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Genetic Reduction or Negative Modulation of $mGlu_7$ Does Not Impact Anxiety and Fear Learning Phenotypes in a Mouse Model of *MECP2* Duplication Syndrome

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Abstract

Rett syndrome and *MECP2* Duplication syndrome are neurodevelopmental disorders attributed to loss-of-function mutations in, or duplication of, the gene encoding methyl-CpG-binding protein 2 (MeCP2), respectively. We recently reported decreased expression and function of the metabotropic glutamate receptor 7 ($mGlu_7$) in a mouse model of Rett syndrome. Positive allosteric modulation of $mGlu_7$ activity was sufficient to improve several disease phenotypes including cognition. Here, we tested the hypothesis that $mGlu_7$ expression would be reciprocally regulated in a mouse model of *MECP2* Duplication syndrome, such that negative modulation of $mGlu_7$ activity would exert therapeutic benefit. To the contrary, we report that $mGlu_7$ is not functionally increased in mice overexpressing MeCP2 and that neither genetic nor pharmacological reduction of $mGlu_7$ activity impacts phenotypes that are antiparallel to those observed in Rett syndrome model mice. These data expand our understanding of how $mGlu_7$ expression and function is affected by changes in MeCP2 dosage and have important implications for the therapeutic development of $mGlu_7$ modulators.

Graphical abstract

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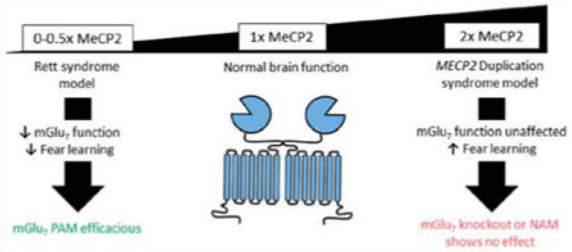

Keywords

MeCP2; MECP2 Duplication syndrome; Rett syndrome; $mGlu_7$; ADX71743; negative allosteric modulator

Introduction

Loss-of-function mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene are present in 95% of patients with the neurodevelopmental disorder Rett syndrome (RTT).[1,2] Conversely, duplication of the *MECP2* locus results in a syndrome characterized by infantile hypotonia, recurrent respiratory infections, limited speech, seizures, intellectual disability, and autism-like behaviors.[3,4] While decades of research have yielded significant insights into the pathophysiology of RTT, our understanding of *MECP2* Duplication syndrome (MDS) remains limited. Recently, Sztainberg et al. reported that antisense oligonucleotide and/or genetic reduction of *MECP2* expression improves phenotypes in *MeCP2-Tg1* mice, a model of MDS,[5] suggesting that symptoms of MDS may be reversible in patients. These data provide rationale to identify processes downstream of *MECP2* dysregulation that can be therapeutically targeted to restore proper neuronal function.

In support of this strategy, we previously identified the metabotropic glutamate receptor 7 ($mGlu_7$) as a potential therapeutic target in a clinical population as well as in a mouse model of RTT.[6] $mGlu_7$ is a G protein-coupled receptor expressed widely throughout the brain at glutamatergic and GABAergic presynaptic terminals.[7,8] $mGlu_7$ activation inhibits neurotransmitter release and plays important roles in synaptic plasticity in the hippocampus and amygdala.[9–13] Additionally, a heterozygous *GRM7* mutation has been reported in a patient with idiopathic autism and homozygous mutations have been linked to severe neurological disease.[14–16] We have demonstrated that *GRM7* expression is activated by MeCP2 binding in vitro and that $mGlu_7$ protein expression is decreased in brain samples from RTT patient autopsies and model mice.[6] Furthermore, positive allosteric modulation of $mGlu_7$ activity restores long-term potentiation at Schaffer Collateral CA1 (SCCA1) synapses in the hippocampus and improves cognition, social interaction and respiratory phenotypes in *Mecp2*$^{+/-}$ female mice.[6]

From our findings in RTT model mice, we hypothesized that MeCP2 overexpression would drive increased $mGlu_7$ expression in *MeCP2-Tg1* mice. This hypothesis was consistent with previous literature reporting that *MeCP2-Tg1* mice display opposing hippocampal phenotypes when compared to RTT model mice. Specifically, slices from *MeCP2-Tg1* mice

have been shown to display increased paired-pulse ratio and enhanced long-term potentiation at SC-CA1 synapses.[5,17] Behaviorally, *MeCP2-Tg1* mice exhibit elevated contextual fear freezing at 20 weeks of age.[17] Another model of MDS, the *Tau-Mecp2* model, also exhibits elevated fear memory, suggesting that overexpression of Mecp2 in the CNS underlies this phenotype.[18] Given that positive modulation of $mGlu_7$ activity restores decreased LTP and contextual fear freezing in *Mecp2*$^{+/-}$ mice to wildtype (WT) levels, we further hypothesized that genetic reduction or negative modulation of $mGlu_7$ activity would normalize hippocampal-dependent phenotypes in *MeCP2-Tg1* animals.

Here, we report that $mGlu_7$ protein expression is upregulated in the hippocampus of *MeCP2-Tg1* mice, but this increase does not translate to a functional increase in $mGlu_7$ activity at SC-CA1 synapses. In contrast to our hypothesis, genetic reduction or negative modulation of $mGlu_7$ activity shows no efficacy in ameliorating exaggerated contextual fear learning or anxiety-like behavior in this mouse model of MDS.

Results and Discussion

To test the hypothesis that MeCP2 overexpression leads to increased $mGlu_7$ expression, we isolated cortex, hippocampus, and striatum samples from 20-week-old *MeCP2-Tg1* mice and WT littermates on a pure FVB/N background for mRNA and protein analysis. *Grm7* mRNA expression detected by quantitative real-time polymerase chain reaction (qRT-PCR) was unchanged in all brain regions examined (Figure 1A). Western blotting confirmed global MeCP2 overexpression by 2–3 fold in samples from *MeCP2-Tg1* mice (Figure 1B,C); however, elevated $mGlu_7$ protein expression was only detected in hippocampal tissue (Figure 1B,D). Representative full blots can be found in Supplementary Figures 1–2.

To characterize the functional effects of elevated $mGlu_7$ protein expression within the hippocampus, we performed ex vivo brain slice electrophysiology at SC-CA1 synapses. Similar to previous reports,[5,17] slices from 20-week-old *MeCP2-Tg1* mice displayed unchanged input–output curves (Figure 2A); however, decreased input-output slope has been reported in younger mice.[19] Increased paired-pulse ratio was observed (Figure 2B), which often corresponds with a decrease in presynaptic glutamate release. Since $mGlu_7$ is a presynaptic regulator of neurotransmitter release, this result was consistent with increased $mGlu_7$ protein expression observed in total protein extracted from hippocampal tissue (Figure 1B,D) and directly opposed our findings in *Mecp2*$^{-/y}$ and *Mecp2*$^{+/-}$ animals.[6]

To test whether $mGlu_7$ receptor activity was increased, we recorded field excitatory postsynaptic potentials (fEPSPs) at SC-CA1 and bath applied the Group III mGlu receptor agonist, LSP4-2022. Application of 100 μM LSP4–2022 depressed synaptic transmission in WT and *MeCP2-Tg1* slices, with no difference in the maximal depression of fEPSP slope (Figure 2C,D). While LSP4-2022 also activates $mGlu_4$ and $mGlu_8$, $mGlu_7$ is believed to be the only presynaptic mGlu receptor at SC-CA1 synapses, making this an ideal synapse to probe $mGlu_7$ function.[20,21] Since an increase in total $mGlu_7$ protein expression did not translate to an increase in $mGlu_7$ activity in this functional readout, we next tested whether $mGlu_7$ expression was increased in synaptosome fractions. In contrast to the total protein Western blots, we did not observe a significant increase in $mGlu_7$ expression in synaptosome

fractions (Figure 2E,F). Taken together, these data suggest that increased cellular $mGlu_7$ does not necessarily lead to increased receptor surface expression and activity at this synapse.

In contrast to *Mecp2*$^{+/-}$ mice, 20-week-old *MeCP2-Tg1* mice have been reported to display increased LTP at SC-CA1 when compared to littermate controls.[5,17] We have previously demonstrated that activation of $mGlu_7$ on GABAergic interneurons is required for the induction of LTP at this synapse; $mGlu_7$ activation decreases GABA release and leads to the disinhibition of excitatory neurons.[11] We therefore hypothesized that negative modulation of $mGlu_7$ activity might normalize LTP in *MeCP2-Tg1* slices despite the lack of increase in receptor function. We induced LTP with high frequency stimulation (two 1s trains at 100 Hz, 20s interstimulus interval) and monitored fEPSPs for 60 min; however, contrary to previous reports,[5,17] we observed no change in LTP between WT and *MeCP2-Tg1* slices (Figure 3).

MeCP2-Tg1 mice exhibit increased anxiety-like behavior and increased contextual fear learning,[17,22] which are phenotypes opposite to those observed in *Grm7*$^{-/-}$ mice and *Mecp2*$^{+/-}$ mice.[23,24] We previously demonstrated that increasing $mGlu_7$ activity with the positive allosteric modulator (PAM) VU0422288 normalizes anxiolytic behavior and fear learning in *Mecp2*$^{+/-}$ mice. We therefore hypothesized that either a genetic or pharmacological reduction of $mGlu_7$ function might rescue behavior in *MeCP2-Tg1* mice in a similar manner. We crossed female *MeCP2-Tg1* mice (FVB/N) with male *Grm7*$^{+/-}$ mice (C57BL/6J) to genetically reduce $mGlu_7$ expression in the context of MeCP2 overexpression and studied the F1 offspring. We first confirmed that $mGlu_7$ protein levels were significantly reduced in total protein and synaptosomal isolates from these animals (Figure 4A,B). We next performed the elevated plus maze to examine anxiety-like behavior and contextual fear conditioning as a measure of hippocampal-dependent memory. Genetic reduction of $mGlu_7$ expression did not mitigate increased anxiety-like behavior in *MeCP2-Tg1* mice as measured by time spent in the open arms of an elevated plus maze (Figure 4C). Furthermore, independent of *Grm7* genotype, mice with the *MECP2* transgene displayed heightened freezing when re-exposed to the conditioning context 24 h post training along with delayed extinction learning when re-exposed to the conditioning context for 7 subsequent days (Figure 4D).

As genetic reduction of $mGlu_7$ could have evoked developmental compensatory mechanisms, we next sought to confirm that acute negative modulation of $mGlu_7$ signaling pharmacologically had no impact on phenotypes in adult *MeCP2-Tg1* mice. To assess anxiety phenotypes, WT and *MeCP2-Tg1* mice were pretreated with either vehicle or the $mGlu_7$ NAM ADX71743[25] and tested in the elevated plus maze assay. Similar to genetic reduction of $mGlu_7$, administration of ADX71743 did not alter anxiety phenotypes in WT and *MeCP2-Tg1* mice (Figure 5A). To determine whether $mGlu_7$ negative modulation had any effect on learning and memory phenotypes, WT and *MeCP2-Tg1* mice were pretreated with a single dose of ADX71743 prior to fear conditioning, and tested for contextual fear memory 24 h later. Relative to vehicle-treated controls, *MeCP2-Tg1* mice treated with vehicle exhibited a significant increase in freezing when re-exposed to the context, and ADX71743 administration had no effect on freezing in *MeCP2-Tg1* mice (Figure 5B). Conversely, WT mice treated with ADX71743 also presented with significantly increased

freezing relative to vehicle-treated controls, and failed to extinguish the fear memory in a manner similar to *MeCP2-Tg1* mice (Figure 5C,D).

To complement our $mGlu_7$ studies in RTT model mice, the experiments here aimed to evaluate the therapeutic potential of $mGlu_7$ receptor negative modulation in a mouse model of MDS. We previously reported that MeCP2 binding to the *GRM7* promoter activates gene transcription in vitro. In brain tissue samples from *Mecp2*$^{-/y}$ mice, $mGlu_7$ mRNA and protein expression were decreased in a brain-region specific manner.[6] Here, we tested the hypothesis that overexpression of MeCP2 in vivo would drive increased $mGlu_7$ expression. Interestingly, the only significant change observed in samples from *MeCP2-Tg1* mice was an increase in total cellular $mGlu_7$ in the hippocampus. These findings suggest that the relationship between MeCP2 dysregulation and $mGlu_7$ expression may be more complex than initially appreciated and dependent on the brain region examined. It is possible that basal expression of $mGlu_7$ is maintained at, or near, maximal levels or that another process independent of MeCP2 is rate-limiting, such that MeCP2 overexpression has no effect on $mGlu_7$ protein levels in some brain regions. Further studies will be needed to fully elucidate the molecular link between MeCP2, *GRM7* mRNA, and $mGlu_7$ protein expression.

Independent of receptor expression, we hypothesized that $mGlu_7$ genetic reduction or negative modulation could be an effective approach to reduce symptoms in *MeCP2-Tg1* mice due to the established role of $mGlu_7$ activation in LTP induction at SC-CA1 synapses.[11] However, despite extensive efforts, we were unable to replicate previous reports that LTP is elevated in slices from *MeCP2-Tg1* animals.[5,17] This could be due to differences in strain, age, genetic drift or technical/experimental differences. Interestingly, *Tau-Mecp2* mice exhibit decreased HFS-induced LTP at SC-CA1 despite exaggerated fear learning.[18] Together, these seemingly discordant results highlight that the effect of MeCP2 overexpression on LTP at SC-CA1 synapses appears to be complex and that, at least for this form of LTP, may not correlate with anxiety or fear memory phenotypes in mouse models of MeCP2 over-expression. In our experiments, one single administration of ADX71743 was given prior to fear conditioning to mimic our previous experiments in RTT model mice; however, if the primary deficit in *MeCP2-Tg1* mice is memory extinction as opposed to acquisition, we do not exclude the possibility that compound administration on test days may be efficacious.

In summary, we tested the hypothesis that $mGlu_7$ expression is reciprocally regulated by MeCP2 such that negative modulation of $mGlu_7$ would be efficacious in a mouse model of MDS. However, $mGlu_7$ expression was increased only in the hippocampus of *MeCP2-Tg1* mice, and behavioral phenotypes were found to be insensitive to changes in $mGlu_7$ expression/activity. Interestingly, Lu et al. recently reported that mouse models of RTT and MDS share similar hippocampal circuit abnormalities including hypersynchronous firing.[26] Our findings further support the idea that mouse models of MDS are not simply antiparallel to RTT models, but instead exhibit a unique pathophysiology that warrants further study.

Methods

Chemicals

ADX71743 and LSP4-2022 were synthesized in-house as described in ref 11. For in vivo experiments, ADX71743 was formulated using 10% Tween 20 as vehicle.

Animals

All animals used in the present study were group housed with food and water given ad libitum and maintained on a 12 h light/dark cycle. Animals were cared for in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All studies were approved by the Institutional Animal Care and Use Committee for Vanderbilt University School of Medicine and took place during the light phase. *MeCP2-Tg1* breeders were obtained from The Jackson Laboratory (FVB-Tg(MECP2)1 Hzo/J, stock no. 008679). Male mice were aged until 20–25 weeks of age for all experiments. *Grm7* knockout mice were cryorecovered from the Mutant Mouse Regional Resource Center (B6.129P2-Grm7^{Tm1Dgen}/Mmnc) and maintained under identical conditions.

Quantitative Real-Time PCR (QRT-PCR)

Cortex, hippocampus, and striatum were microdissected from 20-week-old *MeCP2-Tg1* mice and WT littermates. Total RNA was prepared from tissue samples using TRIzol Reagent (Invitrogen) in accordance with manufacturer's instructions. Total RNA from each brain region was DNase-treated with Roche Turbo DNase kit, and cDNA from 2 μg of total RNA was synthesized using the VILO kit (Invitrogen). RT-qPCR on cDNA from 25 ng of initial RNA template was then run in triplicate using Taqman Fast Reagent Mix (Life Technologies) and Life Technologies gene expression assays for *Grm7* (Mm0118924_m1) and *Gapdh* (Mm03302249_g1). C_t values for each sample were normalized to *Gapdh* expression and analyzed using the delta–delta C_t method as described in Gogliotti et al.[27] Values exceeding two times the standard deviation were classified as outliers. Each value was compared to the average delta-C_t value acquired for wild-type mice and calculated as percent-relative to the average control delta-C_t.

Total and Synaptosomal Protein Preparation

For total protein isolation, tissue samples from 20-week-old *MeCP2-Tg1* mice and WT littermates were homogenized using a hand-held motorized mortar and pestle in radioimmunoprecipitation assay buffer (RIPA) containing 10 mM Tris-HCL, 150 mM NaCl, 1 mM ethyl-enediaminetetraacetic acid (EDTA), 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, and 1% deoxycholate. After homogenization, samples were spun for 20 min at 15 000*g* at 4 °C. The supernatant was then transferred to new tubes, and the protein concentration was determined using a bicinchoninic acid (BCA) protein assay (Pierce). For synaptosome preparations, tissue was homogenized in 9 mL of ice-cold sucrose/HEPES (0.32 M sucrose, 4.2 mM HEPES, pH 7.4) using a Teflon-glass homogenizer (Wheaton Science Products). The homogenate was centrifuged at 1000*g* for 5 min at 4 °C, and the resultant supernatant was centrifuged at 12 000*g* for 15 min at 4 °C. The final pellets

containing synaptosomes were resuspended in RIPA buffer, and protein concentration was determined by BCA assay.

SDS-Page and Western blotting

Proteins (50 μg) were electrophoretically separated using a 4–20% SDS polyacrylamide gel and then transferred onto a nitrocellulose membrane (Bio-Rad). Membranes were blocked with Odyssey blocking buffer (LiCor) for 1 h at room temperature. Membranes were probed with the following primary antibodies: rabbit anti-MeCP2 (1:1000, Millipore 07–013), rabbit anti-$mGlu_7$ antibody (1:1000, Millipore 07–239), mouse anti-Gapdh antibody (1:5000, ThermoFisher MA5-15738), and mouse anti-Tubulin antibody (1:500, Abcam ab44928) overnight at 4 °C. Membranes were washed three times with Tris-buffered saline and Tween 20 (TBS-T, 25 mM Tris, 150 mM NaCl, 0.05% Tween 20) and then incubated with either a goat anti-rabbit fluorescent secondary antibody (800, 1:5000, LiCor) or a goat anti-mouse fluorescent secondary antibody (680, 1:5000, LiCor). Fluorescence was then quantified using Image Studio Light software. Each value for MeCP2 and $mGlu_7$ protein expression was first normalized to the value calculated for Gapdh expression (total protein blots) or Tubulin expression (synaptosome blots).

Extracellular Field Potential Recordings

Coronal brain slices were prepared from 20-week-old *MeCP2-Tg1* mice and WT littermates. Mice were anesthetized with isoflurane and decapitated. Brains were rapidly removed and submerged in ice-cold sucrose cutting buffer containing: 230 mM sucrose, 2.5 mM KCl, 8 mM $MgSO_4$, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 10 mM glucose, and 26 mM $NaHCO_3$ saturated with 95%/5% O_2/CO_2. A block of tissue containing hippocampus was trimmed, embedded in agarose, and coronal slices 400 μm thick were cut using a Compresstome VF-200 (Precisionary Instruments). Slices were transferred to a holding chamber containing *N*-methyl-D-glucamine (NMDG)-HEPES recovery solution (in mM, 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 D-glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 10 $MgSO_4$, 0.5 $CaCl_2$, pH 7.3, 305 mOsm) for 15 min at 32 °C. Slices were then transferred to a room temperature modified artificial cerebral spinal fluid (ACSF) containing (in mM) 126 NaCl, 1.25 NaH_2PO_4, 2.5 KCl, 10 D-glucose, 26 $NaHCO_3$, 2 $CaCl_2$ and 1 $MgSO_4$, and 600 μM sodium ascorbate for at least 1 h. Subsequently, slices were transferred to a submersion recording chamber and continuously perfused (2 mL/min) with ACSF containing (in mM) 126 NaCl, 1.25 NaH_2PO4, 2.5 KCl, 10 D-glucose, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgSO_4$ heated to 32 °C. All solutions were continuously bubbled with 95%/5% O_2/CO_2.

A concentric bipolar stimulating electrode was positioned near the CA3–CA1 border, and paired-pulse field excitatory postsynaptic potentials (fEPSPs) were evoked (100 μs duration, every 20 s) by placing a glass recording electrode in the stratum radiatum of CA1. Input–output curves were generated for each slice, and the stimulation intensity was adjusted to 50% of the maximum response for subsequent experiments. Paired-pulse ratios (PPR) were calculated as the ratio between the slope of the second fEPSP divided by the slope of the first fEPSP. PPRs were calculated at several interstimulus intervals (ISI) ranging from 10 to 500 ms.

For LSP4-2022 and LTP experiments, slopes of three consecutive sweeps were averaged and normalized to the average slope during the baseline period. Data were digitized using a Multiclamp 700B, Digidata 1322A, and pClamp 10 software (Molecular Devices). For LSP4-2022 experiments, paired-pulse fEPSPs were generated with a 20 ms ISI at 0.05 Hz. After 10 min of stable baseline recordings, 100 μM LSP4-2022 was applied for 10 min followed by a 10 min washout period. Long-term potentiation (LTP) was induced by applying two trains of 100 Hz stimulation (HFS, 1 s duration, 20 s intertrain interval (ITI)) after a 15 min baseline. ADX71743 (10 μM) was applied for 10 min prior to and during the application of HFS. fEPSPs were monitored for 60 min after HFS, and percent LTP was quantified as the normalized slope during the last 5 min of recording.

Elevated Plus Maze

Twenty-week-old *MeCP2-Tg1* mice and WT littermates were habituated to the testing room for 1 h prior to the elevated plus maze test. Mice were placed on the elevated plus maze and allowed to explore freely for 5 min. Time spent exploring each arm was measured using AnyMaze tracking software. For ADX71743 experiments, mice were dosed intraperitoneally (i.p., 10 mL/kg) with 60 mg/kg ADX71743 or vehicle (10% Tween 80) 15 min prior to being placed in the maze.

Fear Conditioning

Twenty-week-old *MeCP2-Tg1* mice and WT littermates were habituated to the testing room for 2 h on the day prior to training and the morning of training. On training day, mice were injected i.p. 15 min prior to conditioning with either vehicle (10% Tween 80) or 60 mg/kg ADX71743. Mice were then placed into an operant chamber with a shock grid (Medassociates Inc.) in the presence of a 10% vanilla odor cue. Following a 3 min habituation period, mice were exposed to two 1 s, 0.7 mA foot shocks spaced 30 s apart. For experiments using the offspring of *MeCP2-Tg1* and *Grm7*$^{+/-}$ mice, only one shock was administered due to higher freezing observed in mice of that genetic background. Mice remained in the context for an additional 30 s after the second foot shock. Mice were placed back into the same shock chamber with a 10% vanilla odor cue and the percent of time spent freezing during a 3 min testing period was assessed for up to 7 days after conditioning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

LTP	long-term potentiation
MDS	*MECP2* Duplication syndrome
MeCP2	methyl CpG binding protein 2
$mGlu_7$	metabotropic glutamate receptor 7

NAM	negative allosteric modulator
PAM	positive allosteric modulator
RTT	Rett syndrome
SC-CA1	Schaffer collateral-CA1
WT	wild type

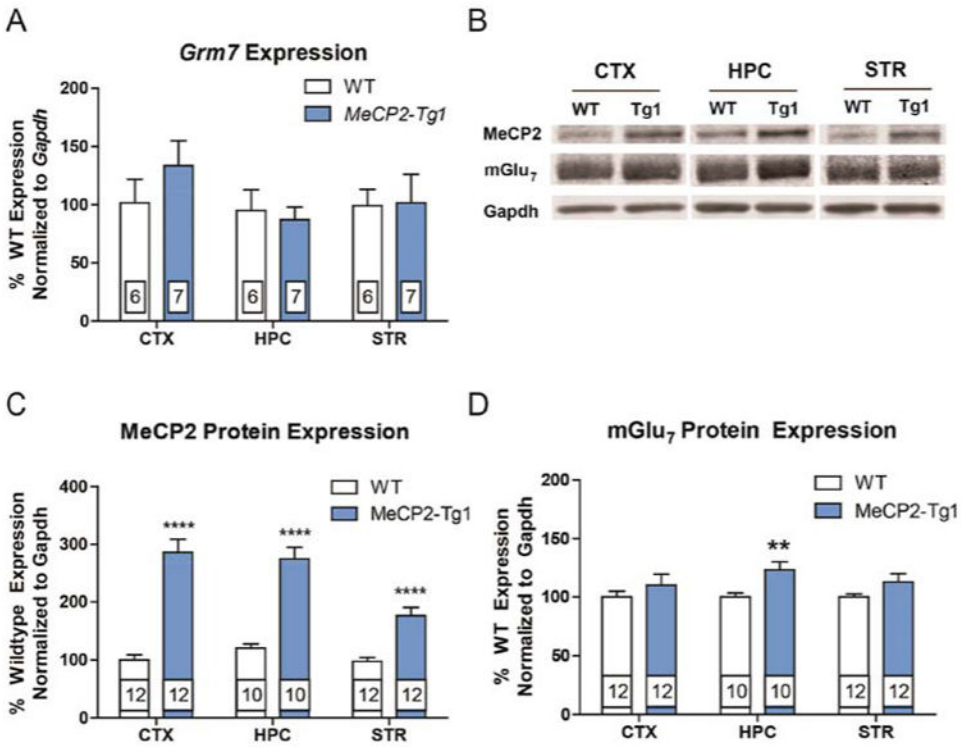

Figure 1.

Total $mGlu_7$ protein expression is increased in the hippocampus of *MeCP2-Tg1* mice. (A) *Grm7* mRNA expression is not changed in samples from *MeCP2-Tg1* mice ($N = 6–7$ samples per genotype). (B) Representative Western blots showing total $mGlu_7$ and MeCP2 protein expression. (C) Quantification of MeCP2 expression from total protein isolates: Cortex (WT 100.1 ± 8.7% ($N = 12$) vs *MeCP2-Tg1* 286.6 ± 22.5% ($N = 12$), ****$p < 0.0001$), hippocampus (WT 110 ± 6.2% ($N = 10$) vs *MeCP2-Tg1* 232.3 ± 17.6% ($N = 10$), ****$p < 0.0001$), striatum (WT 97.5 ± 6.7% ($N = 12$) vs 176.6 ± 13.8% ($N = 12$), ****$p < 0.0001$). Student's *t* tests for each region. (D) Quantification of $mGlu_7$ expression from total protein isolates: Cortex (WT 100 ± 4.8% ($N = 12$) vs MeCP2-Tg1 110.2 ± 9.2% ($N = 12$)), hippocampus (WT 100.0 ± 3.5% ($N = 10$) vs 123.0 ± 7.2% ($N = 10$), **$p < 0.01$), striatum (WT 100 ± 2.5% ($N = 12$) vs MeCP2-Tg1 112.7 ± 7.1% ($N = 12$)); Student's *t* tests for each region.

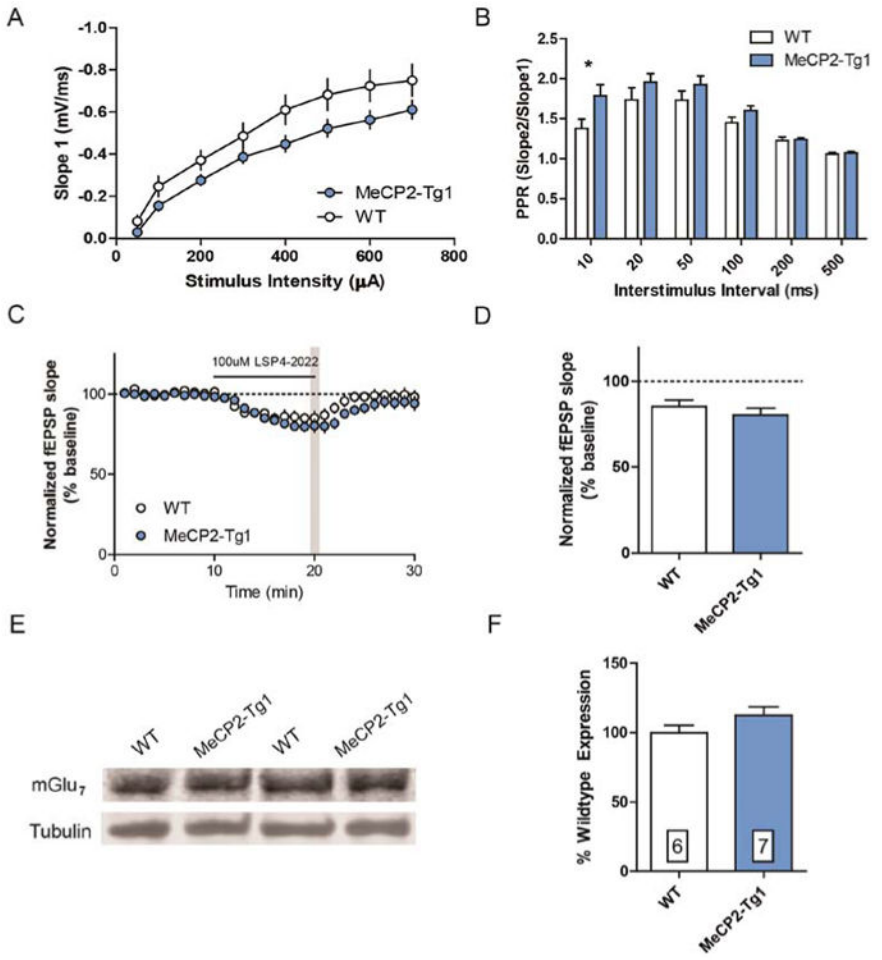

Figure 2.

$mGlu_7$ function is not affected by *MECP2* duplication at SC-CA1 synapses in mice. (A) Input–output curves were not significantly different between slices derived from 20-week-old WT and *MeCP2-Tg1* mice ($N = 8$ slices/5–6 mice). Two-way ANOVA. (B) Paired pulse ratios were significantly increased at a 10 ms interstimulus interval in *MeCP2-Tg1* slices ($N = 7$ slices/7 mice, $^*p < 0.05$). Two-way ANOVA with Bonferroni post hoc comparisons. (C, D) Depression by 100 μM LSP4–2022 at minute 20 (gray bar) was not significantly different between genotypes. WT 85.4 ± 3.8 ($N = 8$ slices, 5 mice) vs *MeCP2-Tg1* 80.2 ± 4.1 ($N = 8$ slices, 6 mice), Student's *t* test. (E) Representative $mGlu_7$ Western blots from synaptosomal protein isolates. (F) Quantification of $mGlu_7$ protein expression was not significantly different between genotypes. WT 100 ± 5.2% ($N = 6$ mice) vs *MeCP2-Tg1* 112.4 ± 6.2% ($N = 7$ mice). Student's *t* test.

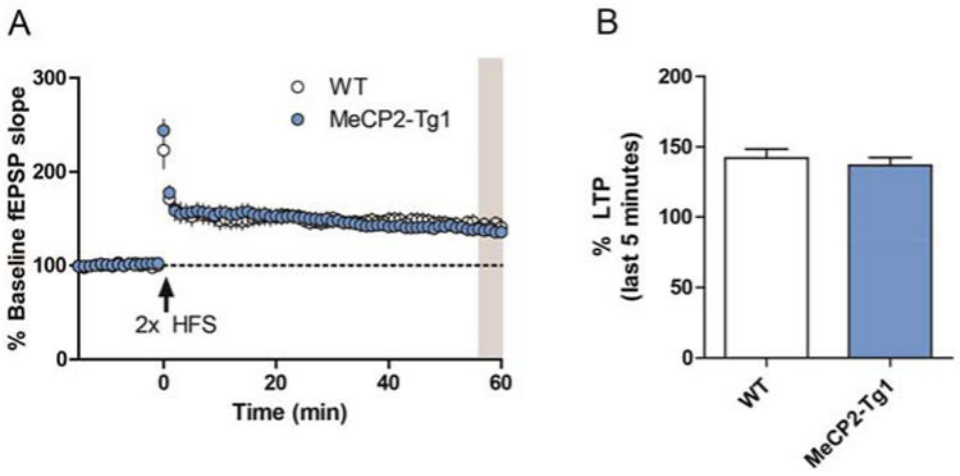

Figure 3.

LTP induced by HFS is unchanged at the SC-CA1 synapses in slices from 20-week-old *MeCP2-Tg1* mice. (A) After a 15 min baseline recording, LTP was induced by two trains of HFS, and fEPSPs were monitored for 60 min. (B) Quantification of percent LTP during the last 5 min of recording (gray bar). WT 142.1 ± 6.4 (N = 8 slices/9 mice) vs *MeCP2-Tg1* 137.0 ± 5.4 (N = 13 slices, 9 mice), Student's t test.

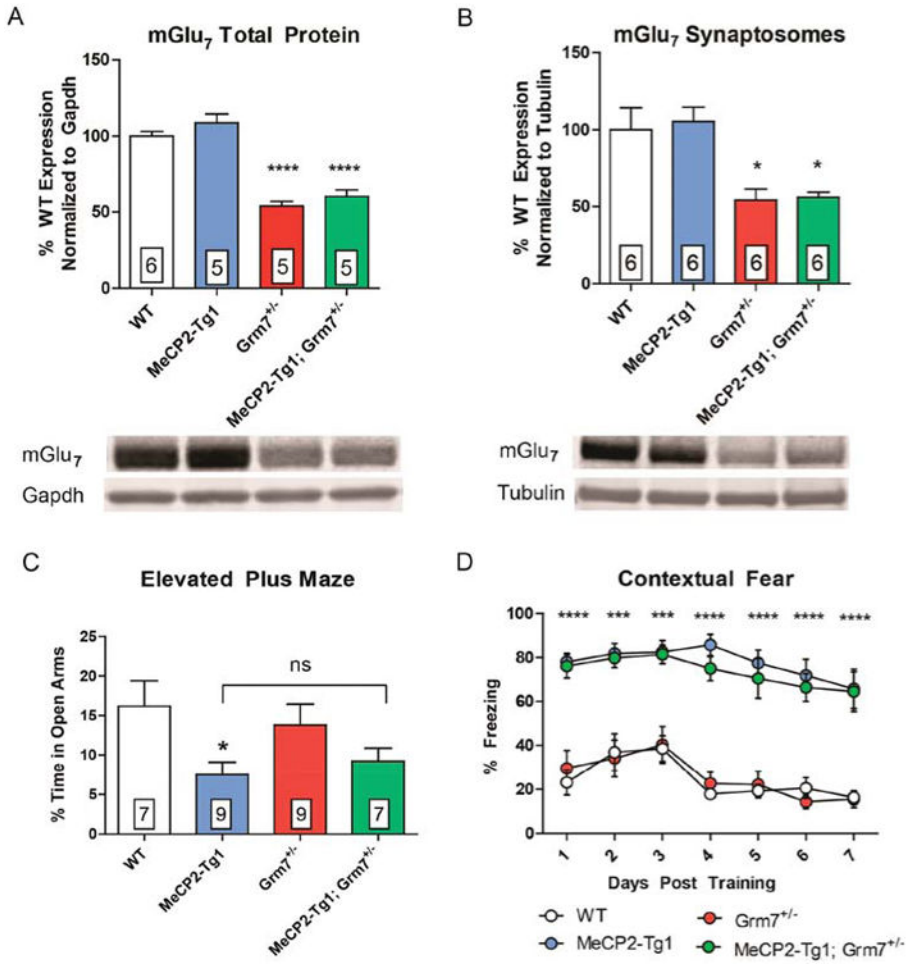

Figure 4.

Genetic reduction of $mGlu_7$ does not affect anxiety or fear behavior in *MeCP2-Tg1* mice. (A) $mGlu_7$ expression in total protein isolates from hippocampal tissue in *MeCP2-Tg1* mice compared relative to WT (WT 100.0 ± 3.1%, *MeCP2-Tg1* 108.6 ± 5.8%, *Grm7*$^{+/-}$ 54.0 ± 3.0%, *MeCP2-Tg1; Grm7*$^{+/-}$ 60.2 ± 4.5%, $N = 5–6$ mice per genotype, ****$p < 0.0001$, one-way ANOVA, Bonferroni comparisons relative to WT). (B) $mGlu_7$ expression from synaptosomal isolates from hippocampal tissue (WT 100.0 ± 14.1, *MeCP2-Tg1* 105.3 ± 9.3, *Grm7*$^{+/-}$ 54.2 ± 7.3, *MeCP2-Tg1; Grm7*$^{+/-}$ 55.8 ± 3.7, $N = 6$ mice per genotype, *$p < 0.05$, one-way ANOVA, Bonferroni comparisons relative to WT). (C) *MeCP2-Tg1* mice spend less time in the open arms of an elevated plus maze relative to WT mice, regardless of *Grm7* genotype ($N = 7–9$ mice per genotype, *$p < 0.05$, one-way ANOVA, Bonferroni comparisons). (D) *MeCP2-Tg1* mice exhibit increased contextual fear freezing compared to those that do not, regardless of *Grm7* genotype ($N = 7–10$ mice per genotype, ***$p < 0.001$, ****$p < 0.0001$, two-way ANOVA, Bonferroni comparisons to WT).

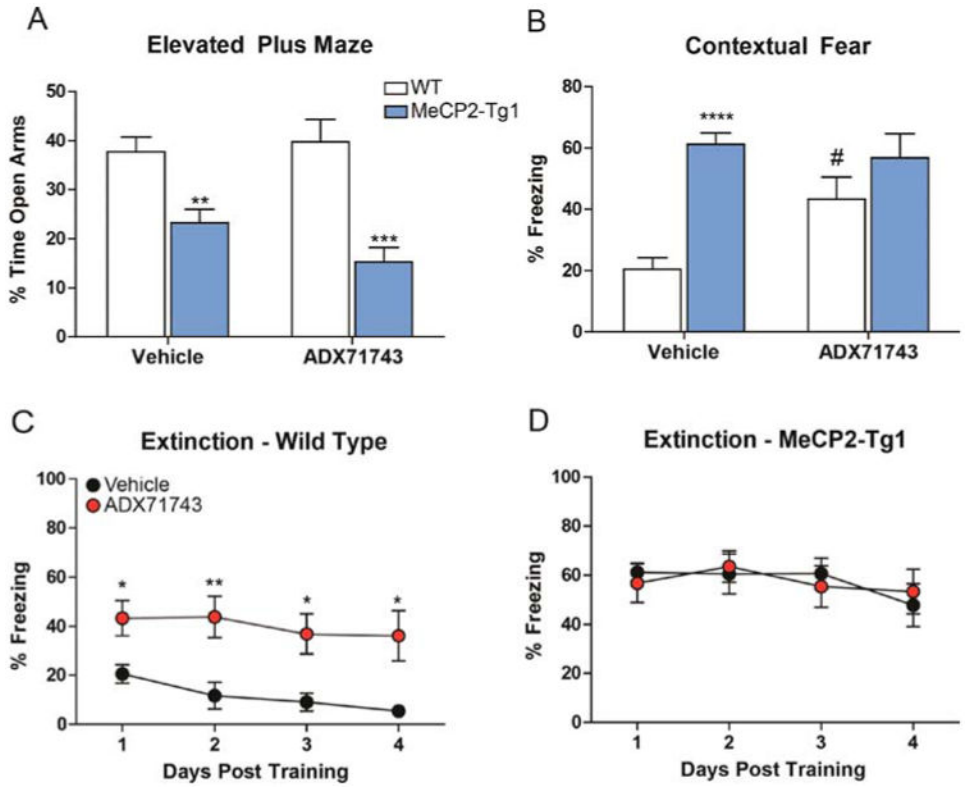

Figure 5.

$mGlu_7$ negative allosteric modulation does not affect anxiety or fear behavior in *MeCP2-Tg1* mice. (A) Vehicle-treated *MeCP2-Tg1* mice spent significantly less time in the open arms of the elevated plus maze relative to WT controls (WT: 37.8 ± 3.0% (N = 16) vs *MeCP2-Tg1*: 23.2 ± 2.8% (N = 18), $^{**}p < 0.01$), and ADX71743 (60 mg/kg) administration had no effect on this phenotype (WT: 39.8 ± 4.6% (N = 9) vs *MeCP2-Tg1*: 15.3 ± 3.0%, (N = 7) $^{***}p < 0.001$). Two-way ANOVA with Bonferroni post hoc analysis. (B) Relative to WT mice, *MeCP2-Tg1* mice treated with vehicle exhibited an enhanced contextual fear freezing response (WT: 20.5 ± 3.7% (N = 22) vs *MeCP2-Tg1:* 61.2 ± 3.7% (N = 17), $^{****}p < 0.0001$). ADX71743 pretreatment had no effect in *MeCP2-Tg1* mice relative to the vehicle-treated mice of the same genotype (MeCP2-Tg1:56.8 ± 7.9% (N = 11)); however, treatment with ADX71743 resulted in a significant increase in freezing in WT mice (WT: 43.3 ± 7.2% (N = 12), $^{\#}p$ 0.05, # denotes within genotype comparison. Two-way ANOVA with student's t test post analysis). (C,D) Wild-type mice treated with ADX71743 during training demonstrated a significantly attenuated ability to extinguish fear memory relative to vehicle-treated controls ($^{*}p < 0.05$, $^{**}p < 0.01$). ADX71743 administration had no effect on extinction in *MeCP2-Tg1* mice. Two-way ANOVA with Bonferroni post hoc analysis.