares were used as markers for placing the barriers in different maze configurations as described by Rabinovich and Rosvold (1951) [71] and to define error zones. Removable barriers (10 cm high) were created using black opaque Plexiglas and each was supported by two permanent bases (2.5 cm X 2.5 cm).

Hebb-Williams Maze with landmarks—The H-W maze apparatus was identical to the standard condition, except for the addition of landmarks. For intra-maze cues, each test maze included six images of simple geometric shapes (e.g. circle, square, triangle) surrounded by a white background were used as landmarks (10 cm X 10 cm). An identical set of six shapes was used in each maze configuration. Each set of shapes was the same

color as that of the test maze with the white background clearly distinguishing the shape from the maze wall. The landmarks were distributed within a test maze such that at least one image was visible from any given position within the maze. Landmarks were laminated and adhered to the interior of the maze with double-sided tape.

Procedure. The procedure used in the current study was the same as that of MacLeod and colleagues (2010). The protocol consisted of three consecutive phases: habituation, acquisition and testing. Initially, mice were habituated to the maze environment for 20 minutes per day on 4 consecutive days with barriers and doors to the start and goal box removed. During the last two sessions the goal box was baited (Rodent Chow, 100 mg) and mice had *ad lib* access to the food for the duration of the session. Subsequently, mice were trained on 6 acquisition mazes (Figure. 1a.)[71]. On any given day, mice were tested such that they completed five trials for each of two of the 6 acquisition mazes. Mice completed all 6 acquisition mazes in sequence as many times as necessary for them to attain the criterion performance, which was defined as two consecutive sessions completed successfully in less than 30 seconds each. The acquisition phase required an average of 7 days to complete. On each acquisition trial, mice received a small reinforcer (Rodent Chow, 20 mg). Immediately following acquisition, mice were given a selection of the standard test mazes over 4 days [71] according to the same training protocol used during acquisition sessions. None of the acquisition or testing sessions exceeded 180 seconds. Mice completed the Rabinovitch and Rosvold (1951) maze configurations. Latency and number of errors were recorded. Latency was recorded from the moment the barrier at the start box was removed until the animal took its first bite of food. An error was scored each time the animal's two front paws crossed into an error zone (Figure. 1b). Experimenters were blind to the animal genotypes and were never visible to the mice during the runs. The maze was

thoroughly cleaned between trials and all trials were recorded using a closed-circuit camera mounted on the ceiling directly above the maze.

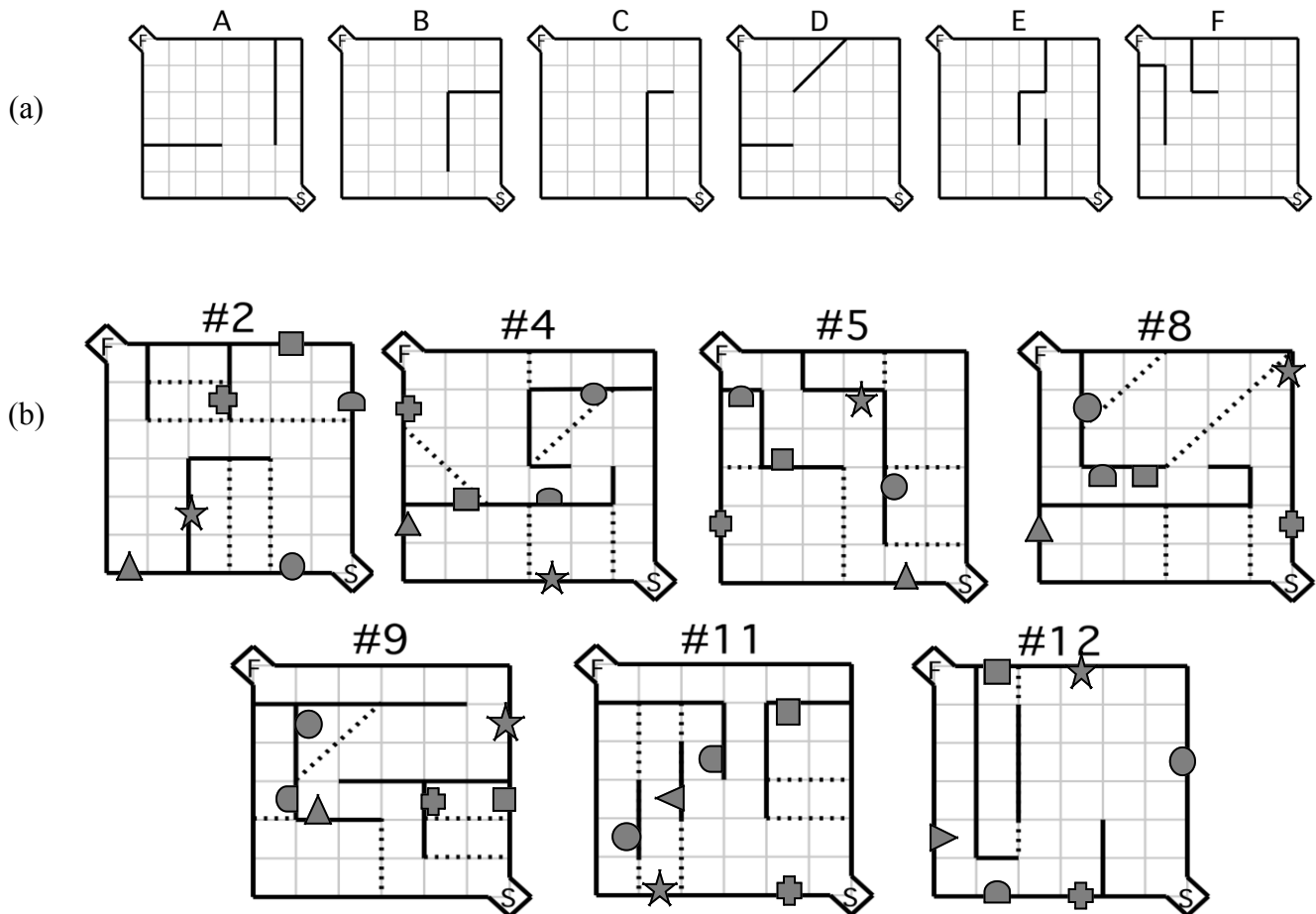


Figure 1. Maze Configurations. (a) Testing was conducted using the six practice mazes (A-F) and (b) the seven test mazes depicted. For each maze configuration, the (S) depicted in the bottom right hand corner represents the start box, and the (F) in the top left corner represents the goal box. Error zones are depicted by the dotted lines. Landmark configurations are depicted by geometric shapes.

Statistical Analyses. The egocentric maze data utilized to form the non-landmark groups was obtained from a previously published study [7]. The number of errors made across maze trials was analyzed using a mixed-design analysis of variance (ANOVA) with Group as between-subjects variable and both Maze and Trial as within-subjects variables.

Latency for completion of maze trials was analyzed in the same manner. Prior to the analyses, latency variables were transformed to square root scores in order to normalize the distribution of the data.

Previous studies suggest that *fmr1* KO mice may exhibit increased activity levels as compared to wild type mice [e.g. 72]. Thus, we assessed activity levels by obtaining a count of the number of line crosses per unit of time for trial 1 of maze 12. The latter maze was chosen because it has the least number of partitions thus allowing for the clearest observation of locomotion. We restricted our analysis to trial 1 because performance on this trial is independent of learning and memory and reflects exploratory behavior. All statistical analyses were conducted using IBM Statistics 20.0 (SPSS Inc., Chicago, USA).

Results

Latency

A 4 X 7 X 5 ANOVA was conducted with Group (*fmr1* KO landmark, wild-type landmark, *fmr1*KO non-landmark, wild-type non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6, 234} = 3.825, p < 0.001, \text{partial } \eta^2 = .089$) and Trial ($F_{4, 156} = 26.273, p < 0.0001, \text{partial } \eta^2 = .403$) but not for Group ($F_{1, 19} = .823, p = 0.49, \text{partial } \eta^2 = .06$). However, a significant three-way interaction among Maze, Trial and Group was found ($F_{72, 936} = 1.324, p < 0.05, \text{partial } \eta^2 = .092$). Two way interactions between Maze X Group ($F_{18, 234} = 1.681, p < 0.05, \text{partial } \eta^2 = .114$) and Maze X Trial ($F_{24, 936} = 3.825, p < 0.01, \text{partial } \eta^2 = .046$) were also significant. Given the significant three-way interaction, the source of the difference was sought through post-hoc analyses described below.

Post Hoc Analyses

Fmr1 KO landmark vs. fmr1 KO non-landmark

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1*KO landmark, *fmr1*KO non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6, 114} = 8.778, p < 0.0001, \text{partial } \eta^2 = .316$) and Trial ($F_{4, 76} = 26.737, p < 0.0001, \text{partial } \eta^2 = .585$) but not for Group ($F_{1, 19} = .300, p = 0.59, \text{partial } \eta^2 = .016$), indicating that the latency to complete the mazes did not differ significantly between the *fmr1*KO landmark and *fmr1*KO-non landmark group (Figure 2a). However, significant two-way interactions between Maze and Group ($F_6 = 2.894, p = .012, \text{partial } \eta^2 = .132$), and Maze and Trial ($F_{24, 456} = 2.133, p = .002, \text{partial } \eta^2 = .101$) were found. Alpha levels for *post-hoc* analyses of the interaction were adjusted using a Bonferroni correction to control for familywise error rate across X ($\alpha = .05/7 = 0.007$). *Post-hoc* independent samples t-tests revealed only one large significant pairwise difference attributable to longer latencies for the *fmr1* KO non-landmark mice on Maze 4 ($t_{19} = -3.702, p = 0.002$).

Fmr1 KO landmark vs. wild-type control landmark

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1* KO landmark, wild type landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6, 108} = 21.505, p < 0.0001, \text{partial } \eta^2 = .544$), Trial ($F_{4, 72} = 26.279, p < 0.0001, \text{partial } \eta^2 = .593$) and Group ($F_{1, 18} = 9.477, p = 0.006, \text{partial } \eta^2 = .345$), indicating that on average, wild-type mice took significantly longer than *fmr1* KO mice. Furthermore, a three-way interaction between Maze, Trial and Group was found ($F_{24, 432} = 1.890, p = 0.007, \text{partial } \eta^2 = .095$)(Figure 3a). *Post hoc* independent samples t -tests showed significant pairwise difference between groups on trial 3 ($t_{20} = 3.003, p = 0.008$) and trial 5 ($t_{20} = 2.515, p = 0.022$) of maze 12, trial 5 of Maze 8 ($t_{20} = 4.267, p < 0.0001$), trial 3 ($t_{20} = 2.357, p = 0.030$) of Maze 4, trial 4 Maze 9 ($t_{20} = 2.718, p =$

0.014), and trial 1 of Maze 11 ($t_{20}= 2.441, p = 0.014$). However, when the α level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = 0.05/35 = 0.0014$), the only difference that remained significant was trial 5 of Maze 8, with the wild type landmark group taking significantly longer than the *fmr1* KO landmark group to complete the maze.

Wild-type control landmark vs. wild-type control non-landmark

A 2 X 7 X 5 ANOVA was conducted with Group (wild-type landmark, wild-type non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6,120} = 7.857, p < 0.0001$, partial $\eta^2 = .282$), Trial ($F_{4,80} = 28.571, p < 0.0001$, partial $\eta^2 = .588$) but not for Group ($F_{1,20} = 2.833, p = 0.108$, partial $\eta^2 = .124$), indicating that on average, the time it took to complete the mazes did not differ significantly between the wild-type landmark and wild-type non landmark group. However, a significant three-way interaction between Maze, Trial and Group was found ($F_{24, 480} = 3.302, p < 0.0001$, partial $\eta^2 = .142$) (Figure 4a). Post hoc independent samples t -tests showed significant pair-wise difference between groups on trial 3 ($t_{20}= 2.892, p = 0.009$) and trial 4 ($t_{20}= 3.198, p = 0.005$) of Maze 12, trials 1 ($t_{20}= 2.906, p = 0.009$) and trial 2 ($t_{20}= 2.713, p = 0.013$) of Maze 8, trial 1 of Maze 9 ($t_{20}= 2.217, p = 0.038$), and trial 1 of Maze 11 ($t_{20}= 3.669, p = 0.002$). However, when the α level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = 0.05/35 = 0.0014$), these differences were no longer significant.

Error

A 4 X 7 X 5 ANOVA was conducted with Group (*fmr1* KO landmark, wild-type landmark, *fmr1*KO non-landmark, wild-type non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed

significant main effects for Maze ($F_{6, 234} = 26.024, p < 0.0001, \text{partial } \eta^2 = .400$), Trial ($F_{4, 156} = 35.219, p < 0.0001, \text{partial } \eta^2 = .585$) and Group ($F_{1, 39} = .6871, p < 0.001, \text{partial } \eta^2 = .346$). However, a significant three-way interaction among Maze, Trial and Group was found ($F_{72, 936} = 1.803, p < 0.001, \text{partial } \eta^2 = .122$). Two way interactions between Maze X Group ($F_{18, 234} = 2.192, p < 0.01, \text{partial } \eta^2 = .144$) and Maze X Trial ($F_{24, 936} = 4.326, p < 0.001, \text{partial } \eta^2 = .100$) were also significant. Given the significant three-way interaction, the source of the difference was sought through post-hoc analyses described below.

Post Hoc Analyses

fmr1 KO landmark vs. fmr1 KO non-landmark

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1*KO landmark, *fmr1*KO non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6, 114} = 13.784, p < 0.0001, \text{partial } \eta^2 = .420$) and Trial ($F_{4, 76} = 13.659, p < 0.0001, \text{partial } \eta^2 = .418$), but not for Group ($F_{1, 19} = .932, p = 0.347, \text{partial } \eta^2 = .047$), indicating that on average, the number of errors made in the mazes did not differ significantly between the *fmr1*KO landmark and *fmr1* KO-non landmark group (Figure 2b). However, a significant three-way interaction between Maze, Trial and Group was found ($F_{24, 456} = 1.827, p = .01, \text{partial } \eta^2 = .088$). *Post-hoc* independent samples t-tests revealed only two significant pairwise differences attributable to significantly greater errors committed by the *fmr1*KO landmark mice on trial 2 ($t_{19} = 2.902, p = 0.009$) and trial 4 ($t_{19} = 2.262, p = 0.036$) of maze 11. However, when the alpha level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = .05/35 = 0.0014$), this difference was no longer significant.

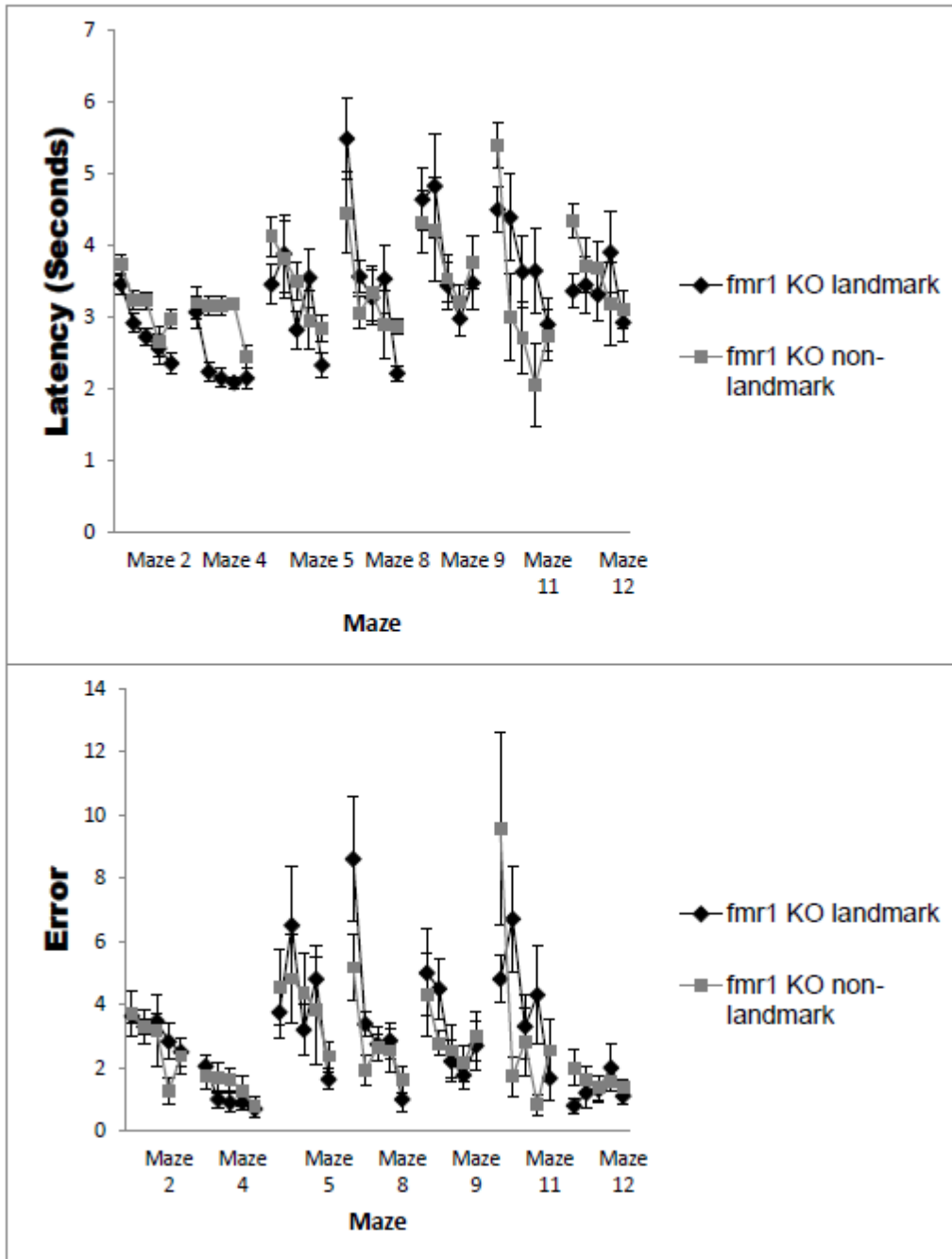


Figure 2: *Fmr1* KO Comparisons. Performance of *fmr1* KO landmark and *fmr1* KO non-landmark mice for each of the seven mazes on each of the five respective trials. Error bars represent the standard error of the mean. (a) Mouse latency. Mean mouse latency measured in seconds for each of the five maze trials. (b) Mouse error. Mean Mouse error for each of the five maze trials.

Fmr1 KO landmark vs. wild-type control landmark

A 2 X 7 X 5 ANOVA was conducted with Group (*fmr1* landmark, wild-type landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6,108} = 23.114, p < 0.0001, \text{partial } \eta^2 = .562$), and Trial ($F_{4,72} = 19.453, p < 0.0001, \text{partial } \eta^2 = .519$), but not for Group ($F_{1,18} = 0.541, p = 0.472$), indicating that on average, the number of errors committed in mazes did not differ significantly between the wild-type landmark and the *fmr1* KO landmark group (Figure 3b). The interaction between Maze and Trial was significant ($F_{24,432} = 4.330, p < 0.0001, \text{partial } \eta^2 = .194$).

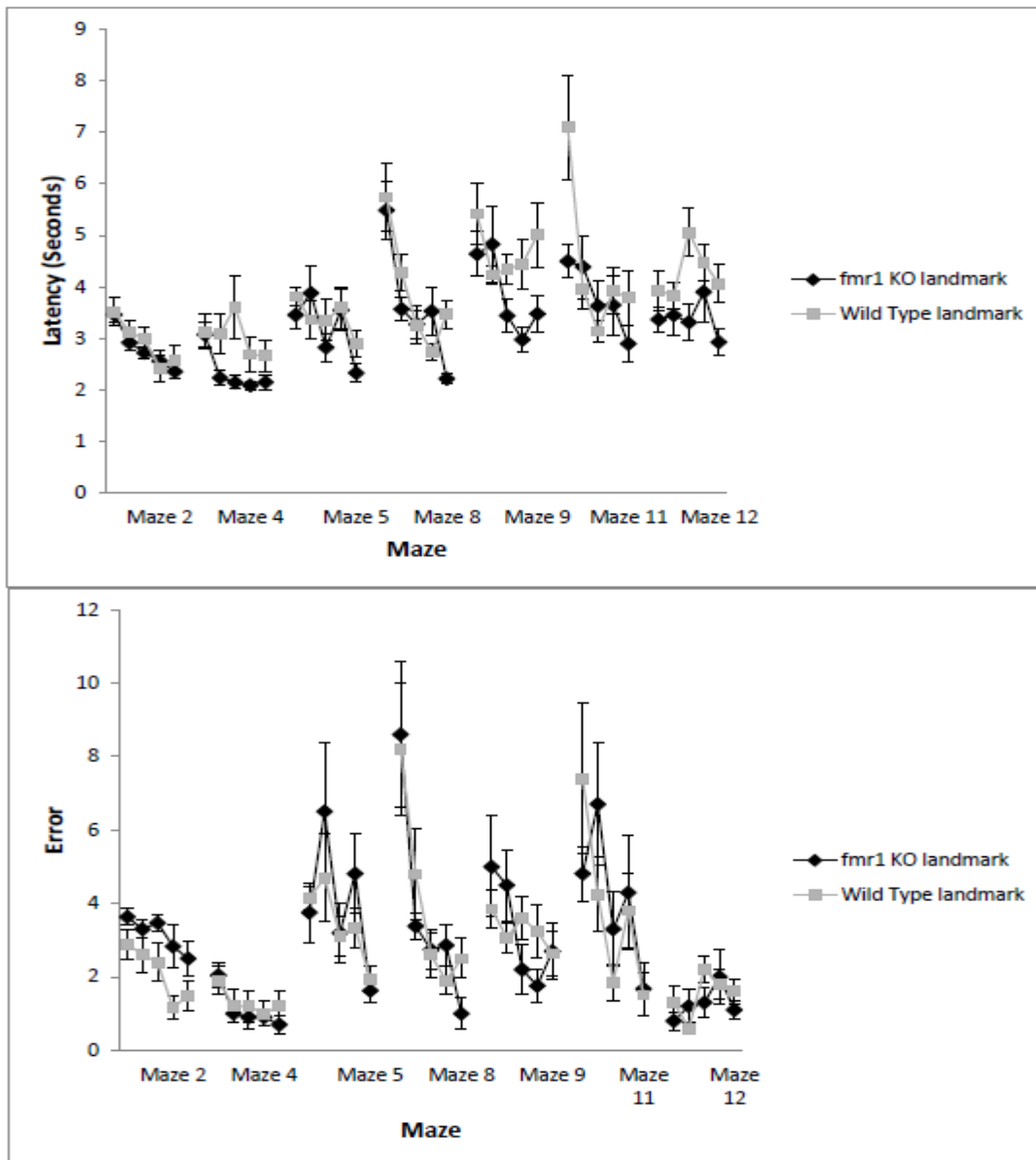


Figure 3. Landmark comparisons. *Fmr1* KO landmark and wild-type control landmark data. Performance of *fmr1* KO and wild-type controls on each of the seven mazes on each of the five respective trials. Error bars represent the standard error of the mean. (a) Mouse latency. Mean mouse latency measured in seconds for each of the five maze trials. (b) Mouse error. Mean Mouse error for each of the five maze trials.

Wild-type control landmark vs. wild-type control non-landmark

A 2 X 7 X 5 ANOVA was conducted with Group (wild-type landmark, wild-type non-landmark) as the independent measures variable and both Maze (7 levels) and Trial (5 levels) as repeated measures variables. The latter revealed significant main effects for Maze ($F_{6,120} = 13.592, p < 0.0001, \text{partial } \eta^2 = .405$), Trial ($F_{4,80} = 26.865, p < 0.0001, \text{partial } \eta^2 = .573$) and Group ($F_{1,20} = 14.380, p < 0.001, \text{partial } \eta^2 = .418$), indicating that on average, the wild-type controls made significantly more errors on the landmark task. Furthermore, a three-way interaction between Maze, Trial and Group was found ($F_{24,480} = 2.435, p < 0.0001, \text{partial } \eta^2 = .109$)(Figure 4b). Post hoc independent samples t -tests showed significant pair-wise difference between groups on trial 3 ($t_{20} = 2.321, p = 0.031$) of Maze 12, trial 1 ($t_{20} = 2.940, p = 0.008$), trial 2 ($t_{20} = 2.247, p = 0.036$) and trial 5 ($t_{20} = 3.015, p = 0.007$) of Maze 8, trial 1 of Maze 9 ($t_{20} = 2.840, p = 0.010$), and trial 1 ($t_{20} = 2.376, p = 0.002$) and trial 4 ($t_{20} = 2.724, p = 0.006$) of Maze 11. However, when the α level was adjusted using a Bonferroni correction to control for familywise error rate ($\alpha = 0.05/35 = 0.0014$), these differences were no longer significant.

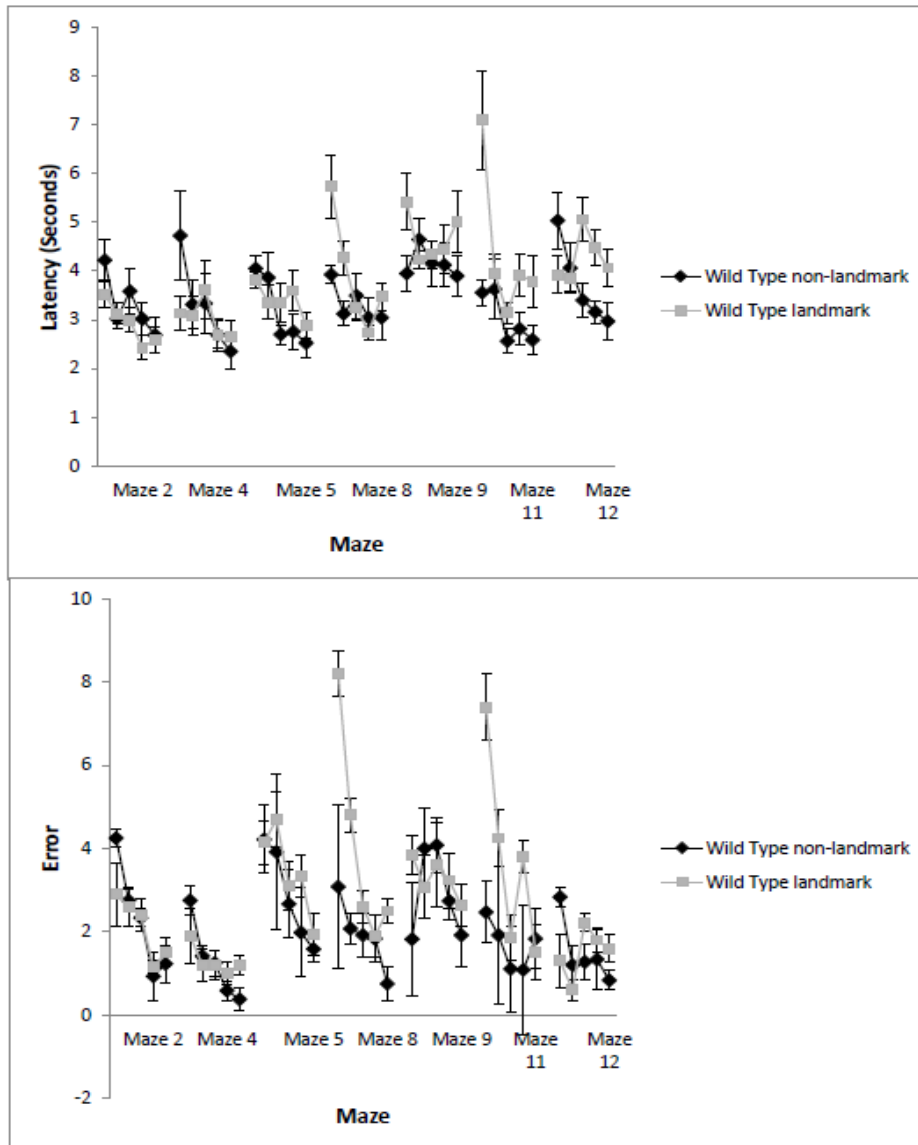


Figure 4: Wild-type control comparisons. Performance of wild-type landmark and wild-type non-landmark mice for each of the seven mazes on each of the five respective trials. Error bars represent the standard error of the mean. (a) Mouse latency. Mean mouse latency measured in seconds for each of the five maze trials. (b) Mouse error. Mean Mouse error for each of the five maze trials.

Activity Levels

Independent samples t-tests revealed no significant differences in activity levels between the comparison groups. ($t = .272, p = .788$)

Discussion

The present study examined the spatial navigation abilities of a murine model of FXS, *fmr1*KO, and wild-type control mice, on two versions of the Hebb-Williams Maze. Results revealed that the performance of the *fmr1* KO mice generally did not differ between landmark and non-landmark groups, indicating that the presence of landmarks neither enhanced nor hindered mouse performance. The one exception to this pattern was Maze 4, where the landmark group was faster than the non-landmark group, without committing additional errors. This suggests that the presence of landmarks actually facilitated mouse performance for this specific maze. Although variations have been used in the literature to establish the difficulty of each of the H-W mazes, with some studies basing this on the total number of errors committed per maze and others creating a ratio of number of errors per trial/number of error zones, it is interesting that Maze 4 has generally been characterized as one of the easiest mazes [6, 73, 74]. It is possible that on mazes that are considered easier and perhaps have a lower cognitive demand, landmarks facilitate performance because subjects are better able to attend to and thus utilize the additional spatial cues. The improvement in performance on Maze 4 is interesting and suggests it may be a good candidate for use in future studies to measure cognitive change.

The finding that the general pattern of results was similar regardless of whether landmarks were present can be interpreted as a replication of the spatial learning and memory deficit observed in *fmr1* KO mice published by MacLeod and colleagues (2010). These authors showed greater errors but not latency in the *fmr1*KO group as compared to

wild type controls. In the present study, there were generally no differences between the landmark *fmr1* KO group and the *fmr1* KO non-landmark group from MacLeod and colleagues (2010), suggesting that the deficit is preserved. Post-hoc analyses comparing *fmr1* KO from the landmark task, to the wild-type controls from the MacLeod and colleagues (2010) study supports this conjecture. Specifically, the *fmr1* KO landmark group have similar latencies but make significantly greater errors than the wild-type group. This adds further strength to the literature demonstrating spatial memory deficits in *fmr1* KO mice, which has at times been inconsistent and difficult to replicate [15,16, 22-26].

In contrast to the *fmr1* KO groups, significant differences in performance variables were observed between the wild-type control groups. Overall, latency did not differ with or without the presence of landmarks, indicating that on average, the time it took for groups to complete the mazes was similar. However, there were group difference on measures of error, with mice generally committing greater errors on the landmark tasks. Performance deficits seemed most apparent on earlier maze trials and did not seem to vary depending on maze difficulty; although both groups performed similarly on maze 5, which is reported to be one of the most challenging maze [7].

We did not expect that the control mice would do worse on the landmark task. However, the type of background strain used may partially account for these results. The FVB background strain used in the present study as compared to the C57BL/6 strain are known to rely relatively less on visual cues for learning [16,20, 22, 24]. Furthermore, *fmr1* KO mice have been shown to display a dampened response to novelty [114], suggesting that their performance was not impacted to the same degree as controls by the presence of novel landmarks within the maze environment. In contrast, the introduction of novel cues directly into the maze environment, may have disrupted control performance due to the tendency

among mice to explore novel stimuli.

In comparing *fmr1* KO to wild-type controls on the landmark tasks, the pattern of results interpreted in isolation could be understood as a reversal of the spatial deficit reported in MacLeod et al (2010). However, viewed in the context of the performance of the other wild type comparison groups, results seem to more accurately reflect the poor performance of controls. That is, the wild-type mice's performance was so poor on the landmark task that when compared to the *fmr1* KO group, the *fmr1* KO mice appeared to demonstrate superior performance.

Contrary to our hypotheses, landmarks had a minimal effect on *fmr1* KO performance but significantly impaired wild-type control performance. This can be reconciled in several ways. In the present study, landmarks were placed within the maze environment, or placed proximally. Research has shown that distally placed landmarks, or landmarks external to the maze environment, may provide a more stable frame of reference because they do not change their relative positions as an animal or individual navigates through a maze environment [49]. Although there is extensive research supporting the use of proximally placed maze cues in both human and animal studies [for example: 75-79], it has been noted that it is more difficult to train animals to use these cues [77, 80, 81]. In contrast, animals quickly adapt to utilizing distal cues [82, 83]. In the present study, mice had only 5 trials to learn how to utilize the proximally placed cues before they were reconfigured for the next maze. Future studies should use a distal placement of landmarks to see if this may facilitate maze learning.

Similarly, it has been shown that intra-maze landmark placement can trigger increased exploratory behavior, which is thought to be necessary for the creation and

updating of allocentric spatial representations [34, 84, 85]. Therefore, altering the configuration of the landmarks for each maze may have increased exploratory reactions by introducing new stimuli or spatial arrangements into a familiar environment [84], which may explain the increases in errors observed in the wild type mice on the landmark task

Furthermore, research has shown that several variables, including the number of landmarks, their location, and the geometric arrangement of cues can influence results [86,-88]. For example, Chamizo and colleagues (2004) found that the control or salience of a landmark is relative to its proximity to the goal [89, 90]. Optimal performance is obtained when landmarks are positioned directly above, or near the platform, whereby the animal can use the cue as a beacon [91]. Similarly, Janzen and van Turenhout, (2004) have demonstrated a selective response in the parahippocampal gyrus to landmarks placed at decision points. Additional landmarks placed at non-decision points and exposure to cues participants reported as being salient did not elicit increased activation [92]. In the present study, it is possible that during testing in one maze, certain landmarks may have been in closer proximity to the goal box or placed at a key decision point, and thus been differentially reinforcing. When we reconfigured landmark placement for the next maze, the landmarks that were moved may have lost their reinforcing or informative properties and thus interfered with learning.

Related to this, there is evidence that presenting landmarks in a consistent relationship is an important factor. Research has demonstrated that animals may use multiple landmarks in a configural pattern [93-98], and that learning, or an association, is shared between cues [99]. This suggests that the geometric arrangement of landmarks as a whole is likely utilized to provide useful spatial information, and our attempt to counterbalance landmark presentation across mazes may have reduced the usefulness of the cues. Lastly, its

well established that mice rely heavily on senses like olfaction and audition more than sight for navigation [100,101]. Although we attempted to control for these confounds by thoroughly cleaning the maze in between each trial and by conducting testing in a quiet environment, it is still possible that the saliency of these sensory cues for mice may have prevented them from using the landmarks to navigate and therefore the task may not have preferentially targeted allocentric processing.

In the present study we were not able to clarify the nature of the spatial deficit in *fmr1* KO mice by utilizing egocentric and allocentric versions of H-W maze paradigm, but it appears we were able to replicate the spatial deficit in *fmr1* KO mice. Demonstrating similar levels of performance between affected mice in the landmark and non-landmark task, suggests that they exhibit the same deficit (e.g. increases in error but not latency) as was found in Macleod and colleagues (2010) study. Post hoc analysis comparing the landmark *fmr1* KO group to the wild-type controls from Macleod and colleagues (2010) lends support to this notion. It is well established that hippocampal functioning is required for spatial navigation, [102-106] and the spatial deficits observed in *fmr1* KO mice may in part be due to underlying hippocampal dysfunction resulting from the reduced expression of FMRP. This hypothesis makes sense in the context of findings that suggest that in typically developing humans and wild type mice, *FMRI/fmr1* mRNA levels are higher in the hippocampus [61, 62]. Similar results were demonstrated in Old World Monkeys [63], suggesting that the hippocampus is an area of the brain that is particularly reliant on and thus sensitive to changes in FMRP expression as occur in FXS. Furthermore, research has shown that there is significantly greater hippocampal activity when good navigators complete a wayfinding task [64] and positive correlations have been demonstrated between hippocampal volume and navigational skills, with good navigators having significantly greater

hippocampal volumes [65-67]. Research has reliably demonstrated that the hippocampus is involved to a greater extent in allocentric navigational tasks, whereas the parietal cortex and by extension, the dorsal stream are involved in egocentric navigation [33,35, 39-43]. For several of the explanations discussed in previous sections, landmark characteristics as used in the current study did not optimally promote an allocentric navigational strategy.

This study makes several important contributions to the literature. First, a clear strength is its novelty. To our knowledge, ours is the first study to examine the utility of landmarks in *fmr1* KO mice on the H-W maze task. An additional strength of the present study is the replication of the spatial deficit in *fmr1* KO mice. This further establishes the validity of the H-W mazes as an ecologically valid behavioural assay able to detect behavioural deficits in mice and strengthens the utility of using H-W virtual mazes to test affected humans. This is important because using a translational approach increases the validity of the murine model as well as strengthens the notion that results reflect a similar underlying spatial deficit that is being targeted and expressed in both species. We previously proposed that H-W mazes could be used as a tool to evaluate therapeutic strategies both during early development in animals and later in clinical trials. Several pharmacologic studies have demonstrated the reversal of aberrant neuroanatomical characteristics as well as behavioral and cognitive impairments in animal models of FXS [107-110]. In addition, pharmacological compounds have been used to treat cognitive and behavioural deficits of FXS [111-113] but there have been some challenges in establishing reliable outcome measures.

Many cognitive measures used previously have been too difficult for the majority of individuals affected by FXS to complete. Furthermore, even if these measures have been administrable they resulted in unacceptable levels of variability [112]. Gross and colleagues

(2012) describe the design of appropriate outcomes measures as an obstacle in clinical trial studies with FXS, and they highlight the need for better FXS-specific outcome measures. A significant barrier is balancing the need for a measure that be both capable of testing a wide range of affected individuals from low to high functioning, while also avoiding the issue of floor or ceiling effects [113]. In this regard, the H-W maze battery is appealing given the range of maze configurations with different levels of difficulty. We identified Maze 4 as a task where landmarks actually facilitated performance in the affected group, which lends strength to its inclusion in the battery of H-W mazes for future studies measuring cognitive change. The current study provides additional evidence of the reliability of the H-W paradigm in its ability to detect spatial deficits. Future research examining maze pathways would help further understand the types of strategies being utilized when solving the mazes and highlight which factors may be contributing to poorer maze performance among *fmr1* KO mice.

Conclusions

The current study revealed that the performance of the *fmr1* KO mice generally did not differ between landmark and non-landmark tasks, indicating that the presence of landmarks neither enhanced nor hindered mouse performance. An interesting exception to this pattern was observed on Maze 4, whereby the *fmr1* KO landmark group acquired learning faster than its non-landmark equivalent, without committing additional errors; suggesting that the presence of landmarks actually facilitated mouse performance for this specific maze. Contrary to our hypotheses, landmarks significantly impaired wild type control performance. The implications of these data have been discussed, as well as possible explanations as to why landmarks, in the manner they have been implemented, do not improve allocentric processing.

The current study provides additional evidence of the reliability of the H-W paradigm in its ability to detect spatial deficits, suggesting it may be a useful outcome measure in treatment studies.

List of Abbreviations

H-W: Hebb-William Maze
KO: Knock out
FXS: Fragile X Syndrome
FMRI: Fragile X Mental Retardation 1 gene
FMRP: Fragile X Mental Retardation protein
M-pathway: magnocellular pathway
LGN: lateral geniculate nuclei
ANOVA: analysis of variance

Competing Interests

The authors declare they have no competing interests

Authors' Contributions

LM contributed to all phases of the manuscript including study conceptualization, data collection, analyses and writing manuscript drafts. CK provided guidance and assistance throughout all phases and contributed significantly to the interpretation and discussion of results as well as reviewing the manuscript. CK is responsible for all correspondence and requests for reprints. CM contributed to the methodological design and data collection. RG contributed to the behavioral testing. MH, as well as all authors contributed to the review and discussion of interpretation of the manuscript. All authors have read and approved the final version of the manuscript.

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Chapter Four: Study III

Study III

Brief report: Behavioral Analysis of performance of individuals with fragile X syndrome on Hebb-Williams maze

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Abstract

A prominent feature of the Fragile X Syndrome (FXS) phenotype is impairment in visual spatial cognition and perceptual processing. MacLeod and colleagues (2010) demonstrated spatial learning and memory deficits in FXS affected humans, however, hypotheses related to strategies employed to solve the maze problems were not tested. The current brief report extends previous research by examining additional dependent variables extracted from the behavioural data cited in MacLeod and colleagues (2010). The results provide further insight into the differential navigational strategies participants affected by FXS may employ as compared to matched controls. Results revealed significant differences in performance variables between individuals, with FXS participants generally performing worse than the comparison group participants. The implications of the pattern of results and navigational strategies will be discussed.

Brief report: Behavioral Analysis of performance of individuals with fragile X syndrome on Hebb-Williams maze

Fragile X Syndrome (FXS) is the most prevalent form of heritable mental retardation (Turner, Webb, Wake, & Robinson, 1996). FXS arises from a mutation in the *FMRI* gene on the X-chromosome (Online Mendelian Inheritance in Man® [OMIM] 309550; Verkerk et al., 1991; Sherman, 2002) and leads to a well-defined phenotype that is characterized by a range of physical, behavioral, neuroanatomical and cognitive deficits. A prominent feature of the FXS phenotype is impairment in visual spatial cognition and perceptual processing (Kogan et al., 2004 a & b, Farzin & Rivera, 2010; Farzin, Rivera, & Whitney, 2008; Cornish, Munir & Cross, 1998; 1999; Crowe & Hay, 1990).

MacLeod and colleagues (2010) demonstrated spatial learning and memory deficits in FXS affected humans and *fmr1* KO mice using both virtual and physical versions of Hebb-Williams mazes (H-W). Although significant differences on performance variables were observed between groups, the FXS participants were able to complete the first trial of the mazes faster than comparison participants while making similar numbers of errors. The authors posited that this finding argued against deficits in visual perceptual processing, as was initially hypothesized. Instead they suggested that the impairments observed in both affected groups appeared to be more consistent with visual spatial learning and memory deficits. To illustrate this notion, for example, it is possible that the FXS group may have completed the first maze trial faster than the controls while committing similar numbers of errors, however, it could be that the affected group took a longer path or more inefficient route. Similarly, controls may have paused longer and more frequently in trial 1, which may have enabled better encoding of spatial information ultimately translating to better overall maze learning (i.e., significantly better performance in later trials).

The finding that FXS participants were able to complete the first trial of the mazes faster than comparison participants while making similar numbers of errors is similar to the pattern of impairment found by Dobkin and colleagues (2000), who observed that in early trials, *fmr1* KO mice exhibited a higher rate of correct responses as compared to controls. However, across trials, performance of *fmr1* KO did not improve and at the completion of testing their performance did not match the final performance of controls. To account for these results, the authors suggested that *fmr1* KO mice might have approached navigating the

maze in a different manner (Dobkin et al., 2000), such that their strategy may have been conducive to solving the maze problem initially, but may not have transferred across trials. Unfortunately, hypotheses related to strategies employed to solve the maze problems were not tested by Dobkin and colleagues (2000) or MacLeod and colleagues (2010). Therefore, the current brief report extends this previous research by examining different dependent variable extracted from the behavioural data cited in MacLeod and colleagues (2010). Results obtained are hypothesized to provide further insight into the differential navigational strategies participants affected by Fragile X Syndrome may employ as compared to matched controls.

There are several examples in the literature describing how different populations utilize unique approaches to solving maze problems. A consistent finding has been gender differences in spatial navigation tasks (Galea & Kimura, 1993; Gron et al., 2000; Harris, 1978; Voyer, Voyer, & Bryden, 1995). For example, women tend to rely on landmark cues and verbally mediated route learning to solve spatial problems whereas men tend to use both geometric and landmark cues (Dabbs, Chang, Strong, & Milun, 1998; Harris, 1978; Lawton, 1994; Sandstorm, Kaufman & Huettel, 1998). On the H-W mazes, women were found to pause more frequently and generally performed less efficiently across mazes trials compared to men (Shore et al., 2000). Authors surmised that women tended to perceive more decision points than men and thus may have paused more frequently to inspect their environment at these points. Gron, Wunderlich, Spitzer, Tomczak and Riepe (2000) provided neuroimaging results indicative of gender differences in approaches to a spatial processing task. They found that whereas for women the right parietal and right prefrontal areas of the brain were activated, left hippocampal regions were activated in men. Similarly, healthy individuals who use spatial strategies to navigate (strategies dependent on learning the relationship between features in the environment) have been found to have significantly greater activity in the hippocampus (Bohbot et al., 2004) and grey matter density in this region (Bohbot et al., 2007). In contrast, individuals who use non-spatial strategies (those that are less flexible and depend on stimulus response type learning) have been shown to have less hippocampal activation but significantly elevated activity in the caudate nucleus (Bohbot et al., 2004), as well as greater grey matter density in this region (Bohbot et al., 2007). Interestingly, individuals who first utilized a spatial strategy but switched to a non-spatial strategy, also

showed a corresponding decrease in hippocampal activity (Bohbot et al., 2004). Taken together, these findings highlight that different areas of the brain appear to be involved in different approaches to solving maze problems and also point to methods for enhancing performance. For example, whereas men might possibly benefit from prior knowledge of goal box locations to facilitate the development of cognitive maps, which are thought to be hippocampal dependent (Nadel, 1980), women might benefit from additional exploratory time (Shore et al., 2001) to enhance activation of spatial working memory areas of their brain (Jonides et al., 1993).

Lesion studies in animals have also demonstrated different approaches to maze problem solving. Experiments using the Morris Water maze (MWM) have shown that rats with parietal lesions compensate by attempting to locate the hidden platform using a unique circular trajectory from the wall until they find the goal (Kolby & Walker 1987). Similarly, control but not fimbria-fornix lesioned rats were found to make occasional, direct, high velocity returns to the maze start position while exploring (Whishaw, Hines, & Wallace, 2001). Although similarities in place learning ability were found between control and lesion rats, Save and Poucet (2000) observed differences in the search behavior utilized to solve the problem, with lesioned rats directing less search behavior to the platform than controls. Finally, in a cross-species study, Shore and colleagues (2000) demonstrated not only difference in several performance variables between humans and mice, but also showed that mice tended to avoid the middle of the maze, or open spaces, while humans moved directly to the goal box.

Learning can be assessed several different ways and although traditional variables such as latency and error provide useful and necessary information, additional variables such as the route taken to arrive at the goal may be needed to demonstrate how participants are solving the problems. The results of the MacLeod and colleagues study (2010) were compelling, however, the variables used may not have completely captured the visual spatial memory or learning deficits that were found. . Therefore, the current brief report extends previous research by generating digital maze pathways of H-W maze performance as well extracting more detailed behavioural data. These measures were selected to provide further insight into the navigational strategies participants might use to solve the H-W mazes.

We hypothesize that as a result of visual-spatial memory and learning difficulties,

individuals affected by FXS as compared to the typically-developing mental age matched comparison participants will exhibit poorer results on all performance variables. In addition, affected individuals will exhibit differences in behavioural strategies employed to solve the H-W mazes, which will be evidenced by the pattern of pathways taken to solve the maze. Given the well-established hippocampal abnormalities found in affected individuals, we predict that they will display a less flexible style of visual spatial navigation. This may include a stimulus response style of learning or a performance profile similar to that of females, which is characterized by greater exploration and pausing.

Method

The Hebb-Williams virtual maze design and testing protocol used for the current study was previously described in MacLeod and colleagues (2010). All maze trials generated from participants of MacLeod and colleagues (2010) study were digitized to allow for detailed behavioural analysis of maze navigation. Fifteen male participants with FXS (mean chronological age = 24 years, SD = 4.9, mean verbal mental age = 7.57 years, SD = 1.92) were recruited from patient contact lists at Rush University Medical Center, Chicago, IL. All had a DNA-confirmed diagnosis of FXS. Seventeen male typically-developing comparison participants (mean chronological age = 5 years, SD = 2.9, mean verbal mental age = 8.07 years, SD = 2.03) were individually matched according to the verbal mental age. Wolfram Mathematica version 8 was programmed to extract relevant data by using the X-Y positional coordinates of the participants during a given trial to generate a digital image of the maze pathway. This generated a visual representation of the pathway the participant took to solve the maze, which allowed for an assessment of how the participant spent their time in the maze and approached the maze problem. Additionally, custom computer programming allowed for the generation of raw data from each maze trail. Variables that were generated included the total distance travelled across maze trials, and frequency and mean duration of time spent immobile (e.g. pausing/inspecting).

Statistical Analyses

The maze data utilized for this study was obtained from a previously published study (MacLeod et al., 2010). The length of pathways across maze trials was analyzed using a mixed-design analysis of variance (ANOVA) with Group as between-subjects variable and both Maze and Trial as within-subjects variables. The frequency of pauses and mean duration

of pauses for each maze trial were analyzed in the same manner. Prior to the analyses, variables were transformed to square root scores in order to normalize the distribution of the data. All statistical analyses were conducted using IBM Statistics 20.0 (SPSS Inc., Chicago, USA).

Results

Comparing FXS Human and Comparison Performance

Distance Travelled

A $2 \times 7 \times 3$ ANOVA was conducted with group (FXS, comparison participants) as the independent measures variable and both maze (seven levels) and trial (three levels) as repeated measures variable. The latter revealed significant main effects for Maze ($F_{6, 138} = 13.237, p < .0001$), and Trial ($F_{2, 46} = 16.790, p < .0001$) but not for Group ($F_{1, 23} = 1.350, p = 0.257$). The interactions between Group and Trial ($F_{2, 60} = 11.851, p < .0001$) and Maze and Group were significant ($F_{6, 60} = 3.164, p = .006$) (Figure 1). Alpha levels for *post-hoc* analyses of the Group and Trial interaction were adjusted using a Bonferroni correction to control for familywise error rate across trials ($\alpha = .05/9 = .006$). We examined differences within each group to assess for significant improvements across trials. For the comparison group, post-hoc paired samples t-tests revealed significant decreases in distance travelled to reach the goal box between trial 1 and trial 2 ($t_{11} = 3.507, p = .005$), and trial 1 and trial 3 ($t_{11} = 7.926, p < .0001$). In contrast, in the FXS group there was only a significant difference between distance travelled between trial 1 and trial 3 ($t_{12} = 2.604, p = .023$). It is important to note that independent samples t-tests only revealed significant differences in distance travelled between groups for trial 3 (trial 3: $t_{23} = -2.47, p = .02$). This means the decrease in distance travelled on trial 3 for the FXS group (mean = 22.41) was still significantly longer than the decrease observed on trial 3 in the comparison group (mean = 18.15). This suggests that although the FXS group improved across trials, they still performed significantly worse than comparison participants (See Table 1. for group means).

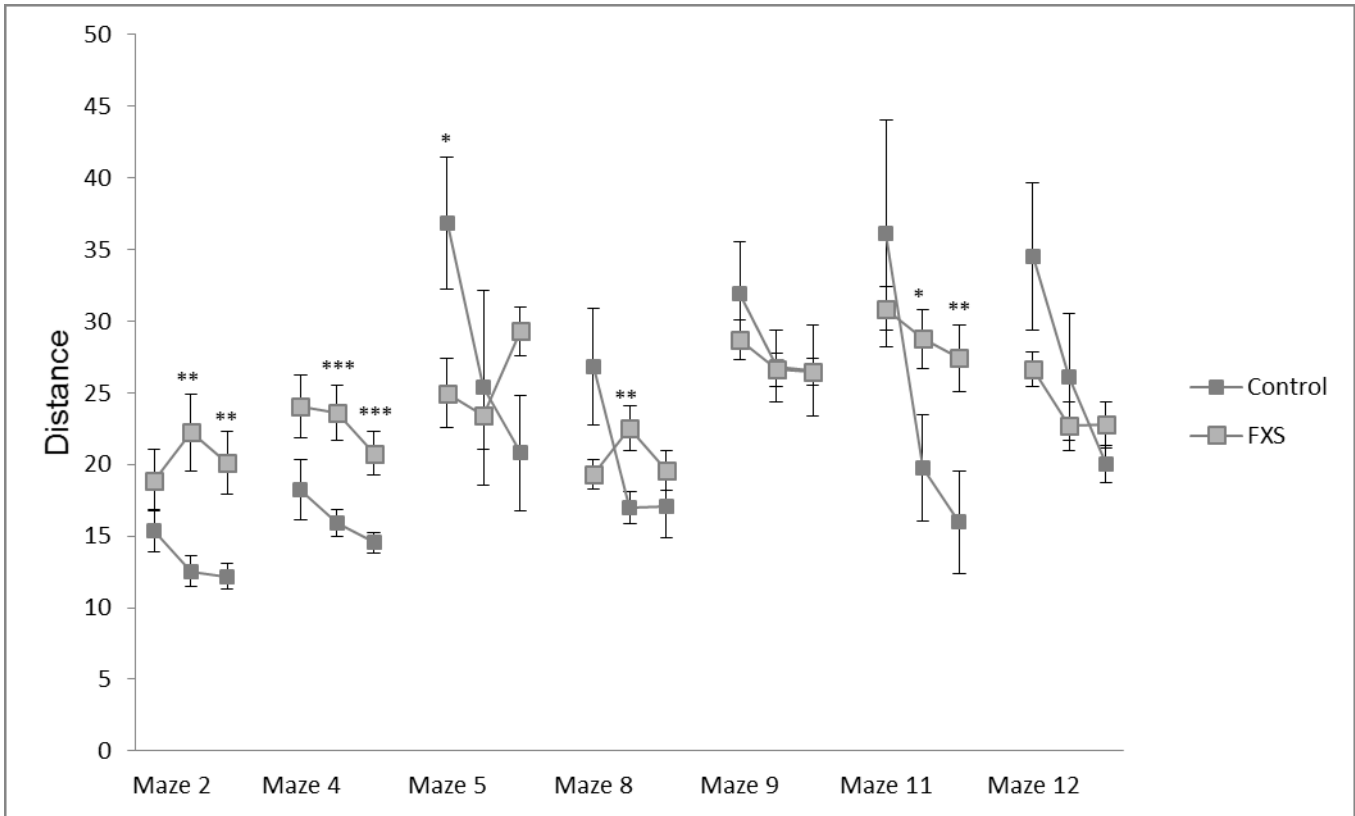


Figure 1. Distance travelled. Performance of comparison and FXS individuals on each of the three trials on each of the seven Hebb Williams mazes. Error bars represent the standard error of the mean. * = $p < .05$, ** = $p < .01$, *** = $p < .001$

Table 1. Average Distance Travelled for FXS and Control Participants

	Control (SEM)	FXS SEM)
Maze 2		
Trial 1	15.38(1.47)	18.88(2.15)
Trial 2	12.55 (1.06)	22.23(2.69)
Trail 3	12.16 (0.89)	20.01(2.16)
Maze 4		
Trial 1	18.26(2.12)	24.03(2.17)
Trial 2	15.88(0.95)	23.60(1.95)
Trial 3	14.53(0.72)	20.76(1.52)
Maze 5		
Trial 1	36.83(4.60)	24.96(2.43)
Trial 2	25.36(6.81)	23.39(2.30)
Trial 3	20.80(4.05)	29.28(1.71)
Maze 8		
Trial 1	27.81(4.05)	19.28(1.02)
Trial 2	16.98(1.10)	22.54(1.54)
Trail 3	17.03(2.19)	19.57(1.38)
Maze 9		
Trial 1	31.96(3.61)	28.70(1.38)
Trial 2	26.84(2.50)	26.60(1.15)
Trail 3	26.55(3.20)	26.49(0.95)
Maze 11		
Trial 1	36.16(7.91)	30.88(1.51)
Trial 2	19.73(3.70)	28.78(2.05)
Trail 3	15.96(3.56)	27.41(2.33)
Maze 12		
Trial 1	34.50(5.16)	26.62(1.19)
Trial 2	26.09(4.44)	22.69(1.70)
Trail 3	20.02(1.28)	22.78(1.61)

Frequency of Pauses

A $2 \times 7 \times 3$ ANOVA was conducted with group (FXS, comparison participants) as the independent measures variable and both maze (seven levels) and trial (three levels) as repeated measures variables. This revealed significant main effects for Group ($F_{1,23} = 17.514$, $p < .0001$), illustrating that the FXS group paused a significantly greater number of times than the comparison group. The interaction between Group and Trial ($F_{2,60} = 6.036$, $p = .005$) was also significant. Alpha levels for *post-hoc* analyses for the interaction were adjusted using a Bonferroni correction to control for familywise error rate across trials ($\alpha = .05/9 =$

.006). Independent samples t-tests revealed significant differences in the frequency of pauses between groups on trial 2 ($t_{23} = -4.019, p < .001$) and trial 3 ($t_{23} = -4.362, p < .001$), with the FXS group pausing more frequently than the comparison group (Figure 2).

We examined differences within each group to assess for significant improvements across trials. For the comparison group, post-hoc paired samples t-tests revealed significant decreases in the number of pauses to reach the goal box between trial 1 and trial 3 ($t_{11} = 8.40, p < .001$). In contrast, for the FXS group there were no significant differences in the frequency of pauses between any of the trials (trial 1 vs. trial 2: $t_{12} = -.13, p = .900$; trial 1 vs. trial 3: $t_{12} = .350, p = .73$; trial 2 vs. trial 3: $t_{12} = .500, p = .620$).

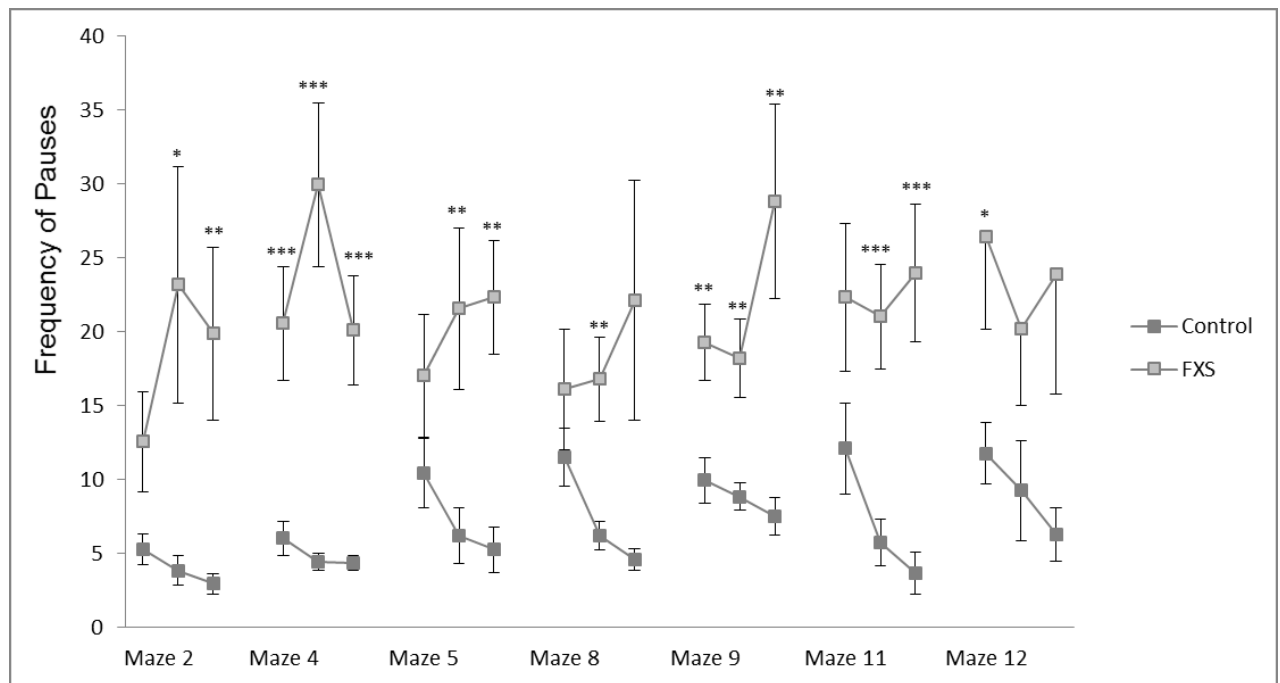


Figure 2. Frequency of Pauses. Performance of comparison and FXS individuals on each of the three trials on each of the seven Hebb-Williams mazes. Error bars represent the standard error of the mean. * = $p < .05$, ** = $p < .01$, *** = $p < .001$

Mean Duration of Pauses

A $2 \times 7 \times 3$ ANOVA was conducted with group (FXS, comparison controls) as the independent measures variable and both maze (seven levels) and trial (three levels) as repeated measures variable. This revealed a significant main effect for Group ($F_{1,23} = 82.878, p < .0001$) with FXS on average pausing for a longer duration than the comparison group (Figure 3).

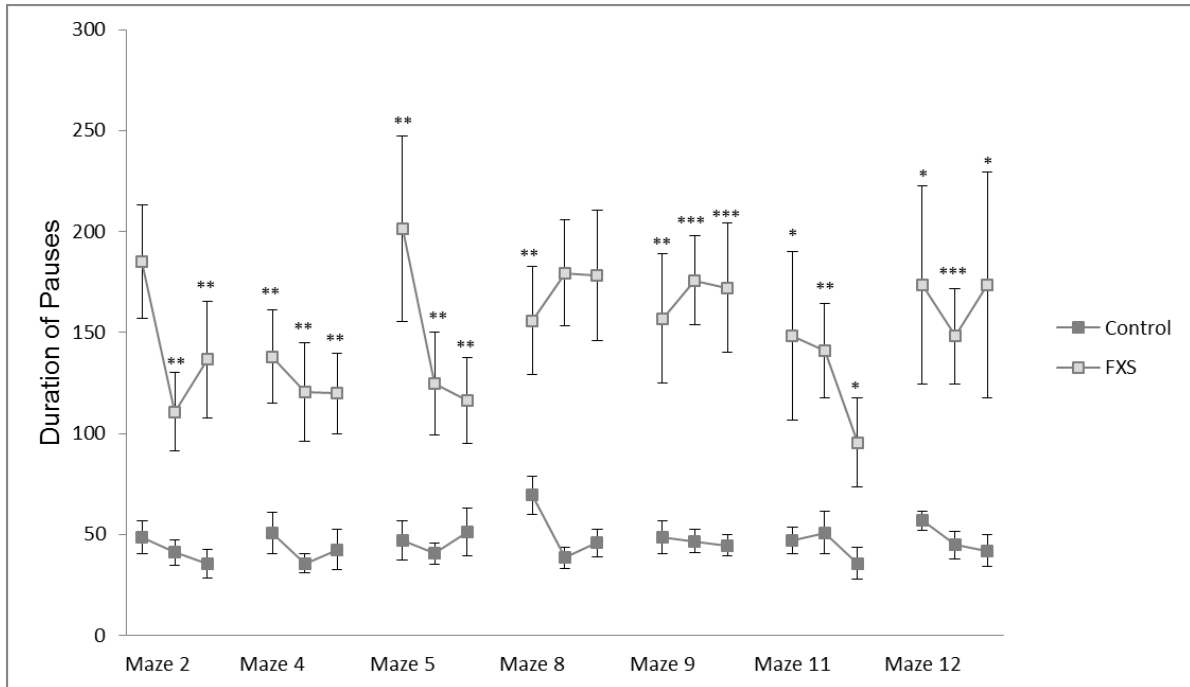


Figure 3. Average Pause Duration. Mean duration of pauses. Performance of comparison and FXS individuals on each of the three trials on each of the seven Hebb-Williams mazes. Error bars represent the standard error of the mean. * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

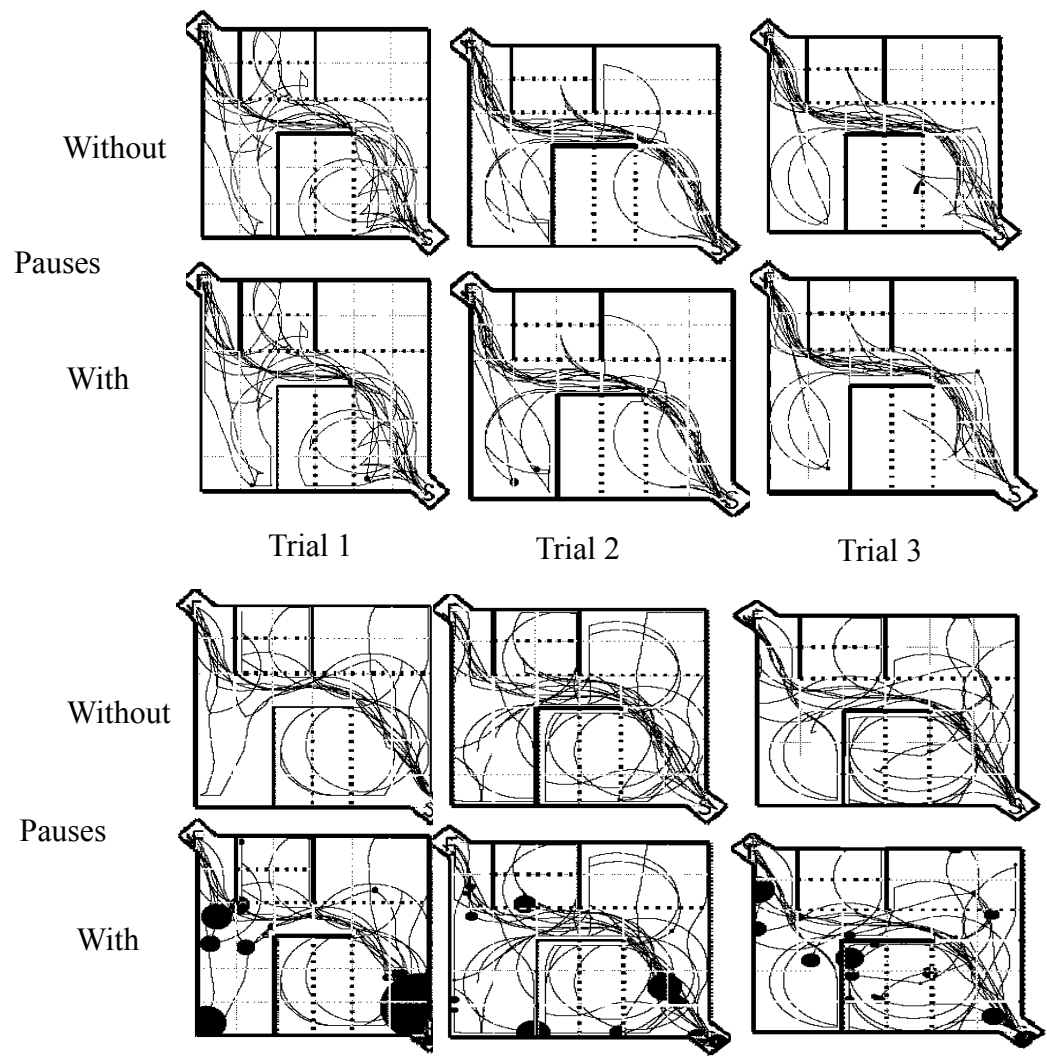


Figure 4. Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 2. The black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines. (See Appendices E through K for other H-W mazes).

Discussion

The present brief report provided a more detailed examination of the spatial navigation abilities of individuals affected by FXS as measured by performance on the H-W mazes. Results revealed significant differences in performance variables between groups, with FXS participants generally performing worse than the comparison group participants. This pattern of general impairment supports our hypothesis and is consistent with results from Macleod and colleagues (2010). In contrast to the FXS group, the comparison group paused less frequently and for shorter durations. Although the distance travelled by the FXS group decreased across trials, they still performed significantly worse than comparison participants even by trial 3. Furthermore, the comparison group demonstrated improvement across trials as indicated by a significant decrease in the frequency of pausing. The affected group's tendency to pause more frequently and for a longer duration (see Figure 4 and Appendices), is similar to the pattern described by Shore and colleagues (2000) with regards to gender differences, which the authors suggested may be indicative of participants perceiving more choice or decision points in the maze and therefore, stopping more frequently to examine the environment.

This notion is also consistent with Tolman's theory of *vicarious trial and error* (VTE), which hypothesizes that learning occurs through active investigation (Tolman, 1948). Behaviorally, this type of learning is observed at choice points, and appears as vacillation between two potential options (Muenzinger 1938; Tolman 1938; 1939). Tolman emphasized several features of VTE, including that increased VTE behaviors typically occur immediately before dramatic improvements in task performance, VTE decreases as performance reaches ceiling, and VTE reappears if the task is suddenly changed or becomes more difficult (Tolman 1938, 1939). The maze pathways of the present study are consistent with this theory (see Appendices A-G). It appears that on the first trial, both controls and FXS participants pause frequently (Maze 12, Maze 4). However, across trials, controls appear to have integrated relevant information, such that they no longer need to pause and are able to move through the maze using a direct pathway (Maze 12, Maze 4). When the maze configuration is changed, particularly for more difficult mazes, comparison participants begin pausing again on trial 1, but this behavior generally decreases by trial 2 (example, Maze 8). In contrast, the pattern of results for FXS participants suggests that they continue

using VTE across trials, as is demonstrated by their tendency to continue pausing. Maintaining this initial search strategy across trials may reflect an inability of affected individuals to adaptively integrate or apply the knowledge obtained on previous trials, which may be indicative of an underlying deficit in learning and/or memory.

Alternatively, it is possible that with greater number of trials, FXS participants may have begun to demonstrate similar levels of performance as controls. In the animal literature, it has been shown that it is more challenging to train subjects to use cues within the maze environment (Gothard, Skaggs, Moore, & McNaughton, 1996; Gould-Beierle, & Kamil, 1996; Teroni, Portenier, & Etienne, 1987), as opposed to cues placed distally (Harvey et al., 2008; Harker, & Whishaw, 2002). In the present study, participants only had three trials to learn how to navigate the test maze before they moved on to a new maze configuration. Future studies should extend maze trials and consider employing distally placed landmarks to determine if these may facilitate maze learning in affected individuals.

Deficits in maze learning may also result from a failure to adhere to rules and to adjust response set to modified environments (Canavan, 1983; Milner, 1965). In order to efficiently solve the H-W mazes, an essential rule is to avoid blind alleys (Winocur & Moscovitch, et al., 1990) with impaired spatial learners repeating incorrect responses. Performance of affected individuals illustrates this deficit, as is clearly depicted in maze 5,4, 2 and 8 (see Appendices). Typically, on trial 1 both comparison participants and FXS participants tend to enter blind alley or error zones. However, comparison participants generally exhibit dramatic improvement by trial 2 and only occasionally exhibit entries by trial 3. FXS participants appear to continue entering error zones across trials. The tendency of affected individuals to repeat incorrect responses may be related to learning or memory deficits or may reflect non-adaptive behaviors associated with FXS. Individuals with FXS are known to engage in verbal perseveration, perseveration of previously successful strategies, stereotypic behaviors and to display obsessive compulsive symptoms (Rogers et al., 2001; Bregman et al., 1988; Hagerman et al., 1994; Hagerman; 2005; Kogan et al, 2010 JIDR), which may account for the pattern of repeated responses.

An alternative explanation for an inability to integrate spatial knowledge may be related to impaired hippocampal functioning. It is well established that individuals with FXS exhibit hippocampal abnormalities (Reiss, Lee & Freund, 1994; Kates, Abrams, Kaufmann,

Breiter & Reiss, 1997; Greco et al., 2011) and the hippocampus plays a critical role in aspects of spatial learning and memory, including cognitive map formation (O'Keefe, & Dostrovsky, 1971; Morris, Garrud, Rawlins & O'Keefe, 1982; Rogers & Kesner, 2006; Hunsaker, Tran & Kesner, 2008). As an individual or animal navigates through its environment it creates an internal representation of the environment in the form of a cognitive map (O'Keefe & Nadel, 1978). Interestingly, subjects with hippocampal damage can learn simple spatial discrimination tasks but exhibit impaired performance when certain parameters of the procedure are changed (Corkin, et al., 1997; Morris, Garrud, Rawlings & O'Keefe, 1982). For example, in the MWM, rats with hippocampal lesions are able to find the hidden platform from one release point but are impaired if the release point is changed (Morris, Garrud, Rawlings & O'Keefe, 1982). These findings suggest that the hippocampus may be involved in more complex spatial behavior such as organizing spatial information and expressing spatial memory flexibility (as discussed by Eichenbaum, 2003). In the present study, maze configurations are altered after three test trials, which would also require cognitive flexibility. This notion is consistent with the pattern of results from the present study and suggests that perhaps FXS participants are utilizing an adaptive strategy on trial one but are unable to organize or adapt responses on subsequent trials.

Related to this, it is well established that initial exploratory activity is necessary for the formation and updating of spatial representations (O'Keefe & Nadel, 1978; Poucet, Chapuis, Durup & Thinus-Blanc, 1986, Thinus-Blanc et al., 1987; Thinus-Blanc et al., 1998). In particular, spatially manipulating or changing the location of objects within an environment is thought to renew exploratory behavior (Thinus-Blanc et al., 1987; Cheng & Spetch 1998). Based on this, one would predict an efficient H-W maze learner would display increased exploration, or longer maze pathways on trial 1, and shorter maze pathways on subsequent trials. Changes in maze configuration would result in re-emergence of this pattern. In the present study, results for distance travelled are consistent with this. Both groups travelled significantly shorter distances across trials. However, by trial 3 FXS participants were still traveling significantly longer to reach the goal than those from the comparison group. On trial 1 of new maze configurations, both groups exhibit longer maze pathways. This suggests that although the FXS group improved across trials, they did not approach the comparison level of performance. It may be that FXS participants are continuing to explore across trials,

whereas controls quickly learn the most direct route.

An alternative explanation for what appears to be increased exploratory behavior exhibited by affected individuals may reflect hyperactivity. Attention Deficit Hyperactivity Disorder (ADHD) is commonly observed in individuals affected by FXS (Turk & Cornish, 1998; Chen & Toth, 2001). Individuals with FXS may already be more susceptible to engaging in exploratory behaviors and altering the maze configuration after only 3 trials may have increased exploratory reactions. We attempted to control for this by obtaining a detailed medical history from participants and fixing at a constant the velocity as participants navigated through the virtual environment. Despite efforts to control for hyperactivity, it is possible that even mild hyperactivity of inattention across trials may have impeded learning or encoding of solution.

The current brief report extends previous research by providing further insight into the navigational strategies participants may use to solve the H-W mazes. Although impaired performance was observed in FXS participants, it is important to highlight that though their performance did not reach levels observed in comparison participants, they did demonstrate some improvement across trials. It is possible that changing the maze configuration after only 3 trials did not allow affected individuals enough time to integrate spatial information. Future studies would benefit by extending the number of trials to determine if additional practice facilitates performance. Related to this, the finding that FXS participants performed similarly to controls on trial one for distance traveled and that they were faster on trial 1 in MacLeod and colleagues (2010) study, underlines the importance of using multiple trials to tease out possible spatial learning and memory impairments.

Despite several contributions of the present study, it could be strengthened by analyzing where the groups paused within the maze to determine if they occurred at choice points or in different locations for the two groups. Studies have shown that landmarks placed at decision points, or intersections, are more likely to be remembered than those placed at simple turns (non-decision points) (Janzen & Van Turennout, 2004; Janzen, Jansen, & Van Turennout, 2008). If the affected group is pausing more frequently than controls, but their pauses occur at decision making points, this would provide telling information on the nature of the affected group's spatial learning deficits and provide further direction on where to focus treatment strategies. Unfortunately, due to a number of factors including the open field-

type design and the lack of information related to participants' orientation (e.g. facing forward or backwards), the current version of the H-W virtual maze does not lend itself to this type of analyses.

Similarly, the present study focused on exploring affected human maze performance. To provide further insight into the neurobiological phenotype of FXS and strengthen the notion that our results reflect underlying spatial deficits, future research should evaluate whether similar patterns of impairments are found in murine models of the disorder, *fmr1* KO mice. Research suggests that landmarks may promote greater flexibility in utilizing alternative navigational strategies, which may preferentially target different underlying neuroanatomical structures (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Nadel & Hardt, 2004). Future studies exploring navigational strategies and performance variables on H-W mazes containing landmarks would provide further insight into the pattern of spatial memory deficits characteristic of FXS.

In conclusion, the results of this brief report generally support the hypothesis that there was greater impairment in performance for individuals affected by FXS as compared to controls. This impairment was evident in the pattern of pathways taken to solve H-W mazes, consistent with the notion that affected individuals employed different behavioral strategies. Future research exploring maze performance in versions of H-W mazes with landmarks as well as translational research examining maze pathways between *fmr1* KO mice and controls would help further understand if this pattern of impairment is consistent across species. This may also further clarify which factors may be contributing to poorer maze performance among affected individuals.

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Chapter Five: General Discussion

Introduction

Fragile X syndrome (FXS) is the most prevalent form of heritable mental retardation (Turner, Webb, Wake, & Robinson, 1996). It is a single gene disorder that arises from a trinucleotide expansion of the Fragile X Mental Retardation 1 gene (*FMR1*) on the X chromosome (Online Mendelian Inheritance in Man® [OMIM] 309550; Verkerk *et al.*, 1991; Sherman, 2002) that reduces levels of Fragile X Mental Retardation protein (FMRP) (Verkerk *et al.*, 1991). The lack of expression of *FMR1* in somatic cells (Pieretti *et al.*, 1991) leads to a well-defined phenotype that is characterized by a range of behavioral and cognitive deficits, including impairments in visual-spatial cognition (Macleod *et al.*, 2010; Cornish, Munir & Cross, 1998; 1999; Crowe & Hay, 1990). Previous studies have demonstrated a deficit in basic visual perceptual processing in individuals with FXS (Kogan *et al.*, 2004 a & b; Farzin & Rivera, 2010; Farzin, Rivera, & Whitney, 2011). Kogan and colleagues (2004 a, b) were the first to provide neurobiological and behavioral evidence that individuals with FXS have an early visual processing deficit that impacts dorsal stream functioning and contributes to the visual-motor deficits observed in affected individuals. However, how such a deficit translated into observed visual spatial navigation deficits remained unknown.

Studies of *fmr1* knockout mice, a murine model of FXS, had also demonstrated deficits that at times mirror those observed in individuals affected by FXS (Paradee *et al.*, 1999; Chen & Toth, 2001; Mineur, Sluyter, De Wit, Oostra & Crusio, 2002; Mineur, Huynh & Crusio, 2006; Spencer, Alekseyenko, Serysheva, Yuva-Paylor & Paylor, 2005). Despite some concordance, results from tests focusing on replicating the spatial processing deficit observed in affected humans were mixed (D'Hooge *et al.*, 1997; Dobkin, *et al.*, 2000; The Dutch-Belgian Fragile-X Consortium 1994; Kooy *et al.*, 1996; Mineur *et al.*, 2002; Paradee *et al.*, 1999; Peier *et al.*, 2000). It therefore remained controversial as to whether the murine model of FXS exhibited a similar spatial deficit as was documented for affected humans.

In an effort to resolve the ambiguity in the literature and extend previous research, this dissertation employed a novel experimental approach to evaluate spatial learning and memory in FXS using Hebb-Williams mazes (H-W). H-W mazes are a well-established measures of spatial cognition in animals (Hebb & Williams, 1946; Rabinovitch & Rosvold, 1951; Shore, Stanford, MacInnes, Klein, & Brown, 2001). The the design of a computerized

version of these mazes allowed for a translational study to evaluate visual-spatial deficits in individuals with FXS, as well as *fmr1* knockout mice (KO), under comparable testing conditions, a criterion not met in previous studies.

This dissertation includes three separate but related studies. Across all studies, either the performance of people affected by FXS and/or *fmr1* KO mice was compared to comparison controls on seven H-W mazes of increasing difficulty. Study one employed the traditional configuration of the H-W mazes to evaluate performance variables that include latency to complete the maze and number of the errors. Study two used the same H-W maze configuration and performance measures as study one, but further expanded on the findings by adding landmarks to the maze environment to evaluate how these might impact spatial learning and memory in *fmr1* KO mice. Lastly, study three entailed a more in-depth behavior analysis of maze navigation performance for FXS individuals from study 1. This general discussion is structured by first presenting and discussing the findings of each study individually. Following this, the strengths, limitations and implications of this dissertation for future investigation and clinical practice are discussed.

Study 1: A comparative study of the performance of individuals with Fragile X syndrome and *fmr1* knockout mice on Hebb-Williams mazes

In the first study, I compared the performance of individuals affected by FXS to typically-developing mental age-matched comparison participants on seven H-W mazes of increasing difficulty levels. I also compared the performance of *fmr1* KO mice to wild-type control mice on the same maze problems. I hypothesized that individuals affected by FXS and *fmr1* KO mice as compared with their respective control groups would exhibit poorer performance on mazes deemed more difficult. Specifically, I hypothesized that impairments in H-W performance indicative of abnormal basic visual functioning would manifest as significantly greater latencies and error rates across all maze problems for affected humans and *fmr1* KO mice for the first trial with persisting deficits observed on subsequent trials.

The results of study 1 revealed significant differences in performance for both human and animal experiments. In contrast to the FXS participants, performance of the comparison participants improved as indicated by significantly fewer errors across trials. A similar pattern of results was observed when latency across trials was analyzed. Although significant

differences between participants was found for all trials, on trial 1 the FXS participants completed mazes faster than their peers while committing a similar number of errors. That participants affected by FXS were able to successfully complete the mazes suggested that basic visual functioning necessary for solving a novel spatial task was intact in these individuals. However, with subsequent trials, the FXS group appear unable to either learn or recall the maze solution and continue to use a trial and error strategy to find the goal box.

Given the evidence that abnormalities in spatial learning can be partially attributed to hippocampal deficits (Morris *et al.*, 1982; Logue, Paylor, & Wehner, 1997); Ekstrom *et al.*, 2003; Ghaem *et al.*, 1997, Iaria *et al.*, 2003; O'Keefe, & Dostrovsky, 1971) we speculated that performance of individuals affected by FXS on the virtual H-W maze may be more suggestive of impairment in hippocampal function rather than to a visual-perceptual deficit. *Fmr1* KO mice were found to exhibit impairments similar to those observed in humans affected by FXS for the same H-W mazes. We found that overall wild type control mice made significantly fewer errors than the *fmr1* KO group. However, the latency to complete the mazes for both groups was not significantly different. Similar to the human data, we speculate that impaired performance of *fmr1* KO mice on the H-W mazes observed here is attributable to abnormal processing in the hippocampus.

Taken together, the results of study one revealed significant differences in performance for FXS affected individuals as compared to mental age-matched comparison individuals and support the hypothesis that a selective deficit in spatial learning and memory characteristic of the FXS phenotype can be observed in the murine model of FXS, the *fmr1* KO if equivalent tasks are employed in testing humans and mice.

Study 2: Landmarks and Hebb-Williams maze performance: Evaluating visual-spatial learning of *fmr1* knockout mice

In the second study, I used the same Hebb-Williams maze configurations and performance measures as study one, but included distinct spatial cues to the maze environment that could be used for allocentric encoding. Incorporating landmarks into the H-W mazes was hypothesized to provide additional spatial cues that would facilitate the use of alternative navigational strategies only to wild type mice. Research has demonstrated that landmarks tend to favor hippocampal processing, suggesting that their utilization in a

navigational task should reveal deficits in allocentric processing. Therefore, we hypothesized that only wild type mice will benefit from the introduction of landmarks. Due to hippocampal abnormalities in *fmr1* KO mice caused by lack of FMRP, it was expected they would show relatively poorer performance as compared to wild type mice on landmark tasks because they would be less effective in developing a cognitive map of the mazes.

Contrary to our hypotheses, landmarks significantly impaired wild type control performance. In addition, results revealed that the performance of the *fmr1* KO mice generally did not differ between landmark and non-landmark tasks, indicating that the presence of landmarks neither enhanced nor hindered mouse performance. An interesting exception to this pattern was observed on Maze 4, whereby the *fmr1* KO landmark group acquired learning faster than its non-landmark equivalent, without committing additional errors; suggesting that the presence of landmarks actually facilitated mouse performance for this specific maze. Maze 4 has generally been characterized as one of the easiest mazes (Macleod et al., 2010; Pereira et al., 2005; Meunier, Saint-Marc & Destrade, 1986), and we conjectured that on mazes that are considered easier, and perhaps have a lower cognitive demand, landmarks facilitate performance because subjects are better able to attend to and thus utilize the additional spatial cues. Lastly, we suggested that finding that the general pattern of results for *fmr1* KO mice was similar regardless of whether landmarks were present can be interpreted as a replication of the spatial learning and memory deficit observed in *fmr1* KO mice found in study 1.

Study 3: Brief report: Behavioral Analysis of performance of individuals with fragile X syndrome on Hebb-Williams maze

In study three, I conducted a more in-depth analysis of maze navigation performance for FXS individuals from study 1. All maze trials generated from Study 1 were digitized, allowing for additional performance measures to be created and providing for further insight into how individuals were solved the mazes. The additional performance measures included the total distance travelled across maze trials, and the frequency and duration of time spent immobile (e.g. pausing/inspecting). I hypothesized that as a result of visual-spatial memory and learning difficulties, individuals affected by FXS as compared to the typically-

developing mental ages matched comparison participants would exhibit poorer results on all performance variables. In addition, affected individuals would exhibit differences in behavioral strategies employed to solve the H-W mazes, which would be evident by the pattern of pathways taken to solve the maze.

Consistent with the hypotheses and findings from study 1, results revealed significant differences in performance variables between individuals, with FXS participants generally performed worse than the comparison group participants. In contrast to the FXS group, the comparison group paused less frequently and for a shorter duration. Although the distance travelled by the FXS group decreased across trials, evidence of some learning, they still performed significantly worse than comparison participants even by trial 3. Furthermore, the comparison group demonstrated improvement across trials as indicated by a significant decrease in the frequency of pausing.

In contrast to the controls, FXS participants appeared to maintain their initial search strategy across trials, which we speculated could reflect an inability of affected individuals to adaptively integrate or apply the knowledge obtained on previous trials, which may be indicative of an underlying deficit in learning and/or memory. Although impaired performance was observed in FXS participants, it is important to highlight that though their performance did not reach levels observed in comparison participants, they did demonstrate some improvement across trials. Surprisingly, when interpreting the results of study 3 in the context of findings from study 1, the faster latency of FXS participants on trial 1 did not appear in any of the additional dependent variables extracted. After careful inspection of the data, the only possible hypotheses we can suggest to account for these conflicting findings may be the differences in data treatment in each study. In study 1, the raw data did not meet the assumption of normality and were transformed, whereas the same treatment of data was not required in study 3. It is possible that the manner in which the data was handled could account for the difficulty generalizing findings across studies. The studies also consisted of small sample sizes and it is possible that including additional participants in future research may help clarify the interpretation of the results.

Taken together, the results of study 3 generally supported the hypothesis that there was greater impairment in performance for individuals affected by FXS as compared to controls. This impairment was evident in the pattern of pathways taken to solve H-W mazes,

consistent with the notion that affected individuals employed different behavioral strategies.

Implications for H-W-Mazes as Behavioral Assay: Treatment Studies and Translational Aspect

A clear strength of this dissertation is its novelty. Specifically, there have been few studies comparing human and rodent performance on the same task (Shore *et al.*, 2001) and to our knowledge, none that have directly compared individuals affected by FXS to a murine model of the disorder on the same task. The utilization of the novel H-W experimental paradigm in this dissertation offered several distinct advantages. First, this paradigm allowed for a comparison across species to directly evaluate performance of individuals affected by FXS to *fmr1* KO mice. The similarity between tasks lends strength to the notion that this paradigm is tapping similar cognitive domains between human and animals, thus further supporting the construct validity of H-W mazes. Second, H-W mazes are reported to be sensitive to detecting alterations of hippocampal dependent spatial abilities (Winocur & Moscovitch, 1990) better than radial or water maze tasks (Pereira, Cosquer, Schimchowitsch, & Cassel, 2005). Third, establishing that behavioral assays, such as the H-W maze, are able to detect behavioral deficits in both human and mice is advantageous because they can be used to evaluate pharmacological and behavioral interventions to reverse or mitigate the symptoms of FXS. In addition, the H-W paradigm and protocol have been standardized and are supported by an immense literature from which to draw comparisons (Shore *et. al.*, 2001). Finally, findings of comparable deficits among affected individuals and *fmr1* KO mice on a spatial navigation task provides further insight into the neurobiological basis of the FXS phenotype.

The establishment of reliable behavioral assays to test neurodevelopmental disorders is important for testing the validation of new therapeutics. We propose that H-W mazes could be used as a tool to evaluate therapeutic strategies both during early development in animals and later in clinical trials. Significant evidence suggests that changes in spinal properties and morphology may impact neuronal plasticity, which may underlie cognitive deficits in learning and memory that characterize FXS (Huber *et al.*, 2002; Hayashi and Majewska 2005; Segal, 2005; Yuste and Bonhoeffer, 2004). Given the current understanding of the molecular role of FMRP, many treatment studies have been conducted using

pharmacological compounds aimed at targeting receptors such as mGluR. Several studies have demonstrated the reversal of aberrant neuroanatomical characteristics as well as behavioral and cognitive impairments in animal models of FXS (Liu et al, 2011; Mines et al, 2010; Yuskaitis et al, 2010; Bilousova et al, 2009) and some of the cognitive and behavioral deficits observed in individuals with FXS (Jacquemont et al, 2011; Berry-Kravis *et al.*, 2009; 2006; 2004).

Although pharmacological compounds have been used to treat cognitive and behavioral deficits of FXS (Berry-Kravis *et al.*, 2006; 2004) there have been some challenges in establishing reliable outcome measures. Many cognitive measures used previously have been too difficult for the majority of individuals with FXS to complete and further have produced unacceptable levels of variability (Berry-Kravis *et al.*, 2006). Gross and colleagues (2012) describe the design of appropriate outcomes measures as an obstacle in clinical trial studies with FXS, and they highlight the need for better FXS-specific outcome measures. A significant barrier is balancing the need for a measure that is both capable of testing a wide range of affected individuals from low to high functioning, while also avoiding the issue of floor or ceiling effects (Gross et al., 2012). Recent work by Kogan and colleagues (2009) has produced compelling results using a comparative neuropsychological (CN) approach, where an experimental paradigm used to evaluate cognitive abilities in animals was modified to enable testing of individuals affected with FXS. The movement towards the CN approach is exciting and creates opportunities for conducting a cross-species study using this apparatus.

Given the obstacles that exist within this domain of research, the ability of this dissertation to overcome these speaks to its strength and unique contributions to the literature. In this regard, the H-W maze battery is appealing due to the range of maze configurations with different levels of difficulty. For example, in study 2, we identified Maze 4 as a task where landmarks actually facilitated performance in the affected group, which lends strength to its inclusion in the battery of H-W mazes for future studies measuring cognitive change. Additional studies investigating the reliability of the H-W paradigm is its ability to detect spatial deficits, as well the stability of performance measures over time would be beneficial. Establishing this is important not only for individuals with FXS but also for other developmental disorders, such as autism, that share similarities with FXS in the underlying molecular pathways (Awadalla et al, 2010; Pinto et al, 2010). Researchers

speculate that treatment strategies optimizing functioning in FXS, will be far reaching and will also benefit individuals with autism spectrum disorders and other disorders with similarly affected neural pathways (as reviewed by Gross, Berry-Kravis, & Bassell, 2012).

Related to this, in this dissertation we have established that affected individuals, with a range of intellectual deficiency, can successfully perform the H-W maze tasks and have shown variability in performance of test mazes depending on maze difficulty. The range of difficulty of the H-W mazes suggests it could be utilized for testing pre-mutation carriers and affected females. Individuals with a pre-mutation (50-200 CGG repeats) generally have near normal levels of FMRP expression (Devys, Lutz, Rouyer, Bellocq, & Mandel, 1993) and often display average levels of intellectual functioning (Cornish et al., 2005) with subtle neuropsychological impairments (e.g., Kogan & Cornish, 2010), including a selective spatial deficit (Hocking, Kogan & Cornish, 2012). Females being homozygous for the X chromosome, typically possess one unaffected X chromosome, which enables some FMRP expression (Dobkin et al., 2000). In general, this results in a less severe FXS symptomatology with the most common symptoms in the female population being deficits in executive function skills and social anxiety (Bennetto et al., 2001). Ongoing research continues to provide evidence demonstrating correlations between the neuroanatomical abnormalities observed and varying degrees of functional impairment in individuals with FXS. For example, Keri and Benedek (2009; 2010) demonstrated a similar visual deficit in both affected individuals and pre-mutation carriers, however, the deficit was more pronounced for those individuals with full mutation. Interestingly, in recent research using healthy male volunteer subjects, investigators showed a positive association between peripheral FMRP levels and performance on tasks (Keri & Benedek, 2011), which may lend support to the notion of a possible dose dependent relationship between the CGG repeat on the *FMR1* gene and the deficit in visual processing for FXS. As well as testing affected females and pre-mutation carriers, establishing that affected individuals can complete the H-W paradigm creates opportunities to test other neurodevelopmental syndromes such as Down syndrome, Turner Syndrome and Williams Syndrome.

Taken together, this dissertation further establishes the validity of the H-W mazes as an ecologically valid behavioral assay that is able to detect behavioral deficits in humans and mice. In addition, we propose that the H-W mazes may be used as a tool to evaluate

therapeutic strategies both during early development in animals and later in clinical trials

Implications for FXS Phenotype: Spatial Learning & Memory

The results of this dissertation contribute to the literature aimed at delineating the FXS phenotype by providing further evidence of the visual spatial learning impairment in FXS. To our knowledge, this is the first study to not only specifically explore navigation abilities in the affected population, but to also demonstrated a similar pattern of results in a murine model of the disorder, *fmr1* KO mice. Although we hypothesized that individuals affected by FXS would perform worse on the virtual H-W mazes because spatial navigation relies in part on intact vision (Tees *et al.*, 1981), and therefore efficient processing within the dorsal visual stream, our findings argued against a visual-perceptual deficit as the sole explanation for observed differences on subsequent trials. Contrary to our hypothesis in study 1, that participants affected by FXS were able to successfully complete the mazes suggests that basic visual functioning necessary for solving a novel spatial task is intact in these individuals. However, with subsequent trials, the FXS group appear unable to either learn or recall the maze solution and continue to use a trial and error strategy to find the goal box.

These results do not negate the previous studies that have demonstrated a deficit in basic visual perceptual processing in individuals with FXS (Kogan *et al.*, 2004 a & b Farzin & Rivera, 2010; Farzin, Rivera, & Whitney, 2011). Many of these studies (e.g. Kogan *et al.* (2004 a,b) evaluated visual function at perceptual threshold, whereas the H-W mazes utilized in this study are presented at supra-threshold values (i.e., stimuli are clearly visible). It is possible that the early visual processing deficit demonstrated in affected individuals does not translate into observed visual spatial navigational deficits as evaluated in this dissertation.

There is a consensus that FXS is best characterized by a constellation of strengths and weaknesses within the cognitive domain that differentiate the FXS profile from that of other disorders of mental retardation (Van der Molen *et al.*, 2010; Cornish *et al.*, 2005). Over the past decade several lines of research have helped define the unique phenotype of FXS. Our results are consistent with research showing individuals affected by FXS experience difficulties on visual-spatial tasks including those requiring participants to manipulate objects in space (Crowe *et al.*, 1990; Cornish, Munir & Cross, 1998; 1999; Freund & Reiss,

1991; Mazzocco, Singh Bahtia, & Lesniak-Karpiak, 2006). We speculated that the deficits in learning and memory we observed in this dissertation may be due to impairment in hippocampal function. Animals with hippocampal damage can learn simple spatial discrimination tasks but have impaired performance when certain parameters of the procedure are changed. Similarly, in the Morris Water Maze rats with lesioned hippocampi are able to find the hidden platform from one release point but demonstrate an impaired performance if the release point is changed (Morris, Garrud, Rawlings & O'Keefe, 1982). Given that the hippocampus is likely involved in more complex spatial behavior such as organizing spatial information and expressing spatial memory flexibility (as discussed by Eichenbaum, 2003), it is not surprising that a paradigm such as H-W mazes present challenges for participants with impaired hippocampal functioning. If impaired performance on H-W mazes is attributable to abnormal processing in the hippocampus, future studies should examine whether these deficits are attributable to impairment in encoding, storage, retrieval or some combination thereof.

The H-W maze task as utilized in this dissertation can be understood as tapping many different cognitive domains including response flexibility, inhibiting previous learned responses, and planning. The mazes require participants to develop self-ordered search strategies to solve each individual maze configuration, hold the rules/goal of the task online and update it as they move on to a new maze. To reduce the cognitive demand/flexibility required to complete the full battery of H-W mazes and further delineate the nature of the spatial deficit, future studies should limit the test mazes and focus on administering the easier H-W maze configurations (Maze 4, Maze 2 & Maze 12) for a greater number of trials. In this dissertation we tested participants on 7 different configurations of the H-W mazes and to mitigate for fatigue in humans, presented each for relatively few trials (3 for human, 5 for mice). If impaired performance is due to learning and memory, it is possible that with greater repetition in the number of trials, FXS participants may have begun demonstrating similar levels of performance as controls. In study 2, we showed that the *fmr1* KO landmark group acquired learning faster than its non-landmark equivalent, without committing additional errors; suggesting that the presence of landmarks actually facilitated mouse performance for this specific maze. Although study 2 was conducted with mice, it is interesting that on one of the easier mazes, with additional spatial cues that may encourage greater associative

learning, performance was improved. The improvement in performance on Maze 4 is interesting and suggests it may be a good candidate for use in future studies to measure cognitive change.

Individuals affected with FXS have been found to perform poorly on tasks of working memory (Munir et al., 2000), and tasks that place a higher demand on executive function (as reviewed by Hooper et al., 2008). Van der Molen and colleagues (2010) evaluated a range of cognitive abilities for individuals with FXS, and demonstrated that their performance was relatively spared on associative learning tasks, or tasks that may place less demand on executive control than working memory tasks. Interestingly, Krueger and colleagues (2011) have demonstrated that prefrontal cortex-dependent forms of learning are altered in *Fmr1* KO mice. Specifically, they found that deficits in cognitive flexibility, with *fmr1* KO mice performing similarly to controls on the acquisition of an appetitive instrumental response task but demonstrating significant impairment on later acquisition of a visuospatial discrimination task. These authors described the results as consistent with an inability to flexibly respond to changing reward contingencies and shift attention from one perceptual dimension (spatial location) to another (visual cue). They suggest that behavioral assays that draw on areas of the brain involved in cognitive flexibility may be useful in evaluating pharmacological tools (Krueger et al., 2011). Given the number of cognitive domains that likely are utilized in solving the full battery of H-W task, the latter could be a potential candidate.

Having established in this dissertation that visual-spatial learning and memory impairments exist in both humans affected by FXS and *fmr1* KO mice, the next steps would be to determine if these deficits are specific to FXS over and above the effects of non-specific mental retardation. Researchers have hypothesized that certain developmental disorders with visual deficits (developmental dyslexia, Williams Syndrome, Autism Spectrum disorder) have an impairment in dorsal stream functioning (as reviewed by Grinter, Maybery, & Badcock 2010; Braddick et al., 2003). Future research should include a comparison group with a similar IQ profile for the identification of a FXS specific cognitive profile. Possible candidates for comparison are Turner Syndrome and Down Syndrome (DS).

Previous research has shown that visuospatial deficits exist in Turner Syndrome (Alexander et al., 1966; Cornoldi et al., 2001) as well as FXS (Cornish et al., 1998; 1999;

Kogan et al., 2004). In comparison to the Turner Syndrome group, individuals with FXS displayed greater difficulty locating the target objects; an ability dependent on the “where” or dorsal pathway of visuospatial processing (Mazzocco, Singh Bhatia, & Lesniak-Karpiak, 2006). Similarly, individuals with DS have been shown to exhibit a unique cognitive profile with strengths in visuospatial cognition and weaknesses in declarative memory (Wang & Bellugi 1994; Jarrold *et al.* 1999; Laws 2002) and verbal working memory (Nadel 2003). In comparison to DS, Kogan and colleagues (2009) showed that individuals with FXS were impaired on object discrimination learning aspects of a task as well as reversal tasks. The development of animal models (DS) (Sago et al., 2000) makes this population especially appealing, given the opportunities to conduct translational studies using the H-W mazes. Taken together, one would predict that the visual perceptual impairments characteristic of FXS will result in a syndrome-specific deficit in visual-spatial navigation.

Implications for FXS KO Mice & Behavioral Phenotype FXS

A clear contribution of this dissertation is its uniqueness in comparing human and rodent performance on the same task, as to our knowledge, very few studies have conducted cross-species comparisons and when this dissertation was completed, no studies have directly compared behavioral performance of individuals affected by FXS to a murine model of the disorder on the same task. This type of research is important because using such a translational approach increases the validity of the murine model as well as strengthens the notion that our results reflect a similar underlying spatial deficit that is being targeted and expressed in both species. Studies of *fmr1* knockout mice have demonstrated deficits that under certain conditions mirror those observed in individuals affected by FXS, with impairments having been described for a range of behavioral tasks including Conditioned Fear Response (Paradee *et al.*, 1999), Prepulse Inhibition (Chen & Toth, 2001), Open Field Activity (Mineur, Sluyter, De Wit, Oostra & Crusio, 2002), and Social Interaction tasks (Mineur, Huynh & Crusio, 2006; Spencer, Alekseyenko, Serysheva, Yuva-Paylor & Paylor, 2005). Despite some concordance, results from tests of the murine model of FXS focusing on replicating the spatial processing deficit observed in affected humans have been mixed (D’Hooge *et al.*, 1997; Dobkin, *et al.*, 2000; The Dutch-Belgian Fragile-X Consortium 1994; Kooy *et al.*, 1996; Mineur *et al.*, 2002; Paradee *et al.*, 1999; Peier *et al.*, 2000). It therefore

remained controversial as to whether the murine model of FXS exhibits a similar spatial deficit as has been documented for affected individuals.

This dissertation adds strength to the utility of *fmr1* KO mice for use in studies measuring spatial learning and memory as we were able to show that *fmr1* KO mice exhibit impairments similar to those observed in the FXS group for the same H-W mazes. Thus, providing support for the face validity of the *fmr1* KO model. Similar to the human data, increased errors observed in *fmr1* KO mouse performance seems to more accurately reflect a learning or memory deficit, rather than non-specific factors or visual-perceptual ability *per se*. Importantly, evidence of this visual spatial learning and memory deficit was found not only in study 1, but later replicated in study 2. Replication of the spatial deficit and establishment of its stability overtime has important implications for treatment studies aimed at reversing cognitive deficits. In addition to the benefits related to FXS, further delineating the *fmr1* KO phenotype creates opportunities for utilizing this animal model to study other disorders. Interestingly, *fmr1* KO mice have been shown to display autistic-like behaviors, which suggests that the KO mice may also be a useful mouse model for autism (Bernardet and Crusio, 2006).

Despite the wealth of knowledge that is obtained from behavioral testing with animals, very few studies have conducted cross-species comparisons. One of the reasons for this is that there are several confounds and challenges to overcome when testing genetically modified animals. Some of the inconsistencies that have been found among studies may be due to several factors including: differences in background strain used (Spencer et al., 2011; 2006), number of generations of back crossing (Gu et al., 2002), pre/post natal environmental factors (Spencer et al., 2011), maternal variables (Francis, Szegda, Campbell, Martin, & Insel, 2003) and the choice of the spatial task employed as well as the equivalency of such tasks to those of human spatial cognition. For example, a significant challenge is the use of negative reinforcement in many behavioral paradigms, (e.g. escape water in MWM) whereas the same reinforcement contingencies are not present in the human equivalent tasks (Hunsaker, 2012). The H-W maze may be viewed as a relatively non-aversive task with similar reward contingencies for both humans and mice. However, most paradigms currently used, including the H-W mazes, require the animal to be removed from their home cage and physically transported to the testing environment. It is well established that this causes

significant distress in the animal (as discussed by Baker 2011). Given the obstacles that exist in animal testing, it is not surprising that successfully conducting translational studies possesses many challenges.

There is concern regarding the disconnect between rapid technological advances in creating mouse models and the development of sensitive behavioral techniques required to assess the behavioral phenotype (as discussed by Hunsaker, 2012). Several technological advances have been developed that create exciting possibilities which may help reduce some of the variability in results that are often observed in behavioral paradigms. The development of innovative imaging technologies has allowed for *in vivo* MRI analyses of the brain in mouse models of genetic disorders (Ellegood *et al.*, 2010; Kooy *et al.*, 1999; Kovacevic *et al.*, 2005), which will add strength to cross-species comparison of neuropathological features *in vivo*. In addition, new monitoring systems permit the observation and recording of several behaviors (e.g. activity, eating, drinking, head bobbing, and grooming) to be obtained while the animal remains within its home cage (Goulding, 2008, Clever Sys in Reston, Virginia). Home cages have also been modified to allow for different types of instrumental testing within the animals' environment (Noldus Information Technology, Netherlands). Lastly, researchers are exploring alternative genetically modified animal models to test genetic manipulation on behavior, including rat models of disease. Callaway (2011) describes the innovative work of Richard Paylor who is using knockout rats to study autism. In addition, Sigma-Aldrich currently offers knockout rats for schizophrenia, Parkinson's disease and autism (Baker, 2011). Using rats for this type of research makes sense, since many of the currently utilized behavioral test paradigms were designed for use with rats and because these animals demonstrate more complex behaviors. Thus, they may be good models for teasing out subtle behavioral changes associated with genetic manipulations (Baker 2011). With the range of these new innovative technologies, it is hopeful that more a consistent picture will begin to emerge in terms of the implications to humans of data acquired from animal research.

Overall Limitations

Although this dissertation makes several important contributions to the literature, it does have limitations. One of these may be the differences in the composition of the

comparison groups in study 1 used to interpret the data from the two species. For the human study, a mental age matched comparison group was used, conversely, in the animal study, the *fmr1* KO group was compared to chronologically matched controls. Conversely, in the animal study the *fmr1* KO group was compared to chronologically matched controls. It is possible that the composition of the animal groups could have increased the likelihood of observing a significant difference between the two groups tested. In the literature, studies of individuals affected by FXS primarily compare their performance to individuals who are typically-developing but score similarly on a given measure of intellectual functioning. Therefore, the comparison participants are almost always chronologically younger than the affected individuals in order to avoid the confound of intelligence. As there is no established methodology for determining mental age of either KO or wild type mice, matching on this variable was not possible. A survey of the literature on *fmr1* KO mice reveals that studies either fail to inform the reader about the age of the subjects or use chronological age matching.

Similarly, study 1 and study 3 included three participants with mosaicism of the *FMRI* gene. Given that individuals with a mosaic tend to exhibit less severe impairments across most cognitive measures (Cohen, Nolin, Sudhalter, Ding, Dobkin, & Brown, 1996; McConkie-Rosell *et al.*, 1993; Merenstein *et al.*, 1996), inclusion of these individuals in the current study could have minimized the likelihood of observing a significant difference between the two groups tested. Despite their inclusion, a significant differences in the FXS individuals were observed, as compared to mental-age matched peers. Furthermore, a review of the data obtained from individuals with mosaicism revealed that their performance was similar to that of the participants with the full-mutation.

In study 1 and study 2, a limited number of data points were included in our mouse hyperactivity analyses. This precludes us from making definitive conclusion that activity level is not a factor in the observed differences in error rates between the groups. Increases in both hyperactivity and reductions in neophobia characteristic of *fmr1* KO mice may have led to increased numbers of entries in to error zones, which in turn, resulted in poor learning or encoding of inefficient solutions to the mazes. We conducted a *post-hoc* analysis in both study 1 and study 2 of activity levels between the murine groups to test this hypothesis, and consistent with previous reports, our results indicate that there are no significant differences

in activity levels between *fmr1* KO and control mice (The Dutch-Belgian Fragile X Consortium, 1994, Mineur et al., 2002, Peier, 2000; Spencer, 2005; Zupan and Toth, 2008). Future analyses of the specific paths taken by the respective groups to reach the goal box would help establish whether increased errors committed by the *fmr1* KO mice is a cause or consequence of a learning deficit. Similarly, it is also possible that the greater number of errors committed by the *fmr1* KO mice relate to higher rates of perseveration for incorrect solutions to a given maze problem. Additional studies exploring the paths taken to solve the mazes may be able to address this issue.

Similarly, study 3 of this dissertation could be strengthened by analyzing the location of where the groups paused within the maze to determine if they occurred at choice points or in different locations for the two groups. Studies have shown that landmarks placed at decision points, or intersections, are more likely to be remembered than those placed at simple turns (non-decision points) (Janzen & Van Turenout, 2004; Janzen, Jansen, & Van Turenout, 2008). If the affected group is pausing more frequently than controls, but their pauses occur at decision making points, this would provide telling information on the nature of the affected group's spatial learning deficits and provide further direction on where to focus treatment strategies.

Finally, in study 2, the finding that landmarks generally did not facilitate performance could be due to several factors that should be taken into consideration in future studies. Future studies should carefully consider the placement of landmarks and perhaps use distally placed cues to see if this facilitates maze learning. Research has shown that distally placed landmarks, may provide a more stable frame of reference because they do not change their relative positions as an animal or individual navigates through a maze environment (Parron, Poucet, & Save, 2004). In the present study, landmarks were placed proximally and it has been noted that it is more difficult to train animals to use these cues (Gothard, Skaggs, Moore, & McNaughton, 1996; Gould-Beierle, & Kamil, 1996; Teroni, Portenier, & Etienne, 1987). Second, research has shown that the number of landmarks, their location, and consistency in presentation can influence results (Fenton et al., 1994; Prados, 2000; Prados & Trobalon, 1998). In study 2 it is possible that certain landmarks may have been in closer proximity to the goal box or placed at a key decision point, and thus been differentially reinforcing. Third, research has demonstrated that animals may use multiple landmarks in a

configural pattern (Benhamou & Poucet, 1998; Collett et al., 1986; Gallistel & Cheng, 1985; Maurer & Derivaz, 2000; Roberts & Pearce, 1999), and that learning, or an association, is shared between cues (Shettleworth, 2005). This suggests that the geometric arrangement of landmarks as a whole is likely utilized to provide useful spatial information, and our attempt to counterbalance landmark presentation across mazes may have reduced the usefulness of the cues because the landmarks that were moved may have lost their reinforcing or informative properties and thus become confusing for subjects. For these, and possible other reasons, the landmarks as they were utilized in study 2 generally did not appear to optimally promote an allocentric navigational strategy.

Summary

This Dissertation explored visual spatial learning and memory in Fragile X syndrome (FXS). Across all studies, either the performance of individuals affected by FXS and/or *fmr1* KO mice was compared to comparison controls on seven H-W mazes of increasing difficulty levels. Several important findings were generated. First, the results of study 1 revealed significant differences in performance for FXS affected individuals as compared to mental age-matched comparison individuals and support the hypothesis that a selective deficit in spatial learning and memory characteristic of the FXS phenotype can be observed in the murine model of FXS, the *fmr1* KO if equivalent tasks are employed in testing humans and mice. Second, in Study two, contrary to our hypotheses, landmarks significantly impaired wild type control performance and surprisingly, the performance of the *fmr1* KO mice was neither enhanced, or hindered by the presence of landmarks. We suggested that finding that the general pattern of results for *fmr1* KO mice was similar regardless of whether landmarks were present can be interpreted as a replication of the spatial learning and memory deficit observed in *fmr1* KO mice found in study 1. Finally, in study three, consistent with the hypotheses and findings from study 1, results generally supported the hypothesis that there was greater impairment in performance for individuals affected by FXS as compared to controls. This dissertation has presented the strengths, limitations and implications of the results for future investigation and clinical practice.

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Appendix A
Consent form for participants affected by Fragile X Syndrome

Title of Study:

Navigational Abilities in Individuals Affected by Fragile X Syndrome

Investigators:

XXXXXX, Assistant Professor, School of Psychology, University of Ottawa, 613-XXX-XXXX

My son, _____ (name), has been invited to participate in a research study conducted by XXX. This study will evaluate navigational abilities in boys and males affected by the Fragile X syndrome (FXS) and Down syndrome. Individuals affected by FXS appear to have difficulty perceiving motion information. Since perception of visual motion is necessary for navigating through the environment, we wish to explore the hypothesis that individuals with FXS demonstrate poorer navigational abilities when comparing their performance to that of individual affected by a different form of mental retardation, Down Syndrome. This research will allow us to determine if individuals affected by Fragile-X syndrome display a different profile of performance on the navigation task than individuals affected by Down's syndrome. This research will also us determine if the the gene involved in Fragile-X syndrome plays is important for the development of navigational abilities.

My son will be asked to complete a vocabulary test and the navigation tasks. The vocabulary task requires that he point to a picture that corresponds to a word. The navigation tasks require that the participant navigate through 3-dimensional mazes rendered on the computer. The participant will navigate through the maze using a joystick or keys on the keyboard. This will take about one hour and a half.

We will ask you to fill in a health questionnaire that will be used to describe individuals who participated in our study in any ensuing publication arising from this research. The information provided in the questionnaire is confidential and will not be used to identify your son personally.

The study does not subject the participant to any known risk other than potential boredom and difficulties with completing the tasks. Due to the nature of the 3-dimensional mazes there is a small chance of dizziness, headaches or mild nausea. My son will be encouraged to express himself and the testing will be stopped immediately if any of these symptoms are experienced. The experimenter will at all times during the testing ensure to the best of his/her knowledge that the participant is comfortable. If the experimenter sees that my son is fatigued or is experiencing discomfort, the testing will be stopped immediately.

My son's results will be kept confidential and he will not be identified in any way when the results are published. My son's data will be recorded in a computer and stored according to a codified name that includes only his initials and the day of testing. If for whatever reason my son's results are not used for this study, they may be used for another study as a comparison for another condition.

I will receive \$50 as compensation plus remuneration for parking expenses. My son can keep the rewards he will receive during the study. Payment is not dependent on whether or not he is able to complete the study; payment will be made when the study is either

completed or terminated.

My son's participation is voluntary and he is under no obligation to participate in this study. He may withdraw from the study at any time without any negative consequences.

I have been provided with a copy of this consent form and I have had the opportunity to ask any questions that I may have about the study, and all of my questions have been answered.

Having thoroughly read, understood, and had full explanation of this consent form, I voluntarily consent to my child participating in this research study conducted by Drs. XXX and XXX. If I have any questions, I may contact Dr. XXX. If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, XXXXXXXXXX or ethics@uottawa.ca.

There are two copies of the consent form, one of which is mine to keep.

Experimenter

Signed

Name of Child

Name of Parent

Signed

Date

Appendix B

Consent form for Control Participants

Title of Study:

Navigational Abilities in Individuals Affected by Fragile X Syndrome and Down's syndrome

Investigators:

Dr. XXX, Assistant Professor, School of Psychology, University of Ottawa, 613-XXX-XXXX

My son, _____ (name), has been invited to participate in a research study conducted by Dr. XXX. This study will evaluate navigational abilities in boys and males affected by the Fragile X syndrome (FXS) and Down syndrome. Individuals affected by FXS appear to have difficulty perceiving motion information. Since perception of visual motion is necessary for navigating through the environment, we wish to explore the hypothesis that individuals with FXS demonstrate poorer navigational abilities when comparing their performance to that of individual affected by a different form of mental retardation, Down Syndrome. This research will allow us to determine if individuals affected by Fragile-X syndrome display a different profile of performance on the navigation task than individuals affected by Down's syndrome. This research will also us determine if the the gene involved in Fragile-X syndrome plays is important for the development of navigational abilities.

My son will be asked to complete a vocabulary test and the navigation tasks. The vocabulary task requires that he point to a picture that corresponds to a word. The navigation tasks require that the participant navigate through 3-dimensional mazes rendered on the computer. The participant will navigate through the maze using a joystick or keys on the keyboard. This will take about one hour and a half.

I will be asked to fill in a health questionnaire that will be used to describe individuals who participated in our study in any ensuing publication arising from this research. The information provided in the questionnaire is confidential and will not be used to identify **my** son personally.

The study does not subject my son to any known risk other than potential boredom and difficulties with completing the tasks. Due to the nature of the 3-dimensional mazes there is a small chance my son may experience dizziness, headaches or mild nausea. My son will be encouraged to express himself and the testing will be stopped immediately if any of these symptoms are experienced. The experimenter will at all times during the testing ensure to the best of his/her knowledge that the participant is comfortable. If the experimenter sees that my son is fatigued or is experiencing discomfort, the testing will be stopped immediately.

My son's results will be kept confidential and he will not be identified in any way when the results are published. My son's data will be recorded in a computer and stored according to a codified name that includes only his initials and the day of testing. If for whatever reason my son's results are not used for this study, they may be used for another

study as a comparison for another condition.

I will receive \$25.00 as compensation plus remuneration for parking expenses. My son can keep the rewards he will receive during the study. Payment is not dependent on whether or not he is able to complete the study; payment will be made when the study is either completed or terminated.

My son's participation is voluntary and he is under no obligation to participate in this study. He may withdraw from the study at any time without any negative consequences.

I have been provided with a copy of this consent form and I have had the opportunity to ask any questions that I may have about the study, and all of my questions have been answered.

Having thoroughly read, understood, and had full explanation of this consent form, I voluntarily consent to my child participating in this research study conducted by Drs. XXX and XXX. If I have any questions, I may contact Dr. XXX. If I have any questions regarding the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, XXXXX or ethics@uottawa.ca.

There are two copies of the consent form, one of which is mine to keep.

Experimenter

Signed

Name of Child

Name of Parent

Signed

Date

Appendix C

Assent Form

We will be asking you to play a type of game on the computer today. You will be asked to find a toy on the computer. You will have to use the joystick (or keyboard) to walk through a maze to find a toy. When you find the toy, you will be given a small gift that you may keep after the study. This experiment will help researchers understand how humans find things in a maze. Pls let me know if you don't feel good, if you feel nauseous, or dizzy. You can stop the game at any time you want. You can also take a break at any time. You can also ask questions anytime.

If that is okay with you to play the game please make an X in the box below.

Experimenter

Signed

Date

Appendix D

Date: _____

History Questionnaire

Participant Information:

Name: _____

Date of Birth (D/M/Y): _____

Age: _____

Handedness: (1) Left (2) Right (3) Both

Parent Information:

Name: _____

Home Address: _____

Home Phone: _____

Signature of Responsible Party: _____

We are interested in your son's personal history because it may help us to better understand the results of our study. Your answers to a few short questions will aid us in this effort. Please do not hesitate to skip questions you do not feel comfortable answering or for which you do not know the answer. All answers will be kept strictly confidential. Thank you for your help.

Language:

Place of Birth: _____

Languages Spoken (in order of fluency): _____

Primary Language: _____

(If you answered English, go directly to Question 1.)

At what age did your child first learn English? _____

At what age did he become fluent in English? _____

Medical History:

1. Does your child suffer from visual problems YES / NO

Last visit to the optometrist: _____

Circle all of the following that apply:

(A) Nearsighted / Farsighted

(B) Glasses / Contact Lenses

(C) Colour Blind

(D) Strabismus (Lazy eye)

2. Has your child been unconscious, had a head injury or had blackouts in the last year? YES / NO

If yes: Cause: _____
Duration: _____
Treatment: _____
Outcome: _____

3. Has your child been seriously ill or hospitalized in the past 6 months? YES / NO

If yes: Cause: _____
Duration: _____

4. Has your child ever suffered from seizures? YES / NO

If yes, what was the Age of onset: _____
Frequency: _____
Treatment: _____
Nature: _____

5. Is your child suffering from diabetes? YES / NO

If yes, what was the Age of onset: _____

6 Any injuries to or motor difficulties with the arms and/or hands? YES / NO

If yes, what is/was the nature? _____

7. Is your child currently taking medication? YES / NO

If yes, pls list ALL medications

8. If your child is taking medication for ADHD, pls indicate when was the last time he took his medication.

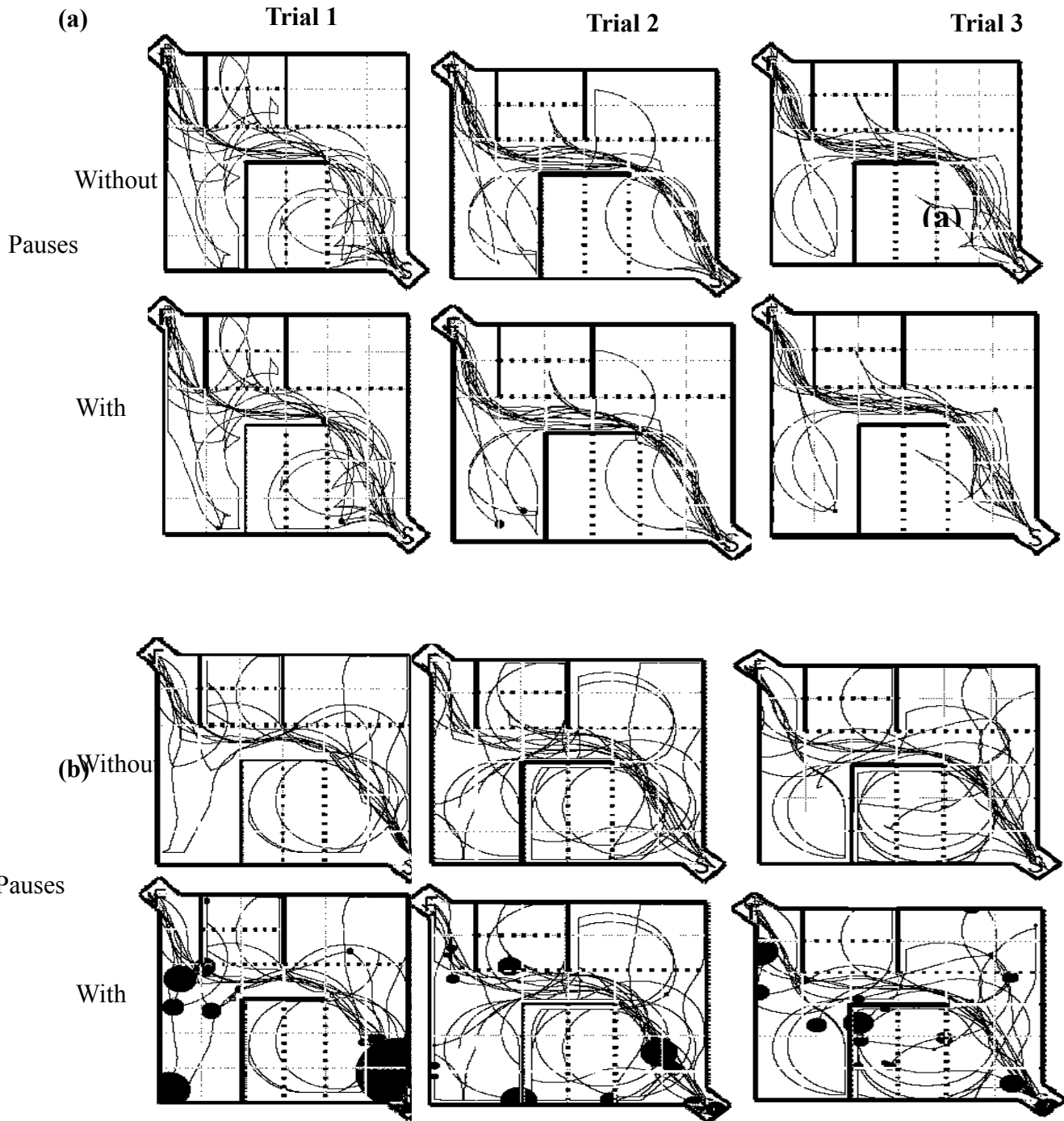
9. Does your child currently have any medical problems? YES / NO

If yes, pls explain

10. Please provide us with any additional information you feel may influence your child's performance in this study, including events that may have taken place today.

Appendix E-Maze 2

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 2. The dark black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.



Appendix F-Maze 4

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 4. The dark black dots indicate pause points, with larger dots representing a longer duration of pause.

Error zones are depicted by the black dotted lines.

Trial 1

Trial 2

Trial 3

(a)

Without

Pauses

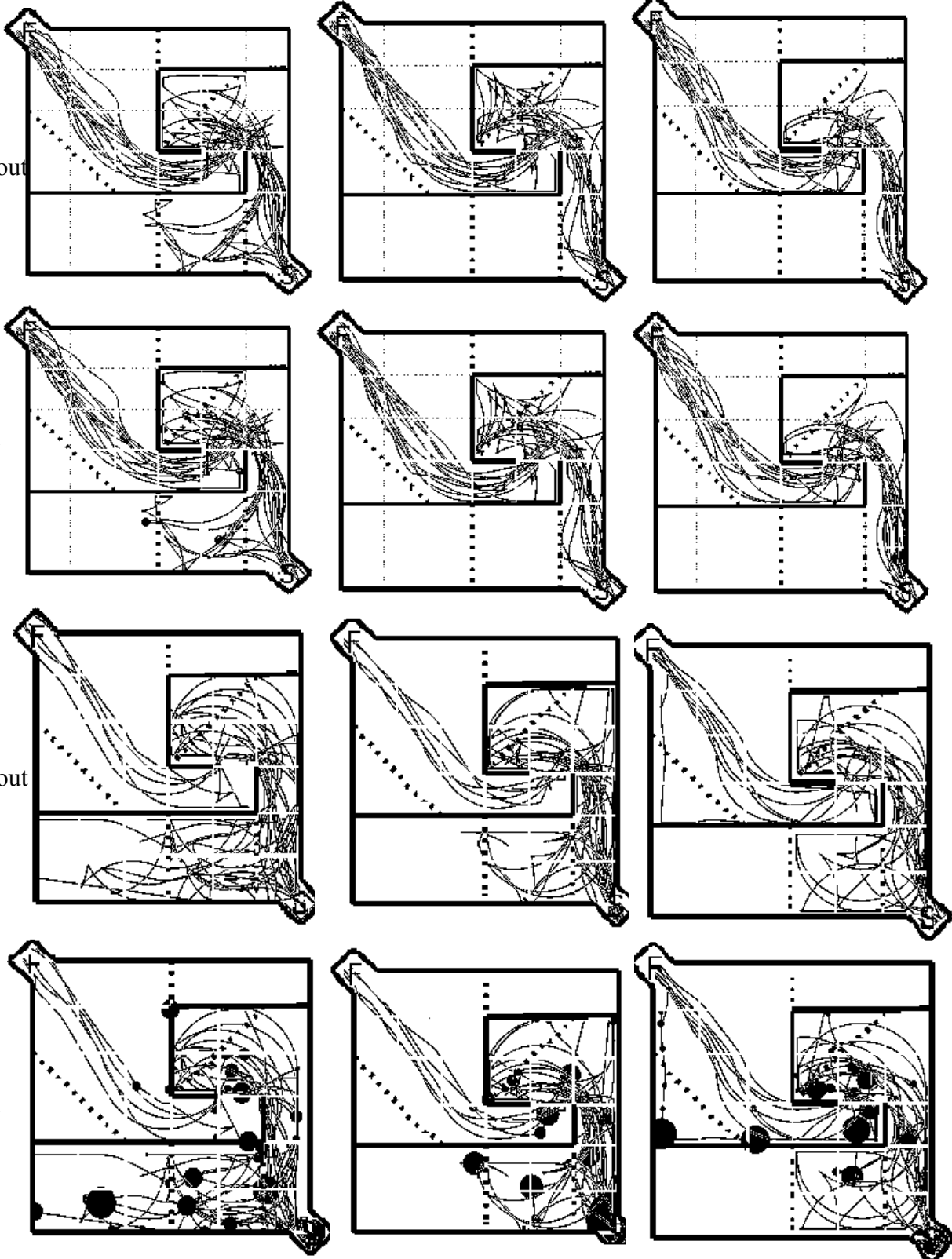
With

(b)

Without

Pauses

With



Appendix G-Maze 5

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 5. The dark black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.

Trial 1

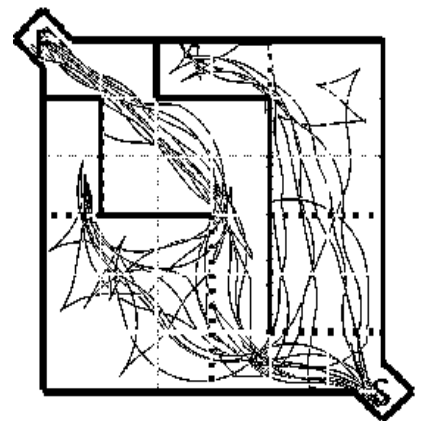
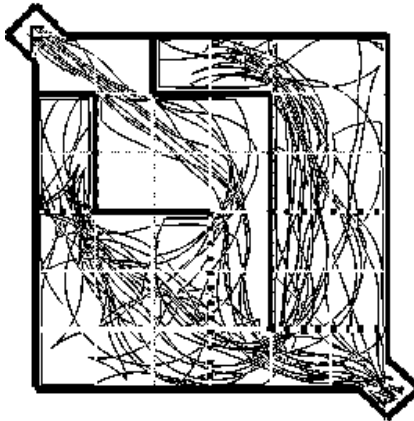
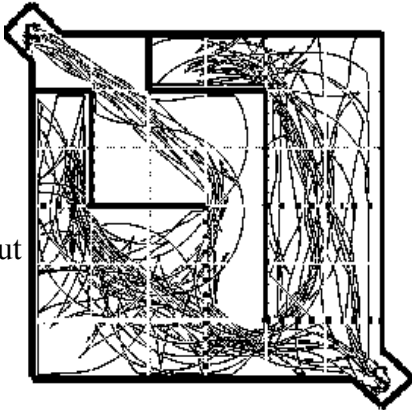
Trial 2

Trial 3

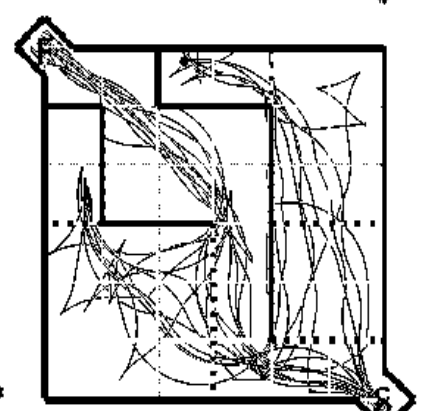
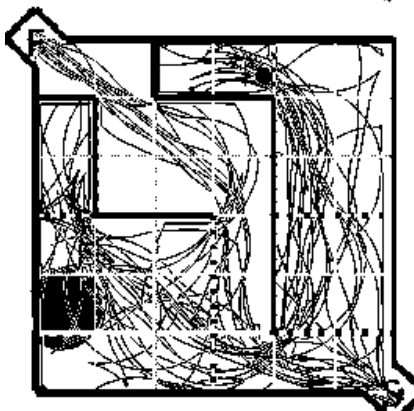
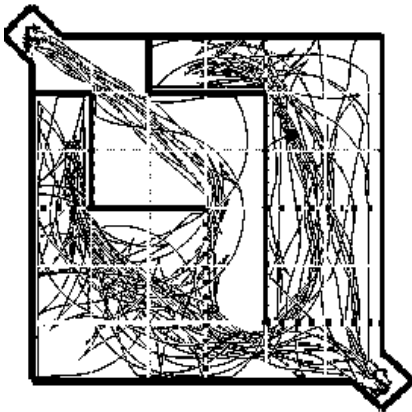
(a)

Without

Pauses



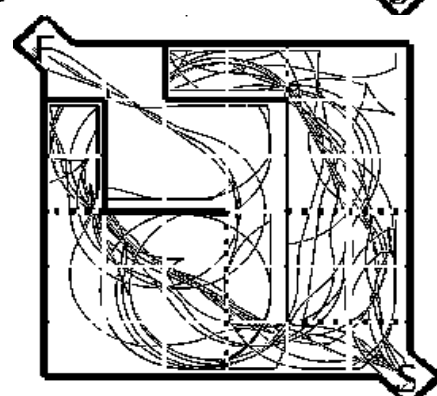
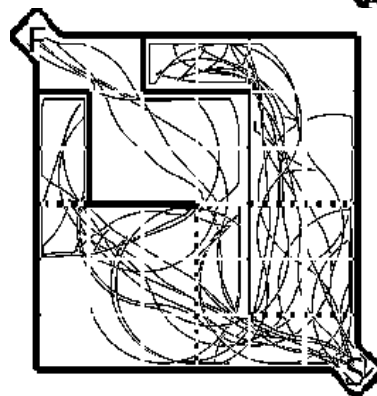
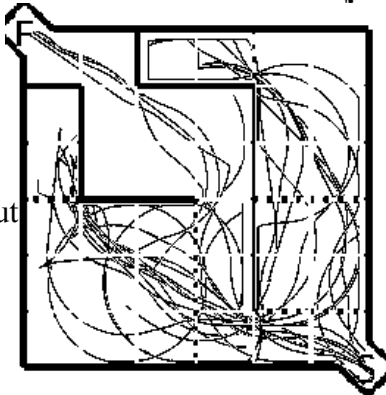
With



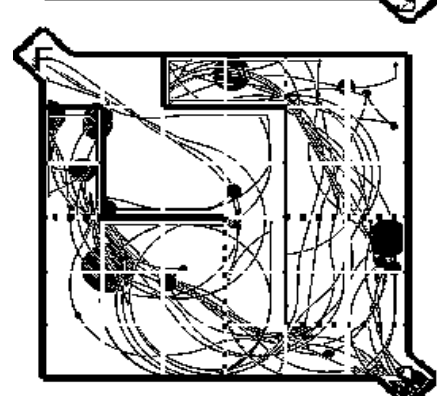
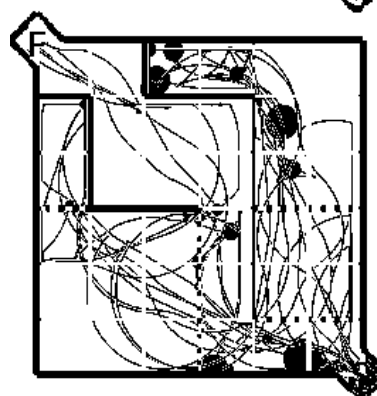
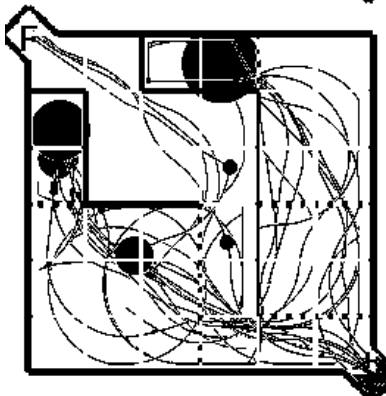
(b)

Without

Pauses

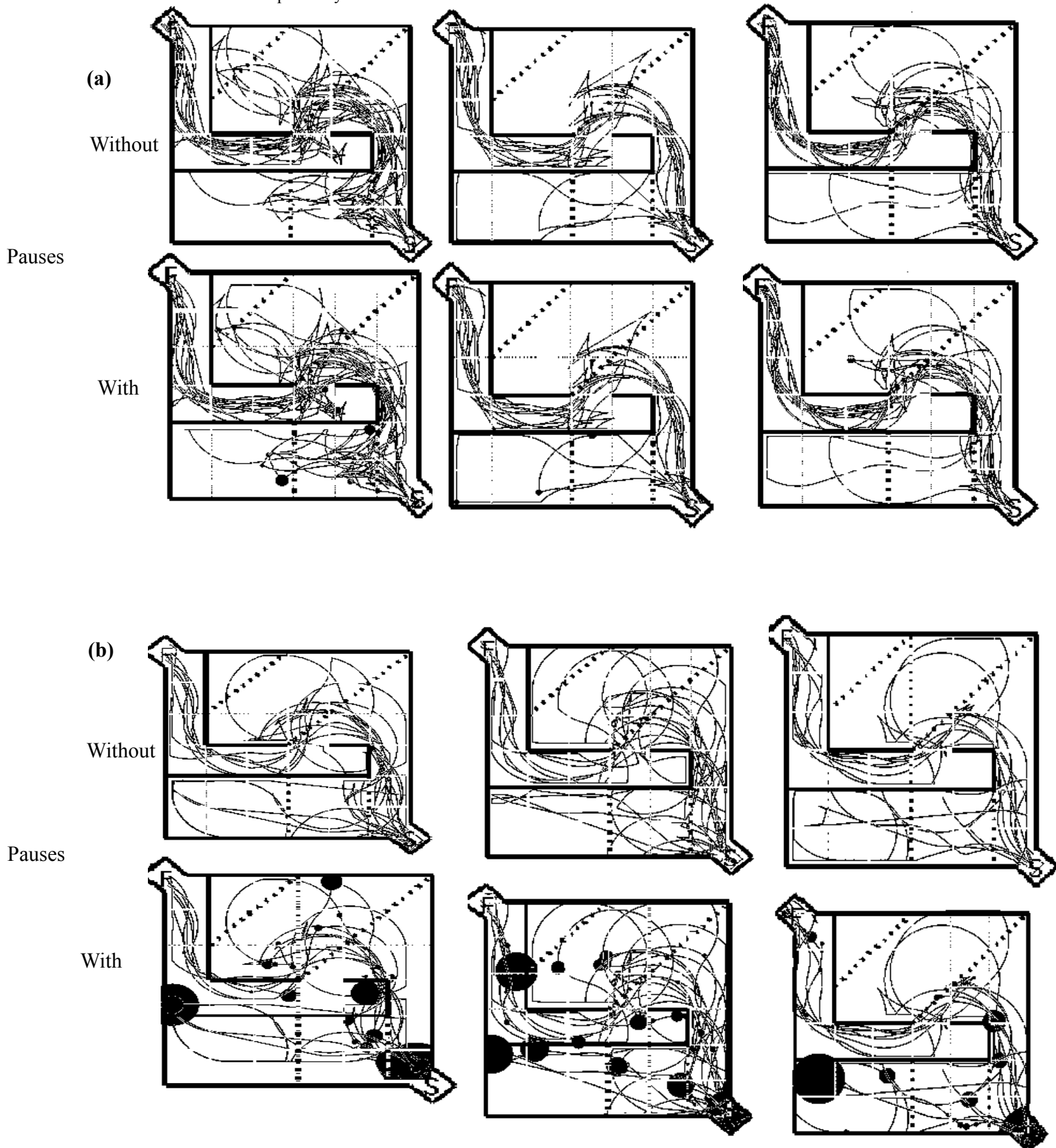


With



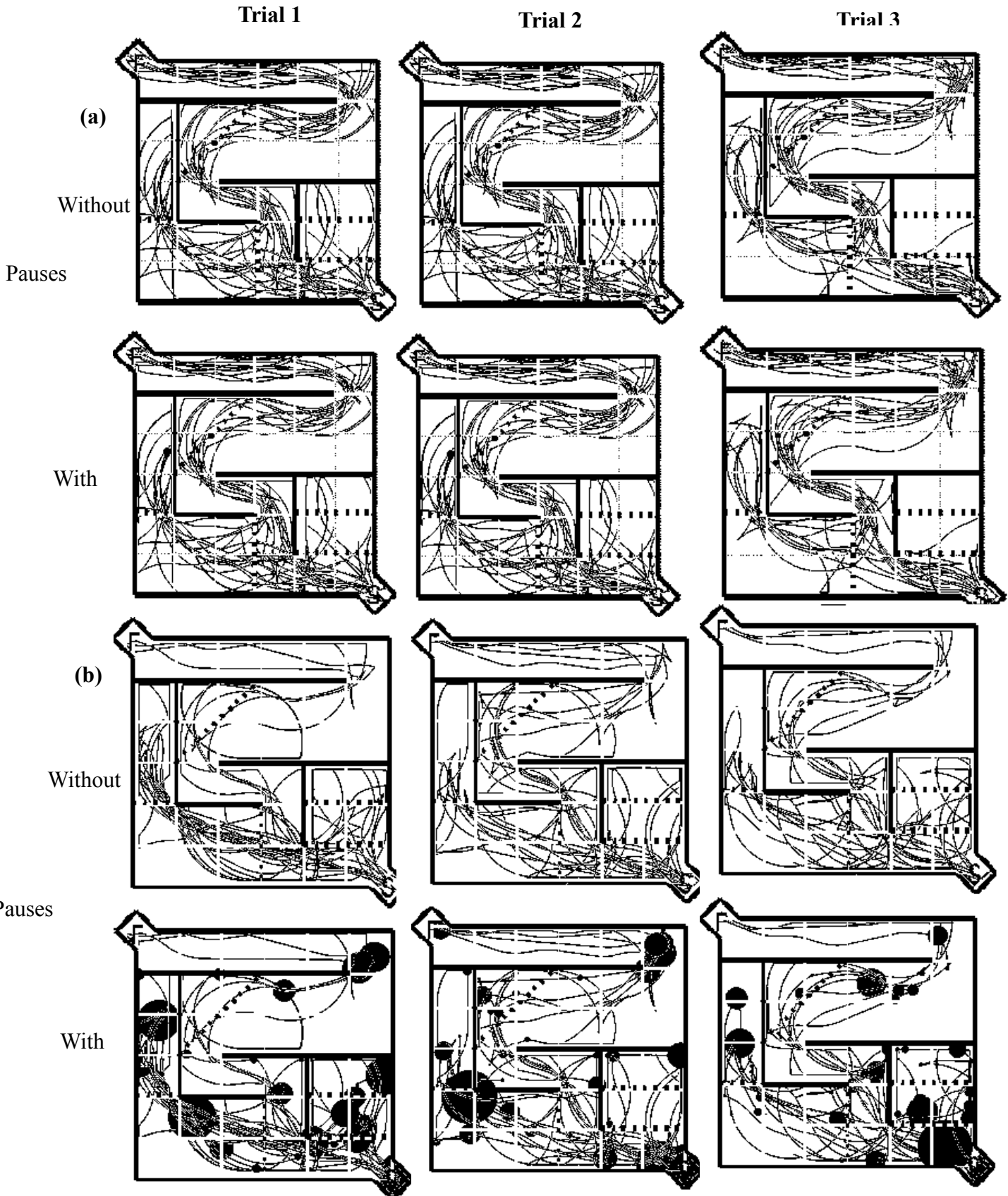
Appendix H-Maze 8

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 8. The dark black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.



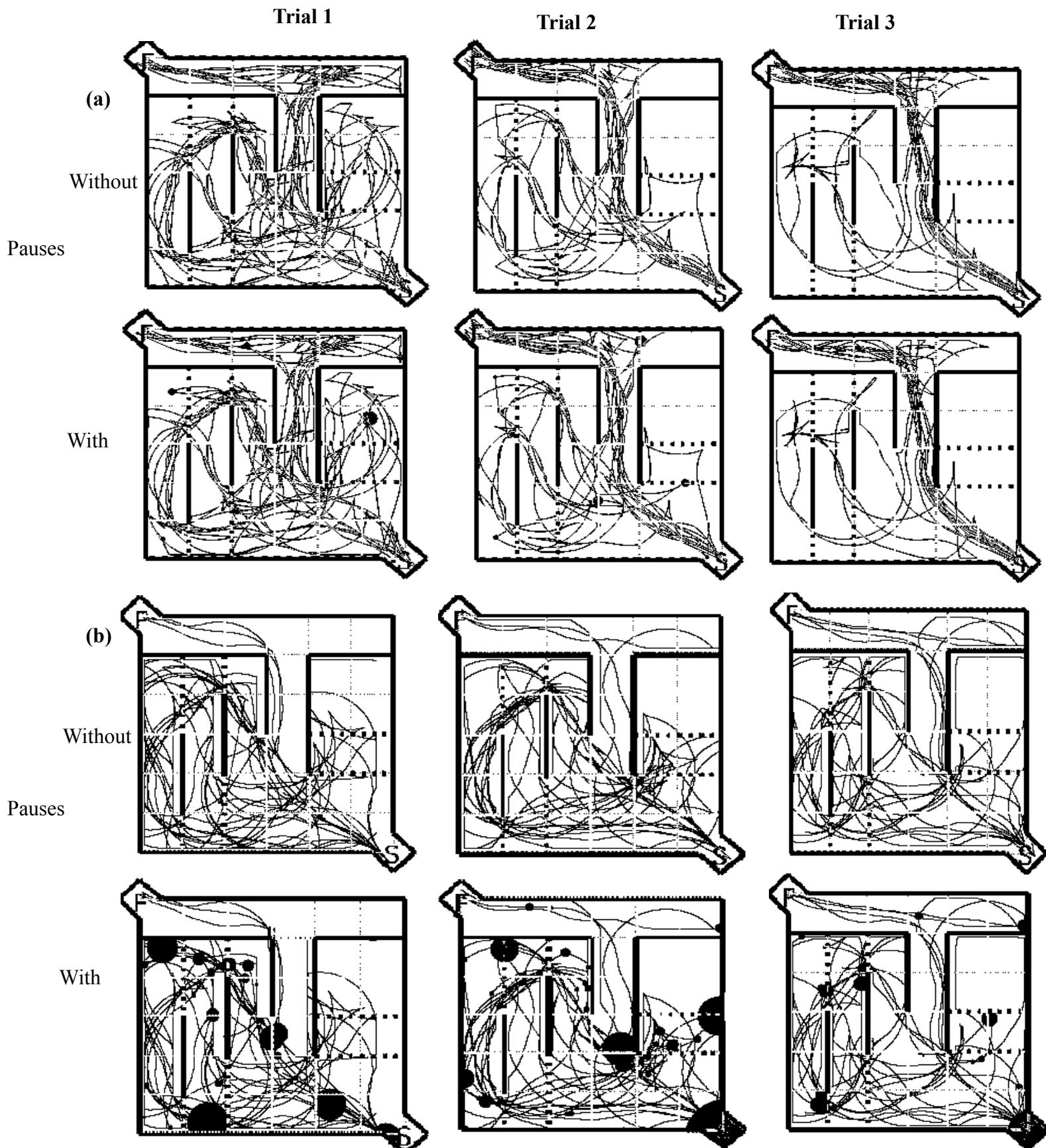
Appendix I-Maze 9

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 9. The dark black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.



Appendix J-Maze 11

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 11. The dark black dots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.



Appendix K- Maze 12

Superimposed maze pathways for controls (a) and FXS participants (b) across trials (1-3) of Hebb-Williams Maze 12. The dark blackdots indicate pause points, with larger dots representing a longer duration of pause. Error zones are depicted by the black dotted lines.

