A Role for the NMDA Receptor in Synaptic Plasticity in the Hippocampus of the *Fmr1* Transgenic Mouse Model of Fragile X Syndrome

by

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Supervisory Committee

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Abstract

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Fragile-X syndrome (FXS) is the most common form of inherited intellectual impairment. Caused by the transcriptional repression of the *Fmr1* gene on the X chromosome, FXS results in the loss of the Fragile-X Mental Retardation Protein (FMRP). Human female patients with FXS are heterozygous for the *Fmr1* mutation whereas males are hemizygous. FXS has been studied far less in females than in males due to a generally less severe clinical phenotype. Previous research has implicated the metabotropic glutamate receptor (mGluR) in synaptic plasticity alterations in the *cornu ammonis* area 1 (CA1) region of the juvenile male *Fmr1* knock-out (KO) hippocampus. In contrast, our investigations into the young adult dentate gyrus (DG) subfield of the hippocampus have revealed *N*-methyl-D-aspartate receptor (NMDAR)-associated impairments in synaptic plasticity. The current study sought to extend these investigations to the young adult female *Fmr1* heterozygous (Het) and *Fmr1* KO mouse as well as investigate NMDAR- and mGluR-mediated long-term depression (LTD) in the DG and CA1 of the young adult male *Fmr1* KO mouse. Input-output curves and paired pulse measures of short-term plasticity were also evaluated in all genotypes. Field electrophysiology revealed a significant impairment in long-term potentiation (LTP) and LTD in male *Fmr1* KO and female *Fmr1* Het mice that was associated with NMDAR alteration. A more robust synaptic protocol was not able to rescue LTP in the male *Fmr1* KO DG. Paired-pulse low-frequency stimulation and (RS)-3,5-dihydroxyphenylglycine (DHPG)-induced mGluR-LTD was intact in all genotypes and brain regions examined. Although further investigation will be required to expand our understanding of FXS and to fully elucidate the mechanisms behind intact synaptic plasticity in the female *Fmr1* KO mouse, our results suggest that NMDARs may be poised as important contributors to hippocampal pathophysiology in FXS.

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List of Abbreviations

ACSF	Artificial cerebral spinal fluid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APV	2-amino-5-phosphonopentanoic acid (NMDA receptor antagonist)
BIC	Bicuculline methiodide (GABAA receptor antagonist)
CA1	*Cornu Ammoni* area 1
CA2	*Cornu Ammoni* area 2
CA3	*Cornu Ammoni* area 3
CaMKII	Calmodulin-dependent protein kinase
CGG	Cytosine-guanine-guanine
CS	Conditioning Stimulation
DAG	Diacylglycerol
DG	Dentate gyrus
EC	Entorhinal cortex
EPSC	Excitatory postsynaptic current
ERK	Extracellular signal-related kinase
fEPSP	Field excitatory postsynaptic potential
Fmr1	Fragile-X Mental Retardation Syndrome 1
FMRP	Fragile-X Mental Retardation Protein
FXR1P	Fragile X Mental Retardation Syndrome-Related Protein 1
FXR2P	Fragile X Mental Retardation Syndrome-Related Protein 2
FXS	Fragile-X Syndrome
GABA	γ-aminobutyric acid
HFS	High frequency stimulation
IP_3	Inositol trisphosphate
KH	K homology domain
LFS	Low frequency stimulation
LPP	Lateral perforant path
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
mGluR	Metabotropic glutamate receptor
miRNA	MicroRNA
MPP	Medial perforant path
mRNP	Messenger ribonucleoprotein
mTor	Mammalian target of rapamycin
NLS	Nuclear localization signal
NES	Nuclear export signal
NMDAR	N-methyl D-aspartate receptor
PI3K	Phosphoinositide 3-kinase
PFC	Prefrontal Cortex
PKC	Protein kinase C
PLC	Phospholipase C
PPD	Paired-pulse depression

PPF	Paired-pulse facilitation
PP-LFS	Paired-pulse low frequency stimulation
RGG box	Arginine-glycine-glycine box

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1. Introduction

1.1 Fragile-X Syndrome – Clinical Manifestations

As the most common form of inherited intellectual impairment (Bagni and Greenough, 2005), Fragile-X Syndrome (FXS) affects approximately 1 in 4000 males and 1 in 8000 females (Turner et al., 1996). FXS is characterized by mild to severe intellectual disability. Behavioural phenotypes include hyperarousal in situations with excess auditory, visual, or tactile stimuli, increased susceptibility to seizures, hyperactivity and attention deficits, shyness and social anxiety, aggressive outbursts, and autistic features such as hand-flapping, perseveration in speech, poor eye contact, and biting (Simko et al., 1989; Hagerman and Hagerman, 2002; Hersh and Saul, 2011). Physical phenotypes of FXS include facial dysmorphology, with an elongated face, large or protruding ears, a prominent forehead and jaw, a high arched palate, and macroorchidism in males (Simko et al., 1989; Garber et al., 2008; Hersh and Saul, 2011). Connective tissue problems may also be a factor contributing to ophthalmologic, orthopedic, and skin manifestations of FXS including strabismus, hyperextensible joints, flat feet, and soft velvet-like skin as well as to the appearance of otitus media, cardiac malformation, and hypertension (Hagerman and Hagerman, 2002; Simko et al., 1989).

1.1.1 Genetic Cause - CGG Repeat Expansion

The most common mutation leading to FXS is an expanded cytosine-guanine-guanine (CGG) repeat tract in the Fragile X Mental Retardation Syndrome 1 (*Fmr1)* gene that, while being highly polymorphic in normal individuals, normally contains between 6 – 54 repeats (Fu et al., 1991). A CGG repeat expansion between 43 – 200 repeats is referred to as a premutation (Fu et al., 1991) and premutations of 55 – 200 repeats are meiotically unstable during maternal germline transmission (Fu et al., 1991). As the CGG repeat length increases within the premutation range, dysregulation of neuronal function can occur as a result of toxic increases in *Fmr1* mRNA (Handa et al., 2005). A CGG

repeat expansion in the range of 200 or more repeats will most often cause hypermethylation of CpG islands (cytosine guanine dinucleotides) in the promoter region and CGG repeat (Pieretti et al., 1991; Hansen et al., 1992). Hypermethylation will lead to transcriptional silencing of the *Fmr1* gene and a loss of its protein product, the Fragile-X Mental Retardation Protein (FMRP) (Pieretti et al., 1991; Hansen et al., 1992). When cells from individuals affected with the full mutation are karyotyped in folate-deficient medium the location of the *Fmr1* gene on the X chromosome appears pale and thin, or fragile, leading to the terminology now associated with this disorder (Sutherland, 1977). The expanded CGG repeat accounts for >99% of mutations causing FXS, however this syndrome also results from other mutations. One such mutation that causes severe FXS is a missense point mutation of an isoleucine to asparagine within the *Fmr1* gene (De Boulle et al., 1993).

1.1.2 Fragile X Mental Retardation Protein (FMRP)

The roots of FXS lay in the loss of FMRP, a protein with 17 exons and 12 different splice variants that has a spectrum of involvement in FXS (Ashley et al., 1993a). FMRP is an RNA binding protein whose activity can be modulated by phosphorylation (Ceman et al., 2003; Narayanan et al., 2008). FMRP has been shown to play an important role in the trafficking of mRNA from the nucleus to the cytoplasm and distal postsynaptic sites (Zalfa et al., 2003; Bassell and Warren, 2008; Dictenberg et al., 2008) as well as in mRNA translational control at polyribosomes (Darnell et al., 2005, 2011). FMRP has been found to associate with the RNA-induced silencing (RISC) complex, suggesting that FMRP may act through microRNAs (miRNAs) to selectively regulate RNA translation (Caudy et al., 2002; Ishizuka et al., 2002; Jin et al., 2004). The association of miRNAs with polyribosomes may also be condusive to translational regulation of FMRP target RNAs (Kim et al., 2004). Data supporting the association of FMRP with the RISC complex include the association of FMRP with Dicer and AGO1 (Jin et al., 2004). In addition, FMRP may be essential for the effects of miR-125b and miR-132 on spine morphology and may act through miR-125b to translationally regulate the GluN2A subunit of the NMDAR (Edbauer et al., 2010). FMRP may also act to regulate the

initiation of mRNA translation through the inhibition of 80S ribosomal complex assembly, as mRNA in the 80S fraction is significantly reduced *in vitro* upon the addition of exogenous FMRP when compared to truncated FMRP (Laggerbauer et al., 2001). Importantly, the association of FMRP with kinesin binding partners (Dictenberg et al., 2008) enables it to function in the trafficking of mRNAs to distal post-synaptic sites where it can function to locally regulate mRNA translation (Comery et al., 1997; Irwin et al., 2000).

FMRP contains six important functional domains: a nuclear localization signal (NLS), a nuclear export signal (NES), two coiled-coil domains, two K Homology domains (KH1 and KH2), and an arginine-glycine-glycine (RGG) box. The presence of a NLS and a NES suggests that FMRP plays a role in the transport of bound mRNAs from the nucleus to the cytoplasm (Eberhart et al., 1996), and indeed FMRP has been shown to be located both in the nucleus and the cytoplasm and has been captured using immunogold electron microscopy in the probable process of shuttling through nuclear pores (Feng et al., 1997). FMRP's coiled coil domains are thought to be involved in protein-protein interactions. The first coiled coil domain has been shown to interact with FMRP, Fragile X Mental Retardation Syndrome-Related Protein 1 (FXR1P) and FXR2P while the second coiled coil domain has been shown to bind to the 60S large ribosomal subunit (Siomi et al., 1996). The ability of FMRP to bind RNA relies on 3 binding domains that may mediate RNA-protein interactions: two KH domains and one RGG box (Bardoni et al., 2006; Melko and Bardoni, 2010), which together bind approximately 4% of the mRNA in the mammalian brain, including FMRP's own mRNA (Ashley et al., 1993b; Brown et al., 1998).

Taken together these findings suggest FMRP is highly involved in the trafficking and translational regulation of mRNAs and give credence to the hypothesis that FMRP forms a messenger ribonucleoprotein (mRNP) complex with mRNA in the nucleus and functions in the trafficking of polyribosomes. Thus FMRP may influence the transport as well as the translation of bound mRNA.

1.2 Hippocampal Neuropathology in FXS Patients

In human fetal brains, the hippocampus is one of the most strongly labeled structures for *Fmr1* mRNA (Abitbol et al., 1993). The hippocampus, in particular the granular layer of the dentate gyrus, is also one of the highest *Fmr1* mRNA expressing areas of the brain in mice (Hinds et al., 1993; See below for a detailed overview of the hippocampus). This suggests that the *Fmr1* gene plays an important role in the hippocampus, a region known to play a critical role in declarative, episodic, and spatial learning and memory (See Squire, 1992 and Eichenbaum, 2003 for a review), including memory trace fixation, brain arousal, and modulation of attention needed to process and register information (Vinogradova, 2001). In agreement, females with FXS have been found to have a reduced activation of the hippocampus during visual memory encoding as well as deficits in episodic memory (Greicius et al., 2004).

Athough there is still some controversy over gross hippocampal structural changes in patients with FXS (Jäkälä et al., 1997; Kates et al., 1997; Hazlett et al., 2009; Lightbody and Reiss, 2009; Greco et al., 2011), differences in hippocampal volume measurements between studies have been suggested to be due to abnormal brain development in FXS and a need for more discriminating methodologies to reveal subtle atypical hippocampal morphology (reviewed in Lightbody and Reiss, 2009).

1.2.1 The Hippocampal Formation

The hippocampus is a C-shaped structure elongated along the dorsal-ventral (also referred to as septotemporal) axis of the brain from the septal nuclei (dorsally) to the temporal lobe (ventrally) (see Figure 1 for a gross morphological localization of the hippocampus in the rodent brain). The term hippocampus was derived from the Greek word for seahorse and was first coined in 1587 by the Greek anatomist Arantius due to a striking resemblance of this brain region to the seahorse (Andersen et al., 2006). Other anatomists compared the arched hippocampal structure of the hippocampus to a ram's horn and in 1742, De Garengeot named the hippocampus *cornu ammonis*, or Ammon's

horn, after the ram symbol for the mythological Egyptian god Amun Kneph (Andersen et al., 2006). Although the hippocampus has retained its initial name, subdivisions of its structure are commonly termed as abbreviations of *Cornu Ammoni* areas 1 (CA1), 2 (CA2), and 3 (CA3). The hippocampal formation includes the hippocampus proper (CA1, CA2, and CA3 sub-regions), the dentate gyrus (DG), subiculum, parasubiculum, presubiculum, and entorhinal cortex (EC) (Andersen et al., 2006; Figure 1).

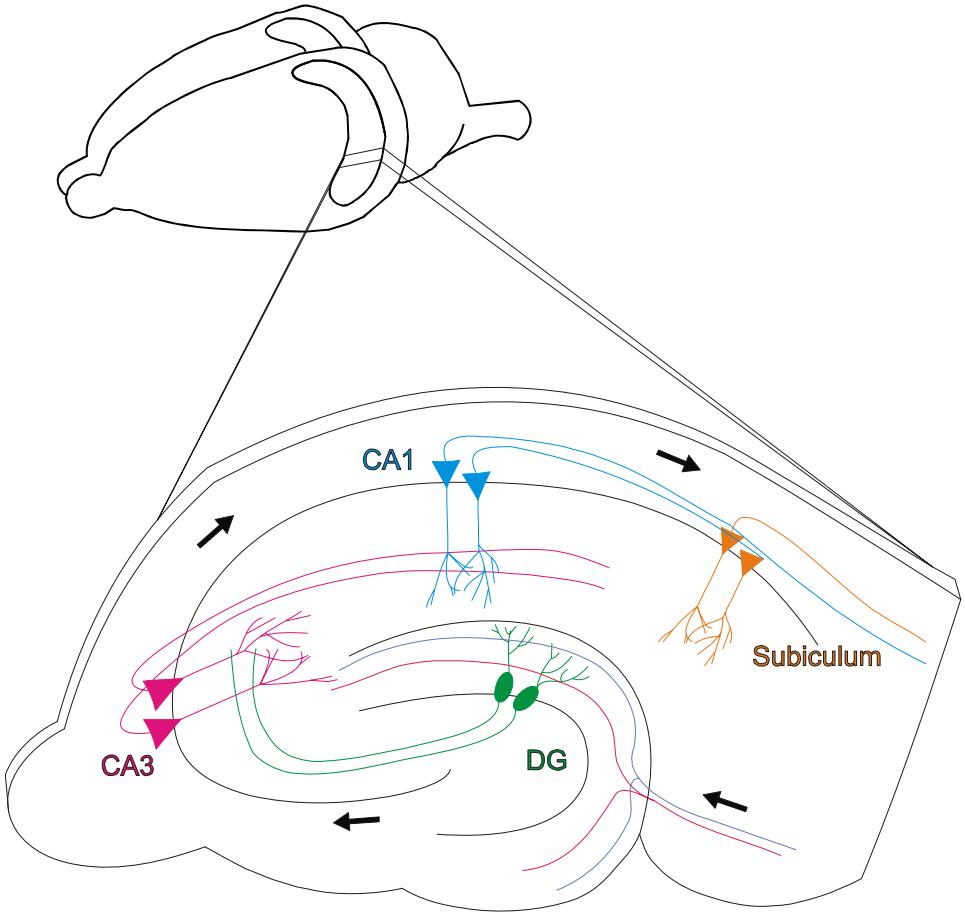

Figure 1. Gross morphological localization of the rodent hippocampus and basic anatomy of the *in vitro* hippocampal slice. Abbreviations: dentate gyrus (DG); *cornu ammonis* area 3 (CA3); *cornu ammonis* area 1 (CA1). Arrows denote the predominantly unidirectional flow of information through the hippocampus. Information will travel from the entorhinal cortex projections to the granule cells in the DG (via the perforant pathway); from the DG to pyramidal cells of the CA3 (via the mossy fiber pathway); from the CA3 to pyramidal cells in the CA1 (via the schaffer collateral pathway); from

the CA1 to pyramidal cells in the subiculum; and finally from both the CA1 and subiculum to the entorhinal cortex (not shown) (adapted from Andersen et al., 1971).

1.2.1.1 Functional Connectivity

The functional connections of the hippocampus flow in a predominantly unidirectional direction starting in the EC (Figure 1). Efferent fibers from the EC form a compact structure called the angular bundle that travels into the hippocampal formation (Amaral and Whitter, 1989; Andersen et al., 2006). These fibers form what is known as the perforant pathway, which is the major path in which neocortical information reaches the hippocampus (Andersen et al., 2006). The perforant pathway will bend into the transverse plane of the hippocampus and travel through the pyramidal layer of the subiculum to enter the dentate gyrus (Amaral and Whitter, 1989). Perforant pathway fibers bound to innervate the DG molecular layer will bifurcate, sending projections to the suprapyramidal (upper) and infrapyramidal (lower) blades of the DG (Andersen et al., 2006). The main cell type in the DG is the granule cell, which extends its dendrites into the molecular layer where three main tracts of information exist: the medial perforant path, the lateral perforant path, and the commissural associational path (Andersen et al., 2006). The medial perforant path originates from the medial aspects of the EC and synapses onto distal dendrites of granule cells in the middle region of the molecular layer (Amaral and Whitter, 1989; Andersen et al., 2006; Hunsaker et al., 2007). The lateral perforant path from the lateral regions of the EC will innervate the granule cell distal apical dendrites that lie closer to the hippocampal fissure (denoted as the space between the DG and CA1 region in Figure 1) (Amaral and Whitter, 1989; Andersen et al., 2006). The commissural associational path is the third path of information traveling to the DG and originates from efferent connections from the contralateral DG that target proximal dendrites of granule cells (Andersen et al., 2006).

From the DG, granule cell axons create the mossy fiber pathway that collateralizes into the hilus region (where glutamatergic interneuron mossy cells will then feedback to innervate the molecular layer of the DG) (Scharfman, 2007). In addition to

this “feedback excitation”, granular neurons will also target and thus be modulated by interneuron basket cells in the DG and the hilus (Scharfman, 2007). Basket cells will release gamma-aminobutyric acid (GABA) in response to granule cell input, forming what is known as “feedback inhibition” (Scharfman, 2007). Information flow down perforant pathway fibers will also activate basket cells in the DG, forming what is known as “feedforward inhibition” (Scharfman, 2007). The mossy fiber pathway will continue through the hippocampus to target the CA3 region, creating en passant synapses onto thorny excrescences of CA3 pyramidal cell dendrites (Andersen et al., 2006; Scharfman, 2007; Vogt and Nicoll, 1999).

In the CA3, the axons of pyramidal neurons will give rise to the Schaffer collateral commissural fiber system, which directly innervates the CA1 and collateralizes within the CA3 (collateral axons are termed the longitudinal association bundle) on the ipsilateral and contralateral sides of the brain (Amaral and Whitter, 1989; Andersen et al., 2006; Scharfman, 2007). The CA2 region will also relay CA3 input to the CA1 region and itself receives input from the EC (Bartesaghi and Gessi, 2004; Chevaleyre and Siegelbaum, 2010; Shinohara et al., 2012). Within the CA1 there are again three main tracts of information flow: (1) the stratum lacunosum-moleculare is the layer found most distal to the pyramidal cell bodies (towards the hippocampal fissure) and is a region innervated by EC afferents; (2) the stratum radiatum is one of two layers innervated by the Schaffer Collateral Commissural afferents; and (3) the stratum oriens, located above the pyramidal cell layer, is also innervated by Schaffer Collateral Commissural afferents (Andersen et al., 2006). Axonal fibers from CA1 pyramidal cells will project to pyramidal cells in the subiculum, which also receives input from the EC (Andersen et al., 2006). From here, axonal projections will be sent to the presubiculum and parasubiculum, and finally, back to the EC (Andersen et al., 2006).

1.2.1.2 The Trisynaptic Circuit and the Lamellar Hypothesis

The flow of information from the EC through the DG, CA3, and CA1 has historically been referred to as the trisynaptic circuit, in reference to the three main sites

of synaptic connections this pathway creates (Andersen et al., 1971; Amaral, 1993) . In 1971, Per Andersen et al. noted that it was possible to cut thin hippocampal slices in a direction transverse to the hippocampal longitudinal axis in such a way as to preserve its fiber orientation. This idea that the hippocampal circuit was organized into strips of functionally independent parallel circuits was the basis of the lamellar hypothesis (Andersen et al., 1971). As Amaral and Witter (1989) reviewed, the major hippocampal fibers are more divergent than separate distinct lamellae (except possibly the mossy fiber pathway) and we now know that many transverse connections do exist in the hippocampus. The hippocampal lamella hypothesis was revisited by Per Andersen et al. in 2000, where they concluded that cells located within a lamellae in the hippocampus will be the most activated by afferent connections within this transverse plane. Although there may be divergent connections outside of this transverse axis of the hippocampus, the lamellar hypothesis and the relatively intact connections that exist along the transverse plane of the hippocampus have allowed for the extensive use of *in vitro* slice preparations to investigate hippocampal functioning.

1.3 Synaptic Plasticity

One of the key contributions to our understanding of possible memory mechanisms was the discovery of long-term potentiation (LTP) by Lomo and Bliss in the laboratory of Per Anderson (Bliss and Lomo, 1973; Lømo, 2003). LTP and its reverse, long-term depression (LTD), are now known as forms of synaptic plasticity, defined as the ability of synapses to alter the efficiency of their synaptic communication. A model whereby the connections between neurons could be strengthened by activity was first popularized by Hebb (1949) and synaptic plasticity is now thought of as the leading neurobiological model for the mechanisms underlying learning and memory. Although a consensus has not yet been attained regarding the exact contribution of synaptic plasticity for learning and memory, LTP in particular has been postulated to play a role in memory formation as an attention or arousal device, rather than a mechanism *per se* for memory retrieval and storage (Shors and Matzel, 1997).

1.3.1 Glutamatergic Receptors

There are three major receptors that are involved in synaptic plasticity in the hippocampus. These include the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), the *N*-methyl-D-aspartate receptor (NMDAR), and the metabotropic glutamate receptor (mGluR). As glutamate is the main excitatory neurotransmitter in the hippocampus, it is no surprise that all three receptors bind glutamate to modulate their activity (Andersen et al., 2006).

The AMPAR is composed of four subunits (GluA1-4) and gates a cation-selective channel that fluxes sodium and potassium (Hollmann and Heinemann, 1994). AMPARs can be developmentally regulated (Pagliusi et al., 1994) and are impermeable to calcium in postnatal life (Kumar et al., 2002). In hippocampal pyramidal neurons AMPARs are either GluA1/2 or GluA2/3 tetramers (Wenthold et al., 1996). AMPARs exhibit fast binding kinetics, a high open probability with rapid deactivation, and are important for the majority of fast excitatory neurotransmission in the brain (Andersen et al., 2006; Kumar et al., 2002; Wenthold et al., 1996).

The NMDAR is an ionotropic glutamate receptor that mainly exists as a heteromeric tetramer, possessing two obligatory GluN1 subunits and two regulatory GluN2 (GluN2A-D) or GluN3 (GluN3A-B) subunits (Cull-Candy and Leszkiewicz, 2004). In the hippocampus, GluN3 subunits do not appear to play an important role in synaptic plasticity (Anderson et al., 2006). While the regulatory GluN2 subunits contain the binding site for glutamate, the obligatory GluN1 subunits contain the binding site for the co-agonist glycine or D-serine (Cull-Candy and Leszkiewicz, 2004; Paoletti and Neyton, 2007). Along with permeability of monovalent cations such as sodium and potassium, NMDARs are also highly permeable to calcium ions and therefore play a large role in many forms of synaptic plasticity. A unique feature of the NMDAR is it functions as a molecular coincidence detector, as its channel will only open if glutamate binds to the receptor while depolarization of the post-synaptic membrane occurs (Cull-Candy and Leszkiewicz, 2004). This voltage dependence arises from an intrinsic

magnesium block within the NMDAR channel pore (Johnson and Ascher, 1990). The necessity of dual input allows the NMDAR to function as a molecular coincidence detector, only responding to signals occurring when both pre-synaptic glutamate release and post-synaptic depolarization occur simultaneously (as happens during synaptic transmission) (Cull-Candy and Leszkiewicz, 2004). As such, NMDARs are able to distinguish concurrent signals, allowing for greater signaling specificity.

The NMDAR is a critical receptor for a variety of processes. Deletion of the gene encoding the GluN1 subunit of the NMDAR leads to respiratory failure and death shortly after birth (Lee et al., 2005). NMDARs are also critical for the development and proper orientation of pre-synaptic terminal arbors as well as post-synaptic dendritic branching (Lee et al., 2005), thus playing a crucial role in the proper development of neuronal circuitry. The subunit composition of the NMDAR may vary across different brain regions and as a function of animal age and neuronal activity levels (Cull-Candy and Leszkiewicz, 2004). The GluN1 subunit is obligatory and almost ubiquitously expressed in the brain (Moriyoshi et al., 1991). The hippocampus expresses large amounts of the GluN2A and GluN2B subunits (Monyer et al., 1994). During early neuronal development the GluN2B subunit is predominantly expressed and as neuronal development progresses GluN2A expression increases, resulting in GluN2A becoming the predominant subunit at maturity (Monyer et al., 1994; Lopez de Armentia and Sah, 2003).

GluN1 subunits have eight functional isoforms (each made up of three splice varients), which differentially influence the inhibition of the NMDAR by protons and zinc or its enhancement by polyamines (Cull-Candy and Leszkiewicz, 2004). Complexes with either GluN1 or GluN2 subunits alone do not form functional channels (Pérez-Otanõ et al., 2001). GluN2A and GluN2B subunits act to functionalize NMDARs in the DG, regulating such properties as channel kinetics (deactivation time), affinity for glutamate, sensitivity to magnesium, and calcium permeability (Cull-Candy and Leszkiewicz, 2004). The GluN2A and GluN2B subunits of the NMDAR are thought to play important roles in synaptic plasticity (Yashiro and Philpot, 2008) and are able to

interact with different intracellular signalling pathways through their cytoplasmic domains (Kim et al., 2005).

mGluRs are also glutamate-binding receptors but are distinct from ionotropic AMPARs and NMDARs. mGluRs contain seven transmembrane domains and are coupled to G proteins that mediate intracellular signaling cascades (Kunishima et al., 2000). mGluRs are found as dimers that exist in a dynamic equilibrium between an active and an inactive state; binding of a glutamate molecule will stabilize the active state (Kunishima et al., 2000). mGluRs have three distinct subtypes. Group I mGluRs (mGluR1 and mGluR5) are mainly found at the post-synaptic membrane in the perisynaptic region (Lujan et al., 1996). Their activation elicits excitatory effects through a phospholipase C (PLC) pathway that can activate inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Coutinho and Knopfel, 2002). However, depending on the conditions, group I mGluRs may also act to depress excitatory postsynaptic currents (Rodríguez-Moreno et al., 1998). Group II (mGluR2 and mGluR3) and group III (mGluR4, 6, 7, and 8) receptors are mainly located in pre-synaptic membranes (Coutinho and Knopfel, 2002) and act to decrease excitatory transmission (Bushell et al., 1996; Macek et al., 1996; Coutinho and Knopfel, 2002).

1.3.2 Long-term Synaptic Plasticity Mechanisms

The mechanisms leading to the expression of LTP start with the synaptic release of glutamate that will diffuse across the synaptic cleft to bind to AMPARs and NMDARs. Binding of glutamate will cause a conformational change in the AMPAR that permits the flow of monovalent cations into the intracellular space (Nakagawa et al., 2005), thus depolarizing the postsynaptic membrane and releasing the intrinsic magnesium block of the NMDAR. This depolarization in addition to the binding of glutamate and D-serine will allow the NMDAR to undergo a conformational change, causing the influx of monovalent cations and calcium (Jahr and Stevens, 1987). This local rise in calcium will activate protein kinases, such as calcium calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), which will phosphorylate Ser-

831 on the GluA1 subunit of the AMPAR (Barria et al., 1997; Lee et al., 2000). Phosphorylation of Ser-831 will increase AMPAR conductance as well as AMPAR insertion into the post-synaptic membrane from an intracellular pool (Lu et al., 2001; Malenka and Bear, 2004). Pre-synaptic mechanisms may also contribute to LTP, such as a change from 'kiss-and-run' vesicle neurotransmitter release to full fusion of the vesicle with the pre-synaptic membrane (Lisman and Raghavachari, 2006).

Neurons must be able to bidirectionally modify the strength of their synaptic connections in order to respond effectively to changes in synaptic activity, and as such, they also have the capacity to undergo LTD in addition to LTP. This form of synaptic plasticity may also require both AMPAR and NMDAR activation, however a slow and small change in post-synaptic calcium levels (as opposed to the quick rise necessary for the induction of LTP) will induce LTD (Mulkey and Malenka, 1992). This calcium influx may preferentially activate protein phosphatases, such as the calcium sensitive Ca^{2+}/calmodulin-dependent protein phosphatase (calcineurin) which activates protein phosphatase 1 by inactivating inhibitor-1 (Mulkey et al., 1994). Interestingly, LTD is associated with the dephosphorylation of Ser-845 of the GluA1 subunit of the AMPAR, a PKA substrate (Lee et al., 2000; Malenka and Bear, 2004). Dephosphorylation of Ser845 will decrease AMPAR channel open probability (Banke et al., 2000) and activate clathrin and dynamin-mediated mechanisms to internalize AMPARs (Lee et al., 2002).

mGluRs can also be involved in LTD, further decreasing AMPAR post-synaptic expression and leading to a reduction in the ability of the post-synaptic cell to respond to stimuli. During LTD, group I mGluRs mediate the activation of PLC through G-protein coupled signaling, causing the production of DAG and IP_3 (Schoepp et al., 1994; Gladding et al., 2009). DAG can lead to activation of PKC while IP_3 will activate calcium release from internal stores (Pin and Duvoisin, 1995). In the hippocampus, group I mGluR activation has been shown to activate a pathway involving a phosphoinositide 3-kinase (PI3K), the serine/threonin-specific protein kinase Akt, and the mammalian target of rapamycin (mTor), in addition to a parallel pathway involving extracellular signal-related kinase (ERK) activation (Gallagher et al., 2004; Hou and Klann, 2004). Another

cascade involved in the induction of mGluR-mediated LTD in the hippocampus may involve a mitogen-activated protein kinase (P38 MAPK) signaling pathway (Bolshakov et al., 2000; Huang et al., 2004). Retrograde signaling may also help to induce mGluR-LTD in the hippocampus, such as the diffusion of endocannabinoids across the synaptic cleft acting to reduce the probability of presynaptic neurotransmitter release (Varma et al., 2001).

1.3.3 Short-term Synaptic Plasticity

In addition to post-synaptic modulation of long-term plasticity, the pre-synaptic terminal can also play a role in synaptic plasticity. Here, neurotransmitter release will occur in response to calcium influx through voltage-dependent calcium channels (Zucker and Regehr, 2002). Once neurotransmitters are released into the synaptic cleft, calcium ions in the presynaptic cytoplasm will be disposed of by calcium buffers and extrusion mechanisms. However, this can take several minutes to achieve (Andersen et al., 2006). Successive stimulation of the pre-synaptic afferents can therefore increase the effectiveness of transmitter release due to an additive effect of calcium influx and residual calcium in the pre-synaptic terminal (Zucker and Regehr, 2002). This increase in the effectiveness of transmitter release in response to successive stimulation will be influenced by the probability of release from the readily releasable pool of neurotransmitters located near the pre-synaptic membrane. This can be influenced by previous incomplete fusion of vesicles with the pre-synaptic membrane in response to an initial stimulation (Andersen et al., 2006; Zucker and Regehr, 2002).

At many synapses, synaptic depression, rather than facilitation, can occur in reponse to rapid successive stimulation of the pre-synaptic terminal. A depression of the response can be observed if the readily-releasable pool of vesicles has been depleted, the release of modulatory substances from pre-synaptic or post-synaptic terminals act to inhibit the release of neurotransmitter, or ligand-gated receptors desensitize, thereby reducing the ability of the pre-synaptic terminal to respond to activity (Zucker and Regehr, 2002). Pre-synaptic vesicle transmitter release thus has an ability to undergo

short-term plasticity, or use-dependent plasticity, in response to stimulation. These short-term changes in vesicle release probability may last up to a few minutes and have the ability to increase or decrease transmission (Andersen et al., 2006).

1.4 The *Fmr1* KO Mouse

An important advance in the understanding of FXS was the development of the *Fmr1* knock-out (KO) mouse by the Dutch-Belgium Fragile X Consortium (1994). This transgenic mouse was created using homologous recombination in embryonic stem cells of a vector containing a neomycin cassette targeted to exon 5 of the *Fmr1* gene (Bakker et al., 1994). The *Fmr1* KO mouse lacks FMRP but maintains normal fertility and health (Bakker et al., 1994). Adding to the success of this mouse model is the fact that the *Fmr1* gene is highly conserved between human and mouse, having 97% homology at the amino acid level (Ashley et al., 1993b). At the nucleic acid level, the human and murine *Fmr1* genes have 95% homology, both possessing the same number of introns and exons (Kirkpatrick et al., 2001). Though intron size varies, exon size is identical between the murine and human *Fmr1* gene with the exception of exons 1, 11, and 17 (Kirkpatrick et al., 2001). Exons 1 and 17 correspond to the first and last exon and have not been found to correspond to the functional motifs FMRP uses (Kirkpatrick et al., 2001). Exon 11 also does not correspond to functional motifs of FMRP and has been shown to not affect RNA-binding activity *in vitro* (Price et al., 1996; Kirkpatrick et al., 2001). Importantly, the expression level and pattern of *Fmr1* mRNA and FMRP is similar between humans and mice (Hinds et al., 1993), making the *Fmr1* KO mouse a good model in which to study the neurobiological ramifications of FXS.

Though *Fmr1* KO mice lack FMRP they still contain an intact *Fmr1* gene promotor. This may lead to aberrant transcription and the production of abnormal RNA species in this mouse model (Bakker et al., 1994; Yan et al., 2004). It must be remembered however that although there is *Fmr1* mRNA produced in the *Fmr1* KO mouse model, a large fraction of human subjects with FXS may also have varying levels of *Fmr1* mRNA even though a full length hypermethylated CGG repeat expansion is

present with little to no FMRP being produced (Tassone et al., 2001). Although previous studies have not shown mRNA produced in human subjects, mRNA was found to be present with a more robust PCR protocol (Tassone et al., 2001). The CGG repeat expansion was found to also be resistant to cleavage by enzymes that are sensitive to DNA methylation (Tassone et al., 2001). This suggests that enzyme resistance and absence of FMRP may not necessarily indicate a transcriptionally silent *Fmr1* gene and therefore the *Fmr1* KO mouse model is still a viable model to use for FXS research.

1.4.1 Hippocampal Behavioral Deficits in the *Fmr1* KO mouse

Fmr1 KO mice show clear alterations in behaviours associated with proper functioning of the hippocampus. These behavioural alterations include a decrease in basal anxiety levels (Peier et al., 2000; Qin et al., 2002; Spencer et al., 2005; Bilousova et al., 2009; Eadie et al., 2009; Liu and Smith, 2009; Min et al., 2009; Yuskaitis et al., 2010), an increase in anxiety in response to social situations (Spencer et al., 2005), and abnormalities in social interactions with lower levels of response to social novelty (Spencer et al., 2005; Liu and Smith, 2009). Passive avoidance is another task that has been linked to hippocampal functioning (Isaacson and and Wickelgren, 1962; Lorenzini et al., 1996) and has been shown to be impaired in *Fmr1* KO mice (Liu and Smith, 2009; Qin et al., 2002; Yuskaitis et al., 2010 but also see Bakker et al., 1994). Another behavioural task that requires the hippocampus is contextual fear conditioning (Phillips and LeDoux, 1992; McEchron et al., 1998). Although Paradee et al. (1999) found impaired contextual fear conditioning in *Fmr1* KO mice these results have not been supported by more recent research (Dobkin et al., 2000; Peier et al., 2000; Van Dam et al., 2000).

Other tasks dependent on hippocampal functioning include spatial and contextual learning and memory. While *Fmr1* KO mice have been found to have deficits in spatial learning and memory (Van Dam et al., 2000; Mineur et al., 2002), conflicting results exist. In the Morris water maze spatial learning task (Morris et al., 1982; Morris, 1984), reversal learning seems to be more affected by the loss of FMRP (Bakker et al., 1994;

D'Hooge et al., 1997; Gantois et al., 2001), suggesting reduced behavioural flexibility of *Fmr1* KO animals. However, later studies have not found spatial learning deficits in the Morris water maze task using *Fmr1* KO animals (Paradee et al., 1999; Peier et al., 2000; Eadie et al., 2009) and differences in genetic background may contribute to these discrepancies (Paradee et al., 1999; Dobkin et al., 2000). Finally, *Fmr1* KO mice also show impairments in context dependent learning and memory (Eadie et al., 2010). Interestingly, the context discrimination task is normally dependent on functional NMDARs in the DG (McHugh et al., 2007), a region of the hippocampus that shows abnormalities in NMDAR function in the *Fmr1* KO mouse (Eadie et al., 2010; Yun and Trommer, 2011).

In addition to alterations in hippocampal dependent cognitive functioning, the male *Fmr1* KO mouse also shows other phenotypic similarities to FXS in human individuals, including progressive macroorchidism with testicular development (Bakker et al., 1994; Kooy et al., 1996; Slegtenhorst-Eegdeman et al., 1998). Although genetic background may influence the behavioural phenotype of FXS in the mouse, a dysregulation of neurologic function as a result of a loss of FMRP is similar to that seen in human individuals, supporting the use of the *Fmr1* KO mouse in the assessment of hippocampal functioning in FXS.

1.4.2 Structural and Synaptic Plasticity Dysregulation in the *Fmr1* Null Mouse Hippocampus

The DG region of the hippocampus is one of the few regions of the brain where neurogenesis (the creation of new cells from a pool of multipotent progenitor cells) continues to occur throughout adulthood (Altman and Das, 1965). As granular neurons mature, the complexity and size of their dendrites increases as they connect within the existing hippocampal network (Zhao et al., 2006). As neurogenesis may be subtly impacted by the loss of FMRP (Eadie et al., 2009), so too may the normal development of neuronal circuits that are crucial for proper cognitive functioning. Indeed, structural deficits have been revealed in the hippocampus of *Fmr1* KO mice, including an immature

dendritic spine morphology and increased spine density (Grossman et al., 2006, 2010; Levenga et al., 2011).

1.4.2.1 Long-Term Synaptic Plasticity in the CA1

In the CA1 subfield of the *Fmr1* KO hippocampus, high-frequency and theta burst stimulation- (TBS) induced LTP appears intact (Godfraind et al., 1996; Paradee et al., 1999; Li et al., 2002; Larson et al., 2005; Lauterborn et al., 2007; Zhang et al., 2009; Connor et al., 2011; Lee et al., 2011). The threshold for the induction of LTP in this hippocampal region may be altered however. While 10 trains of TBS does not reveal impairments in LTP in the CA1, 5 trains of TBS have been shown to unmask LTP deficits (Lauterborn et al., 2007; Lee et al., 2011). Alterations have also been found in other forms of synaptic plasticity, such as impairments in glycine-induced LTP (Shang et al., 2009) and increases in β-adrenergic-dependent excitatory heterosynaptic LTP (Connor et al., 2011).

The loss of FMRP may also disproportionately impair synaptic plasticity in the CA1 of juvenile *Fmr1* KO mice. Impaired LTP in the CA1 region of the hippocampus of young *Fmr1* KO mice has been revealed in association with decreased GluA1 AMPAR subunit expression (Hu et al., 2008). Pilpel et al. (2009) also found increased NMDAR-dependent LTP associated with a decreased AMPA/NMDA ratio using a low-frequency stimulation pairing protocol in *Fmr1* KO2 mice at 2 weeks postnatal. These changes in the *Fmr1* KO2 mouse were lost by 6-7 weeks of age (Pilpel et al., 2009). In the CA1, mGluR priming of LTP shows similar results in both *Fmr1* KO and WT slices, although priming requires protein synthesis only in WT slices (Auerbach and Bear, 2010). In 2002, Bear and colleagues discovered normal low-frequency-induced LTD in the CA1 of the juvenile *Fmr1* KO mouse but abnormally increased metabotropic glutamate receptor-5 (mGluR5)-dependent LTD (Huber et al., 2002). This finding has been supported (Hou et al., 2006; Zhang et al., 2009; Sharma et al., 2010) and recently extended to adult animals (5 – 6 and 9 – 11 months old) (Choi et al., 2011). In the CA1 region of *Fmr1* KO mice mGluR-mediated LTD is protein synthesis independent (Nosyreva and Huber, 2006;

Zhang et al., 2009), which is in contrast to WT animals (Hou et al., 2006; Nosyreva and Huber, 2006; Zhang et al., 2009). Increased M1 muscarinic acetylcholine receptor-mediated LTD has also been found in the *Fmr1* KO mouse CA1(Volk et al., 2007).

1.4.2.2 Long-Term Synaptic Plasticity in the DG

Recent experiments focusing on the DG region of the hippocampus in the young adult male *Fmr1* KO mouse have shown robust alterations in synaptic plasticity. Significant impairments have been found in HFS and TBS-induced LTP (Eadie et al., 2010; Yun and Trommer, 2011) as well as an attenuation of LFS-induced LTD (Eadie et al., 2010). Interestingly, the impairments in LTP and LTD were shown to be dependent on NMDARs (Eadie et al., 2010; Yun and Trommer, 2011) and have been associated with NMDAR hypofunction in this brain region (Eadie et al., 2010; Yun and Trommer, 2011).

1.4.2.3 Short-Term Plasticity and Measures of Cellular Function

Pre-synaptic and short term plasticity changes have also been found in the *Fmr1* KO mouse. In the CA1 of 2 week old *Fmr1* KO mice, a less developed presynaptic phenotype has been revealed with a smaller synaptic structure, a reduced number of vesicles per cluster surface, and an increased fraction of docked vesicles per total number of vesicles (Klemmer et al., 2011). Accelerated vesicle recycling and larger vesicle pools have also been revealed in the CA1, with an increase in cumulative calcium influx through voltage gated calcium channels (Deng et al., 2011). At lower stimulus intensities however, paired-pulse facilitation was found to be decreased in *Fmr1* KO mice (Klemmer et al., 2011). Idrissi et al (2010) also found abnormal paired pulse plasticity in the CA1 of *Fmr1* KO slices, which revealed paired-pulse depression at a comparable inter-stimulus interval that evoked paired-pulse facilitation in WT slices. However, no significant difference in paired-pulse plasticity has been found in other studies (Huber et

al., 2002; Larson et al., 2005; Hou et al., 2006; Zhang et al., 2009; Choi et al., 2011; Deng et al., 2011). Short-term synaptic depression has also been shown to be decreased in amplitude in *Fmr1* KO slices (Deng et al., 2011) while short term potentiation is not altered (Godfraind et al., 1996). Although there has not been extensive research into presynaptic afferents that may modulate synaptic plasticity in the DG of *Fmr1* KO mice, paired-pulse plasticity has so far been found to be comparable to controls (Eadie et al., 2010; Yun and Trommer, 2011). However, the loss of presynaptic FMRP may result in a reduction in functional excitatory connections from the DG to the CA3 region (Hanson and Madison, 2007).

Braun and Segal (2000) have examined basal synaptic function in the *Fmr1* KO mouse and revealed intact miniature AMPAR-mediated currents once primary hippocampal neuronal cultures had established synaptic connections. Other laboratories have also found no differences in baseline synaptic responses (Huber et al., 2002; Larson et al., 2005; Hou et al., 2006; Zeier et al., 2009; Zhang et al., 2009; Choi et al., 2011; Klemmer et al., 2011). Another study using organotypic hippocampal slice cultures found a slight alteration in mEPSC frequency as well as AMPAR and NMDAR-mediated EPSCs only when pairs of cells were patched within the same slice (*Fmr1* KO and post-synaptic FMRP expressing *Fmr1* KO neurons) (Pfeiffer and Huber, 2007). Early postnatal *Fmr1* KO2 mice have been shown to have a decrease in AMPAR-mediated EPSCs in the CA1 when compared to controls, which is not found when investigated in adulthood (Pilpel et al., 2009). When examining DG efferent connections to the CA3 there seem to be altered distribution and sizes of both intrapyramidal mossy fiber terminal fields (mossy fibers terminating within the pyramidal cell layer) and infrapyramidal mossy fiber terminal fields (mossy fibers terminating below the pyramidal cell layer to mainly basal dendrites) in *Fmr1* KO mice (Ivanco and Greenough, 2002; Mineur et al., 2002). Recent work in the DG itself has revealed intact responses to increasing stimuli strength as well as intact AMPAR-mediated currents (Eadie et al., 2010; Yun and Trommer, 2011).

1.5 Objectives

As our laboratory recently revealed impaired bidirectional synaptic plasticity in the DG region of the male *Fmr1* KO mouse model (Eadie et al., 2010), this thesis aimed to extend field electrophysiological investigation to the DG region of female *Fmr1* Het and *Fmr1* KO mice. Synaptic plasticity deficits previously shown in the CA1 region of the *Fmr1* KO male mouse may be due to an increased threshold for proper activation of otherwise intact circuitry (Chen et al., 2010). This thesis therefore made use of a more sustained HFS conditioning stimulation protocol to determine whether impairments in LTP in the DG could be rescued by a more powerful stimulation protocol.

Although mGluR-mediated LTD has been intensely investigated in the juvenile *Fmr1* KO CA1 hippocampus, at the time of experimentation there was a lack of research into mGluR-mediated LTD in the adult hippocampus. This thesis therefore aimed to investigate mGluR-mediated LTD in the CA1 region of the young adult hippocampus in both male and female *Fmr1* mutant animals. These investigations were also extended into the DG region of the hippocampus in male and female animals.

Further investigation will be required to expand our understanding of FXS and fully elucidate the mechanisms behind altered synaptic plasticity in the hippocampus. Our results suggest that NMDARs may be poised as important contributors to hippocampal pathophysiology in FXS.

2. Materials and Methods

2.1 Transgenic Mice

C57Bl/6J male *Fmr1* KO and WT littermate mice were generated by breeding a *Fmr1* Het female with either a WT or *Fmr1* KO male mouse from our well established breeding colony. Founders for our breeding colony were originally provided by Dr. Mark Bear (Massachusetts Institute of Technology, Cambridge, Massachusetts, USA) and are a C57Bl/6 backcrossed strain originally derived from the *Fmr1* KO mouse detailed in Bakker et al. (1994). C57Bl/6J female *Fmr1* KO and *Fmr1* Het mice were generated by breeding a *Fmr1* Het female mouse with a *Fmr1* KO male mouse. WT and *Fmr1* Het female mice were generated by breeding a *Fmr1* Het female mouse with a WT male mouse. The C57Bl/6J strain was used as it shows robust hippocampal synaptic plasticity (Nguyen, 2006). All mice were sexed, weaned, and ear-punched at post-natal day 24 and housed with minimal enrichment (tubes and nesting material). Animals were genotyped using a standard genotyping protocol (see below). The experimenter was blinded to the group identity of all mice throughout the course of the experiments. All experiments were carried out in accordance with international standards on animal welfare and guidelines set by the Canadian Council on Animal Care, and the Animal Care Committees at the University of British Columbia and the University of Victoria. All efforts were made to minimize pain and discomfort for all animals.

2.2 Genotyping

Genotyping was performed as previously described (Eadie et al., 2010). Ear or tail tissue from each animal was obtained and stored at -20^{o}C until processing. Briefly, tissue was placed in 180 µl digestion buffer and 20 µl Proteinase K in a DNAse and RNase-free tube and incubated overnight in a thermomixer at 55^{o}C (while agitated at 300 RPM). The samples were then centrifuged at 21,000 RCF for a total of 3 minutes. The supernatants were removed and transferred into a new tube, followed by the addition of 20 µl of

RNase A. Samples were then vortexed and incubated for 2 minutes at room temperature. 200 µl of lysis buffer was then added to each tube followed by the addition of 200 µl of 100% ethanol and vortexing. The lysate was transferred to a new spin column and centrifuged at room temperature for 1 minute at 9300 RCF. The spin column was then placed in a fresh tube and washed by adding 500 µl of Wash Buffer I and centrifuging for 1 minute at 9300 RCF at room temperature. This process was repeated with 500 µl of Wash Buffer II and the spin column was centrifuged for 3 minutes at 21,000 RCF at room temperature. 100 µl of Elution Buffer was then added and incubated at room temperature for 1 minute followed by centrifugation for 1 minute at 21,000 RCF using new collection tubes. DNA in collection tubes was used directly in PCR or stored at -20^{o}C.

The PCR reaction was performed by mixing 2 µl DNA with 2.5 µl 10X PCR Reaction Buffer, 11 µl nuclease-free H_2O, 2.0 µl (2.5 mM) dNTP, 2.5 µl (50 mM) $MgCl_2$, 1.25 µl (1 µM) of each forward and reverse primer, and 0.5 µl Taq DNA polymerase (Invitrogen, Burlington, Ontario, Canada). The cycling parameters for PCR were as follows: One cycle of 5 minutes at 94^{O}C was followed by 35 cycles of 60 seconds at 94^{O}C, 90 seconds at 65^{O}C and 150 seconds at 72^{O}C. This was followed by an infinite hold at 4^{O}C. Primers M2= 5′ ATCTAGTCATGCTATGGATATCAGC 3′ and N2 = 5′ GTGGGCTCTATGGCTTCTGAGG 3′ were used to probe for the *Fmr1* KO allele (fragments amplified were 800 base pairs). Primers S1 = 5′ GTGGTTAGCTAAAGTGAGGATGAT 3′ and S2 = 5′ CAGGTTTGTTGGGATTAACAGATC 3′ were used to probe for the WT allele (amplified fragments were 465 base pairs). PCR products were run on a 1.5% agarose gel. 10,000x SYBR-safe was used (1:13,333 in 1x TAE) and DNA bands were visualized using a BioRad Gel-Doc trans-illuminator (BioRad, Mississauga, Ontario, Canada). A WT band without a KO band indicated homozygosity for the WT allele; a WT band and a KO band indicated heterozygosity; and a KO band without a WT band indicated homozygosity for the KO allele. Animals with ambiguous bands were re-genotyped.

2.3 Electrophysiology

Young adult (2-4 month old) male and female mice were briefly anesthetized with inhalant isoflurane, immediately decapitated, and their brains removed directly into oxygenated (95% O_2/5% CO_2), ice-cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaHPO4, 25 NaHCO3, 2 CaCl2, 1.3 MgCl2, and 10 dextrose at a pH of 7.3.

2.4 Estrus Cycle

Variation in blood levels of gonadal hormones across the estrus cycle has been well-documented in humans and rodents, and it is becoming increasingly apparent that gonadal hormones influence structural and functional plasticity in the brain (Woolley, 1998).

To ensure that all female mice used in these experiments were in the same stage of their estrus cycle, animals were subjected to vaginal lavage immediately before decapitation followed by Papanicolaou (PAP) histochemistry. Vaginal lavages were performed with 0.9% saline and samples were immediately transferred onto superfrost ® plus microscope slides (Erie Scientific Company, Portsmouth, New Hampshire, U.S.A.) and left to dry for a minimum of 24 hours. Staining consisted of a 30 second – 1 minute immersion under a minimal stream of lukewarm tap water after which slides were immersed into Gills hemotoxylin solution 1 for 3 minutes, washed in tap water, immersed in Scott's tap water substitute (0.3 % sodium bicarbonate and 2 % anhydrous magnesium sulfate in distilled water) for 20 seconds, and then immersed in PAP stain (equal parts of Orange G6 and Eosin-azure 50; Sigma-Aldrich, Oakville, Ontario, Canada) for 3 minutes. The slides were then immersed two times in 100% ethanol for 30 seconds each followed by 2 5-minute incubations in a xylen substitute (Citrisolv; Fisher Scientific Company, Ottawa, Ontario, Canada). Coverslipping was performed using permount mounting medium (Electron Microscopy Sciences, Burlington, Ontario, Canada).

All samples were analyzed by conventional light microscopy using an Olympus microscope (Model BX51TF, Olympus Corporation, Center Valley, Pennsylvania, U.S.A., using a 20X objective lens. Pictures were obtained with a Q-colour camera and Image Pro Plus software (MediaCybernetics Inc., Bethesda, Maryland, U.S.A.). Picture colour and contrast was enhanced using CorelDraw X3 Graphics Suite (Corel Corporation, Ottawa, Ontario, Canada). As proestrus has been associated with alterations in structural and functional plasticity (Warren et al., 1995), all female mice in proestrus were removed from the experiments and subsequent analyses.

2.5 Preparation of Sections

Following removal of the brain, the cerebrum was longitudinally hemi-sected and blocked for sectioning (frontal lobe and cerebellum were removed). Transverse hippocampal slices (350 μm) were obtained using continuously oxygenated ACSF that was maintained at 4^{o}C using a cooled Vibratome 1500 (Ted Pella Inc., Redding, California, U.S.A.). Slices were incubated in continuously oxygenated ACSF at 30^{o}C and kept in order using a custom modified 24-well plate. Slices were allowed to rest for a minimum of 1 hour before recordings commenced.

2.6 Electrophysiological Recordings

All recordings were obtained in oxygenated ACSF at 30^{o}C. Slices were perfused at a rate of approximately 2 ml / minute. All drugs used were dissolved in ACSF before bath application. Medial perforant path and schaffer collateral commissural path evoked field excitatory post-synaptic potentials (fEPSPs) were obtained using a concentric bipolar stimulating electrode (FHC Inc., Bowdoin, Maine, USA) and a glass recording electrode (0.5-1.5 MΩ) filled with ACSF and placed in the medial molecular layer or the stratum radiatum, respectively. Field EPSPs were collected using an Axon MultiClamp 700B amplifier connected to a Windows computer running Clampex 10.2 software (Molecular Devices, Sunnyvale, California, U.S.A.). Electrodes were placed under the

visual guidance of a microscope. The slope from a single fEPSP trace was calculated from the initial slope of the fEPSP relative to the slope of the 10 ms interval immediately preceding afferent stimulation. The current magnitude (10-50 μA) was delivered through a digital stimulus isolation amplifier (Getting Instruments Inc., San Diego, California, USA) and set to elicit a fEPSP approximately 40-50% of maximum for synaptic potentiation experiments and 50-60% for synaptic depression experiments. A stable baseline (for a minimum of 20 minutes) was obtained by delivering single pulse stimulation at 15 second interstimulus intervals. In male animals, 30 or 15 second interstimulus intervals were utilized during chemically-induced mGluR-mediated LTD and 30 second interstimulus intervals were utilized during synaptically induced LTD). The medial perforant pathway was distinguished from the lateral perforant pathway by source-and-sink analysis, where the stimulating electrode was placed briefly into the lateral perforant path and the recording electrode into the medial perforant path. This elicited an upward slope of the fEPSP as ions moved from the external solution surrounding the recording electrode into the dendrites of the lateral perforant path and thus ensured proper placement of electrodes in the medial perforant path during electrophysiological recordings.

2.7 Synaptic Plasticity Induction Protocols and Basal Measures of Physiological Parameters

LTP of fEPSPs was induced using a conditioning stimulus (CS) consisting of 4 trains of 50 pulses at 100 Hz, 30 s apart (high-frequency stimulation; HFS). An additional experiment with an HFS conditioning protocol of 4 trains of 100 pulses at 100 Hz, 60 s apart was employed to assay the response of male *Fmr1* KO slices to a stronger HFS stimulation protocol. LTD of fEPSPs was induced using a low-frequency conditioning stimulus (LFS) consisting of 900 pulses delivered at 1Hz over 15 minutes. HFS-LTP and LFS-LTD in the DG were conducted in the presence of 5 μM bicuculline methiodide (BIC; Sigma-Aldrich, Oakville, Ontario, Canada) to block the inhibitory effects of the gamma-aminobutyric acid receptor type A ($GABA_A$) on synaptic plasticity in this region of the hippocampus. BIC was bath applied for a minimum of 10 minutes prior to and

during the CS. Experiments assessing the role of the NMDAR in HFS-LTP and LFS-LTD were also conducted in the presence of (2R)-amino-5-phosphonovaleric acid (APV; Sigma-Aldrich, Oakville, Ontario, Canada). BIC (5 μM) was bath applied for a minimum of 5 minutes before the application of a mixture of both BIC (5 μM) and APV (50 μM) (which was then bath applied for a minimum of 5 minutes before and during the respective CS).

mGluR-mediated LTD was examined using both a chemical and synaptic conditioning stimulation induction protocol. Chemical induction of mGluR-mediated LTD was performed with a 5 minute bath application of 100 μM (RS)-3,5-Dihydroxyphenylglycine (DHPG; Tocris Bioscience (cedarlane), Burlington, Ontario, Canada) and 50 μM APV. Synaptic CS induction of mGluR-mediated LTD was conducted using 900 pairs of stimuli (50 ms interstimulus interval) delivered at 1 Hz over 15 minutes (PP-LFS). For PP-LFS induction of LTD, APV (50 μM) was bath applied to the slice to antagonize NMDARs for a minimum of 5 minutes before and during the application of PP-LFS CS.

Input-output experiments were conducted to measure basal dendritic excitation in response to increasing applied current in ACSF (using an increasing pulse width from 30 to 300 μs with 30 μs interstimulus intervals). Paired-pulse recordings were also utilized to measure presynaptic release probability in naive slices. Paired-pulse recordings consisted of 5 sets of two pulses each with an interpulse interval of 50 μs (20 s between paired stimuli) delivered in ACSF. Any slices which showed population spikes in the fEPSP were removed from the paired-pulse dataset and any subsequent analyses in order to measure presynaptic release probability without the confounds of feedback inhibition or excitation.

2.8 Analyses of Electrophysiological Recordings

Evoked fEPSP responses were digitized and the initial slope of the fEPSP was analyzed using pClamp 10 software (Molecular Devices, Union City, California, USA).

All data are represented as percentage change from the initial average baseline fEPSP slope, which was defined as the average slope obtained for the 20 minutes prior to CS application. Percentage potentiation or depression was calculated as follows: (average fEPSP 50 to 60 minutes post-CS) / (average fEPSP between -20 to 0 minutes pre-CS) x 100. A single recording was considered a single sample due to the relatively high variability between recordings.

2.9 Statistical Analyses

Data are presented as means ± standard error of the mean (SEM). Differences between the mean values of experimental groups were compared with a one or two-tailed Student's *t* test or the nonparametric Mann-Whitney U Test using Statistica 7.0 software (StatSoft, Tulsa, OK, USA). The same electrophysiological data obtained from female WT slices was analysed with *Fmr1* Het and with *Fmr1* KO data in different statistical tests. Input-output curves were compared using a repeated-measures analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$.

3. Results

3.1 Synaptic plasticity in the DG subfield of WT and *Fmr1* KO Mice.

3.1.1 Basic Physiological Parameters in the Dentate Gyrus of Male WT and *Fmr1* KO Mice.

Paired-pulse stimulation and input/output curves were utilized to gain an understanding of whether basal physiological parameters were altered in the dentate gyrus of the male *Fmr1* KO mouse. Paired-pulse stimulation was assessed in ACSF and used as a measure of presynaptic neurotransmitter release. Paired-pulse depression was seen in WT animals in the medial perforant path (Pulse 2 = 92.9 ± 2.4 % of pulse 1; n = 12; Figure 2) but in *Fmr1* KO animals the same paired-pulse protocol induced paired pulse facilitation (Pulse 2 =107.2 ± 3.1 % of pulse 1; n = 22; Figure 2). There was a significant difference between WT and *Fmr1* KO responses to paired-pulse assay of presynaptic release probability (*t* test; $t_{(32)} = -3.150$, $P = 0.004$) that suggests *Fmr1* KO animals may have a lower probability of neurotransmitter release.

Input/Output (IO) functions were performed to assess stimulus response curves in ACSF in the medial perforant path (MPP) of the DG region of the hippocampus in young adult (2 - 4 months old) WT and *Fmr1* KO littermate male mice (Figure 2). The slope of the fEPSP increased significantly when the applied current was elevated from a pulse width of 30 to 300 μs (WT n = 12, KO n = 11; repeated measures ANOVA: $F_{(8,168)} = 213.700$, $P < 0.001$). No statistical differences were found between genotypes (repeated measures ANOVA: $F_{(1,21)} = 0.000$, $P = 0.833$).

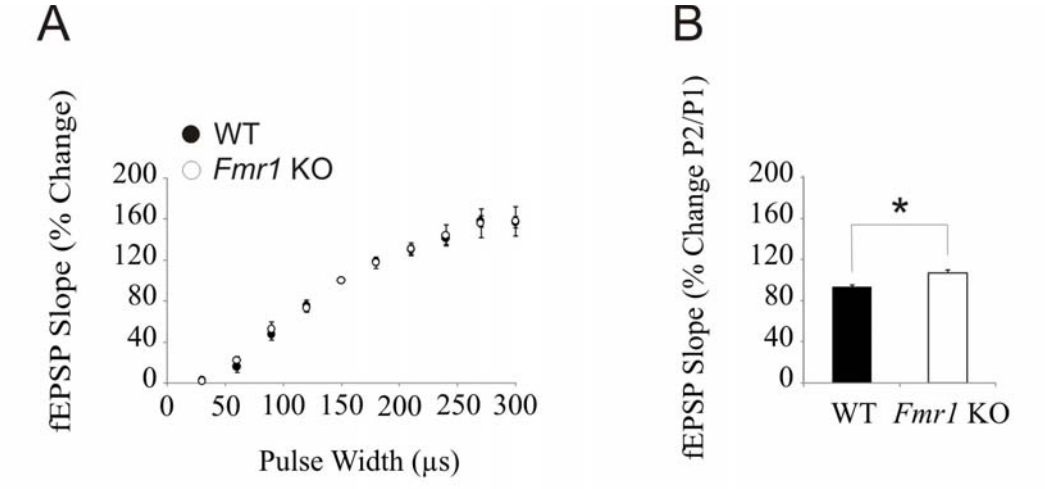

Figure 2. Input/output functions and paired-pulse stimulation in the DG of male WT and *Fmr1* KO mice. (**A**) Synaptic responses to single pulse stimuli at predetermined incremental intensities in 2 – 4 month old *Fmr1* KO animals are not significantly different from those observed in WT animals. (**B**) Conversely, *Fmr1* KO male mice are more likely to show a small degree of paired-pulse facilitation in the medial perforant path than their WT counterparts *(P*<0.05).

3.1.2 Synaptic Plasticity in the Dentate Gyrus of Male WT and *Fmr1* KO Mice.

To determine whether young adult (2 - 4 months old) male *Fmr1* KO animals had the same capacity for LTP and LTD in the DG as control animals, a high frequency conditioning stimulus (4 trains of 50 pulses at 100 Hz) and a low frequency conditioning stimulus (900 pulses given at 1 Hz over 15 minutes) were applied. HFS was delivered in the presence of 5 μM BIC (applied for a minimum of 10 minutes prior to and during the CS) and produced a robust LTP of the slope of the fEPSP in WT animals when measured 50 to 60 minutes after conditioning stimulation (WT = 69.8 ± 16.2 % of baseline response to stimulation; n = 8; Figures 3A/B). The same conditioning stimulation protocol induced significantly less LTP in *Fmr1* KO animals (KO: the size of the response increased by 19.0 ± 13.4 %; n = 7; Figures 3A/B; Mann-Whitney U Test: U(13) = 7.000, Z = -2.430, *P* = 0.015). The bath application of the NMDAR antagonist APV (50 μM) for a minimum of 5 minutes before and during the HFS conditioning stimulation

protocol (applied in the presence of 5 μM BIC) attenuated LTP to the same level in both WT (WT: 3.5 ± 6.7 % response; n = 4) and *Fmr1* KO animals (KO = 3.7 ± 2.4 % response; n = 5; Mann-Whitney U Test: U(7) = 10.000, Z = 0.000, *P* = 1.000; Figure 3C). This data suggests that the LTP induced through HFS in both WT and *Fmr1* KO slices was predominantly NMDAR dependent.

LFS produced LTD of the slope of the fEPSP in WT animals when measured 50 to 60 minutes after conditioning stimulation (WT = the size of the response decreased by 26.2 ± 4.1 %; n = 9; Figures 3D/E). The same conditioning stimulation protocol induced significantly less LTD in *Fmr1* KO animals (KO: the size of the response decreased by 15.4 ± 3.5 %; n = 11; one-tailed *t* test; $t_{(19)} = 2.049$, *P* = 0.028; Figures 3D/E). The bath application of the NMDAR antagonist APV (50 μM) for a minimum of 5 minutes before and during the LFS conditioning stimulation protocol (applied in the presence of 5 μM BIC) decreased LTD to the same level in both WT (WT = -7.2 ± 6.4 % response; n = 5) and *Fmr1* KO animals (KO = -6.3 ± 3.9 % response; n = 4; *t* test; $t_{(8)} = 0.416$, *P* = 0.688; Figure 3F). This data suggests that the LTD induced through LFS in both WT and *Fmr1* KO slices was predominantly NMDAR dependent.

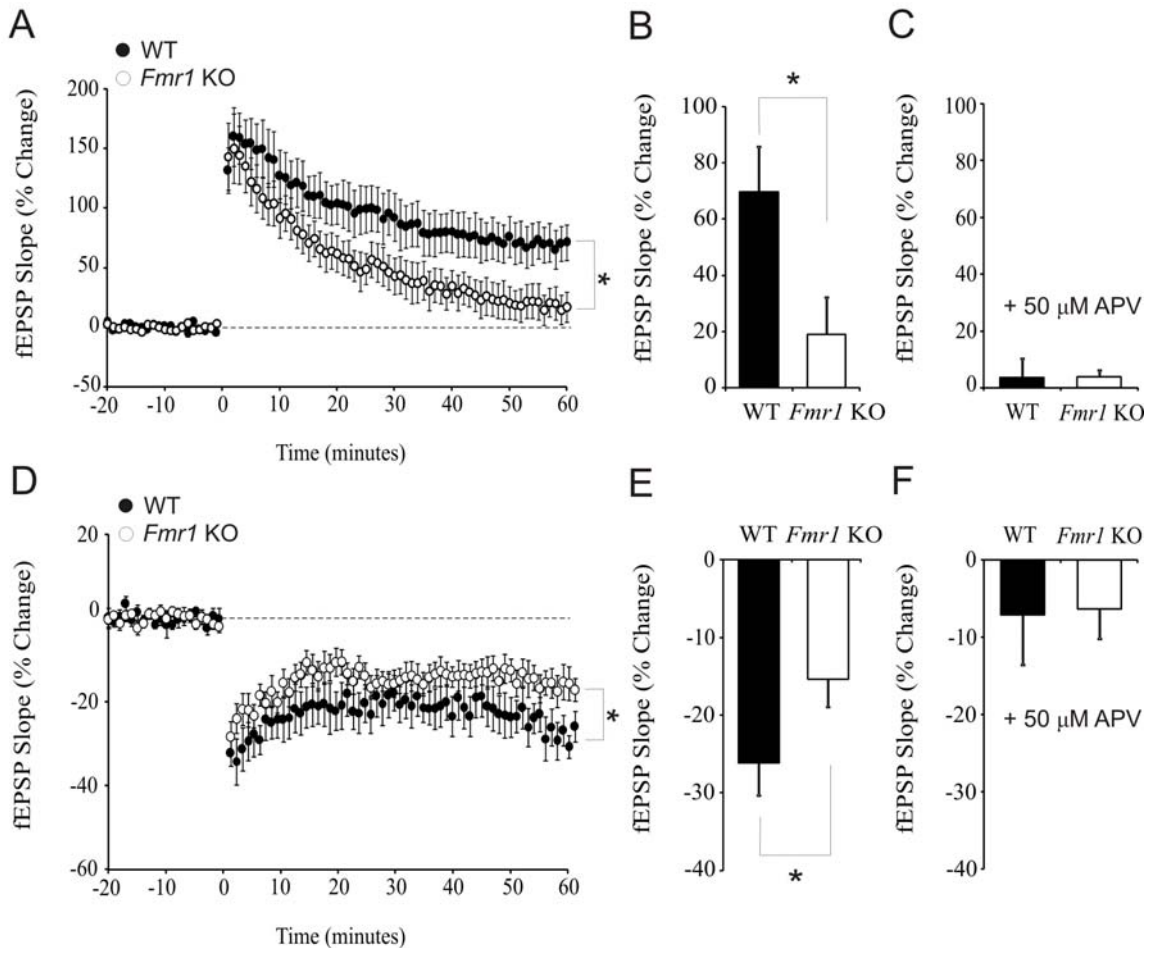

Figure 3. **Male *Fmr1* KO mice exhibit impaired bidirectional synaptic plasticity in the DG. Blockade of the NMDAR abolishes synaptic plasticity differences.** (**A**) The magnitude of LTP (HFS applied at time zero) is significantly decreased in the DG of 2 – 4 month old *Fmr1* KO animals when compared to controls at (**B**) 50 – 60 minutes after conditioning stimulation ($P<0.05$). (**C**) Application of a selective NMDAR antagonist (APV) ameliorates differences between WT and *Fmr1* KO animals. (**D**) The magnitude of LTD (LFS applied at time zero) is significantly decreased in the DG of *Fmr1* KO animals when compared to controls at (**E**) 50 – 60 minutes after conditioning stimulation ($P<0.05$). (**F**) Application of a selective NMDAR antagonist (APV) abolishes differences in LTD between WT and *Fmr1* KO animals.

To determine whether a more robust NMDAR-dependent stimulation protocol may improve the amplitude and reliability of calcium signalling through NMDARs in the male *Fmr1* KO DG, an additional conditioning stimulus protocol was used. This CS protocol was also carried out in 5 μM BIC as previously described and consisted of 4 trains of 100 pulses at 100 Hz, 60 seconds apart (*Fmr1* KO 100 pulses). As seen in Figure 4, increasing the duration of the 100 Hz conditioning protocol did not alter the amount of LTP observed in slices from *Fmr1* KO mice (*Fmr1* KO 50 pulses: 19.0 ± 13.4 % of baseline reponse to stimulation; n = 7; *Fmr1* KO 100 pulses: 24.6 ± 14.8 % of baseline reponse to stimulation; n = 8; Mann Whitney U Test: U(13) = 25.000, Z = 0.347, P = 0.728; Figure 4).

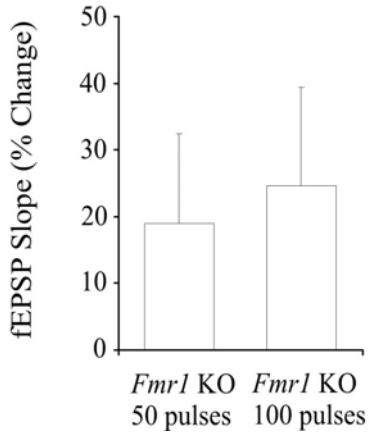

Figure 4. Increased intensity of HFS stimulation does not alter LTP in the DG of *Fmr1* KO male mice. A more robust HFS CS consisting of 4 trains of 100 pulses given at 100 Hz (*Fmr1* KO 100 pulses) was not able induce a greater magnitude of LTP in 2 – 4 month old *Fmr1* KO animals than a 50 pulse CS paradigm when measured 50 – 60 minutes after the CS.

A chemical induction using a solution of 100 μM DHPG (a type I mGluR agonist) and 50 μM APV was bath applied for 5 minutes to examine mGluR-mediated LTD in the DG of the male *Fmr1* KO mouse without the influence of NMDARs. mGluR-LTD was elicited in the DG of both WT and *Fmr1* KO slices (Figures 5A/B) and analyzed 50 - 60 minutes after application of the DHPG/APV solution (WT: = -19.7 ± 4.1 % of baseline

response to stimulation; n = 17; KO = -19.004 ± 3.532 % of baseline response to stimulation; n = 11; Figure 5B). The same chemical induction protocol induced equivalent levels of mGluR-mediated LTD in WT and Fmr1 KO slices (Mann Whitney U Test: U(26) = 90.000, Z = -0.165, P = 0.869).

Separate experiments were carried out to investigate mGluR-mediated LTD in the *Fmr1* KO mouse with the use of a synaptic paired-pulse LFS (PP-LFS) CS protocol (Figure 5C/D). 50 μM of APV was bath applied for a minimum of 5 minutes before and during PP-LFS, which consisted of 900 pairs of stimuli (50 ms interstimulus interval) delivered at 1 Hz. The application of the PP-LFS CS was also able to induce LTD in the DG of both WT and *Fmr1* KO slices (WT: = -16.8 ± 5.7 % of baseline response to stimulation; n = 9; KO = -19.6 ± 10.4 % of baseline response to stimulation; n = 12; Figures 5C/D) and no significant differences were observed between WT and *Fmr1* KO slices when measured at 50 - 60 minutes post CS (Mann-Whitney U Test: U(19) = 58.000, Z = 0.132, *P* = 0.895; Figure 5D). This suggests that mGluR-mediated LTD is intact in the DG of the male *Fmr1* KO animal.

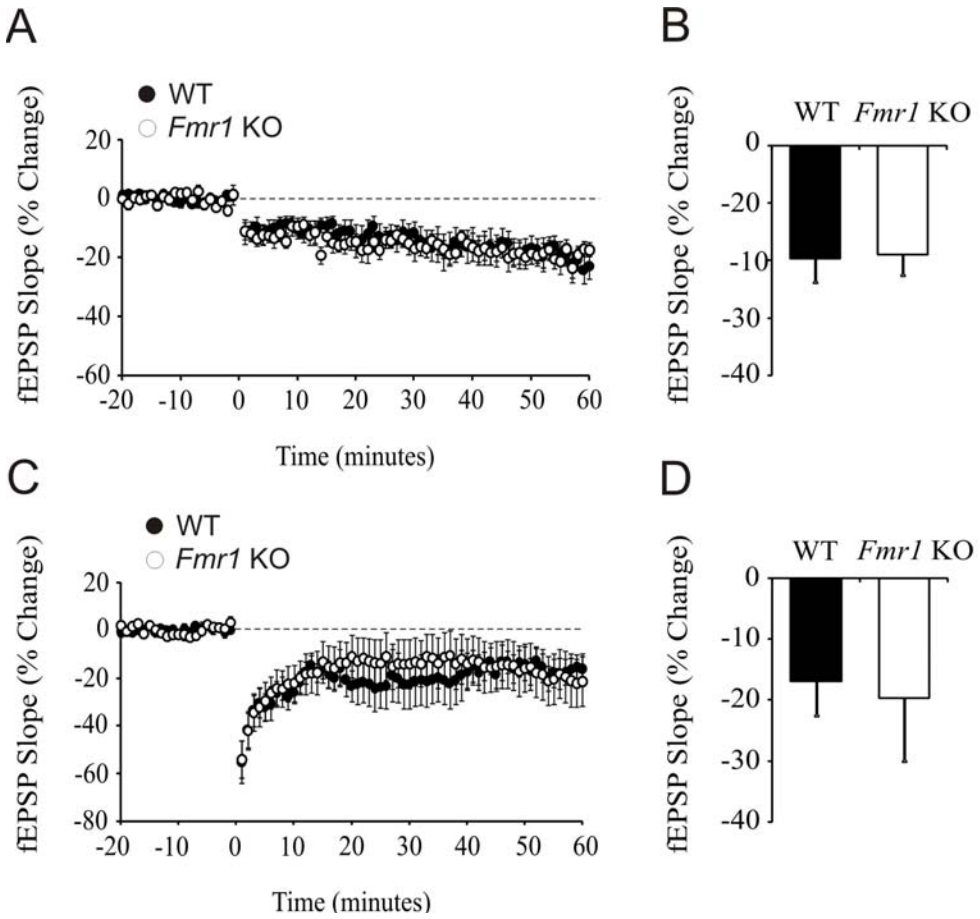

Figure 5. Intact DHPG and PP-LFS induced mGluR-mediated LTD in the DG of male *Fmr1* KO animals. (A) The magnitude of LTD induced by a 5 minute application of DHPG and APV at time zero in 2 – 4 month old animals is similar in both *Fmr1* KO and control slices when compared at (**B**) 50 – 60 minutes after conditioning stimulation. (**C**) The application of PP-LFS at time zero in the presence of the selective NMDAR antagonist APV (50 μM) also reveals similar LTD in the *Fmr1* KO slices when compared to controls.

3.2 Synaptic plasticity in the CA1 subfield of WT and *Fmr1* KO Mice.

3.2.1 Basic Physiological Parameters in the CA1 subfield of WT and *Fmr1* KO Male Mice.

IO functions to measure basal dendritic excitation in response to increasing applied current in the Schaffer Collateral Commissural Pathway (SCP) was performed in ACSF in the stratum radiatum of the CA1 region of the hippocampus in young adult (2 - 4 months old) wild-type (WT) and *Fmr1* KO (KO) littermate mice. The slope of the fEPSP significantly increased when the applied current was increased from a pulse width of 30 to 300 μs (WT n = 10, KO n = 10; repeated measures ANOVA: $F_{(8,144)} = 112.700$, $P < 0.001$; Figure 6A). No statistical differences were found between genotypes (repeated measures ANOVA: $F_{(1,18)} = 0.200$, $P = 0.684$; Figure 6A).

Paired-pulse conditioning stimulation (50 μs interpulse interval) was used as a measure of presynaptic neurotransmitter release in ACSF. Paired-pulse facilitation was seen in WT animals in the SCP (Pulse 2 = 136.4 ± 5.1 % of pulse 1; n = 9) as well as in *Fmr1* KO animals (Pulse 2 = 142.3 ± 5.9 % of pulse 1; n = 12). There was no significant difference between WT and *Fmr1* KO responses to paired-pulse assay of presynaptic release probability in the CA1 (Mann-Whitney U Test: U(19) = 52.000, Z = -0.142, P = 0.887; Figure 6B). This data suggests that *Fmr1* KO animals have intact presynaptic release probability as assessed using the paired-pulse stimulation protocol in the CA1 region of the hippocampus.

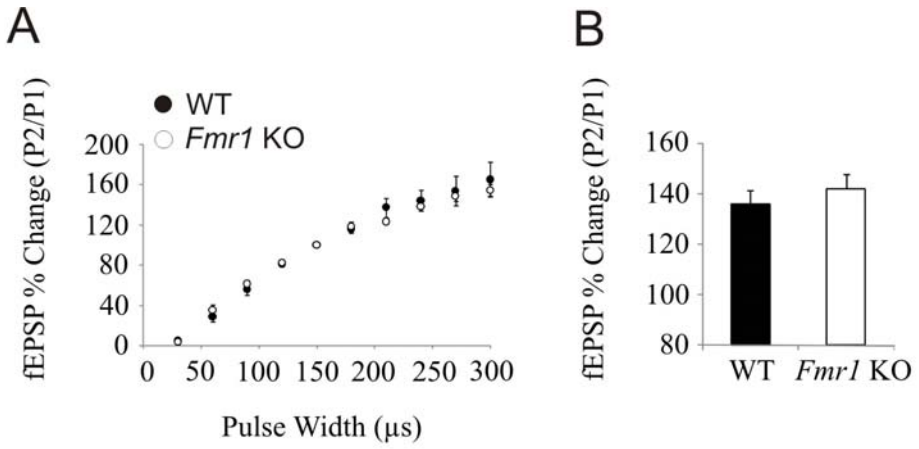

Figure 6. Intact input/output functions and paired-pulse responses in the CA1 of male *Fmr1* KO mice. (**A**) Synaptic responses to single pulse stimuli at predetermined incremental intensities in 2 – 4 month old *Fmr1* KO male animals are not different from those observed in WT animals in the CA1. (**B**) *Fmr1* KO male mice show similar degrees of paired-pulse facilitation in the Schaffer Collateral Pathway when compared to controls.

3.2.2 Synaptic Plasticity in the CA1 of Male *Fmr1* KO Animals.

A HFS of 4 trains of 50 pulses at 100 Hz produced a robust LTP of the slope of the fEPSP in the CA1 SCP (stratum radiatum) of both WT and *Fmr1* KO slices (Figures 7A/B) when measured 50 to 60 minutes after conditioning stimulation (WT = 54.9 ± 13.4% of baseline response to stimulation; n = 8; KO = 75.5 ± 10.2 % of baseline response to stimulation; n = 8; Figure 7B). The same conditioning stimulation protocol induced equivalent levels of LTP in WT and *Fmr1* KO slices (Mann-Whitney U Test: $U(14) = 22.000$, $Z = -1.050$, $P = 0.294$ (Figures 7A/B).

A LFS of 900 pulses given at 1 Hz over 15 minutes induced LTD of the slope of the fEPSP in the CA1 SCP of both WT and *Fmr1* KO slices (Figures 7C/D) when measured 50 to 60 minutes after conditioning stimulation (WT = -13.4 ± 5.1% of baseline response to stimulation; n = 6; KO = -16.1 ± 2.5 % of baseline response to stimulation; n = 6; Figure 7D). The same conditioning stimulation protocol induced equivalent levels of

LTD in WT and *Fmr1* KO animals (Mann-Whitney U Test: U(10) = 17.000, Z = -0.160, *P* = 0.873). This data suggests that HFS induced LTP and LFS induced LTD are intact in the CA1 of the young adult male *Fmr1* KO animal.

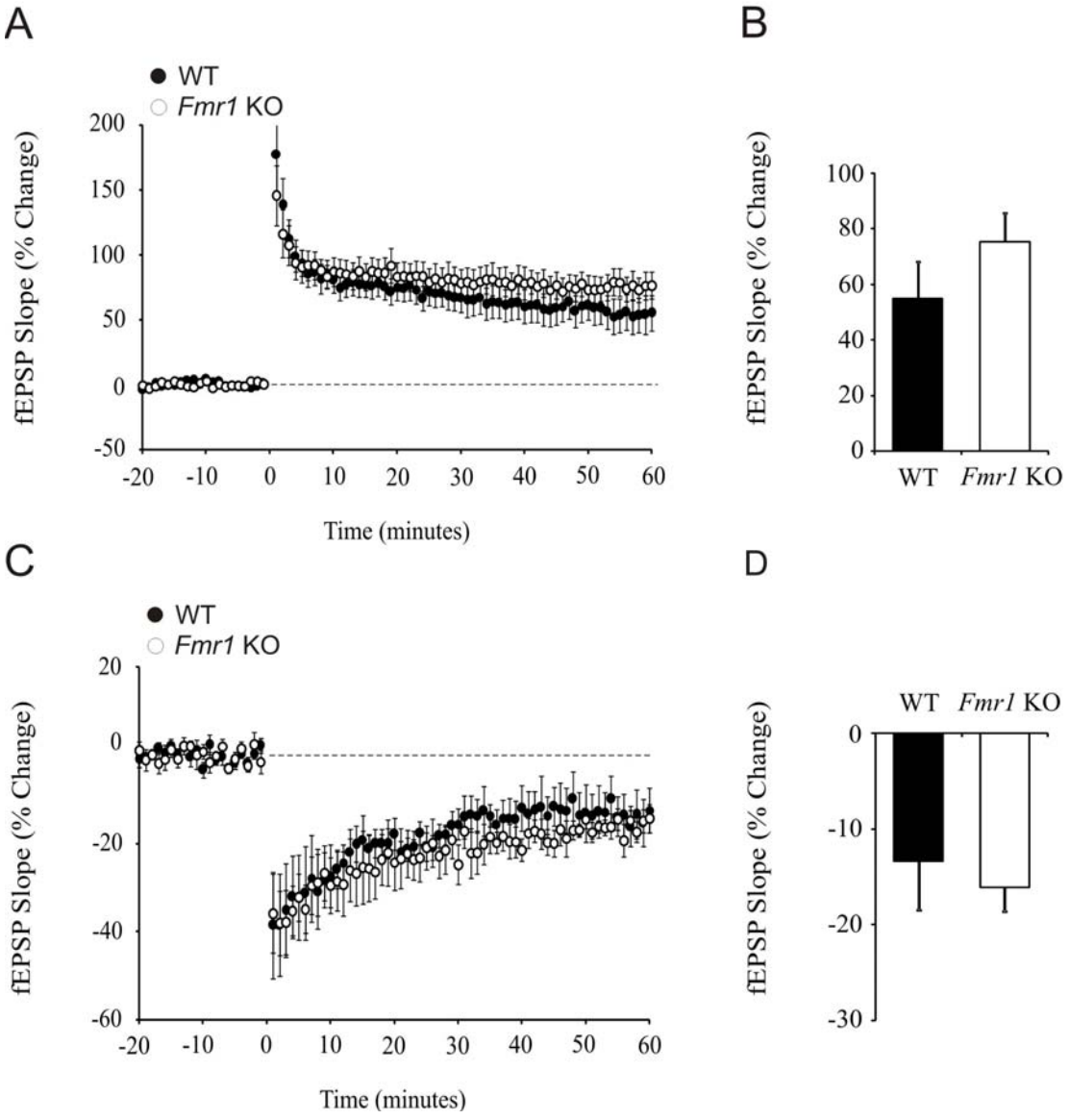

Figure 7. Normal bidirectional synaptic plasticity in the CA1 of male *Fmr1* KO mice. (**A**) The magnitude of LTD induced by HFS CS application at time zero is similar in 2 – 4 month old *Fmr1* KO and WT animals when compared (**B**) 50 – 60 minutes after conditioning stimulation. (**C**) The application of a LFS protocol at time zero also revealed similar LTD in the *Fmr1* KO slices when compared to controls (**D**) 50 – 60 minutes after the CS.

mGluR-mediated LTD was also investigated in the SCP of the CA1 region of the *Fmr1* KO mouse. The previously described (Section 2.7) protocols of chemical and synaptic induction of mGluR-mediated LTD were utilized. Baseline stimulation consisted of a stimulus that was set to induce 50-60% of the maximum response of the tissue given at an interstimulus interval of 15 or 30 seconds for all experiments utilizing DHPG and 30 seconds for all PP-LFS experiments. Chemical induction of mGluR-mediated LTD with DHPG and APV elicited LTD of the slope of the fEPSP in the CA1 of both *Fmr1* KO and control animals (WT: = -19.1 ± 4.0% of baseline response to stimulation; n = 18; KO = -19.1 ± 5.1 % of baseline response to stimulation; n = 16; Figures 8A/B) but was not significantly different between WT and *Fmr1* KO slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test; $t_{(32)} = 0.074$, $P = 0.942$; Figure 8B). The application of PP-LFS CS also induced LTD in the CA1 (WT: = -21.1 ± 5.1 % of baseline response to stimulation; n = 8; KO = -19.0 ± 4.6 % of baseline response to stimulation; n = 8; Figures 8C/D) but was not significantly different between WT and *Fmr1* KO slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test: $t_{(14)} = -0.343$, $P = 0.737$; Figure 8D). This data suggests that mGluR-mediated LTD is intact in the CA1 of the young adult male *Fmr1* KO animal.

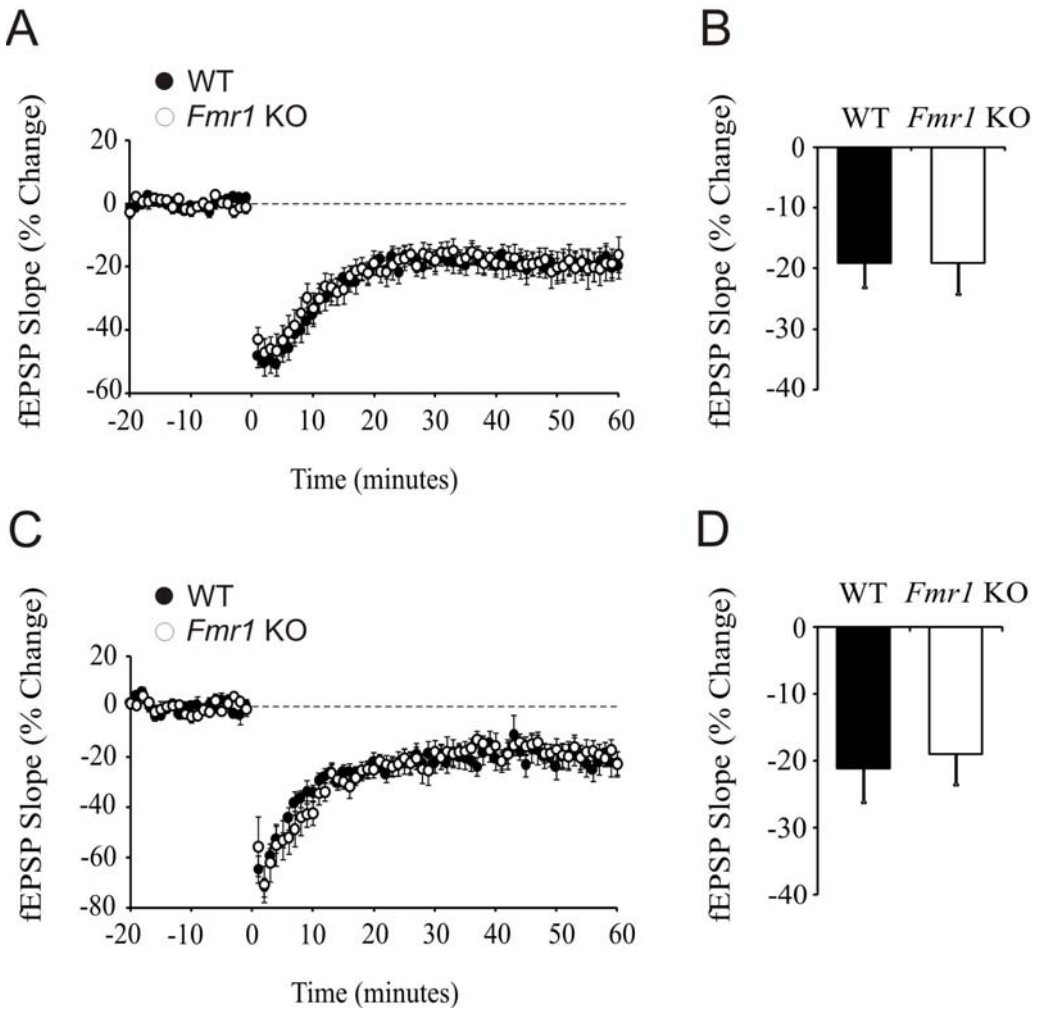

Figure 8. Intact DHPG (A/B) and PP-LFS (C/D) induced mGluR-mediated LTD in the CA1 of male *Fmr1* KO animals. (**A**) The magnitude of LTD induced by a 5 minute DHPG and APV application at time zero is similar in 2 – 4 month old *Fmr1* KO and control animals when compared at (**B**) 50 – 60 minutes after conditioning stimulation. (**C**) The application of PP-LFS at time zero in the presence of the selective NMDAR antagonist APV (50 μM) also reveals similar LTD in the *Fmr1* KO slices when compared to WT slices.

3.3 Synaptic Plasticity in Female *Fmr1* Mutant Mice

In this study, we have used two independent female models of FXS to investigate synaptic functioning: *Fmr1* Het female mice and *Fmr1* KO female mice. FXS-affected females exhibit mosaicism due to random cellular X inactivation (McMahon et al., 1981). As such, *Fmr1* Het female mice will contain variable expression of FMRP depending on which tissue has preferential activation of the mutant or wild-type *Fmr1* gene on the X chromosome. To attain a more in depth picture of the role of FMRP in synaptic plasticity in the female brain, we have also used *Fmr1* KO female mice in which the *Fmr1* gene is inactivated in all tissues.

3.3.1 Determination of Estrus Cycle in Female Mice.

To ensure that animals used in these experiments were not in the proestrus stage of their estrus cycle, which has been associated with alterations in functional plasticity (Warren et al., 1995), all female animals were subjected to vaginal lavage immediately before decapitation while under the influence of inhalent isoflurane. Vaginal lavages were processed for the PAP staining and representative examples of each stage of the estrus cycle are shown in Figure 9. All female animals that were in proestrus at the time of decapitation were excluded from all subsequent experiments and analyses.

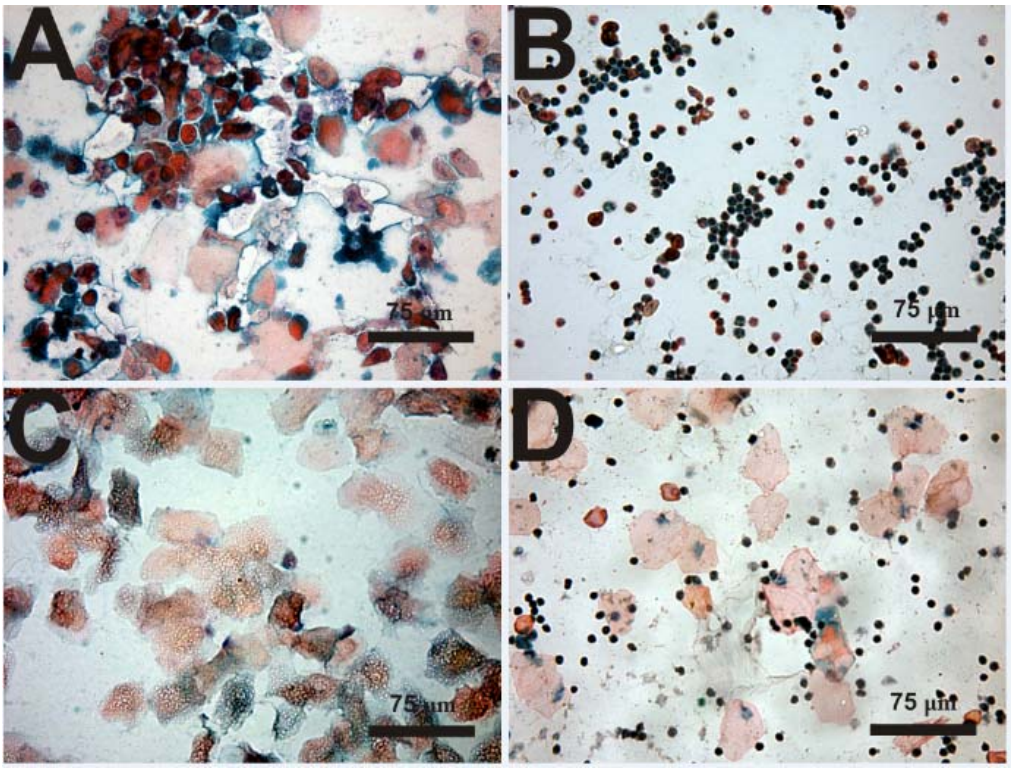

Figure 9. Female Estrus cycle. Representative photomicrographs of the (**A**) proestrus, (**B**) diestrus, (**C**) estrus, and (**D**) metestrus phases of the mouse estrus cycle obtained from 2 – 4 month old mice. Scale bar denotes 75 µm.

3.3.2 Determination of Input/Output curve and Paired-Pulse Analysis in Female WT and *Fmr1* Mutant Mice.

IO functions to measure basal excitation in response to increasing applied current were performed in ACSF in the MPP of the hippocampal DG in young adult (2 - 4 months old) WT and *Fmr1* Het littermate female mice. The slope of the fEPSP significantly increased when the applied current was increased from a pulse width of 30 to 300 µs (WT n = 7, Het n = 6; repeated measures ANOVA: $F_{(8,88)} = 109.300$, $P < 0.001$; Figure 10A). No statistical differences were found between genotypes (repeated measures ANOVA: $F_{(1,11)} = 0.100$, $P = 0.805$; Figure 10A).

Paired-pulse conditioning stimulation (50 µs interpulse interval) was used as a measure of presynaptic neurotransmitter release. Paired-pulse depression was seen in WT

animals in the medial perforant path (Pulse 2 = 97.7 ± 1.9 % of pulse 1; n = 6) but in *Fmr1* Het animals paired pulse responses to the first and second pulse were approximately equivalent (Pulse 2 = 98.4 ± 3.3 % of pulse 1; n = 3; Figure 10B). There was no significant difference between WT and *Fmr1* Het responses to paired-pulse assay of presynaptic release probability (Mann-Whitney U Test: U(7) = 10.000, Z = 0.114, P = 0.909; Figure 10B), suggesting that WT and *Fmr1* Het female mice have an equal probability of neurotransmitter release.

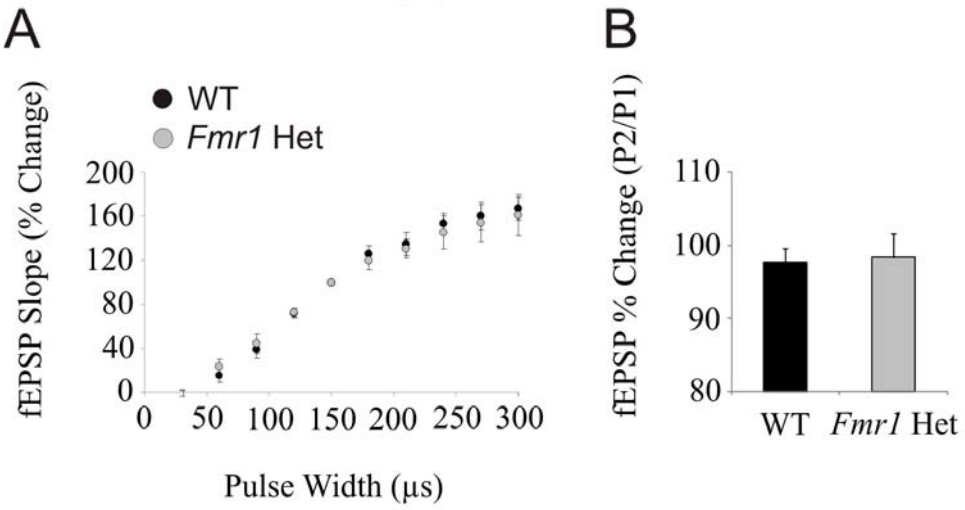

Figure 10. Intact basal synaptic transmission and short-term synaptic plasticity as assessed by a paired pulse conditioning stimulus paradigm in the DG of *Fmr1* Het female mice. (**A**) Synaptic responses to single pulse stimuli at predetermined incremental intensities in 2 – 4 month old *Fmr1* Het animals are not different from those observed in WT animals. (**B**) *Fmr1* Het mice show a similar degree of paired-pulse response to paired-pulse stimulation in the medial perforant path as controls.

IO functions to measure basal excitation in response to increasing applied current were performed in ACSF in the MPP of the DG in young adult female WT and *Fmr1* KO littermate mice. The slope of the fEPSP significantly increased when the applied current was elevated from a pulse width of 30 to 300 μs (WT n = 7, KO n = 9; repeated measures ANOVA: $F_{(8,112)}$ = 278.300, P < 0.001; Figure 11A). No statistical differences were

found between genotypes (repeated-measures ANOVA: $F_{(1,14)} = 0.100$, $P = 0.764$; Figure 11A).

Paired-pulse conditioning stimulation (50 μs interpulse interval) was used as a measure of presynaptic neurotransmitter release in ACSF. Paired-pulse depression was seen in WT animals in the medial perforant path (Pulse 2 = 97.7 ± 1.9 % of pulse 1; n = 6; Figure 11B) but in *Fmr1* KO animals paired pulse responses to the first and second pulse were approximately equivalent (Pulse 2 = 97.3 ± 8.6 % of pulse 1; n = 5; Figure 11B). There was no significant difference between WT and *Fmr1* Het responses to paired-pulse assay of presynaptic release probability (Mann-Whitney U Test: U(9) = 15.000, Z = 0.406, $P = 0.685$; Figure 11B), suggesting that WT and *Fmr1* KO animals have an equal probability of neurotransmitter release.

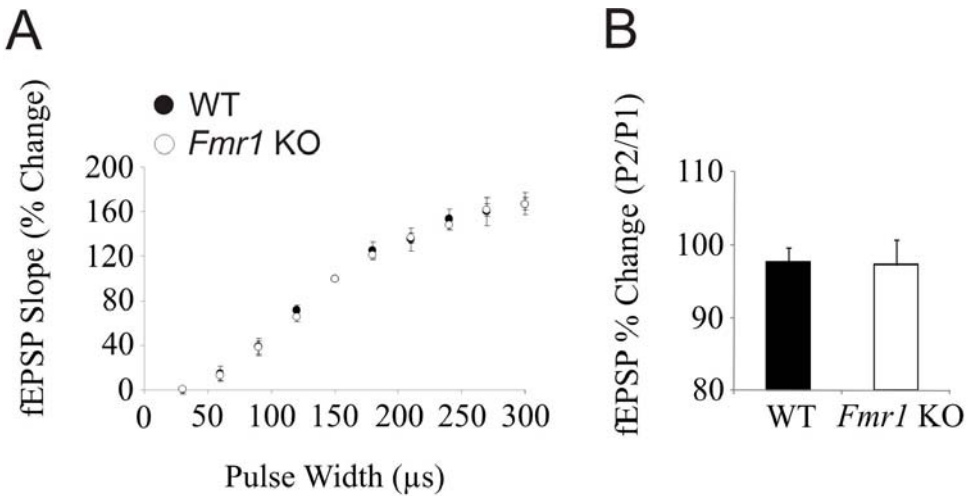

Figure 11. Intact basal synaptic transmission and short-term synaptic plasticity as assessed by a paired pulse conditioning stimulus paradigm in the DG of *Fmr1* KO female mice. (**A**) Synaptic responses to single pulse stimuli at predetermined incremental intensities in 2 – 4 month old *Fmr1* KO female mice are not different from those observed in WT female mice. (**B**) *Fmr1* KO female mice show a similar degree of paired-pulse response to paired-pulse stimulation in the medial perforant path as their WT counterparts.

3.3.3 Synaptic Plasticity in the Dentate Gyrus of WT and *Fmr1* Female Mice

A HFS consisting of 4 trains of 50 pulses at 100 Hz produced a robust LTP of the slope of the fEPSP in WT animals (WT = 92.3 ± 15.3% of baseline response to stimulation; n = 10; Figures 12A/B). The same conditioning stimulation protocol induced significantly less LTP in female *Fmr1* Het animals when measured 50 to 60 minutes after conditioning stimulation (Het: 40.7 ± 6.5 % of baseline response to stimulation; n = 10; Mann-Whitney U Test: U(18) = 16.000, Z = 2.570, *P* = 0.010; Figure 12A/B). The bath application of the NMDAR antagonist APV (50 μM) for a minimum of 5 minutes before and during the HFS conditioning stimulation protocol (applied in the presence of 5 μM BIC) attenuated LTP to the same level in both WT (WT = -4.3 ± 11.4 % of baseline response to stimulation; n = 7) and *Fmr1* Het animals (Het = -4.3 ± 4.6 % of baseline response to stimulation; n = 7; *t* test: $t_{(11)}$ = -1.778, *P* = 0.103; Figure 12C). This data indicates that the LTP induced through HFS in both WT and *Fmr1* KO slices was predominantly NMDA receptor dependent.

A LFS consisting of 900 pulses given at 1 Hz over 15 minutes produced LTD of the slope of the fEPSP in WT animals (WT = -29.7 ± 4.1 % of baseline response to stimulation; n = 11; Figures 12D/E). The same conditioning stimulation protocol induced significantly less LTD in *Fmr1* Het animals when measured 50 to 60 minutes after conditioning stimulation (Het: -12.2 ± 4.1% of baseline response to stimulation; n = 9; Mann-Whitney U Test: U(19) = 8.000, Z = -3.153, *P* = 0.002; Figure 12D/E). This suggests that, similar to that seen in male *Fmr1* KO animals, bidirectional synaptic plasticity is also impaired in the DG of the female *Fmr1* Het animal.

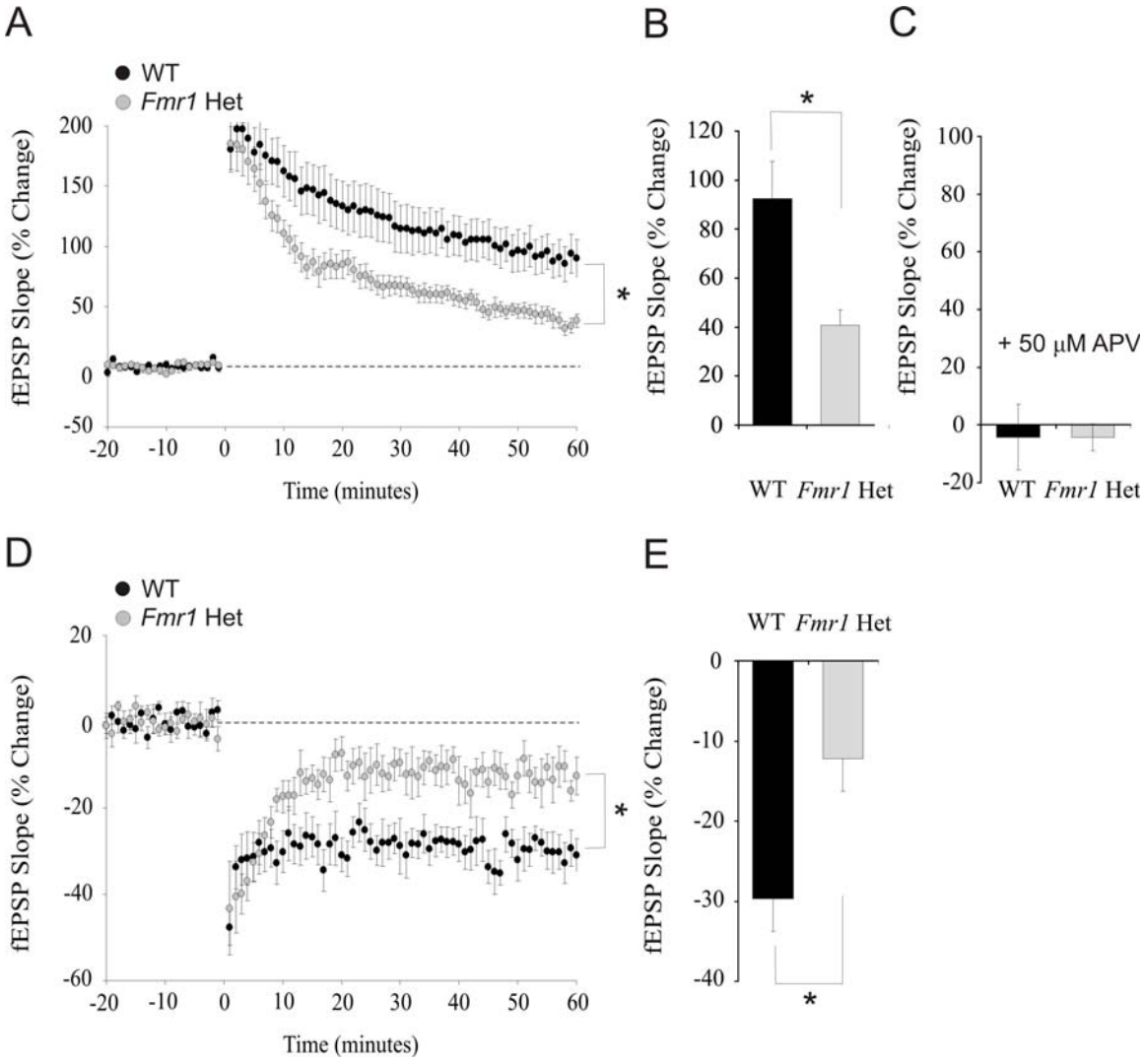

Figure 12. Female *Fmr1* Het mice exhibit impairments in bidirectional synaptic plasticity in the DG. Blockade of the NMDAR abolishes differences in LTP. (**A**) The magnitude of LTP (HFS applied at time zero) is significantly decreased in the DG of 2 – 4 month old *Fmr1* Het female animals when compared to controls at (**B**) 50 – 60 minutes after conditioning stimulation ($P<0.05$). (**C**) Application of the selective NMDAR antagonist (APV) ameliorates differences between WT and *Fmr1* Het animals. (**D**) The magnitude of LTD (LFS applied at time zero) is significantly decreased in the DG of *Fmr1* Het animals when compared to controls at (E) 50 – 60 minutes after conditioning stimulation ($P<0.05$).

HFS (4 trains of 50 pulses at 100 Hz) produced a robust LTP of the slope of the fEPSP in both WT and *Fmr1* KO female slices (WT = 92.3 ± 15.3 % of baseline response to stimulation; n = 11; KO = 73.6 ± 11.9 % of baseline response to stimulation; n = 15; Figures 13A/B). The same conditioning stimulation protocol induced equivalent levels of LTP in female WT and *Fmr1* KO animals when measured 50 to 60 minutes after conditioning stimulation (two-tailed *t* test; $t_{(24)} = 0.746$, $P = 0.462$; Figure 13B). The bath application of the NMDAR antagonist APV (50 μM) for a minimum of 5 minutes before and during the HFS conditioning stimulation protocol (applied in the presence of 5 μM BIC) attenuated LTP to the same level in both WT (WT = -4.3 ± 11.4 % of baseline response to stimulation; n = 7) and *Fmr1* KO animals (KO = -5.7 ± 6.1 % of baseline response to stimulation; n = 7; *t* test; $t_{(12)} = 0.114$, $P = 0.911$; Figure 13C). This data suggests that LTP induced through HFS in both WT and *Fmr1* KO slices was predominantly NMDA receptor dependent.

LFS (900 at 1 Hz) produced a robust LTD of the slope of the fEPSP in both WT and *Fmr1* KO female slices (WT = -29.7 ± 4.1 % of baseline response to stimulation; n = 11; KO = -25.3 ± 6.3 % of baseline response to stimulation; n = 10; Figures 13D/E). The same conditioning stimulation protocol induced equivalent levels of LTD in WT and *Fmr1* KO animals when measured 50 to 60 minutes after conditioning stimulation (Mann-Whitney U Test: U(19) = 50.000, Z = 0.352, $P = 0.725$; Figure 13E). In contrast to the impaired bidirectional synaptic plasticity seen in the DG of male *Fmr1* KO and female *Fmr1* Het animals, this data suggests that the magnitude of bidirectional synaptic plasticity in the DG is normal in female *Fmr1* KO animals.

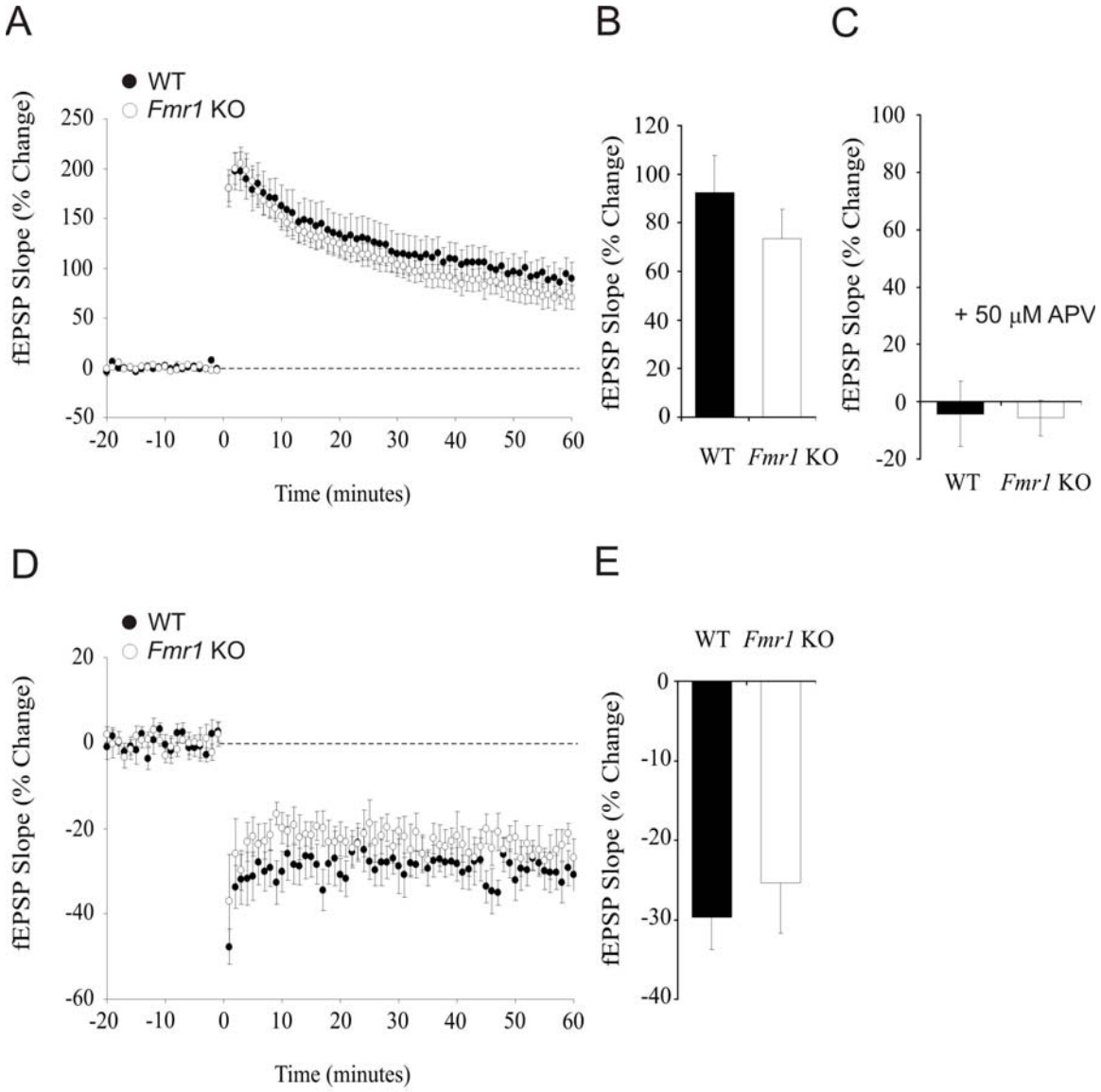

Figure 13. Female *Fmr1* KO mice exhibit normal bidirectional synaptic plasticity in the DG. Blockade of the NMDAR attenuates LTP. (**A**) The magnitude of LTP (HFS applied at time zero) is similar in the DG of 2 – 4 month old *Fmr1* KO female animals when compared to controls at (**B**) 50 – 60 minutes after conditioning stimulation ($P<0.05$). (**C**) Application of the selective NMDAR antagonist (APV) attenuates LTP in both WT and *Fmr1* KO animals. (**D**) The magnitude of LTD (LFS applied at time zero) is also similar in the DG of *Fmr1* Het animals when compared to controls at (E) 50 – 60 minutes after conditioning stimulation ($P<0.05$).

A chemical induction with DHPG was used to elicit mGluR-mediated LTD in the MPP of the DG of WT, *Fmr1* Het, and *Fmr1* KO female mice. A solution of 100 μM DHPG and 50 μM APV was bath applied for a total duration of 5 minutes as the CS. Baseline stimulation consisted of a stimulus set to induce 50 – 60% of the maximum response of the tissue given at an interstimulus interval of 30 seconds.

LTD of the slope of the fEPSP was observed in the DG of both WT and *Fmr1* Het slices (WT = -12.0 ± 4.0 % of baseline response to stimulation; n = 8; Het = -12.7 ± 3.2 % of baseline response to stimulation; n = 12; Figures 14A/B). The same chemical induction protocol elicited equivalent levels of mGluR-mediated LTD in WT and *Fmr1* Het slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test: $t_{(18)} = 0.141$, $P = 0.890$; Figure 14B). A chemical induction with DHPG was also used to elicit mGluR-mediated LTD in the MPP of the DG in *Fmr1* KO mice. LTD of the slope of the fEPSP was observed in the DG of WT and *Fmr1* KO slices (WT = -12.0 ± 4.0 % of baseline response to stimulation; n = 8; KO = -13.8 ± 4.0 % of baseline response to stimulation; n = 9; Figures 14C/D). The same chemical induction protocol induced equivalent levels of mGluR-mediated LTD in the DG of WT and *Fmr1* KO slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test: $t_{(15)} = 0.359$, $P = 0.725$; Figure 14D). This data suggests that mGluR-mediated LTD is intact in the DG of female *Fmr1* transgenic animals.

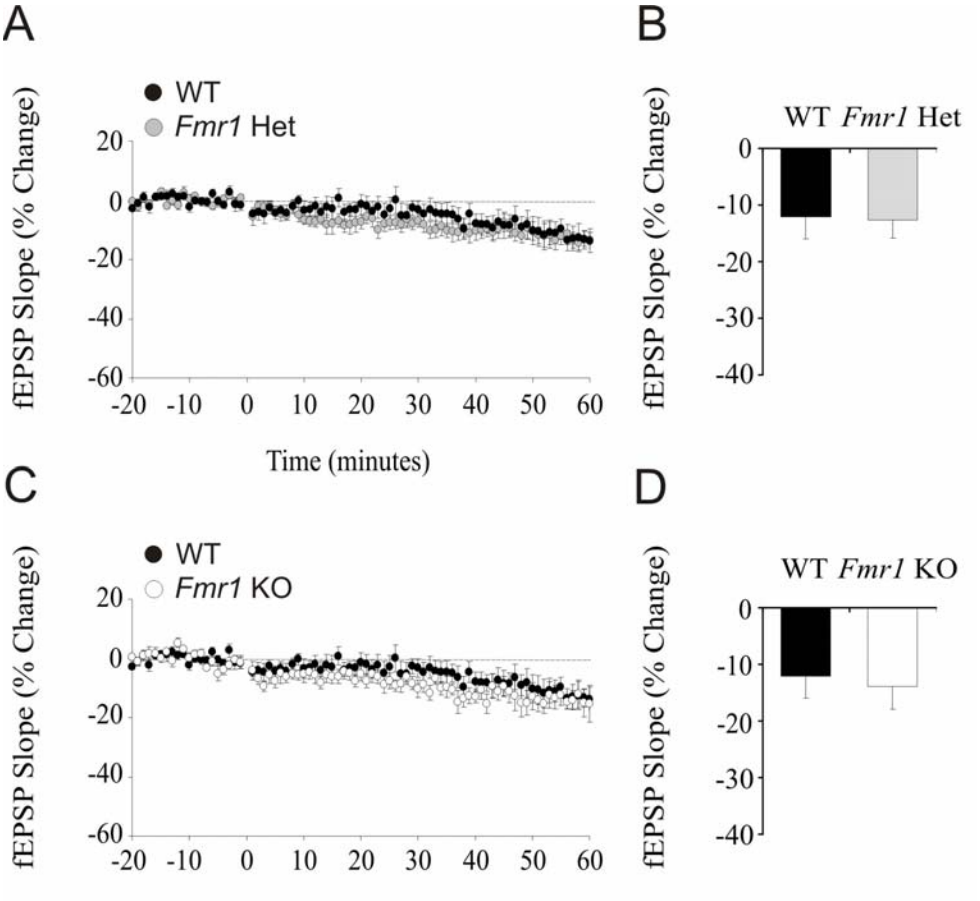

Figure 14. Intact DHPG induced mGluR-mediated LTD in the MPP of the DG of female Fmr1 Het and KO mice. (**A**) The magnitude of LTD induced by a 5 minute DHPG (100 μM) and APV (50 μM) co-application at time zero is similar in 2 – 4 month old female *Fmr1* Het and WT animals when compared at (**B**) 50 – 60 minutes after conditioning stimulation. (**C**) The co-application of DHPG and APV at time zero also reveals similar LTD in 2 – 4 month old female *Fmr1* KO slices when compared to controls (**D**) 50 – 60 minutes after conditioning stimulation.

3.4 Synaptic Plasticity in the CA1 of WT and *Fmr1* Female Mice

3.4.1 Basic Physiological Parameters in the CA1 of WT and *Fmr1* Female Mice

IO functions to measure basal dendritic excitation in response to increasing applied current in the SCP were performed in ACSF in the stratum radiatum of the CA1 region of the hippocampus in young adult (2 - 4 months old) WT and *Fmr1* Het littermate female mice. The slope of the fEPSP significantly increased when the applied current was elevated from a pulse width of 30 to 300 μs (WT n = 6, Het n = 7; repeated measures ANOVA: $F_{(8,88)} = 190.100$, $P < 0.001$; Figure 15A). No statistical differences were found between genotypes (repeated measures ANOVA: $F_{(1,88)} = 0.000$, $P = 0.894$; Figure 15A).

IO functions to measure basal dendritic excitation in response to increasingly applied current in the SCP were also performed in young adult (2 - 4 months old) WT and *Fmr1* KO littermate female mice. The slope of the fEPSP significantly increased when the applied current was increased from a pulse width of 30 to 300 μs (WT n = 6, KO n = 7; repeated measures ANOVA: $F_{(8,88)} = 145.000$, $P = 0.931$; Figure 15B). No statistical differences between genotypes were found (repeated measures ANOVA: $F_{(1,11)} = 0.000$, $P = 0.931$; Figure 15B).

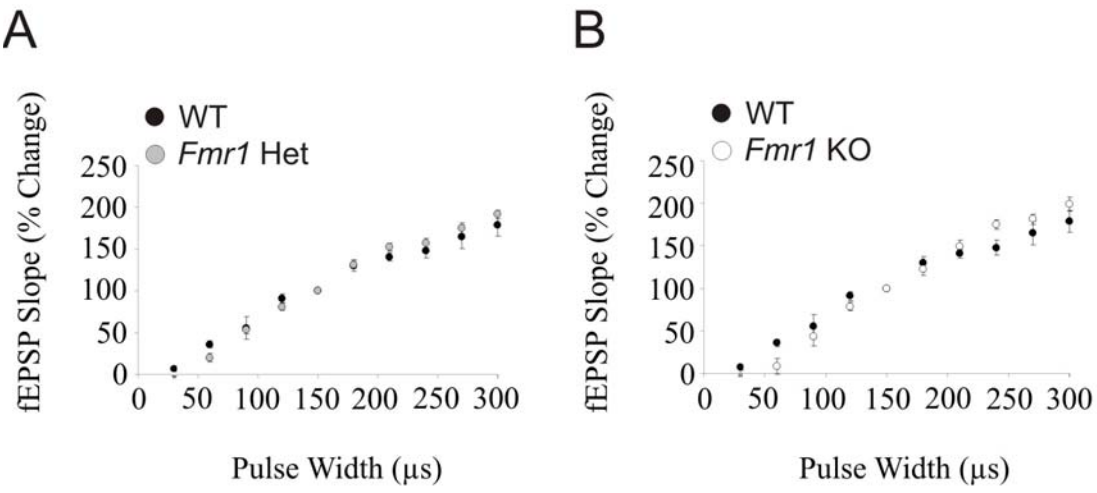

Figure 15. Intact basal synaptic transmission in the CA1 of *Fmr1* Het and *Fmr1* KO female mice. Synaptic responses to single pulse stimuli at predetermined incremental intensities in 2 – 4 month old (**A**) *Fmr1* Het and (**B**) *Fmr1* KO animals are not different from those observed in WT animals in the Schaffer Collateral Pathway of the CA1.

3.4.2 Synaptic Plasticity in the CA1 of WT and *Fmr1* Female Mice

A chemical induction using DHPG was used to elicit mGluR-mediated LTD in the SCP of the CA1 in WT, *Fmr1* Het, and *Fmr1* KO mice. A solution of 100 µM DHPG with 50 µM of APV was bath applied to the slice for 5 minutes as the CS. Baseline stimulation consisted of a stimulus that was set to induce 50 – 60% of the maximum response of the tissue given at an interstimulus interval of 30 seconds. LTD of the slope of the fEPSP was observed in the CA1 of both WT and *Fmr1* Het slices (WT = -24.374 ± 8.694 % of baseline response to stimulation; n = 7; Het = -19.9 ± 6.4 % of baseline response to stimulation; n = 7; Figures 16A/B) and was not significantly different between WT and *Fmr1* Het slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test; $t_{(12)} = 0.432$, $P = 0.674$; Figure 16B). LTD of the slope of the fEPSP was also observed in the CA1 of WT and *Fmr1* KO slices (WT = -24.4 ± 8.7 % of baseline response to stimulation; n = 7; KO = -21.8 ± 4.8 % of baseline response to stimulation; n = 8; Figures 16C/D) and was not significantly different between WT and

Fmr1 KO slices when measured 50 - 60 minutes after application of the CS (two-tailed *t* test; $t_{(13)} = -0.281$, $P = 0.783$; Figure 16D). This data suggests that mGluR-mediated LTD is intact in the CA1 of female *Fmr1* transgenic animals.

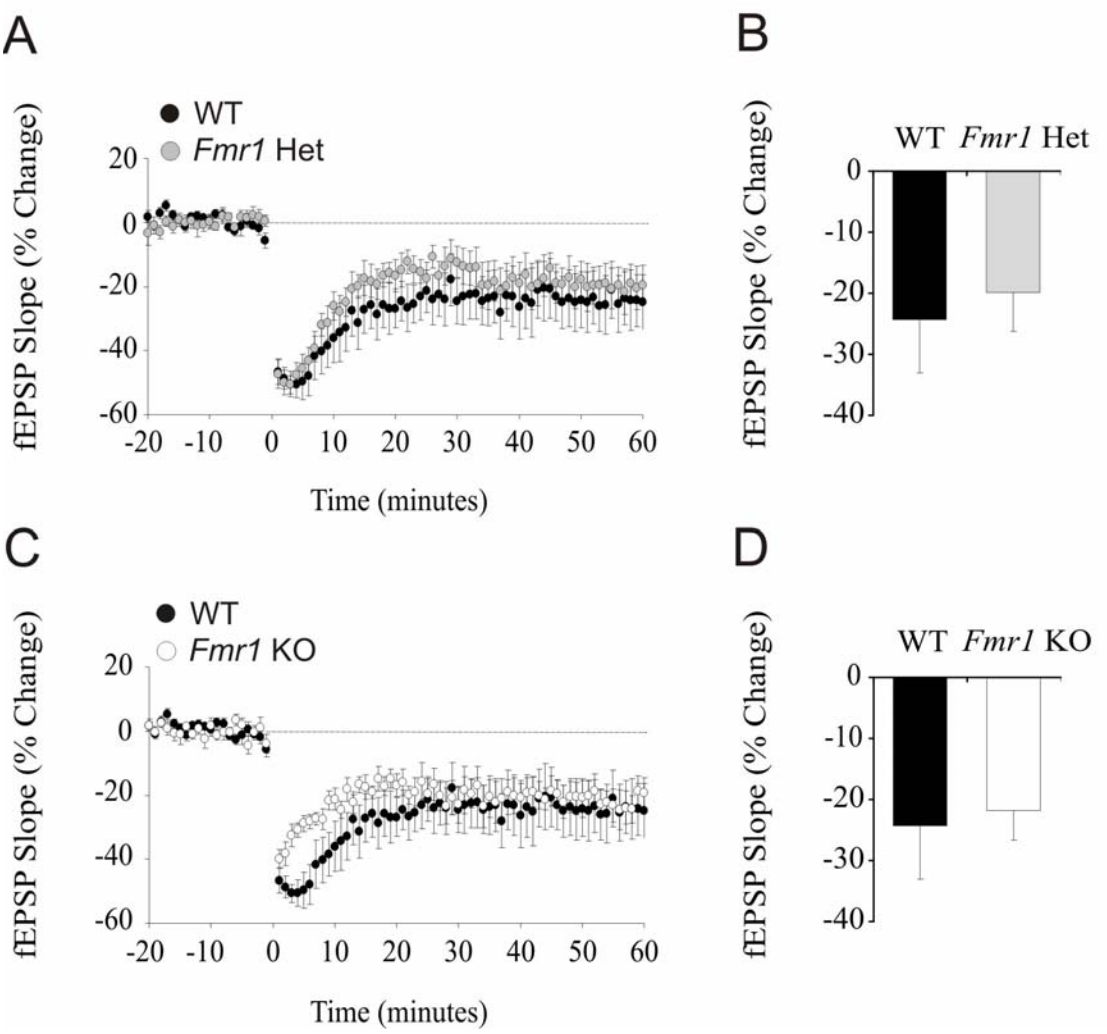

Figure 16. Normal DHPG induced mGluR-mediated LTD in the CA1 of female *Fmr1* Het and *Fmr1* KO mice. (**A**) The magnitude of LTD induced by a 5 minute DHPG (100 μM) and APV (50 μM) co-application at time zero is similar in 2 – 4 month old female *Fmr1* Het and control animals when compared at (**B**) 50 – 60 minutes after conditioning stimulation. (**C**) The co-application of DHPG and APV at time zero also reveals similar LTD in 2 – 4 month old female *Fmr1* KO slices when compared to controls (**D**) 50 – 60 minutes after conditioning stimulation.

4. Discussion

4.1 NMDAR-Mediated Synaptic Plasticity in the Dentate Gyrus

Although males are hemizygous for the *Fmr1* mutation, human females with FXS are usually heterozygous for this mutation. This causes the symptoms of FXS to be characterized by a wide range of phenotypic severity in females. Thus, in this thesis we have investigated bidirectional synaptic plasticity in the DG of male *Fmr1* KO mice as well as female mice that were either heterozygous or homozygous for the Fmr1 mutation. These experiments are the first to examine and to observe selective abnormalities in the capacity for DG plasticity in a young adult female mouse model of FXS. In particular, we have shown that female *Fmr1* Het mice display reduced LTP that is dependent on the NMDA receptor. Female *Fmr1* Het mice also display a reduced LTD when compared to WT controls. Surprisingly however, female *Fmr1* KO animals reveal normal levels of synaptic plasticity.

4.2 Synaptic Plasticity in the Dentate Gyrus of male *Fmr1* KO and female *Fmr1* Het Mice

NMDA receptors have recently come into the spotlight as a novel target in the search for cognitive dysfunction in FXS (Eadie et al., 2010; Krueger et al., 2011; Yun and Trommer, 2011). FMRP has been found to traffick and stabilize key molecules involved in the maintenance of synaptic structure and function, including the NMDAR subunits GluN1, GluN2B (Schütt et al., 2009), and GluN2A (Edbauer et al., 2010). Although we (Figure 7) and others have found normal NMDAR-dependent bidirectional synaptic plasticity in the CA1 subregion of the hippocampus in *Fmr1* KO mice (Godfraind et al., 1996; Huber et al., 2002), the loss of FMRP may have greater functional consequences on NMDAR-dependent plasticity in the DG. In agreement with this hypothesis, we found that both male *Fmr1* KO animals and female *Fmr1* Het animals

show impaired NMDAR-dependent LTP and LTD in this brain region (Figure 3 and Figure 12, respectively). Importantly, prior research has also revealed impaired NMDAR-mediated currents in the DG of young adult male *Fmr1* KO mice (Eadie et al., 2010; Yun and Trommer, 2011).

It may be that FMRP is particularily important during the development of hippocampal circuitry. In agreement, impaired NMDAR-dependent LTP has been found in the CA1 of 2 week old (Hu et al., 2008; Pilpel et al., 2009), but not 6 – 7 week old, *Fmr1* KO mice (Pilpel et al., 2009). As new neurons continue to mature and integrate into DG circuitry throughout young adulthood in both male and female mice (van Praag et al., 2002), FMRP may continue to play a crucial role in the proper functioning of this hippocampal subregion after development.

Previous research in the mouse prefrontal cortex (PFC) found that attenuated spike-timing-dependent LTP in *Fmr1* KO animals was rescued by stronger post-synaptic activity (Meredith et al., 2007). However, in our hands, a stronger HFS conditioning stimulus protocol was not able to increase the amount of LTP in the *Fmr1* KO DG (Figure 4; 100 pulses given at 100 Hz). This may be due to differences in protein expression levels of the NMDAR, as current research in our laboratory has revealed a global deficit in the expression of NMDARs (GluN1, GluN2A, and GluN2B subunits) in the DG of male *Fmr1* KO mice using Western-Blotting (unpublished observations). This decrease in NMDAR numbers may underlie decreases in NMDAR-mediated currents and synaptic plasticity deficits seen in the DG of male *Fmr1* KO animals (Eadie et al., 2010; Yun and Trommer, 2011; Figure 3) and female *Fmr1* Het animals (Figure 12). A reduction in NMDARs may create a ceiling effect on the amount of LTP that can be maintained in the *Fmr1* KO system. Further research warrants the use of a more robust NMDAR-dependent stimulation protocol to conclusively determine whether more powerful activation may improve the amplitude and reliability of calcium signalling through NMDARs in the male *Fmr1* KO DG.

4.3 Paired-Pulse Plasticity in the Dentate Gyrus

Our findings of intact neuronal responses to increasing stimulus strength in the male *Fmr1* KO mouse support previous research (Eadie et al., 2010; Yun and Trommer, 2011; Figure 2) and we have now extended these findings to female *Fmr1* transgenic mice (Figure 10; Figure 11). However, the results obtained for presynaptic release probability as assessed through response to a paired-pulse plasticity induction protocol in the male *Fmr1* KO mouse, diverge from the results obtained by Eadie et al. (2010) and Yun and Trommer (2011). Although overall paired-pulse depression was seen in WT slices, *Fmr1* KO animals did not display the paired-pulse depression that was seen in Eadie et al. (2010) and Yun and Trommer (2011). Instead, *Fmr1* KO animals displayed paired-pulse facilitation in response to the same stimulation parameters used in control slices and in Eadie et al. (2010) and Yun and Trommer (2011).

These discrepancies may be due to the number of slices used in Eadie et al. (2010) and the background strain of animals utilized in Yun and Trommer (2011). Yun and Trommer (2011) used the FVB background strain while our study used the C57Bl/6 background strain. As the strain of inbred mice used during electrophysiological examinations of DG functioning can affect the outcome of synaptic plasticity protocols (Bampton et al., 1999), the potential interaction of the background strain in the knock-out phenotype must be taken into account for the interpretation of electrophysiological data. Although the measurement of fEPSP recordings suggests that the mechanisms of paired-pulse facilitation in the perforant path input to the hilus is grossly similar across control inbred mouse strains (Bampton et al., 1999), it is not clear whether the medial perforant path input to granule cell dendrites has similar paired-pulse plasticity in these two genetic strains, as genetic differences between mouse strains can drastically affect the phenotypes induced by the knockout of other genes (Sibilia and Wagner, 1995; Threadgill et al., 1995). Indeed, the silencing of the *Fmr1* gene has been shown to differentially influence a host of phenotypes depending on the background strain examined (Spencer et al.,

2011), including performance on a hippocampal dependent visuospatial task (Paradee et al., 1999; Dobkin et al., 2000).

An important note in reconciling the differences between the paired-pulse ratio found in the present study and the one by Yun and Trommer (2011) is that these authors included fEPSPs with population spikes in their analyses. When the first pulse of a pair of stimuli is of a sufficient strength to evoke a population spike (a positive inflection in the fEPSP derived from the field current change as action potentials are fired from the soma of the cell) feedback inhibition and feedback excitation will contribute to measures of synaptic function (Andersen et al., 2006). As such, the paired-pulse ratios in Yun and Trommer (2011) may reflect feedback inhibition and feedback excitation in addition to pre-synaptic changes in neurotransmitter release probability. In fact, the paired-pulse ratio can be modulated by the use of low or high stimulus intensity, with higher stimulus intensities that generate population spikes eliciting a statistically larger depression of the second fEPSP slope than that seen with low stimulus intensities (Tamura et al., 2011). In this thesis, the low stimulation strength and the removal of responses that showed neuronal firing may better assess the contribution of the silencing of the *Fmr1* gene on pre-synaptic release probability without the extraneous confounds of feedback inhibition or feedback excitation on the interpretation of paired-pulse plasticity results.

A difference between the current study and that of Eadie et al. (2010) may be the use of a pharmacological agent that blocks $GABA_A$ receptors. Eadie et al. (2010) utilized bicuculline methiodide to block the influence of $GABA_A$ receptors on synaptic activity during paired-pulse recordings while such procedure was not employed in the present work. However, studies suggest that $GABA_A$ receptors may not act to modulate the paired-pulse ratio in an *in vitro* slice preparation at low stimulus intensities (Andreasen and Hablitz, 1994; Peterson et al., unpublished results). Although Eadie et al. (2010) utilized the same mouse colony used in the present study, the discrepancies between the paired-pulse plasticity results may be due to sample sizes. The total number of recordings of paired-pulse plasticity in Eadie et al. (2010) included 6 WT and 5 male *Fmr1* KO slices. In contrast, this thesis made use of 12 WT and 22 male *Fmr1* KO slices to assess

paired-pulse plasticity. As the paired pulse plasticity in this thesis contained a larger cohort of animals than that assessed by Eadie et al. (2010), it may be that a larger sample size was necessary to reveal alterations in presynaptic release probability. These alterations observed in male *Fmr1* KO animals may be mediated through alterations in presynaptic NMDARs or voltage-gated calcium channels. Supporting the loss of power with a small sample size is the results of the same PP plasticity paradigm in female *Fmr1* Het animals (with a sample size of only 2 animals and 3 slices). With this sample size no difference was seen in PP plasticity, although these animals mimic male *Fmr1* KO animals in all other aspects of DG plasticity examined.

4.4 Potential Mechanisms Underlying Intact Synaptic Plasticity in the Female *Fmr1* KO Mouse

Although female *Fmr1* Het mice displayed altered synaptic plasticity when compared to their WT counterparts, female *Fmr1* KO mice did not show reduced levels of synaptic plasticity when compared to WT mice. The significance of this result may lie in the estrogen receptor. The estrogen receptor alpha (ERα) and the estrogen receptor beta (ERβ) are thought to mediate the physiological effects of estrogen on the cell (see Hall et al., 2001 for a review) and estrogen's ability to exert rapid effects on synaptic plasticity has been suggested to be mediated by membrane bound ERα and ERβ (Ishii et al., 2007; Mukai et al., 2007).

Importantly, ERα and ERβ are both found in the dentate gyrus (Shughrue et al., 1997) and estrogen can be locally synthesized in adult hippocampal neurons at basal levels 6 – 8 fold higher than that seen in blood plasma (Kawato et al., 2002). Estrogen synthesis will increase in response to synaptic transmission (Kawato et al., 2002; Shibuya et al., 2003; Ish et al., 2007; Mukai et al., 2007) and will act to increase intracellular calcium transients (Pozzo-miller et al., 1999). These findings, in addition to the localization of ERα to spines in the hippocampus, is suggestive of an ability of ERs to rapidly modulate synaptic plasticity in this region in response to activity dependent signaling (Ishii et al., 2007; Mukai et al., 2007). Importantly, estradiol may upregulate the response of NMDARs to stimuli (Weiland, 1992; Woolley et al., 1997; Daniel and

Dohanich, 2001; El-Bakri et al., 2004) and has been found to increase the concentration of GluN1 protein in the hippocampus (Gazzaley et al., 1996). Estrogen has also been found to rescue corticosterone supression of NMDAR synaptic transmission, mimicking the effects of ER agonists (Ooishi et al., 2012). Thus estrogen may act to sensitize the neuronal network to NMDAR-mediated transmission. This may act to decrease the induction threshold for NMDAR-mediated synaptic plasticity in the hippocampus (Pozzo-Miller et al., 1999) of the female *Fmr1* KO mouse, resulting in intact levels of synaptic plasticity.

Upregulation of brain-derived neurotrophic factor (BDNF) may also contribute to intact synaptic plasticity in the female *Fmr1* KO mouse. Studies have validated a putative estrogen-response-element in the gene encoding BDNF (Sohrabji et al., 1995) and ERα has been found to colocalize with BDNF during development of the hippocampus (Solum and Handa, 2002). Estrogen upregulates the expression of BDNF mRNA and protein in numerous brain regions including the DG (Singh et al., 1995) and BDNF is important for hippocampal LTP (Korte et al., 1995). Increases in endogenous BDNF signaling may be able to rescue deficiencies in LTP stabilization (Rex et al., 2007), which has been found to be abnormal in the male *Fmr1* KO mouse (Chen et al., 2010). In fact, application of BDNF has been found to rescue impaired TBS-induced LTP (5 trains) in the CA1 of the *Fmr1* KO mouse (Lauterborn et al., 2007). Thus an increase in endogenous BDNF levels may also act to contribute to the maintenance of synaptic plasticity in the female *Fmr1* KO. Further research is needed to determine whether basal levels of BDNF are in fact altered in the female *Fmr1* KO animal and whether this may contribute to intact synaptic plasticity in the DG.

Of relevance to FXS is the finding that ERα possesses a stable G-quadruplex motif in its mRNA transcript (Balkwill et al., 2009). This G-quadruplex motif is involved in the modulation of the efficiency of its mRNA translation (Balkwill et al., 2009). FMRP is known to recognize and bind with high affinity to G-quartet and G-quadruplex motifs in target mRNAs in order to regulate their translation (Moine and Mandel, 2001; Melko and Bardoni, 2010). Although further research is warranted to determine whether FMRP may bind and translationally regulate the expression of ERα or ERβ, in the female *Fmr1*

Het and WT mice variable expression of FMRP may function to translationally repress ER mRNA. In the female *Fmr1* KO mice however, a loss of FMRP may allow for abnormally increased expression of the ERα and ERβ, leading to a compensatory mechanism that may act to bolster synaptic plasticity. Further experiments are warranted to determine whether the levels of ERα and ERβ in the hippocampus of the female FXS mouse model do indeed differ and whether this could account for the relatively intact synaptic plasticity seen in the hippocampus of the female *Fmr1* KO mouse.

4.5 mGluR-Mediated Plasticity in the DG

The most influential hypothesis in the search for the neurobiological underpinnings of FXS may be the mGluR theory (Bear et al., 2004). This theory posits that activation of type I mGluRs causes the translation of mRNA important for synaptic plasticity. Normally, this translation is limited by the FMRP also produced by mGluR activation. In the *Fmr1* KO mouse, the lack of FMRP leads to the loss of inhibition of protein synthesis, causing aberrant mGluR-dependent synthesis of LTD-related proteins and increased mGluR-dependent LTD in the CA1 region of the hippocampus (reviewed in Bear et al., 2004).

There has been a paucity of research into whether increased mGluR-mediated LTD occurs in regions of the *Fmr1* KO brain other than the CA1 region of the hippocampus and the cerebellum (Huber et al., 2002; Bear et al., 2004; Koekkoek et al., 2005; Hou et al., 2006; Nosyreva and Huber, 2006; Sharma et al., 2010). Importantly, mGluR-mediated LTD has been found to occur in the DG region of the hippocampus through the application of DHPG (Camodeca et al., 1999; Wang et al., 2007). Using the same protocol as Huber et al. (2002) for both DHPG and PP-LFS induced mGluR-LTD, our results suggest that both of these protocols have the ability to induce mGluR-mediated LTD in the DG of male and female animals (Figure 5, 8, 14, and 16). As the induction of LFS-induced LTD in the DG may also involve the activation of group 1 mGluRs, intact mGluR-mediated LTD in the DG of *Fmr1* transgenic mice suggests that

impaired LFS-induced synaptic plasticity in this hippocampal region may be due to NMDAR hypofunction and not alterations in group I mGluRs.

4.6 Synaptic Plasticity in the CA1

In agreement with previous research, no changes in NMDAR-mediated LTD or LTP were seen in the CA1 of male *Fmr1* KO animals (Huber et al., 2002; Figure 7). Similarily, no changes were seen in either female or male genotypes in the ability of neurons to respond to increasing stimuli strength or on a paired-pulse measure of pre-synaptic function in the CA1 region (Hou et al., 2006; Huber et al., 2002; Choi et al., 2011; Figure 6; Figure 15). Interestingly, intact mGluR-mediated LTD was found in all genotypes and brain regions investigated.

4.7 mGluR-Mediated Plasticity in the CA1

The majority of research behind the mGluR theory of FXS has focused on aberrant mGluR-dependent LTD in the CA1 region of the juvenile *Fmr1* KO hippocampus (Huber et al., 2002; Bear et al., 2004; Hou et al., 2006). Interestingly, only two studies have characterized mGluR-LTD in the CA1 of young adult *Fmr1* KO animals (Choi et al., 2011; Westmark et al., 2011). These authors found enhanced mGluR-LTD in adult *Fmr1* KO animals with application of DHPG. In this thesis, mGluR-mediated LTD was examined in the CA1 and DG of young adult male and female animals. There was no difference seen in the levels of mGluR-LTD obtained in FXS mouse models when compared to control animals using PP-LFS with APV or when using the co-application of DHPG and APV. This is notable, as when mGluR-mediated LTD is chemically induced by DHPG in juvenile animals, LTD is enhanced in *Fmr1* KO animals whether the induction is carried out in the presence or absence of APV (Huber et al., 2002; Hou et al., 2006; Nosyreva and Huber, 2006; Sharma et al., 2010). This may be due to differences in mGluR-mediated LTD induction mechanisms with age (Kumar and Foster, 2007; Moult et al., 2008). The pharmacology of mGluRs in the CA1 of juvenile animals differs from

that in adults, suggesting a developmental regulation of mGluR subtypes may occur in the CA1 region (Baskys and and Malenka, 1991). Our results suggest that the involvement of NMDARs in mGluR-mediated LTD in the *Fmr1* KO animal may be developmentally regulated.

It has recently come to light that mGluR receptors have the ability to both facilitate and inhibit NMDARs to precisely regulate their contributions to synaptic plasticity (Gerber et al., 2007). Activation of NMDARs can also modulate mGluR activity (Alagarsamy et al., 2002, 2005). Applying an mGluR agonist such as DHPG can lead to an enhancement of NMDAR EPSCs evoked by application of NMDA (Harvey and Collingridge, 1993; Dorri et al., 1997; Awad et al., 2000; Snyder et al., 2001) and a depression of NMDAR-currents assessed through synaptic stimulation (Baskys and Malenka, 1991; Snyder et al., 2001; Watabe et al., 2002; Ireland and Abraham, 2009). Whether evoked through NMDA application or synaptic stimulation, DHPG application will act to depress NMDA responses by 60 minutes after treatment, possibly through NMDAR internalization (Snyder et al., 2001). The likelihood of inducing mGluR-LTD and the magnitude of DHPG-induced mGluR-LTD has been found to be increased by removal of inhibitory input or removal of Mg^{2+} that can act to block the NMDAR channel (Harvey et al., 1996; Palmer et al., 1997). mGluR5 may be the mGluR subtype responsible for altering NMDAR responses (Mannaioni et al., 2001; Kotecha and MacDonald, 2003; Kotecha et al., 2003), and has been found to colocalize and coimmunoprecipitate with GluN2B (Guo et al., 2004). Type 1 mGluRs may also have the ability to regulate the phosphorylation of both GluN2A and GluN2B NMDAR subunits (Heidinger et al., 2002; Guo et al., 2004). Our results suggest a role for NMDARs in mGluR-LTD in the hippocampus of adult *Fmr1* mutant mice.

A developmental regulation of NMDAR involvement in mGluR-mediated LTD may in fact exist. In rats, the same PP-LFS induction protocol that was used in this thesis (50 ms paired-pulse interval, 900 pairs of stimuli) or the application of DHPG have been reported to produce an mGluR-dependent (NMDAR independent) LTD in the adult CA1 (Kemp and Bashir, 1999; Kemp et al., 2000; Lee et al., 2005; Kumar and Foster, 2007).

Conversely, PP-LFS produces an NMDAR dependent mGluR-LTD in juvenile rats less than 50 days old (Kemp et al., 2000). Although mGluR-LTD may still be induced in the presence of APV in juvenile rats (Huber et al., 2000), these findings suggest there may be a developmental down-regulation of NMDAR involvement in mGluR-LTD in the adult rat. Furthermore, in aged rats, mGluR-LTD induced with DHPG not only becomes NMDAR dependent, but also increases in magnitude when compared to adult rats (Kumar and Foster, 2007). The NMDAR-dependence of mGluR-LTD protocols may be different in mice than rats however, as the same PP-LFS protocol was sensitive to NMDAR antagonism in the CA1 of adult mice (Lee et al., 2003) and DHPG-induced LTD was insensitive to NMDAR antagonism in younger mice (Watabe et al., 2002). This suggests that with age, other signaling mechanisms may be involved in the induction of mGluR-LTD. As PP-LFS induced mGluR-LTD occludes DHPG-induced mGluR-LTD, these two forms of mGluR-mediated LTD may utilize a common mechanism (Huber et al., 2001). Although further experiments are needed, our data suggests that in young adult FXS mice, mGluRs may lead to an exaggerated mGluR-mediated LTD through a developmentally regulated NMDAR-dependent mechanism.

5. Conclusions and Future Directions

Our laboratory has recently revealed impaired bidirectional synaptic plasticity in the DG region of the male *Fmr1* KO mouse model (Eadie et al., 2010). Importantly, the research in this thesis has corroborated and extended this finding by also evaluating synaptic plasticity in female *Fmr1* transgenic mice. Eadie et al. (2010) correlated deficits in DG bidirectional synaptic plasticity in male mice with NMDAR hypofunction and impaired performance in a behavioural task thought to depend on NMDAR function in the DG (McHugh et al., 2007). The results of the present study indicate that altered functioning of NMDARs may also lead to impaired synaptic plasticity in the female *Fmr1* Het model of FXS. Further research is warranted to determine whether NMDAR-mediated currents are also altered in *Fmr1* transgenic neurons within the *Fmr1* Het DG.

An increased HFS stimulation protocol was not able to increase LTP in the male *Fmr1* KO DG, suggesting that NMDAR hypofunction may create a ceiling effect on the amount of LTP that can be maintained in this system. However, further research is necessary and may include the use of a more robust NMDAR-dependent stimulation protocol to conclusively determine whether more powerful activation may improve the amplitude and reliability of calcium signalling through NMDARs in the male *Fmr1* KO and female *Fmr1* Het DG.

Interestingly, female *Fmr1* KO mice did not show impaired LTP or LTD in the DG, suggesting the existence of a compensatory mechanism. Future studies are warranted to elucidate these mechanisms. Western blotting or an enzyme-linked immunosorbent assay (ELISA) would allow the investigation of whether estrogen receptor levels or basal BDNF levels are altered in the DG of the female *Fmr1* KO animal. Whole-cell patch clamp recording with excised portions of membrane may also lead to a clearer picture of whether estradiol signaling is able to differentially affect the female *Fmr1* KO system. Application of estradiol to excised patches of membrane and removal of membrane patches to an environment without estradiol may allow a closer look at whether estradiol is able to differentially affect synaptic signalling and NMDAR-mediated currents in the

Fmr1 KO slice when compared to *Fmr1* Het and control animals. If so, this finding may point to altered estrogen and NMDA receptor functionality in this system.

As estrogen may improve the sensitivity of cells to NMDAR signalling through an alteration of NMDAR current or NMDAR numbers, techniques such as western blotting or ELISA using proteins obtained from synaptosomal fractions should be used to determine whether numbers of NMDARs are altered at the synapse. These techniques, used in conjunction with the electrophysiological measurement of NMDAR-mediated charge transfer, would give a clearer picture of whether the current transferred through NMDARs or the number of NMDARs are altered in the female *Fmr1* KO DG, possibly leading to the seemingly normal levels of bidirectional synaptic plasticity found in this region. If estrogen mediated signalling pathways do indeed lead to an increase in the ability of neurons to undergo bidirectional synaptic plasticity, this may have potential for the development of therapeutic interventions involving estradiol to target impaired cognitive functioning in FXS.

Our results also suggest an age-dependent involvement of NMDARs in the aberrant mGluR-mediated LTD previously seen in the CA1 of *Fmr1* KO animals. Further research is warranted to determine whether, in our hands, we also see enhanced mGluR-mediated LTD when an NMDAR antagonist is not included during the induction protocol.

Although further investigation will be required to expand our understanding of this devastating neurological syndrome and fully elucidate the mechanisms behind altered synaptic plasticity in the DG, our results suggest that NMDARs may be important contributors to hippocampal pathophysiology in FXS.

Bibliography

Abitbol M, Menini C, Delezoide A, Rhyner T, Vekemans M, Mallet J (1993) Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. Nature genetics 4:147–153.

Alagarsamy S, Rouse ST, Junge C, Hubert GW, Gutman D, Smith Y, Conn PJ (2002) NMDA-induced phosphorylation and regulation of mGluR5. Pharmacology, biochemistry, and behavior 73:299–306.

Alagarsamy S, Saugstad J, Warren L, Mansuy IM, Gereau RW, Conn PJ (2005) NMDA-induced potentiation of mGluR5 is mediated by activation of protein phosphatase 2B/calcineurin. Neuropharmacology 49:135–145.

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. The Journal of comparative neurology 124:319–335.

Amaral D., Whitter M. (1989) The three-dimensional organization of the hippocampal formation: A review of anatomical data. Neuroscience 31:571–591.

Amaral G (1993) Emerging Principles of Intrinsic Hippocampal Organization. Current opinion in neurobiology 3:225–229.

Andersen P, Bliss TV, Lomo T, Olsen LI, Skrede KK (1971) Lamellar organization of hippocampal excitatory pathways. Acta physiologica Scandinavica 76:222–238.

Andersen P, Soleng AF, Raastad M (2000) The hippocampal lamella hypothesis revisited. Brain Research 886: 165 – 171.

Andersen MR, Amaral D, Bliss T, O'Keefe J (2006) The hippocampus book. Oxford University Press, New York, New York.

Andreasen M, Hablitz JJ (1994) Paired-pulse facilitation in the dentate gyrus: a patch-clamp study in rat hippocampus in vitro. Journal of neurophysiology 72:326–336.

Ashley CT, Surcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST (1993a) Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nature genetics 4:244–251.

Ashley CT, Wilkinson KD, Reines D, Warren ST (1993b) FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262:563–566.

Auerbach BD, Bear MF (2010) Loss of the fragile X mental retardation protein decouples metabotropic glutamate receptor dependent priming of long-term potentiation from protein synthesis. Journal of neurophysiology 104:1047–1051.

Awad H, Hubert GW, Smith Y, Levey I, Conn PJ (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. Journal of neuroscience 20:7871–7879.

Bakker CE, Verheij C, Willemsen R, Helm RVD, Oerlemans F, Vermey M, Bygrave A, Hoogeveen T, Oostra BA, Reyniers E, De Boulie K, D'Hooge R, Cras P, Van Velzen D, Nagels G, Martin J-J, De Deyn P, Darby JK, Willems PJ (1994) Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell 78:23–33.

Balkwill GD, Derecka K, Garner TP, Hodgman C, Flint APF, Searle MS (2009) Repression of translation of human estrogen receptor alpha by G-quadruplex formation. Biochemistry 48:11487–11495.

Bampton ET, Gray R a, Large CH (1999) Electrophysiological characterisation of the dentate gyrus in five inbred strains of mouse. Brain research 841:123–134.

Banke TG, Bowie D, Lee H, Huganir RL, Schousboe a, Traynelis SF (2000) Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. Journal of neuroscience 20:89–102.

Bardoni B, Davidovic L, Bensaid M, Khandjian EW (2006) The fragile X syndrome: exploring its molecular basis and seeking a treatment. Expert reviews in molecular medicine 8:1–16.

Barria A, Derkach V, Soderling T (1997) Identification of the Ca^{2+}/Calmodulin-dependent Protein Kinase II Regulatory Phosphorylation Site in the a-Amino-3-hydroxyl-5-methyl- 4-isoxazole-propionate-type Glutamate Receptor. Journal of biological chemistry 272:32727–32730.

Bartesaghi R, Gessi T (2004) Parallel activation of field CA2 and dentate gyrus by synaptically elicited perforant path volleys. Hippocampus 14:948–963.

Baskys A, and Malenka R. (1991) Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. Journal of Physiology 444:687–701.

Bassell GJ, Warren ST (2008) Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron 60:201–214.

Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. Trends in neurosciences 27:370–377.

Bilousova TV, Dansie L, Ngo M, Aye J, Charles JR, Ethell DW, Ethell IM (2009) Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. Journal of medical genetics 46:94–102.

Bliss TVP, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. Journal of Physiology 232:331–356.

Bolshakov VY, Carboni L, Cobb MH, Siegelbaum S, Belardetti F (2000) Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. Nature neuroscience 3:1107–1112.

De Boulle K, Verkerk AJMH, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van Den Bos F, De Graaff E, Oostra BA, Willems PJ (1993) A Point Mutation in the FMR-1 Gene Associated with Fragile X Mental Retardation. Nature genetics 3:31 - 35.

Braun, K, Segal, M (1991) FMRP involvement in formation of synapses among cultured hippocampal neurons. Cerebral cortex 10:1045 – 1052.

Brown V, Small K, Lakkis L, Feng Y, Gunter C, Wilkinson KD, Warren ST (1998) Purified recombinant Fmrp exhibits selective RNA binding as an intrinsic property of the fragile X mental retardation protein. The Journal of biological chemistry 273:15521–15527.

Bushell TJ, Jane DE, Tse HW, Watkins JC, Garthwaite J, Collingridge GL (1996) Pharmacological antagonism of the actions of group II and III mGluR agonists in the lateral perforant path of rat hippocampal slices. British journal of pharmacology 117:1457–1462.

Camodeca N, Breakwell N, Rowan MJ, Anwyl R (1999) Induction of LTD by activation of group I mGluR in the dentate gyrus in vitro. Neuropharmacology 38:1597–1606.

Caudy AA, Myers M, Hannon GJ, Hammond SM (2002) Fragile X-related protein and VIG associate with the RNA interference machinery. Genes & Development: 2491–2496.

Ceman S, O'Donnell WT, Reed M, Patton S, Pohl J, Warren ST (2003) Phosphorylation influences the translation state of FMRP-associated polyribosomes. Human molecular genetics 12:3295–3305.

Chen LY, Rex CS, Babayan AH, Kramár E a, Lynch G, Gall CM, Lauterborn JC (2010) Physiological activation of synaptic Rac>PAK (p-21 activated kinase) signaling is

defective in a mouse model of fragile X syndrome. Journal of neuroscience 30:10977–10984.

Chevaleyre V, Siegelbaum S (2010) Strong CA2 pyramidal neuron synapses define a powerful disynaptic cortico-hippocampal loop. Neuron 66:560–572.

Choi CH, Schoenfeld BP, Bell AJ, Hinchey P, Kollaros M, Gertner MJ, Woo NH, Tranfaglia MR, Bear MF, Zukin RS, McDonald TV, Jongens T, McBride SMJ (2011) Pharmacological reversal of synaptic plasticity deficits in the mouse model of Fragile X syndrome by group II mGluR antagonist or lithium treatment. Brain research 1380:106–119.

Comery T, Harris JB, Willems PJ, Oostra B, Irwin S, Weiler IJ, Greenough WT (1997) Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proceedings of the National Academy of Sciences of the United States of America 94:5401–5404.

Connor S, Hoeffer C, Klann E, Nguyen PV (2011) Fragile X mental retardation protein regulates heterosynaptic plasticity in the hippocampus. Learning & memory 18:207–220.

Coutinho V, Knopfel T (2002) Metabotropic Glutamate Receptors: Electrical and Chemical Signaling Properties. The Neuroscientist 8:551–561.

Cull-Candy SG, Leszkiewicz DN (2004) Role of distinct NMDA receptor subtypes at central synapses. Science Signalling 16:1-9.

Van Dam D, D'Hooge R, Hauben E, Reyniers E, Gantois I, Bakker CE, Oostra B, Kooy RF, De Deyn PP (2000) Spatial learning, contextual fear conditioning and conditioned emotional response in Fmr1 knockout mice. Behavioural brain research 117:127–136.

Daniel JM, Dohanich GP (2001) Acetylcholine mediates the estrogen-induced increase in NMDA receptor binding in CA1 of the hippocampus and the associated improvement in working memory. Journal of neuroscience 21:6949–6956.

Darnell JC, Driesche SJV, Zhang C, Ying K, Hung S, Mele A, Fraser CE, Stone EF, Chen C, Fak JJ, Wook S, Licatalosi DD, Richter JD, Darnell RB (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146:247–261.

Darnell JC, Fraser CE, Mostovetsky O, Stefani G, Jones TA, Eddy SR, Darnell RB (2005) Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. Genes & Development 3:903–918.

Deng PY, Sojka D, Klyachko V. (2011) Abnormal Presynaptic Short-Term Plasticity and Information Processing in a Mouse Model of Fragile X Syndrome. Journal of neuroscience 31:10971–10982.

Dictenberg JB, Swanger S, Antar LN, Singer RH, Bassell GJ (2008) A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. Developmental cell 14:926–939.

Dobkin C, Rabe A, Dumas R, El Idrissi A, Haubenstock H, Brown WT (2000) Fmr1 knockout mouse has a distinctive strain-specific learning impairment. Neuroscience 100:423–429.

Dorri F, Hampson DR, Baskys A, Wojtowicz JM (1997) Down-regulation of mGluR5 by antisense deoxynucleotides alters pharmacological responses to applications of ACPD in the rat hippocampus. Experimental neurology 147:48–54.

D'Hooge R, Nagels G, Franck F, Bakker CE, Reyniers E, Storm K, Kooy RF, Oostra BC, Willems PJ, De Deyn PP (1997) Mildly impaired water maze performance in male Fmr1 knockout mice. Neuroscience 76:367–376.

Eadie BD, Cushman J, Kannangara TS, Fanselow MS, Christie BR (2010) NMDA receptor hypofunction in the dentate gyrus and impaired context discrimination in adult Fmr1 knockout mice. Hippocampus 22:241-254.

Eadie BD, Zhang WN, Boehme F, Gil-Mohapel J, Kainer L, Simpson JM, Christie BR (2009) Fmr1 knockout mice show reduced anxiety and alterations in neurogenesis that are specific to the ventral dentate gyrus. Neurobiology of disease 36:361–373.

Eberhart DE, Malter HE, Feng Y, Warren ST (1996) The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. Human molecular genetics 5:1083–1091.

Edbauer D, Neilson JR, Foster KA, Wang C-F, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp P, Sheng M (2010) Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron 65:373–384.

Eichenbaum H (2003) The hippocampus, episodic memory, declarative memory, spatial memory…where does it all come together? International Congress Series 1250:235–244.

El-Bakri NK, Islam A, Zhu S, Elhassan A, Mohammed A, Winblad B, Adem A (2004) Effects of estrogen and progesterone treatment on rat hippocampal NMDA receptors: relationship to Morris water maze performance. Journal of cellular and molecular medicine 8:537–544.

Feng Y, Gutekunst C, Eberhart DE, Yi H, Warren ST, Hersch SM (1997) Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. Journal of neuroscience 17:1539–1547.

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Warren ST (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1047–1058.

Gallagher SM, Daly CA, Bear MF, Huber KM (2004) Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. Journal of neuroscience 24:4859–4864.

Gantois I, Bakker CE, Reyniers E, Willemsen R, D'Hooge R, De Deyn PP, Oostra B, Kooy RF (2001) Restoring the phenotype of fragile X syndrome: insight from the mouse model. Current molecular medicine 1:447–455.

Garber KB, Visootsak J, Warren ST (2008) Fragile X syndrome. European journal of human genetics 16:666–672.

Gazzaley H, Weiland NG, McEwen BS, Morrison JH (1996) Differential regulation of NMDAR1 mRNA and protein by estradiol in the rat hippocampus. Journal of neuroscience 16:6830–6838.

Gerber U, Gee CE, Benquet P (2007) Metabotropic glutamate receptors: intracellular signaling pathways. Current opinion in pharmacology 7:56–61.

Gladding CM, Fitzjohn SM, Molna E (2009) Metabotropic Glutamate Receptor-Mediated Long- Term Depression: Molecular Mechanisms. 61:395–412.

Godfraind JM, Reyniers E, De Boulle K, D'Hooge R, De Deyn PP, Bakker CE, Oostra B a, Kooy RF, Willems PJ (1996) Long-term potentiation in the hippocampus of fragile X knockout mice. American journal of medical genetics 64:246–251.

Greco CM, Navarro CS, Hunsaker MR, Maezawa I, Shuler JF, Tassone F, Delany M, Au JW, Berman RF, Jin L-W, Schumann C, Hagerman PJ, Hagerman RJ (2011) Neuropathologic features in the hippocampus and cerebellum of three older men with fragile X syndrome. Molecular autism 2:1-13.

Greicius MD, Boyett-Anderson JM, Menon V, Reiss AL (2004) Reduced basal forebrain and hippocampal activation during memory encoding in girls with fragile X syndrome. NeuroReport 15:1579–1583.

Grossman AW, Aldridge GM, Lee KJ, Zeman MK, Jun CS, Azam HS, Arii T, Imoto K, Greenough WT, Rhyu IJ (2010) Developmental characteristics of dendritic spines in the dentate gyrus of Fmr1 knockout mice. Brain research 1355:221–227.

Grossman AW, Elisseou NM, McKinney BC, Greenough WT (2006) Hippocampal pyramidal cells in adult Fmr1 knockout mice exhibit an immature-appearing profile of dendritic spines. Brain research 1084:158–164.

Guo W, Wei F, Zou S, Robbins MT, Sugiyo S, Ikeda T, Tu JC, Worley PF, Dubner R, Ren K (2004) Group I metabotropic glutamate receptor NMDA receptor coupling and signaling cascade mediate spinal dorsal horn NMDA receptor 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. Journal of neuroscience 24:9161–9173.

Hagerman RJ, Hagerman PJ (2002) Fragile X syndrome: Diagnosis treatment and research (3ed). The John Hopkins University Press, Baltimore, Maryland.

Hall JM, Couse JF, Korach KS (2001) The multifaceted mechanisms of estradiol and estrogen receptor signaling. The Journal of biological chemistry 276:36869–36872.

Handa V, Goldwater D, Stiles D, Cam M, Poy G, Kumari D, Usdin K (2005) Long CGG-repeat tracts are toxic to human cells: implications for carriers of Fragile X premutation alleles. FEBS letters 579:2702–2708.

Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD (1992) Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. Human molecular genetics 1:571–578.

Hanson JE, Madison DV (2007) Presynaptic FMR1 genotype influences the degree of synaptic connectivity in a mosaic mouse model of fragile X syndrome. Journal of neuroscience 27:4014–4018.

Harvey J, Collingridge GL (1993) Signal transduction pathways involved in the acute potentiation of NMDA responses by 1S,3R-ACPD in rat hippocampal slices. British journal of pharmacology 109:1085–1090.

Harvey J, Palmer MJ, Irving AJ, Clarke VRJ, Collingridge GL (1996) NMDA receptor dependence of mGlu-mediated depression of synaptic transmission in the CAl region of the rat hippocampus. British journal of pharmacology 119:1239–1247.

Hazlett HC, Poe MD, Lightbody A, Gerig G, Macfall JR, Ross AK, Provenzale J, Martin A, Reiss AL, Piven J (2009) Teasing apart the heterogeneity of autism: Same behavior, different brains in toddlers with fragile X syndrome and autism. Journal of neurodevelopmental disorders 1:81–90.

Hebb, DO (1949) The organization of behavior: A neuropsychological theory. Wiley, New York, New York.

Heidinger V, Manzerra P, Wang XQ, Strasser U, Yu S-P, Choi DW, Behrens MM (2002) Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current:

mediation through the Pyk2/Src-family kinase pathway in cortical neurons. Journal of neuroscience 22:5452–5461.

Hersh JH, Saul R (2011) Health supervision for children with fragile X syndrome. Pediatrics 127:994–1006.

Hinds LH, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, Schalling M (1993) Tissue specific expression of FMR-1 provides evidence for a functional role in fragile x syndrome. Nature genetics 3:36 - 43.

Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annual review of neuroscience 17:31–108.

Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E (2006) Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. Neuron 51:441–454.

Hou L, Klann E (2004) Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. Journal of neuroscience 24:6352–6361.

Hu H, Qin Y, Bochorishvili G, Zhu Y, van Aelst L, Zhu JJ (2008) Ras signaling mechanisms underlying impaired GluR1-dependent plasticity associated with fragile X syndrome. Journal of neuroscience 28:7847–7862.

Huang CC, You JL, Wu MY, Hsu KS (2004) Rap1-induced p38 mitogen-activated protein kinase activation facilitates AMPA receptor trafficking via the GDI.Rab5 complex. Potential role in (S)-3,5-dihydroxyphenylglycene-induced long term depression. The Journal of biological chemistry 279:12286–12292..

Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proceedings of the National Academy of Sciences of the United States of America 99:7746–7750.

Huber KM, Roder JC, Bear MF (2001) Chemical Induction of mGluR5- and Protein Synthesis − Dependent Long-Term Depression in Hippocampal Area CA1. Journal of Neurophysiology:321–325.

Hunsaker MR, Mooy GG, Swift JS, Kesner RP (2007) Dissociations of the medial and lateral perforant path projections into dorsal DG, CA3, and CA1 for spatial and nonspatial (visual object) information processing. Behavioral neuroscience 121:742–750.

Idrissi, AE, Neuwirth, LS, L'Amoreaux, W (2010) Taurine regulation of short term synaptic plasticity in fragile X mice. Journal of Biomedical Science 17:1-5.

Ireland DR, Abraham WC (2009) Mechanisms of group I mGluR-dependent long-term depression of NMDA receptor-mediated transmission at Schaffer collateral-CA1 synapses. Journal of neurophysiology 101:1375–1385.

Irwin S a, Galvez R, Greenough WT (2000) Dendritic spine structural anomalies in fragile-X mental retardation syndrome. Cerebral cortex 10:1038–1044.

Isaacson, R.L., and Wickelgren WO (1962) Hippocampal Ablation and Passive Avoidance. Science 138:1104–1106.

Ish H, Tsurugizawa T, Ogiue-ikeda M, Asashima M, Mukai H, Murakami G, Kimoto T, Kawato S (2007) Local Production of Sex Hormones and Their Modulation of Hippocampal Synaptic Plasticity. The Neuroscientist 13:323-334.

Ishii H, Tsurugizawa T, Ogiue-ikeda M, Asashima M, Mukai H (2007) Local Production of Sex Hormones and Their Modulation of Hippocampal Synaptic Plasticity. The Neuroscientist 13:323–334.

Ishizuka A, Siomi MC, Siomi H (2002) A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. Genes & Development 16:2497–2508.

Ivanco TL, Greenough WT (2002) Altered mossy fiber distributions in adult Fmr1 (FVB) knockout mice. Hippocampus 12:47–54.

Jahr CE, Stevens CF (1987) Glutamate activates multiple single channel conductances in hippocampal neurons. Nature 325:522–525.

Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, Nelson DL, Moses K, Warren ST (2004) Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nature neuroscience 7:113–117.

Johnson JW, Ascher P (1990) Voltage dependent block by intracellular Mg^{2+} of N-methyl-D-aspartate-activated channels. Biophysical Journal 57:1085–1090.

Jäkälä P, Hänninen T, Ryynänen M, Laakso M, Partanen K, Mannermaa A, Soininen H (1997) Fragile-X: neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. The Journal of clinical investigation 100:331–338.

Kates WR, Abrams MT, Kaufmann WE, Breiter SN, Reiss AL (1997) Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. Psychiatry research 75:31–48.

Kawato S, Hojo Y, Kimoto T (2002) Histological and metabolism analysis of P450 expression in the brain. Methods in enzymology 357:241–249.

Kemp N, Bashir ZI (1999) Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. Neuropharmacology 38:495–504.

Kemp N, McQueen J, Faulkes S, Bashir ZI (2000) Different forms of LTD in the CA1 region of the hippocampus: role of age and stimulus protocol. The European Journal of neuroscience 12:360–366.

Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G (2004) Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proceedings of the National Academy of Sciences of the United States of America 101:360–365.

Kim MJ, Dunah AW, Wang YT, Sheng M (2005) Differential roles of NR2A- and NR2B-containing NMDA receptors in Ras-ERK signaling and AMPA receptor trafficking. Neuron 46:745–760.

Kirkpatrick LL, McIlwain KA, Nelson DL (2001) Comparative genomic sequence analysis of the FXR gene family: FMR1, FXR1, and FXR2. Genomics 78:169–177.

Klemmer P, Meredith RM, Holmgren CD, Klychnikov OI, Stahl-Zeng J, Loos M, van der Schors RC, Wortel J, De Wit H, Spijker S, Rotaru DC, Mansvelder HD, Smit AB, Li KW (2011) Proteomics, ultrastructure, and physiology of hippocampal synapses in a fragile x syndrome mouse model reveal presynaptic phenotype. The Journal of biological chemistry 286:25495–25504.

Koekkoek SK, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, De Graaf W, Smit AE, VanderWerf F, Bakker CE, Willemsen T, Ikeda T, Kakizawa S, Onodera K, Nelson DL, Mientjes E, Joosten M, Dr Schutter E, Oostra BA, Ito M, De Zeeuw CI (2005) Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. Neuron 47:339–352.

Kooy RF, D'Hooge R, Reyniers E, Bakker CE, Nagels G, De Boulle K, Storm K, Clincke G, De Deyn PP, Oostra B A, Willems PJ (1996) Transgenic mouse model for the fragile X syndrome. American journal of medical genetics 64:241–245.

Korte M, Carroll P, Wolf E, Brem G, Thoenent H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proceedings of the National Academy of Sciences of the United States of America 92:8856–8860.

Kotecha S, MacDonald JF (2003) Signaling molecules and receptor transduction cascades that regulate NMDA receptor-mediated synaptic transmission. International review of neurobiology 54:51–106.

Krueger DD, Osterweil EK, Chen SP, Tye LD, Bear MF (2011) Cognitive dysfunction and prefrontal synaptic abnormalities in a mouse model of fragile X syndrome. Proceedings of the National Academy of Sciences of the United States of America 108:2587–2592.

Kumar A, Foster TC (2007) Shift in induction mechanisms underlies an age-dependent increase in DHPG-induced synaptic depression at CA3 CA1 synapses. Journal of neurophysiology 98:2729–2736.

Kumar SS, Bacci A, Kharazia V, Huguenard JR (2002) A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. Journal of neuroscience 22:3005–3015.

Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, Morikawa K (2000) Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature 407:971–977.

Laggerbauer B, Ostareck D, Keidel EM, Ostareck-Lederer a, Fischer U (2001) Evidence that fragile X mental retardation protein is a negative regulator of translation. Human molecular genetics 10:329–338.

Larson J, Jessen RE, Kim D, Fine A-KS, Du Hoffmann J (2005) Age-dependent and selective impairment of long-term potentiation in the anterior piriform cortex of mice lacking the fragile X mental retardation protein. Journal of neuroscience 25:9460–9469.

Lauterborn JC, Rex CS, Kramár E, Chen LY, Pandyarajan V, Lynch G, Gall CM (2007) Brain-derived neurotrophic factor rescues synaptic plasticity in a mouse model of fragile X syndrome. Journal of neuroscience 27:10685–10694.

Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631–643.

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955–959.

Lee HY, Ge WP, Huang W, He Y, Wang GX, Rowson-Baldwin A, Smith SJ, Jan YN, Jan LY (2011) Bidirectional Regulation of Dendritic Voltage-Gated Potassium Channels by the Fragile X Mental Retardation Protein. Neuron 72:630–642.

Lee LJ, Lo FS, Erzurumlu RS (2005) NMDA receptor-dependent regulation of axonal and dendritic branching. Journal of neuroscience 25:2304–2311.

Lee SH, Liu L, Wang YT, Sheng M (2002) Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 36:661–674.

Levenga J, De Vrij FMS, Buijsen R a M, Li T, Nieuwenhuizen IM, Pop A, Oostra BA, Willemsen R (2011) Subregion-specific dendritic spine abnormalities in the hippocampus of Fmr1 KO mice. Neurobiology of learning and memory 95:467–472.

Li J, Pelletier MR, Perez Velazquez J-L, Carlen PL (2002) Reduced cortical synaptic plasticity and GluR1 expression associated with fragile X mental retardation protein deficiency. Molecular and cellular neurosciences 19:138–151.

Lightbody A, Reiss AL (2009) Gene, brain, and behavior relationships in fragile X syndrome: evidence from neuroimaging studies. Developmental disabilities research reviews 15:343–352.

Lisman, J, Raghavachari, S (2006) A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses. Science Signalling 10:1-11.

Liu Z-H, Smith CB (2009) Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. Neuroscience letters 454:62–66.

Lopez de Armentia M, Sah P (2003) Development and subunit composition of synaptic NMDA receptors in the amygdala: NR2B synapses in the adult central amygdala. Journal of neuroscience 23:6876–6883.

Lorenzini CA, Baldi E, Bucherelli C, Sacchetti B, Tassoni G (1996) Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodotoxin functional inactivation study. Brain research 730:32–39.

Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT (2001) Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. Neuron 29:243–254.

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. Journal of neuroscience 17:5196–5205.

Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. The European journal of neuroscience 8:1488–1500.

Lømo T (2003) The discovery of long-term potentiation. Philosophical transactions of the Royal Society of London Series B, Biological sciences 358:617–620.

Macek T, Winder DG, Gereau RW, Ladd CO, Conn PJ (1996) Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. Journal of neurophysiology 76:3798–3806.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5–21.

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ (2001) Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. The Journal of neuroscience 21:5925–5934.

McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S (2007) Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science 317:94–99.

McMahon A, Fosten M, Monk M (1981) Random X-chromosome inactivation in female primordial germ cells in the mouse. Journal of embryology and experimental morphology 64:251–258.

Melko M, Bardoni B (2010) The role of G-quadruplex in RNA metabolism: involvement of FMRP and FMR2P. Biochimie 92:919–926.

Meredith RM, Holmgren CD, Weidum M, Burnashev N, Mansvelder HD (2007) Increased threshold for spike-timing-dependent plasticity is caused by unreliable calcium signaling in mice lacking fragile X gene FMR1. Neuron 54:627–638.

Min WW, Yuskaitis CJ, Yan Q, Sikorski C, Chen S, Jope RS, Bauchwitz RP (2009) Elevated glycogen synthase kinase-3 activity in Fragile X mice: key metabolic regulator with evidence for treatment potential. Neuropharmacology 56:463–472.

Mineur YS, Sluyter F, De Wit S, Oostra BA, Crusio WE (2002) Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 12:39–46.

Moine H, Mandel JL (2001) Biomedicine. Do G quartets orchestrate fragile X pathology? Science 294:2487–2488.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529–540.

Moriyoshi K, Masu M, Takahiro I, Shigemoto R, Mizuno N, Nakanishi S (1991) Molecular cloning and characterization of the rat NMDA receptor. Nature 354:31–37.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. Journal of neuroscience methods 11:47–60.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place Navigation Impaired in Rats with Hippocampal Lesions. 297:681-683.

Moult PR, Correa SA, Collingridge GL, Fitzjohn SM, Bashir ZI (2008) Co-activation of p38 mitogen-activated protein kinase and protein tyrosine phosphatase underlies metabotropic glutamate receptor-dependent long-term depression. The Journal of Physiology 586:2499–2510.

Mukai H, Tsurugizawa T, Murakami G, Kominami S, Ishii H, Ogiue-Ikeda M, Takata N, Tanabe N, Furukawa A, Hojo Y, Ooishi Y, Morrison JH, Janssen WG, Rose JA, Chambon P, Kato S, Izumi S, Yamazaki T, Kimoto T, Kawato S (2007) Rapid modulation of long-term depression and spinogenesis via synaptic estrogen receptors in hippocampal principal neurons. Journal of Neurochemistry 100:950–967.

Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486-488.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Nakagawa T, Cheng Y, Ramm E, Sheng M, Walz T (2005) Structure and different conformational states of native AMPA receptor complexes. Nature 433:545–549.

Narayanan U, Nalavadi V, Nakamoto M, Thomas G, Ceman S, Bassell GJ, Warren ST (2008) S6K1 phosphorylates and regulates fragile X mental retardation protein (FMRP) with the neuronal protein synthesis-dependent mammalian target of rapamycin (mTOR) signaling cascade. The Journal of biological chemistry 283:18478–18482.

Nguyen PV (2006) Comparative plasticity of brain synapses in inbred mouse strains. The Journal of experimental biology 209:2293–2303.

Nosyreva ED, Huber KM (2006) Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile X syndrome. Journal of neurophysiology 95:3291–3295.

Ooishi Y, Mukai H, Hojo Y, Murakami G, Hasegawa Y, Shindo T, Morrison JH, Kimoto T, Kawato S (2012) Estradiol rapidly rescues synaptic transmission from corticosterone-induced suppression via synaptic/extranuclear steroid receptors in the hippocampus. Cerebral cortex. 22:926–936.

Pagliusi SR, Gerrard P, Abdallah M, Talabot D, Catsicas S (1994) Age-related changes in expression of ampa-selective glutamate receptor subunits: Is calcium-permeability altered in hippocampal neurons? Neuroscience 61:429–433.

Palmer MJ, Irving AJ, Seabrook GR, Jane DE, Collingridge GL (1997) The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. Neuropharmacology 36:1517–1532.

Paradee W, Melikian HE, Rasmussen DL, Kenneson A, Conn PJ, Warren ST (1999) Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. Neuroscience 94:185–192.

Peier a M, McIlwain KL, Kenneson A, Warren ST, Paylor R, Nelson DL (2000) (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. Human molecular genetics 9:1145–1159.

Perez-Otano I, Schulteis CT, Contractor a, Lipton SA, Trimmer JS, Sucher NJ, Heinemann SF (2001) Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. Journal of neuroscience 21:1228–1237.

Pfeiffer BE, Huber KM (2007) Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. Journal of neuroscience 27:3120–3130.

Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral neuroscience 106:274–285.

Pieretti M, Zhang F, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of Expression of the Fmr1 gene in Fragile X Syndrome. Cell 66:817–822.

Pilpel Y, Kolleker A, Berberich S, Ginger M, Frick A, Mientjes E, Oostra BA, Seeburg PH (2009) Synaptic ionotropic glutamate receptors and plasticity are developmentally altered in the CA1 field of Fmr1 knockout mice. The Journal of physiology 587:787–804.

Pin JP, Duvoisin R (1995) The Metabotropic Glutamate Receptors: Structure and Functions. Neuropharmacology 34:1–26.

Pozzo-miller LD, Inoue T, Murphy DD (1999) Estradiol Increases Spine Density and NMDA-Dependent Ca^{2+} Transients in Spines of CA1 Pyramidal Neurons From Hippocampal Slices. Journal of neurophysiology 81:1404–1411.

Van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030–1034.

Price DK, Zhang F, Ashley CT, Warren ST (1996) The chicken FMR1 gene is highly conserved with a CCT 5'-untranslated repeat and encodes an RNA-binding protein. Genomics 31:3–12.

Qin M, Kang J, Smith CB (2002) Increased rates of cerebral glucose metabolism in a mouse model of fragile X mental retardation. Proceedings of the National Academy of Sciences of the United States of America 99:15758–15763.

Rex CS, Lin C-Y, Kramár E, Chen LY, Gall CM, Lynch G (2007) Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. Journal of neuroscience 27:3017–3029.

Rodríguez-Moreno A, Sistiaga A, Lerma J, Sánchez-Prieto J (1998) Switch from facilitation to inhibition of excitatory synaptic transmission by group I mGluR desensitization. Neuron 21:1477–1486.

Sabaratnam M, Vroegop PG, Gangadharan SK (2001) Epilepsy and EEG findings in 18 males with fragile X syndrome. Seizure 10:60–63.

Scharfman HE (2007) The dentate gyrus: A comprehensive guide to structure, function, and clinical implications. Elsevier, Jordan Hill, Oxford.

Schoepp DD, Goldsworthy J, Johnson BG, Salhoff CR, Baker SR (1994) 3,5-dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus. Journal of neurochemistry 63:769–772.

Schütt J, Falley K, Richter D, Kreienkamp H-J, Kindler S (2009) Fragile X mental retardation protein regulates the levels of scaffold proteins and glutamate receptors in postsynaptic densities. Journal of biological chemistry 284:25479–25487.

Shang Y, Wang H, Mercaldo V, Li X, Chen T, Zhuo M (2009) Fragile X mental retardation protein is required for chemically-induced long-term potentiation of the hippocampus in adult mice. Journal of neurochemistry 111:635–646.

Sharma A, Hoeffer CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, Zukin RS (2010) Dysregulation of mTOR signaling in fragile X syndrome. Journal of neuroscience 30:694–702.

Shibuya K, Takata N, Hojo Y, Furukawa A, Yasumatsu N, Kimoto T, Enami T, Suzuki K, Tanabe N, Ishii H, Mukai H, Takahashi T, Hattori T, Kawato S (2003) Hippocampal cytochrome P450s synthesize brain neurosteroids which are paracrine neuromodulators of synaptic signal transduction. Biochimica et biophysica acta 1619:301–316.

Shinohara Y, Hosoya A, Yahagi K, Ferecskó AS, Yaguchi K, Sík A, Itakura M, Takahashi M, Hirase H (2012) Hippocampal CA3 and CA2 have distinct bilateral innervation patterns to CA1 in rodents. The European journal of neuroscience 35:702–710.

Shors TJ, Matzel LD (1997) Long-term potentiation: what's learning got to do with it? The Behavioral and brain sciences 20:597–614.

Shughrue PJ, Lane MV, Merchenthaler I (1997) Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. The Journal of Comparative Neurology 525:507–525.

Sibilia M, Wagner EF (1995) Strain-dependent epithelial defects in mice lacking the EGF receptor. Science 269:234–238.

Simko A, Hornstein L, Soukup S, Bagamery N (1989) Fragile X Syndrome: recognition in young children. Pediatrics 83:547–552.

Singh M, Meyer EM, Simpkins JW (1995) The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. Endocrinology 136:2320–2324.

Siomi MC, Zhang YAN, Siomi H, Dreyfuss G (1996) Specific sequences in the fragile X syndrome protein Fmr1 and the Fxr proteins mediate their binding to 60S ribosomal subunits and the interactions among them. Molecular and Cell Biology 16:3825–3832.

Slegtenhorst-Eegdeman KE, De Rooij DG, Verhoef-Post M, Van de Kant HJ, Bakker CE, Oostra B, Grootegoed J, Themmen P (1998) Macroorchidism in FMR1 knockout mice is caused by increased Sertoli cell proliferation during testicular development. Endocrinology 139:156–162.

Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. Nature neuroscience 4:1079–1085.

Sohrabji F, Miranda RC, Toran-Allerand CD (1995) Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. Proceedings of the National Academy of Sciences of the United States of America 92:11110–11114.

Solum DT, Handa RJ (2002) Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus. Journal of neuroscience 22:2650–2659.

Spencer CM, Alekseyenko O, Hamilton SM, Thomas AM, Serysheva E, Yuva-paylor LA, Paylor R (2011) Modifying behavioral phenotypes in Fmr1 KO mice: Genetic background differences reveal autistic-like responses. Autism research 4:40–56.

Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R (2005) Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes, brain, and behavior 4:420–430.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological review 99:195–231.

Sutherland GR (1977) Fragile Sites on Human Chromosomes: Demonstration of Their Dependence on the Type of Tissue Culture Medium Author. Science 197:265–266.

Tamura R, Nishida H, Eifuku S, Nagao K, Fushiki H, Watanabe Y, Ono T (2011) Short-term synaptic plasticity in the dentate gyrus of monkeys. PloS one 6:1-8.

Tassone F, Hagerman RJ, Taylor AK, Hagerman PJ (2001) A majority of fragile X males with methylated, full mutation alleles have significant levels of FMR1 messenger RNA. Journal of medical genetics 38:453–456.

Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC (1995) Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science 269:230–234.

Varma N, Carlson GC, Ledent C, Alger BE (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. Journal of neuroscience 21:1-5.

Vinogradova OS (2001) Hippocampus as comparator: Role of the two input and two output systems of the hippocampus in selection and registration of information. Hippocampus 11:578–598.

Vogt KE, Nicoll R (1999) Glutamate and gamma-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 96:1118–1122.

Volk LJ, Daly CA, Huber KM (2006) Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. Journal of neurophysiology 95:2427–2438.

Volk LJ, Pfeiffer BE, Gibson JR, Huber KM (2007) Multiple Gq-coupled receptors converge on a common protein synthesis-dependent long-term depression that is affected in fragile X syndrome mental retardation. Journal of neuroscience 27:11624–11634.

Wang Q, Chang L, Rowan ML, Anwyl R (2007) Developmental dependence, the role of the kinases p38 MAPK and PKC, and the involvement of tumor necrosis factor-R1 in the induction of mGlu-5 LTD in the dentate gyrus. Journal of neuroscience 144:110–118.

Warren SG, Humphreys AG, Juraska JM, Greenough WT (1995) LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. Brain research 703:26–30.

Watabe AM, Carlisle HJ, Dell TJO (2002) Postsynaptic Induction and Presynaptic Expression of Group 1 mGluR-Dependent LTD in the Hippocampal CA1 Region. Journal of neurophysiology 87:1395–1403.

Weiland G (1992) Estradiol Selectively Regulates Agonist Binding Sites on the N-Methyl-D-Aspartate Receptor Complex in the CA1 Region of the Hippocampus. Endocrinology 131:662–668.

Wenthold J, Petralia S, Niedzielski S (1996) Evidence for Multiple AMPA Receptor Complexes in Hippocampal CA1/CA2 Neurons. The Journal of neuroscience 76:1982–1989.

Westmark CJ, Westmark PR, O'Riordan KJ, Ray BC, Hervey CM, Salamat MS, Abozeid SH, Stein KM, Stodola LA, Tranfaglia M, Burger C, Berry-Kravis EM, Malter JS (2011) Reversal of fragile X phenotypes by manipulation of AβPP/Aβ levels in Fmr1KO mice. PLoS ONE 6: 1–11.

Woolley CS (1998) Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Hormones and behavior 34:140–148.

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA (1997) Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. Journal of neuroscience 17:1848–1859.

Yan QJ, Asafo-Adjei PK, Arnold HM, Brown RE, Bauchwitz RP (2004) A phenotypic and molecular characterization of the fmr1-tm1Cgr fragile X mouse. Genes, brain, and behavior 3:337–359.

Yashiro K, Philpot BD (2008) Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55:1081–1094.

Yokomaku D, Numakawa T, Numakawa Y, Suzuki S, Matsumoto T, Adachi N, Nishio C, Taguchi T, Hatanaka H (2003) Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase and mitogen-activated protein kinase in cultured hippocampal neurons. Molecular endocrinology 17:831–844.

Yun SH, Trommer BL (2011) Fragile X mice: reduced long-term potentiation and N-Methyl-D-Aspartate receptor-mediated neurotransmission in dentate gyrus. Journal of neuroscience research 89:176–182.

Yuskaitis CJ, Mines MA, King MK, Sweatt JD, Miller CA, Jope RS (2010) Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile x syndrome. Biochem Pharmacol 79:632–646.

Zalfa F, Giorgi M, Primerano B, Moro A, Di Penta A, Reis S, Oostra B, Bagni C (2003) The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell 112:317–327.

Zeier Z, Kumar A, Bodhinathan K, Feller JA, Foster TC, Bloom DC (2009) Fragile X Mental Retardation Protein Replacement Restores Hippocampal Synaptic Function in a Mouse Model of Fragile X Syndrome. Gene Therapy 16:1122–1129.

Zhang J, Hou L, Klann E, Nelson DL (2009) Altered hippocampal synaptic plasticity in the FMR1 gene family knockout mouse models. Journal of neurophysiology 101:2572–2580.

Zhao C, Teng EM, Summers RG, Ming G-L, Gage FH (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. Journal of neuroscience 26:3–11.

Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annual review of physiology 64:355–405.