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Pharmacological reversal of synaptic plasticity deficits in the mouse model of Fragile X syndrome by group II mGluR antagonist or lithium treatment

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Abstract

Fragile X syndrome is the leading single gene cause of intellectual disabilities. Treatment of a *Drosophila* model of Fragile X syndrome with metabotropic glutamate receptor (mGluR) antagonists or lithium rescues social and cognitive impairments. A hallmark feature of the Fragile X mouse model is enhanced mGluR-dependent long-term depression (LTD) at Schaffer collateral to CA1 pyramidal synapses of the hippocampus. Here we examine the effects of chronic treatment

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of Fragile X mice *in vivo* with lithium or a group II mGluR antagonist on mGluR-LTD at CA1 synapses. We find that long term lithium treatment initiated during development (5–6 weeks of age) and continued throughout the lifetime of the Fragile X mice until 9–11 months of age restores normal mGluR-LTD. Additionally, chronic short term treatment beginning in adult Fragile X mice (8 weeks of age) with either lithium or an mGluR antagonist is also able to restore normal mGluR-LTD. Translating the findings of successful pharmacologic intervention from the *Drosophila* model into the mouse model of Fragile X syndrome is an important advance, in that this identifies and validates these targets as potential therapeutic interventions for the treatment of individuals afflicted with Fragile X syndrome.

Keywords

Fragile X; FMRP; long-term depression (LTD); group II mGluR; lithium; Fragile X syndrome; metabotropic glutamate receptor; LY341495

Introduction

Fragile X syndrome is the most prevalent heritable cause of intellectual disabilities, affecting 1 in 2,500 births (Hagerman, 2008). Fragile X is caused by the loss of *FMR1* gene function, which leads to a constellation of symptoms including seizures, sleep disorders, anxiety and autism, with the overriding clinical manifestation ranging from mild to severe intellectual disability (Jacquemont et al., 2007). Although the function of FMRP remains to be fully understood, it is known to be enriched both presynaptically and postsynaptically, and is associated with and regulates a number of mRNAs in response to synaptic activity (Bassell and Warren, 2008; Jacquemont et al., 2007; Kelleher and Bear, 2008; Ronesi and Huber, 2008; Wang et al., 2010; Zukin et al., 2009).

A *Drosophila* model for Fragile X syndrome, based on the loss of *dfmr1* expression, exhibits several phenotypes in common with Fragile X-related symptoms (Dockendorff et al., 2002; McBride et al., 2005; Morales et al., 2002; Zhang et al., 2001). Chronic treatment with antagonists of the *Drosophila* metabotropic glutamate receptor (DmGluRA) or with lithium can rescue courtship behavior (social interaction), cognitive defects and neuroanatomical defects in the major learning and memory center of the brain (McBride et al., 2005). The *Drosophila* genome contains a single metabotropic receptor (mGluR) and its characterization indicates it activates signaling pathways downstream of both Group I and Group II mGluRs (Choi et al., 2010; McBride et al., 2005; Pan and Broadie, 2007; Pan et al., 2008). At therapeutic doses lithium has been shown to inhibit inositol trisphosphate synthesis and recycling via inhibition of IPPase and IMPase (Acharya et al., 1998; Baraban et al., 1989; Berridge, 1993; Berridge et al., 1989; Hallcher and Sherman, 1980; Williams et al., 2002) as well as to inhibit GSK-3β activity (Klein and Melton, 1996). Therefore, lithium has activities that are capable of inhibiting the downstream signaling of both the group I and group II mGluRs, thus it is not clear which downstream pathways are relevant to the observed phenotypic rescue (Dolen and Bear, 2005; McBride et al., 2005; Walsh et al., 2008). This is an important point when considering how to translate these results into mammalian systems. Relevant to this point, studies in the mouse model of fragile X (*Fmr1* KO) have shown that genetic reduction or short-term pharmacologic inhibition of group 1 mGluRs or the downstream signaling of group I mGluRs can rescue a wide array of mutant phenotypes (de Vrij et al., 2008; Dolen et al., 2007; Min et al., 2009; Yan et al., 2005). However, it is not known if chronic treatments with lithium or group II mGluR antagonists are effective in the mouse, as they have not been formally tested. This is an important question for guiding potential therapies in humans.

Whereas the behavioral and cognitive deficits displayed by the *Drosophila* Fragile X model are robust (Bolduc et al., 2008; McBride et al., 2005), *Fmr1* KO mice display subtle behavioral and cognitive deficits (Bakker and Oostra, 2003; Bassell and Warren, 2008; Jacquemont et al., 2007; Ronesi and Huber, 2008; Wang et al., 2010; Zukin et al., 2009). The most robust endophenotype to date in the Fragile X mouse model is exaggerated metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) in the CA1 region of the hippocampus at 6–8 weeks of age (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006; Sharma et al., 2010). This is both very interesting and important as it is widely accepted that long-term depression (LTD) as well as long-term potentiation (LTP) are cellular models of learning and memory (Altinbilek and Manahan-Vaughan, 2009; Kelleher et al., 2004; Malenka and Bear, 2004; Whitlock et al., 2006).

In this study, the mGluR-LTD phenotype was examined in acute hippocampal slice preparations at 4–6 months of age and at 9–11 months of age in the *Fmr1* KO mice. Fragile X and control mice were chronically treated with lithium or the selective mGluRII antagonist, LY341495. We establish that chronic administration of lithium in a mammalian model of Fragile X similar can rescue deficits, replicating our previous findings reported in a *Drosophila* model. Moreover, we report that short-term administration of either lithium or mGluR inhibitor during adulthood can ameliorate the enhanced mGluR LTD in *Fmr1* KO mice, indicating their potential therapeutic value.

Results

DHPG-induced mGluR-LTD in WT and *Fmr1* KO mice at 5–6 months of age

Long-term potentiation (LTP) and long-term depression (LTD) have long been thought to provide cellular models of learning and memory, and have recently been shown to be associated with memory *in vivo* (Altinbilek and Manahan-Vaughan, 2009; Whitlock et al., 2006). In this study, we elicited mGluR-dependent LTD by application of acute hippocampal slices with DHPG (100 μM, 10 minutes) (Huber et al., 2000; Huber et al., 2001). DHPG is a relatively selective group I mGluR agonist that at 100 μM stimulates maximal mGluR-LTD in wild-type mice (Huber et al., 2002; Huber et al., 2000; Huber et al., 2001). Consistent with these results, we found in hippocampal slices of WT (wild-type control) mice at 5–6 months of age, DHPG elicited a depression of the baseline synaptic response to 82.9 ± 2.3% (n = 6) and 83.5 ± 2.8% (n = 6) of the average pre-DHPG baseline values at 60 and 80 minutes after induction, respectively (Figure 1A, 1D) (Huber et al., 2002; Huber et al., 2000; Palmer et al., 1997). In hippocampal slices from similarly aged Fragile X (*Fmr1* KO) mice, DHPG elicited a depression in the synaptic responses to 68.3 ± 1.5% (n = 8) and 68.9 ± 1.6% (n = 8) of pre-DHPG baseline values at 60 and 80 minutes after induction, respectively; the magnitude of depression was significantly greater than that measured in slices from WT mice ($p = 0.0009$ at 60 and 80 minutes) (Figure 1A, 1D). These findings demonstrate that the enhanced DHPG-induced mGluR-LTD observed in younger mice (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006) is still present in *Fmr1* KO mice at 5–6 months of age. Also, similar to the previous studies, basal synaptic transmission and paired-pulse facilitation (PPF) did not differ between the WT (n = 6) and *Fmr1* KO (n = 8) mice at 5–6 months of age (WT mice) (Figure 1B and 1C)(Hou et al., 2006; Huber et al., 2002), indicating that the difference in the magnitude of LTD is not due to changes in presynaptic release mechanisms.

mGluR-LTD is enhanced in 9–11 month old Fragile X mice compared to controls

Since Fragile X is generally thought of as a developmental disorder that affects the individual throughout the lifespan, our goal was to start treatment early and to continue throughout the majority of the lifetime of mice to determine if such prolonged treatment was

effective. Before initiating these long-term treatment studies, we next examined DHPG-induced mGluR-LTD in older (9–11 month old) Fragile X mice to determine if and how it was affected vs WT mice. At this age, DHPG-induced LTD in WT mice to 81.1 ± 3.5% (n = 9) and 81.9 ± 3.4% (n = 9) of the average pre-DHPG baseline fEPSP slope values at 60 and 80 minutes, respectively, after induction (Figure 1E, 1H). In age-matched *Fmr1* KO mice, DHPG-induced LTD to 62.9 ± 4.1 (n = 8) and 63.5 ± 3.8% (n = 8) of average pre-DHPG baseline values at 60 and 80 minutes, respectively, after induction (Figure 1E, 1H). Thus at this age there was also a significantly larger decrease in amplitude in the *Fmr1* KO mice than in the WT controls ($p = 0.0001$ at 60 and 80 minutes). There was a trend toward having a larger strength of LTD with age in the *Fmr1* KO mice, but it did not reach the level of significance. There was no difference in basal synaptic transmission or PPF in slices of WT (n = 9) *vs*. *Fmr1* KO (n = 8) mice at 9–11 months of age (Figure 1F, 1G), again indicating that the difference in the magnitude of LTD is not due to changes in presynaptic release mechanisms.

Long-term chronic lithium treatment abrogates the enhanced mGluR-LTD in Fragile X mice

To assess the efficacy of chronic treatment initiated early and continued throughout the lifetime of the animals, lithium chow or control vehicle chow was administered to WT or *Fmr1* KO mice beginning at 5–6 weeks and continued until 10–11 months of age. The effect of such a long-term lithium treatment on synaptic plasticity has not been examined previously in WT or *Fmr1* KO mice. Also as groups of mice were weighed weekly, it remained to be determined if this extra handling of the mice would have any effect on DHPG-induced LTD.

In WT mice, long-term treatment with control chow had no effect on DHPG-induced LTD, with LTD of 81.8 ± 2.9% (n = 5) and 81.1 ± 3.5% (n = 5) at 60 and 80 minutes after induction, which is comparable to DHPG-induced LTD in young control chow treated WT mice (Figures 2A and 3A, $p > 0.05$) and in age matched untreated WT mice (1E, Figure 2A, $p > 0.05$). WT mice that were long-term chronically treated with lithium chow also demonstrated normal LTD at 82.2 ± 1.8% (n = 8) and 84.1 ± 1.9% (n = 8) at 60 and 80 minutes after induction ($p > 0.05$) (Figure 2A, G, H). There was no difference in basal synaptic transmission or PPF between WT mice treated long-term with either lithium chow (n = 8) or control vehicle chow (n = 5) (Figure 2B, C). Thus long-term treatment with lithium had no effect on DHPG-induced LTD in control mice.

Fmr1 KO mice on long-term treatment with control vehicle chow had DHPG-induced LTD of 62.1 ± 4.5% (n = 5) and 61.8 ± 3.8% (n = 5) at 60 minutes and 80 minutes after induction, comparable to findings in young control chow treated *Fmr1* KO mice (3D, $p > 0.05$) and in age matched untreated *Fmr1* KO mice (Figures 1E, 2D, $p > 0.05$) (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006). This DHPG-induced LTD in vehicle treated *Fmr1* KO mice was significantly enhanced compared to age-matched, interleaved, vehicle-treated WT mice which had DHPG-induced mGluR-LTD at 81.8 ± 2.9% and 81.1 ± 3.5% at 60 and 80 minutes after induction ($p = 0.0001$ and $p = 0.0002$ respectively) (Figure 2A, 2D, 2G, 2H). Consistent with our hypothesis, we found that *Fmr1* KO mice that were long-term chronically treated with lithium demonstrated abrogation of the enhanced LTD endophenotype, displaying LTD of 79.3 ± 1.4% (n = 9) and 83.9 ± 1.4% (n = 9) at 60 minutes and 80 minutes of age (Figure 2D, 2G, 2H). Basal synaptic transmission was not altered in *Fmr1* KO mice treated with lithium (n = 9) or control vehicle chow (n = 5) (Figure 2E). Also, chronic long-term treatment of *Fmr1* KO mice with lithium (n = 9) or control vehicle chow (n = 5) did not alter PPF (Figure 2F). Thus long-term treatment of *Fmr1* KO mice rescued the DHPG induced enhanced LTD phenotype.

Chronic adult onset lithium treatment abrogates the enhanced mGluR-LTD in Fragile X mice

The potential for amelioration in adulthood of cognitive phenotypes in animal models associated with mental retardation and autism remains a new concept (Ehninger et al., 2008; McBride et al., 2005; Raymond and Tarpey, 2006; Walsh et al., 2008). We next investigated whether treatments initiated in adulthood and continued over a shorter time period could also rescue exaggerated mGluR-LTD in *Fmr1* KO mice. Lithium chow treatment or control chow treatment was given to WT or *Fmr1* KO mice for 10–12 weeks beginning at 8 weeks of age (adulthood), referred to as short-term chronic treatment. As done in the previous experiment, mice were weighed weekly. In WT mice tested at the end of this treatment (4–5 months of age) control chow had no effect on DHPG-induced LTD, compared to untreated 5–6 month-old mice (Figure 1A), with LTD of 78.7 ± 2.0% (n = 6) and 78.3 ± 1.9% (n = 6) at 60 and 80 minutes after induction (Figure 3A, G, H). Similarly, short-term chronic treatment of WT mice with lithium chow did not detectably alter DHPG-induced mGluR LTD, demonstrating LTD of 83.5 ± 2.1% (n = 8) and 82.6 ± 1.6% (n = 8) at 60 and 80 minutes of age (Figure 3A, G, H). Moreover, neither basal synaptic transmission nor PPF differed significantly in WT mice treated with lithium (n = 8) *vs*. control vehicle chow (n = 6) (Figure 3B, C).

In *Fmr1* KO mice, short-term chronic treatment with control vehicle chow had no effect on DHPG-induced LTD compared to untreated 5–6 month old mutant mice (Figure 1A), with LTD of 66.6 ± 3.1% (n =7) and 66.3 ± 3.1% (n = 7) at 60 and 80 minutes after induction (Figure 3D). This DHPG-induced LTD in *Fmr1* KO mice was significantly enhanced compared to LTD in age-matched, control vehicle chow-treated WT mice at 60 ($p = 0.0071$) and 80 minutes ($p = 0.0077$)(78.7 ± 2.0% and 78.3 ± 1.9%; Figures 3A, 3D, 3G, 3H). Short term chronic treatment of *Fmr1* KO mice with lithium significantly decreased DHPG-induced mGluR-LTD, with depression of fEPSP slopes to 81.8 ± 3.5% (n=8, $p = 0.0003$) and 80.8 ± 2.4% (n=8, $p = 0.0006$) at 60 and 80 minutes, respectively, after DHPG induction (Figure 3D, 3G, 3H). Short term chronic lithium treatment (n = 8) administered to *Fmr1* KO mice did not significantly alter basal synaptic transmission or PPF compared to control chow treated mice (n = 7) (Figure 3E, 3F). Thus, short-term chronic treatment with lithium was sufficient to rescue the enhanced DHPG-induced LTD observed at CA1 synapses of *Fmr1* KO mice, without detectably altering synaptic transmission or mGluR-LTD in WT control mice.

Chronic mGluR antagonist treatment during adulthood corrects mGluR-LTD in Fragile X mice

We next examined whether the group II mGluR antagonist LY431495 administered to adult mice could rescue exaggerated mGluR-LTD (Johnson et al., 1999; Ornstein et al., 1998; Sato et al., 2004). Unlike group I mGluRs antagonists, which impair induction of NMDAR-dependent LTP, and L-LTP (Late phase-LTP), group II mGluR antagonists inhibit only LTD at CA1 synapses. Both L-LTP and LTD require *de novo* protein synthesis and may utilize overlapping cellular machinery involved in protein synthesis (Altinbilek and Manahan-Vaughan, 2007; Altinbilek and Manahan-Vaughan, 2009; Balschun and Wetzel, 2002; Bordi et al., 1997; Kulla and Manahan-Vaughan, 2008; Manahan-Vaughan and Reymann, 1997; ManahanVaughan, 1997; Naie and Manahan-Vaughan, 2005; Neyman and Manahan-Vaughan, 2008; Santschi et al., 2006). Group II mGluRs are expressed predominantly pre-synaptically, but are also expressed post-synaptically (Lujan et al., 1997; Tanabe et al., 1992; Tanabe et al., 1993). LY341495 or vehicle was administered to WT or *Fmr1* KO mice for 8 weeks beginning at 8 weeks of age. The concentration of LY341495 used was the minimal dose previously shown to reverse the in vivo effects of a group II mGluR agonist (Johnson et al., 1999; Ornstein et al., 1998). At the cessation of treatment, the mice were

given a hiatus for 3–5 weeks before being tested for DHPG-induced LTD. This was done to ensure that LY341495 had cleared the system prior to electrophysiological experiments, as it has previously been established that a high concentration of LY341495 can acutely block DHPG-induced LTD (Fernandez et al., 2007; Gong et al., 2004; Huber et al., 2000). It was also done to determine if more permanent changes had occurred, such as in the transcriptional profile, that could maintain rescue of the enhanced LTD (Fernandez et al., 2007; Gong et al., 2004).

In WT mice, treatment with LY341495 vehicle for 8 weeks did not detectably alter DHPG-induced LTD, as compared with untreated WT mice (Figure 1A), with LTD of 83.1 ± 1.8% (n = 5) and 83.5 ± 1.8% (n = 5) at 60 and 80 minutes after induction, respectively (Figure 4A, 4G, 4H). In contrast, compared to LY341495 vehicle treated WT mice, WT mice that were chronically treated with LY341495 demonstrated significantly enhanced LTD of 69.1 ± 2.2% (n = 5) and 69.8 ± 2.8 (n = 5) at 60 and 80 minutes after induction ($p = 0.0001$ for both time points) (Figure 4A, 4G, 4H). Chronic administration of LY341495 to WT mice (n = 5) did not significantly alter basal synaptic transmission or PPF compared to vehicle treated WT mice (n = 5) (Figure 4B, 4C).

In *Fmr1* KO mice, an 8 week treatment with vehicle had no effect on DHPG-induced LTD, as compared with untreated *Fmr1* KO mice of a similar age (Figure 1A), with LTD of 72.6 ± 1.5% (n = 6) and 72.7 ± 1.3% (n = 6) at 60 and 80 minutes after induction compared to untreated *Fmr1* KO mice (Figure 4D). DHPG-induced LTD in vehicle-treated *Fmr1* KO mice was significantly enhanced compared to LTD in interleaved, age-matched, LY341495 vehicle-treated WT mice at 60 and 80 minutes (83.1 ± 1.8% and 83.5 ± 1.8%; $p = 0.0005$ and $p = 0.0004$; Figures 4D and 4A). In contrast, *Fmr1* KO mice that were chronically treated with LY341495 demonstrated abrogation of the enhanced mGluR-LTD at CA1 synapses, with fEPSP slope values of 84.1 ± 1.9% (n = 7) and 81.2 ± 2.0% (n = 7) at 60 and 80 minutes ($p = 0.0001$ and $p = 0.0021$; Figure 4D, 4G, 4H). LY341495 treatment of *Fmr1* KO mice (n = 7) did not detectably alter basal synaptic transmission or PPF compared to LY341495 vehicle treated *Fmr1* KO mice (n = 6) (Figure 4E–F), indicating that correction of mGluR-LTD by LY341495 treatment was not due to changes in presynaptic release mechanisms.

Discussion

DHPG-induced mGluR-LTD is enhanced in aged *Fmr1* KO mice

The endophenotype of enhanced DHPG-induced mGluR-LTD in the CA1 region of the hippocampus in *Fmr1* KO mice was chosen to examine the effects of chronic treatment with lithium and LY341495 because of its proven reproducibility. Previous studies, however, were limited to examining DHPG-induced LTD in young mice between 3–8 weeks of age (Banko et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006). In this study we examined enhanced DHPG-induced mGluR-LTD in older mice and found it to persist well into adulthood as tested at 4–6 and 9–11 months of age in the *Fmr1* KO mice. This finding provides a useful phenotype to examine more long-term effects of pharmacological treatment paradigms

Long-term and short-term lithium treatment effectively restored proper mGluR-LTD in the *Fmr1* KO mouse

In previous studies we observed that the treatment of a fly Fragile X model with lithium during development and/or adulthood rescued social interaction, immediate recall and short-term memory (McBride et al., 2005). These results, especially those demonstrating the efficacy of adult only treatment, provided an impetus for examining the effect of lithium

treatment in the mouse Fragile X model. Moreover, lithium treatment has been found to rescue enhanced audiogenic seizures and ameliorates aberrant behaviors in *Fmr1* KO mice, as assessed by open-field activity, elevated plus-maze, and passive avoidance assays (Min et al., 2009; Yuskaitis et al., 2010). Lithium also ameliorates some behaviors in human subjects with Fragile X, as reported in a recent open label clinical trial (Berry-Kravis et al., 2008).

A novel finding of the present study is that long-term chronic lithium treatment initiated early in life and continued until 10–11 months of age rescues exaggerated DHPG-induced mGluR LTD at the Schaffer collateral to CA1 synapse in Fragile X mice, restoring LTD to the level observed in WT mice. In contrast, long-term lithium treatment does not have any obvious effect on DHPG-induced LTD in WT mice. This demonstrates the efficacy of using lithium treatment continued throughout adulthood to rescue impaired synaptic plasticity in a mouse model of Fragile X syndrome.

The efficacy of the long-term chronic lithium treatment begun early and given throughout adulthood in correcting the enhanced LTD endophenotype in the *Fmr1* KO mice left open the extremely important question of whether a shorter course of adulthood only treatments could also be effective. Here we demonstrate that chronic short-term lithium treatment initiated in adulthood (at 8 weeks of age) corrects the enhanced DHPG-induced LTD endophenotype in *Fmr1* KO mice. These findings are consistent with our previous findings that pharmacologic treatment beginning in adulthood could rescue social interaction, immediate recall and short-term in the fly model of Fragile X (McBride et al., 2005). These findings are also consistent with findings of others that chronic (5-day) treatment of *Fmr1* KO mice with lithium ameliorated aberrant behavior of *Fmr1* KO mice, as assessed by performance in open-field activity, elevated plus-maze, and passive avoidance assays (Yuskaitis et al., 2010). Collectively, these findings together with findings in the present study support further studies of the efficacy of treating some Fragile X symptoms with lithium.

LY341495 treatment effectively restored proper mGluR-LTD in *Fmr1* KO mouse

Initially it was speculated, based on our fly data, that group II mGluR antagonists may have efficacy in treating the Fragile X mouse model and Fragile X patients since antagonism of the *Drosophila* mGluR (DmGluRA) was able to rescue memory and social interaction impairments in the Fragile X fly model and DmGluRA has group II mGluR activity (McBride et al., 2005; Parmentier et al., 1996). LY341495 is a selective mGluR antagonist with 10 fold higher affinity for mGluR2/3 (Group II) than mGluR7/8 (Group III) and a 1,000 fold higher affinity for mGluR2/3 than for group I mGluRs (Fitzjohn et al., 1998; Johnson et al., 1999; Kingston et al., 1998; Linden et al., 2009; Ornstein et al., 1998; Wright et al., 2000). Since elegant work has demonstrated that heterozygous genetic deletion of the group I mGluR5 can rescue many phenotypes in the Fragile X mouse model (Dolen et al., 2007), we tried to minimize the potential of antagonizing group I mGluRs by utilizing the LY341495 at the lowest dose demonstrated to reverse the in vivo effects of a group II mGluR agonist (Johnson et al., 1999; Ornstein et al., 1998). For further discussion on the selectivity and dosing of LY341495 please see the methods.

We reasoned that group II mGluR antagonists would increase cAMP levels in response to synaptic stimulation, owing to the fact that the majority of group II mGluRs are coupled to Gi and localize to both pre- and post-synaptic compartments. A rise in intracellular cAMP would, in turn, promote PKA activity and antagonize GSK-3β activity (Fang et al., 2000; Li et al., 2000; Tanji et al., 2002) (Supplemental Figure 1). Consistent with this, lithium treatment has been shown to abrogate elevated GSK-3β activity in *Fmr1* KO mice (Yuskaitis et al., 2010). There has also been postulated a potential role for decreased cAMP

signaling after synaptic stimulation in the *Drosophila* and mouse models of Fragile X as well as demonstrated decreased levels of cAMP in the brains of the *Drosophila* and mouse models of Fragile X and from samples from patients afflicted with Fragile X (Berry-Kravis and Ciurlionis, 1998; Berry-Kravis et al., 1995; Berry-Kravis and Huttenlocher, 1992; Kelley et al., 2007; McBride et al., 2005)

Mammalian group II mGluRs also to a lesser extent couple to Gq; therefore, group II mGluR antagonists would be expected to decrease activity-dependent, InsP3R-mediated Ca^{2+} release from internal stores (Supplemental Figure 1). Lithium can also inhibit this downstream pathway as it can $InsP_3R$-mediated calcium signaling through inhibition of IMPase and IPPase (Acharya et al., 1998; Baraban et al., 1989; Berridge, 1993; Berridge et al., 1989; Hallcher and Sherman, 1980). Thus although we feel that the results obtained in this study are most likely due to reducing mGluR group II mediated pathways (predominantly via Gi coupling, but to a lesser degree possibly via Gq coupling; Supplemental Figure 1), we cannot fully discount the possibility of some rescue coming from decreases in group I pathways as well, especially considering the novelty of using long term pharmacological treatments.

We found that chronic LY341495 treatment initiated in adulthood and continued for 8 weeks rescued the endophenotype of enhanced DHPG-induced LTD in the Fragile X mouse model. The fact that this rescue was observed after a cessation period of 3–5 weeks after the final treatment indicates that changes at the transcriptional level were likely induced by treatment (Fernandez et al., 2007; Gong et al., 2004). In contrast to the results obtained with the *Fmr1* KO mice, chronic LY341495 treatment enhanced DHPG-induced LTD in WT mice. This is a very interesting finding because it is the opposite of the outcome that was seen in *Fmr1* KO mice. Although it is beyond the scope of the current research, one could speculate that this happened due to compensation in the system given that the neuronal signaling network is set up to maintain an optimal range for cAMP signaling in the brain (Sato et al., 2004).

This work demonstrates that group II mGluR antagonists and lithium (GSK-3β inhibitors) are potential therapeutic agents for the treatment of Fragile X and further illustrates the potential efficacy of lithium as a treatment for Fragile X associated pathology. This is an expansion of the mGluR theory to include group II mGluR receptors (Bear et al., 2004). Lastly we would like to point out that the present study shows the novel finding that chronic LY341495 treatment initiated in adulthood and continued for 8 weeks rescues enhanced DHPG-induced LTD in Fragile X mice for at least 3–5 weeks beyond the last drug treatment. The full duration of the rescue has yet to be determined, but the persistence of this rescue indicates that more lasting changes, possibly at the level of transcription or epigenetic modification of the genome were induced by the LY341495 treatment (Fernandez et al., 2007; Gong et al., 2004). This is a very interesting finding in that it indicates the possibility that some drug treatments can induce more lasting changes in the *FMR1* KO brain. To pursue these studies further we need to determine if other *FMR1* KO phenotypes are rescued by LY341495 treatment and if the rescue of other phenotypes also persists beyond a given drug treatment period and for what period of time. Results from these studies are critically important for exploring the use of similar compounds for clinical treatment of Fragile X patients as they indicate that pharmacological treatments may not require continuous drug treatment thus reducing cost and chance of adverse side effects caused by continuous drug exposure.

Experimental Procedure

Mouse Studies

All animal studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at the Drexel University College of Medicine and Albert Einstein College of Medicine. *Fmr1* KO mice backcrossed onto the FVB background and the appropriate control strain were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in-house. The genotype of animals was confirmed by PCR. All mice were subjected to a 12h:12h light: dark cycle. Food and water were provided ad libitum. Only male mice were used for experimentation in this paper.

Electrophysiology

Mice were deeply anesthetized with isoflurane and decapitated. The brain was collected in ice-cold dissection buffer (in mM: sucrose, 215; $NaHCO_3$, 26; NaH_2PO_4, 1.6; $CaCl_2$, 1; KCl, 2.5; $MgSO_4$, 4; $MgCl_2$, 4; glucose, 20) and the hippocampi were dissected. Transverse sections of the hippocampi (400 μm thickness) were prepared in ice-cold aCSF (in mM: NaCl, 124; $NaHCO_3$, 25; NaH_2PO_4, 1.25; $CaCl_2$, 2.5; KCl, 2.5; $MgCl_2$, 1.3; glucose, 10) using a vibrating slicer (Vibratome 3000EP, Vibratome, MO). The slices were allowed to recover at 33° Celsius for 1 hour prior to transecting the CA3 region and placing them in a submersion-style recording chamber, where they were perfused for at least 1 hour (32° Celsius, 2–2.5 ml/min) prior to recording. Dissection and recording buffers were saturated with a 95% O_2 / 5% CO_2 mixture (pH 7.4).

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of area CA1 using extracellular recording electrodes filled with aCSF (resistances: 1 ~ 2 MOhm). Synaptic responses were evoked by stimulating the Schaffer collateral axons with a 200-μsec pulse using a bipolar stainless steel electrode (Rhodes Medical Instruments, CA). The slices were stimulated every 30 s (0.033Hz) for a minimum of 15 minutes to verify stability of response. Basal synaptic transmission (BST) was measured by stimulating the slices at 8 to 10 different stimulus intensities ranging from 5 μA to 50 μA. Paired-pulse facilitation (PPF) was determined at interstimulus intervals of 15 ms, 30 ms, 50 ms and 100 ms. Baseline responses were evoked by stimulating the slices at 0.033 Hz using 50–60% of maximal stimulating intensity, and the data were averaged every minute for a minimum of 30 minutes. Slices were not further used if their responses drifted beyond 5% of the average baseline response. Long-term depression was induced chemically using 100 μM (R,S)-dihydroxyphenylglycine (DHPG) for 10 minutes.

Extracellular recordings were performed with a Multi-Clamp 700A amplifier (Axon Instruments Inc., Union City, CA). Analysis of data was performed blind to the genotype and experimental group. Results were obtained using at least two different litters of mice for all experimental groups. All experiments were interleaved as appropriate. BST and PPF results are reported as mean ± SD. LTD results are reported as mean ± SEM. Significant differences between groups were determined by using a Student's t-test and ANOVA analysis. Significance was similar for all data analyzed by both the Student's t-test and ANOVA, and therefore only the ANOVA p values are shown.

Drug administration

R, S-DHPG and LY341495 were purchased from Tocris (St. Louis, MO). DHPG and LY341495 were prepared as recommended. Fresh stocks were prepared weekly for DHPG, and biweekly for LY341495 and NaOH vehicle. Mice were weighed weekly to help monitor general well-being.

Lithium—5–6 week old (long term treatment group) or 10–12 week old (short term treatment group) male *Fmr1* KO mice and age-matched male WT control mice were administered custom-made chow containing 2.4 g/kg lithium carbonate or vehicle (Bio-Serv, Frenchtown, NJ) for either 10–12 weeks (short term treatment group) or until they reached 10–11 months of age (long term treatment group). This dose and route of administration have previously been shown to achieve clinically relevant concentrations of serum lithium (0.6–1.2 mM) in mice (Son et al., 2003; Su et al., 2004). The serum levels of lithium obtained ranged from 0.66 to 0.96 mM for lithium treated mice (WT and *Fmr1* KO) and < 0.05 (the level of detection) for control treated mice. Food and water were provided ad-libitum. Serum lithium concentration was determined by collecting trunk blood, isolating the serum by centrifugation (11,000 rpm, 5 mins) and then utilizing services provided by the Hospital of the University of Pennsylvania. The tested samples of trunk blood demonstrated that the serum lithium concentration was in the clinically relevant range for all treated mice and none of the control mice.

LY341495—Eight week old male *Fmr1* KO mice and age-matched male control mice were administered 0.9 % saline containing 0.3 mg/kg LY341495 or vehicle alone via subcutaneous injections once daily for 8 weeks. This dose has previously been to shown to be the lowest dose that reverses the in vivo effects of a group II mGluR agonist (Johnson et al., 1999; Ornstein et al., 1998). The IC 50 of LY341495 for mGluR2 and mGluR3 are 2.3 and 1.3 nM. The efficacy of LY341495 treatment on reversing the in vivo effects of the mGluR2/3 agonist LY354750 appears to be selective at this low dose that we utilize, which is why we chose to utilize this dose (Johnson et al., 1999; Ornstein et al., 1998; Sato et al., 2004). However the IC 50 of LY341495 for mGluR7 and mGluR8 are 173 and 990nM, therefore at 10–100 fold higher concentration it also has a the potential to hit four of the mGluR subtypes, all of which are group II and group III mGluRs (Fitzjohn et al., 1998; Johnson et al., 1999; Kingston et al., 1998; Ornstein et al., 1998; Wright et al., 2000). This ability to antagonize mGluR7 and mGluR8 in addition to mGluR2 and mGluR3 appears to be why additional effects of LY341495 treatment arise at doses of 1–3mg/kg or greater (Bespalov et al., 2008; Chaki et al., 2004; Linden et al., 2009; O'Neill et al., 2003). At even higher doses LY341495 becomes a non-selective mGluR antagonist with an IC50 of 6.8 uM for mGluR1 and 8.2 uM for mGluR5 and antagonization of group I mGluRs has been demonstrated in synaptoneurosome preparations at such doses (Fitzjohn et al., 1998; Johnson et al., 1999; Kingston et al., 1998; Sawtell et al., 1999; Wright et al., 2000). The mice were given at a three to five-week drug and handling hiatus prior to testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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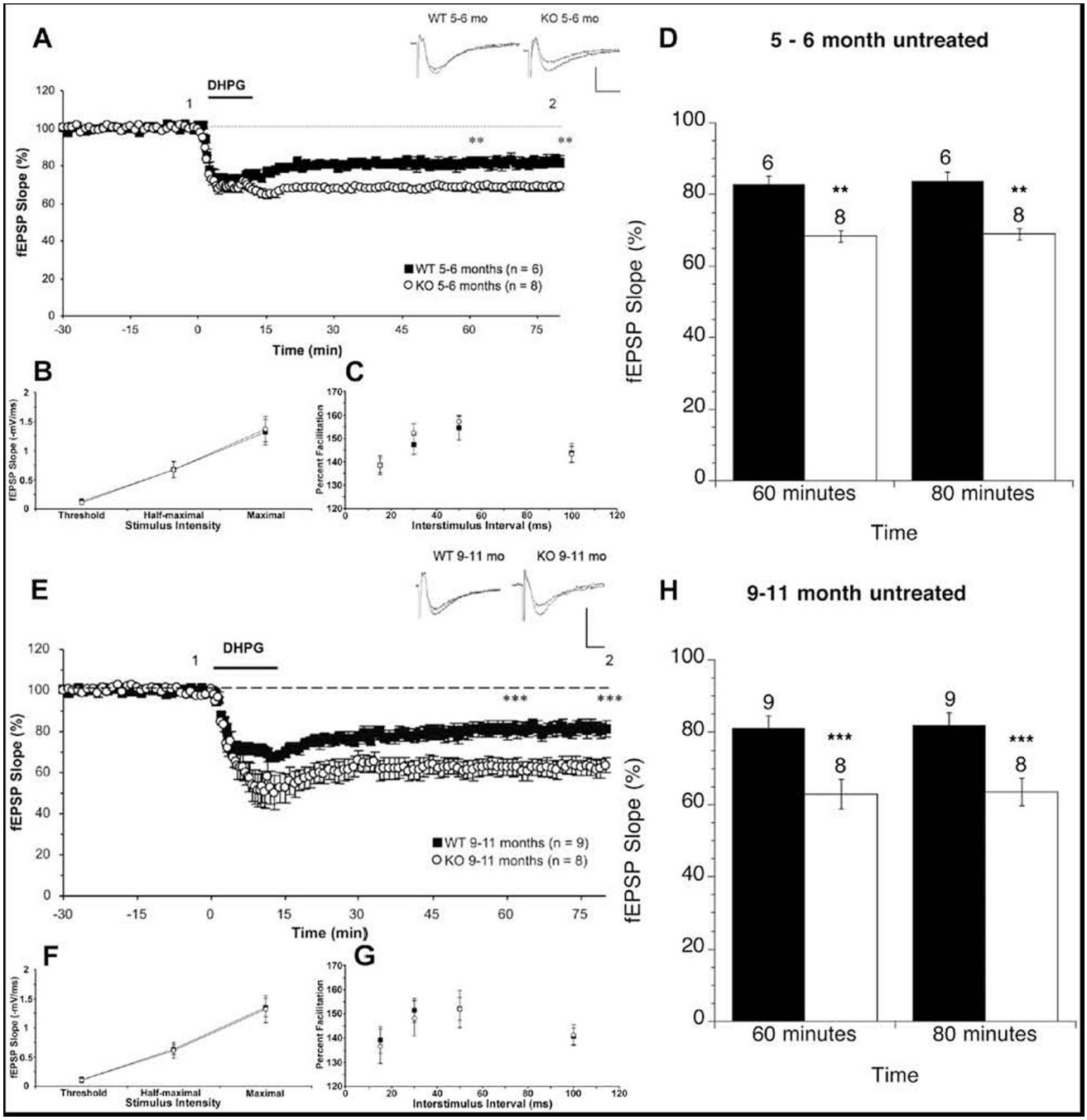

Figure 1. mGluR-LTD is enhanced in *Fmr1* KO mice at 5–6 months of age

(A) In panels A–H *Fmr1* KO (open circles or bars) and interleaved age-matched WT mice (filled squares or bars). Long-term depression of synaptic transmission was induced by brief bath application of the mGluR agonist DHPG (100 μM, 10 min). Mean fEPSP slopes (± SEM) are plotted as a percentage of average pre-induction baseline values. DHPG-induced mGluR-LTD was enhanced in 5–6 month old *Fmr1* KO mice (n = 8 slices) compared to interleaved age-matched WT mice (n = 6 slices) at 60 minutes (WT 5–6 months: 82.9 ± 2.3%; *Fmr1* KO 5–6 months: 68.3 ± 1.5%; $p = 0.0009$) and at 80 minutes (WT 5–6 months: 83.5 ± 2.8%; *Fmr1* KO 5–6 months: 68.9 ± 1.6%; $p = 0.0009$) post-induction.

Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(B) Basal synaptic transmission is normal in *Fmr1* KO mice at 5–6 months of age. Mean evoked fEPSP slopes (± SD) are plotted at three different stimulus intensities. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between 5–6 month old *Fmr1* KO (n = 8) and interleaved age-matched WT mice (n = 6).

(C) Paired-pulse facilitation is normal in *Fmr1* KO mice at 5–6 months of age. Synaptic responses to paired stimulation were evoked at interstimulus intervals ranging from 15 ms to 100 ms. Plotted are mean percent facilitation (± SD), as determined by calculating the ratio of the second fEPSP slope to the first fEPSP slope. At all interpulse intervals, no significant differences were observed in PPF between 5–6 month old *Fmr1* KO mice (n = 8) and interleaved age-matched WT mice (n = 6).

(D) Graphical representation of panel A at 60 and at 80 minutes after DHPG induction of LTD. ** represents $p < 0.001$. The number above each bar denotes the n. Color code as in panel A.

(E) Long-term depression of synaptic transmission was induced and plotted as in panel A. DHPG-induced mGluR-LTD was enhanced in 9–11 month old *Fmr1* KO mice (n = 8 slices) compared to interleaved age-matched WT mice (n = 9 slices) at 60 minutes (WT: 81.1 ± 3.5%; *Fmr1* KO: 62.9 ± 4.1%; $p = 0.0001$) and at 80 minutes (WT: 81.9 ± 3.4%; *Fmr1* KO: 63.5 ± 3.8%; $p = 0.0001$) post-induction. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(F) Basal synaptic transmission is normal in *Fmr1* KO mice at 9–11 months of age and is plotted as in panel B. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between 9–11 month old *Fmr1* KO (n = 8 slices) and interleaved age-matched WT mice (n = 9 slices).

(G) Paired-pulse facilitation is normal in *Fmr1* KO mice at 9–11 months of age and is plotted as in panel C. No significant differences were observed in PPF between 9–11 month old *Fmr1* KO mice (n = 8 slices) and interleaved age-matched WT mice (n = 9 slices).

(H) Graphical representation of panel E at 60 and at 80 minutes after DHPG induction of LTD. *** represents $p < 0.0001$. Color code as in panel E.

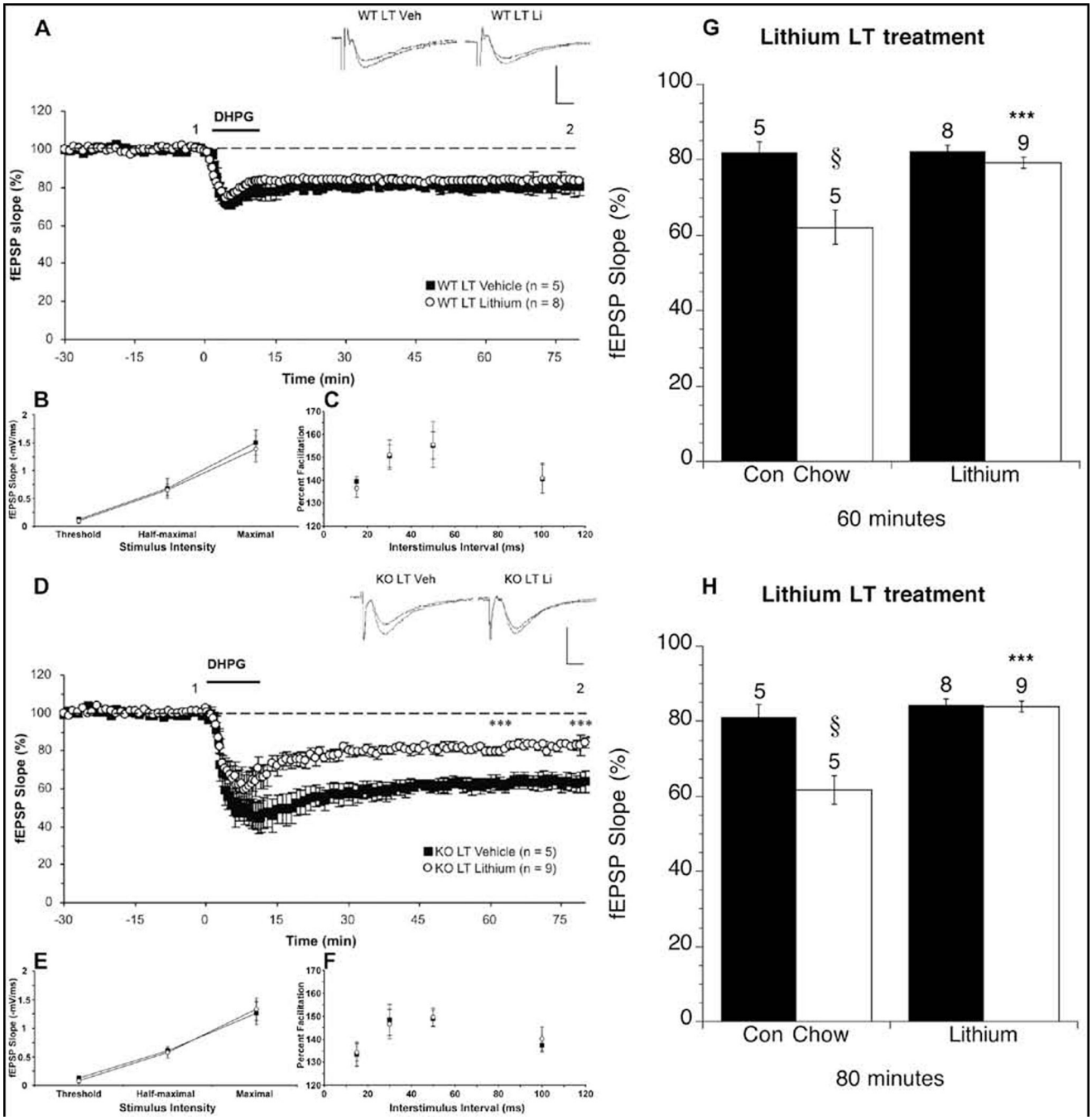

Figure 2. Long-term chronic treatment of WT mice with lithium does not alter mGluR-LTD, while in contrast it abrogates the enhanced mGluR-LTD in Fragile X mice

(A) mGluR-LTD is normal in aged WT after chronic long-term treatment with lithium. 5–6 week old WT mice were administered lithium-containing chow ad libitum throughout adulthood until 10–11 months of age. LTD was induced by brief bath application of the mGluR agonist DHPG (100 μM, 10 min). DHPG-induced mGluR-LTD was not significantly different between long-term lithium-treated WT mice (n = 8 slices, open circles, A–C) and interleaved age-matched vehicle-treated WT mice (n = 5 slices, filled squares, A–C) at 60 minutes (WT LT vehicle: 81.8 ± 2.9%; WT LT lithium: 82.2 ± 1.8%) or at 80 minutes (WT long-term vehicle: 81.1 ± 3.5%; WT long-term lithium: 84.1 ± 1.9%)

post-induction. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(B) Basal synaptic transmission is not affected by chronic long-term lithium treatment in aged WT mice and is plotted as in Figure 1. (vehicle treated WT mice, n = 5; lithium treated WT mice, n = 8)

(C) Paired-pulse facilitation is not affected by chronic long-term lithium treatment in aged WT mice and is plotted as in Figure 1. (vehicle treated WT mice, n = 5; lithium treated WT mice, n = 8)

(D) The enhanced DHPG-induced LTD was abrogated in *Fmr1* KO mice that were administered lithium-containing chow ad libitum beginning at 5–6 weeks of age throughout adulthood until 10–11 months of age. LTD was induced by brief application of the mGluR agonist DHPG (100 μM, 10 min). mGluR-LTD was significantly enhanced in long-term vehicle-treated *Fmr1* KO mice (n = 5 slices, filled squares, D–F) compared to interleaved age-matched vehicle-treated WT mice (2A; n = 5 slices, filled squares) at 60 minutes (WT LT vehicle: 81.8 ± 2.9%; *Fmr1* KO LT vehicle: 62.1 ± 4.5%; $p = 0.0001$) and at 80 minutes (WT LT vehicle: 81.1 ± 3.5%; *Fmr1* KO LT vehicle: 61.8 ± 3.8%; $p = 0.0002$) post induction. Chronic long-term treatment of *Fmr1* KO mice with lithium (n = 9 slices, open circles, D–F) abrogated the enhanced mGluR-LTD phenotype compared to vehicle-treated *Fmr1* KO mice at 60 minutes (*Fmr1* KO LT vehicle 62.1 ± 4.5%; *Fmr1* KO LT lithium: 79.3 ± 1.4%; $p = 0.0001$) and at 80 minutes (*Fmr1* KO LT vehicle: 61.8 ± 3.8%; *Fmr1* KO LT lithium: 83.9 ± 1.4%; $p = 0.0001$) post-induction. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(E) Basal synaptic transmission is normal in long-term lithium treated *Fmr1* KO mice at 10–11 months of age and is plotted as in Figure 1. (vehicle treated *Fmr1* KO mice, n = 5; lithium treated *Fmr1* KO mice, n = 9)

(F) Paired-pulse facilitation is normal in long-term lithium treated *Fmr1* KO mice at 10–11 months of age and is plotted as in Figure 1. (vehicle treated *Fmr1* KO mice, n = 5; lithium treated *Fmr1* KO mice, n = 9)

(G) Chronic long-term treatment of *Fmr1* KO mice with lithium beginning at 5–6 weeks old and continuing throughout adulthood until 10–11 months of age abrogates the enhanced mGluR-LTD phenotype. DHPG-LTD in WT (filled bars) and *Fmr1* KO (open bars) mice treated with control chow or lithium chow at 60 minutes after induction as shown in Figures 2A and 2D. The asterisks *** represent $p < 0.0001$ for comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow ($p = 0.0001$). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values.

(H) DHPG-LTD in WT and *Fmr1* KO mice treated with vehicle or lithium chow at 80 minutes after induction as shown in Figures 2A and 2D. The asterisks *** represent $p < 0.0001$ for comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow ($p = 0.0002$). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values.

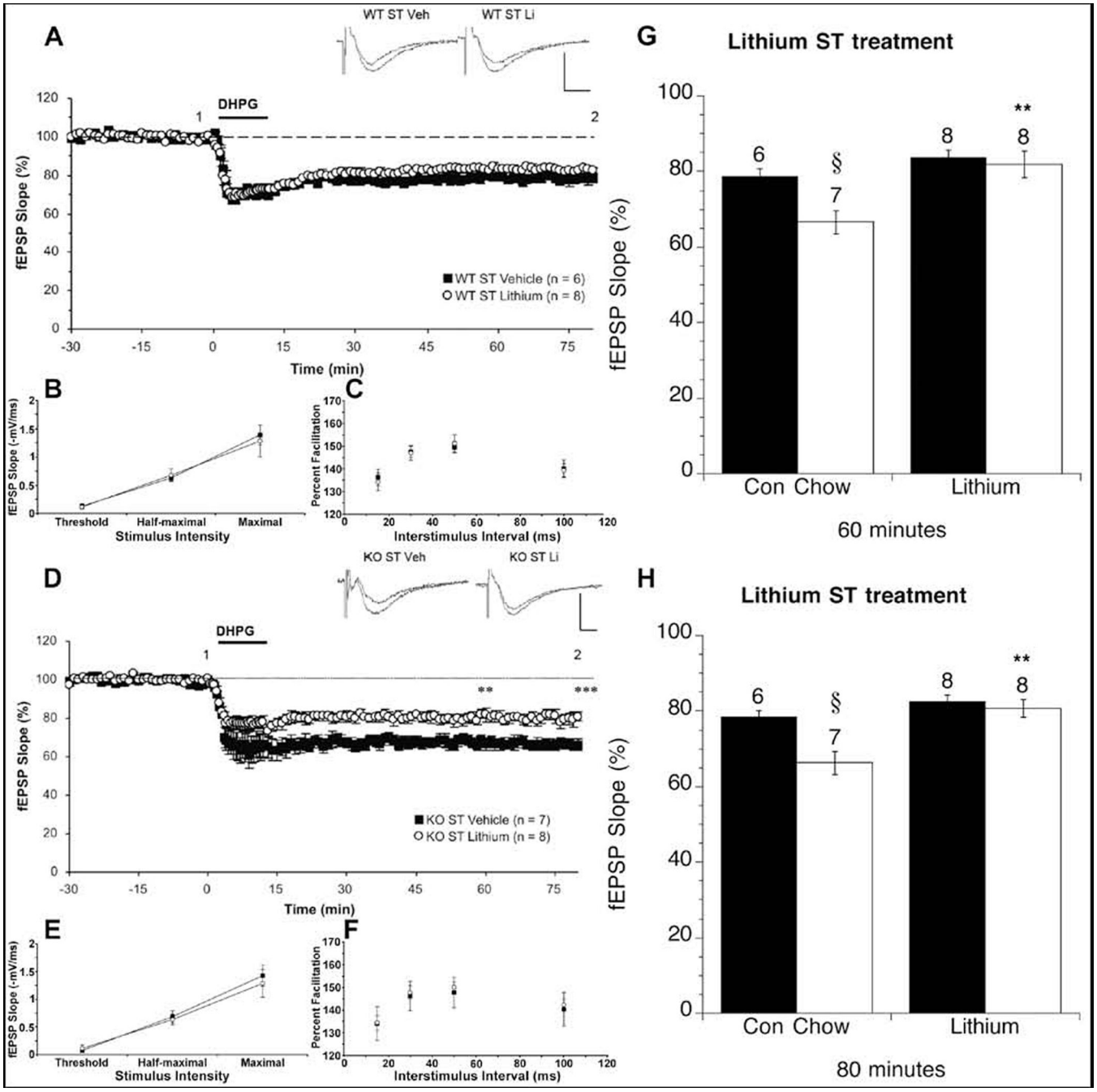

Figure 3. Chronic short-term treatment of WT mice with lithium does not alter mGluR-LTD, while in contrast it abrogates the enhanced mGluR-LTD in Fragile X mice

(A) mGluR-LTD is not changed in WT mice after chronic treatment with lithium. Two-month old WT mice were administered lithium-containing chow ad libitum until 4–5 months of age. LTD was induced by brief bath application of the mGluR agonist DHPG (100 μM, 10 min). DHPG-induced mGluR-LTD was not significantly different in the lithium-treated WT mice (n = 8 slices, open circles) compared to interleaved age-matched vehicle-treated WT mice (n = 6 slices, filled squares) at 60 minutes (WT vehicle: 78.7 ± 2.0%; WT lithium: 83.5 ± 2.1%) or at 80 minutes (WT vehicle: 78.3 ± 1.9%; WT lithium: 82.6 ± 1.6%) post-induction. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-

induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(B) Basal synaptic transmission is not affected by chronic short-term lithium treatment in WT mice. Mean evoked fEPSP slopes (± SD) are plotted at three different stimulus intensities. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between lithium-treated WT mice (open circles) and interleaved age-matched vehicle-treated WT mice (filled squares). (vehicle treated WT mice, n = 6; lithium treated WT mice, n = 8)

(C) Paired-pulse facilitation is not affected by chronic short-term lithium treatment in WT mice. Synaptic responses to paired stimulation were evoked at interstimulus intervals ranging from 15 ms to 100 ms. Plotted are mean percent facilitation (± SD), as determined by calculating the ratio of the second fEPSP slope to the first fEPSP slope. At all interpulse intervals, no significant differences were observed in PPF between lithium-treated WT mice (open circles) and interleaved age-matched vehicle-treated WT mice (filled squares). (vehicle treated WT mice, n = 6; lithium treated WT mice, n = 8)

(D) Chronic short-term treatment of *Fmr1* KO mice with lithium abrogates the enhanced mGluR-LTD phenotype. Eight week old *Fmr1* KO mice were administered lithium-containing chow ad libitum until 4–5 months of age. LTD was induced by brief application of the mGluR agonist DHPG (100 μM, 10 min). mGluR-LTD was significantly enhanced in vehicle-treated *Fmr1* KO mice (n = 7 slices, filled squares) compared to interleaved age-matched vehicle-treated WT mice (Figure 3A; n = 6 slices, filled squares) at 60 minutes (WT vehicle: 78.7 ± 2.0%; *Fmr1* KO vehicle: 66.6 ± 3.1%; $p = 0.0071$) and at 80 minutes (WT vehicle: 78.3 ± 1.9%; *Fmr1* KO vehicle: 66.3 ± 3.1%; $p = 0.0077$) post-induction. Chronic treatment of *Fmr1* KO mice with lithium (n = 8 slices, open circles) abrogated the enhanced mGluR-LTD phenotype compared to vehicle-treated *Fmr1* KO mice at 60 minutes (*Fmr1* KO vehicle: 66.6 ± 3.1%; *Fmr1* KO lithium: 81.8 ± 3.5%; $p = 0.0003$) and at 80 minutes (*Fmr1* KO vehicle: 66.3 ± 3.1%; *Fmr1* KO lithium: 80.8 ± 2.4%; $p = 0.0006$) post-induction. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(E) Basal synaptic transmission is not affected by chronic short-term lithium treatment in *Fmr1* KO mice. Mean evoked fEPSP slopes (± SD) are plotted at three different stimulus intensities. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between lithium-treated *Fmr1* KO mice (open circles) and interleaved age-matched vehicle-treated *Fmr1* KO mice (filled squares). (vehicle treated *Fmr1* KO mice, n = 7; lithium treated *Fmr1* KO mice, n = 8)

(F) Paired-pulse facilitation is not affected by chronic short-term lithium treatment in *Fmr1* KO mice. Synaptic responses to paired stimulation were evoked at interstimulus intervals ranging from 15 ms to 100 ms. Plotted are mean percent facilitation (± SD), as determined by calculating the ratio of the second fEPSP slope to the first fEPSP slope. At all interpulse intervals, no significant differences were observed in PPF between lithium-treated *Fmr1* KO mice (open circles) and interleaved age-matched vehicle-treated *Fmr1* KO mice (filled squares). (vehicle treated *Fmr1* KO mice, n = 7; lithium treated *Fmr1* KO mice, n = 8)

(G) Chronic short-term treatment of *Fmr1* KO mice with lithium beginning in adulthood until 4–5 months of age abrogates the enhanced mGluR-LTD phenotype. DHPG-LTD in WT (filled bars) and *Fmr1* KO (open bars) mice treated with vehicle or lithium at 60 minutes after induction as shown in Figures 3A and 3D. The asterisks ** represent $p < 0.001$ for comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow ($p = 0.0071$). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values.

(H) DHPG-LTD in WT and *Fmr1* KO mice treated with vehicle or lithium at 80 minutes after induction as shown in Figures 3A and 3D. The asterisks *** represent $p < 0.0001$ for

comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow (p = 0.0077). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values.

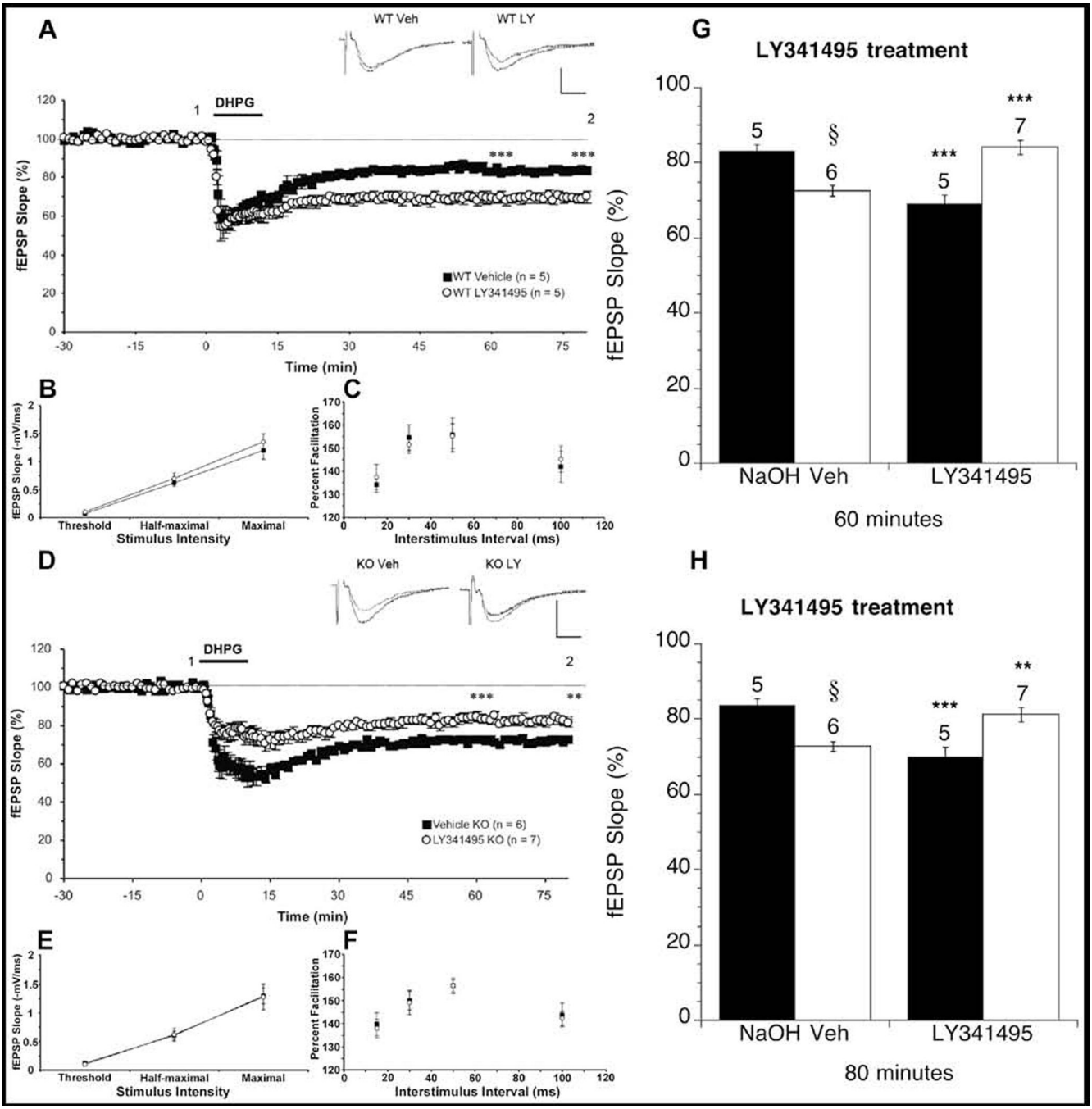

Figure 4. Chronic treatment of WT mice with the mGluR antagonist LY341495 enhances mGluR-LTD, while in contrast it abrogates the enhanced mGluR-LTD in Fragile X mice

(A) Chronic treatment of WT mice with the group II mGluR antagonist LY341495 enhances mGluR-LTD. Eight week old WT mice were administered daily injections of LY341495 for 8 weeks followed by a hiatus of 3–5 weeks. LTD was induced by brief bath application of the mGluR agonist DHPG (100 μM, 10 min). mGluR-LTD was significantly enhanced in LY341495-treated WT mice (n = 5 slices, open circles) compared to interleaved age-matched vehicle-treated WT mice (n = 5 slices, filled squares) at 60 minutes (WT vehicle: 83.1 ± 1.8%; WT LY341495: 69.1 ± 2.2%; $p = 0.0001$) and at 80 minutes (WT vehicle: 83.5 ± 1.8%; WT LY341495: 69.8 ± 2.8%; $p = 0.0001$) post-induction. Plotted are average fEPSP

slope values (± SEM) as a percentage of average pre-induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 1.5 mV and 5 ms.

(B) Basal synaptic transmission is not affected by chronic LY341495 treatment in WT mice. Mean evoked fEPSP slopes (± SD) are plotted at three different stimulus intensities. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between the LY341495-treated WT mice (open circles) and interleaved age-matched vehicle-treated WT mice (filled squares). (vehicle treated WT mice, n = 5; LY341495 treated WT mice, n = 5)

(C) Paired-pulse facilitation is normal in WT mice after chronic LY341495 treatment. Synaptic responses to paired stimulation were evoked at interstimulus intervals ranging from 15 ms to 100 ms. Plotted are mean percent facilitation (± SD), as determined by calculating the ratio of the second fEPSP slope to the first fEPSP slope. At all interpulse intervals, no significant differences were observed in PPF between LY341495-treated WT mice (open circles) and interleaved age-matched vehicle-treated WT mice (filled squares). (vehicle treated WT mice, n = 5; LY341495 treated WT mice, n = 5)

(D) Chronic treatment of *Fmr1* KO mice with the group II mGluR antagonist LY341495 abrogates the enhanced mGluR-LTD phenotype. Eight week old *Fmr1* KO mice were administered daily injections of LY341495 for 8 weeks followed by a hiatus of 3–5 weeks. LTD was induced by brief bath application of the mGluR agonist DHPG (100 μM, 10 min). mGluR-LTD was significantly enhanced in vehicle-treated *Fmr1* KO mice (n = 6 slices, filled squares) compared to interleaved age-matched vehicle-treated WT mice (Figure 4A; n = 4 slices, filled squares) at 60 minutes (WT vehicle: 83.1 ± 1.8%; Fmr1 KO vehicle: 72.6 ± 1.5%; $p = 0.0005$) and at 80 minutes (WT vehicle: 83.5 ± 1.8%; Fmr1 KO vehicle: 72.7 ± 1.3%; $p = 0.0004$) post-induction. Chronic treatment of *Fmr1* KO mice with LY341495 (n = 7 slices, open circles) abrogated the enhanced mGluR-LTD phenotype compared to vehicle-treated *Fmr1* KO mice at 60 minutes (*Fmr1* KO vehicle: 72.6 ± 1.5%; *Fmr1* KO LY341495: 84.1 ± 1.9%; $p = 0.0001$) and at 80 minutes (*Fmr1* KO vehicle: 72.7 ± 1.3%; *Fmr1* KO LY341495: 81.2 ± 2.0%; $p = 0.0021$) post-induction. Plotted are average fEPSP slopes (±SEM) as a percentage of average pre-induction baseline values. Representative traces of field potentials are from times indicated by the numbers on the graph (1 and 2). Calibration bars depict 2 mV and 5 ms.

(E) Basal synaptic transmission is not affected by chronic LY341495 treatment in *Fmr1* KO mice. Mean evoked fEPSP slopes (± SD) are plotted at three different stimulus intensities. Synaptic responses at threshold, half-maximal and maximal stimulus intensities were not significantly different between LY341495-treated *Fmr1* KO mice (open circles) and interleaved age-matched vehicle-treated *Fmr1* KO mice (filled squares). (vehicle treated *Fmr1* KO mice, n = 6; LY341495 treated *Fmr1* KO mice, n = 7)

(F) Paired-pulse facilitation is not affected by chronic LY341495 treatment in *Fmr1* KO mice. Synaptic responses to paired stimulation were evoked at interstimulus intervals ranging from 15 ms to 100 ms. Plotted are mean percent facilitation (± SD), as determined by calculating the ratio of the second fEPSP slope to the first fEPSP slope. At all interpulse intervals, no significant differences were observed in PPF between LY341495-treated *Fmr1* KO mice (open circles) and interleaved age-matched vehicle-treated *Fmr1* KO mice (filled squares). (vehicle treated *Fmr1* KO mice, n = 6; LY341495 treated *Fmr1* KO mice, n = 7)

(G) Chronic treatment of *Fmr1* KO mice with lithium throughout adulthood until 5–6 months of age abrogates the enhanced mGluR-LTD phenotype. DHPG-LTD in WT (filled bars) and *Fmr1* KO (open bars) mice treated with vehicle or lithium at 60 minutes after induction as shown in Figures 4A and 4D. The asterisks *** represent $p < 0.0001$ for comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow ($p = 0.0005$). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average preinduction baseline values.

(H) DHPG-LTD in WT and *Fmr1* KO mice treated with vehicle or lithium at 80 minutes after induction as shown in Figures 4A and 4D. The asterisks ** represent $p < 0.001$ and *** represent $p < 0.0001$ for comparisons of drug versus vehicle treatment within the same genotype. The § represents a significant difference between WT and *Fmr1* KO mice on vehicle control chow ($p = 0.0004$). The number above each bar denotes the n. Plotted are average fEPSP slopes (± SEM) as a percentage of average pre-induction baseline values.