

GABA_A Receptor Antagonism Ameliorates Behavioral and Synaptic Impairments Associated with MeCP2 Overexpression

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Methyl-CpG-binding protein 2 (MeCP2) is a ubiquitously expressed transcriptional regulator with functional importance in the central nervous system. Loss-of-function mutations in *MECP2* results in the neurodevelopmental disorder, Rett syndrome, whereas increased expression levels are associated with the neurological disorder, *MECP2* duplication syndrome. Previous characterization of a mouse line overexpressing *Mecp2* demonstrated that this model recapitulated key behavioral features of *MECP2* duplication syndrome with specific deficits in synaptic plasticity and neurotransmission. Alterations in excitation/inhibition balance have been suggested to underlie neurodevelopmental disorders with recent data suggesting that picrotoxin (PTX), a GABA_A receptor antagonist, rescues certain behavioral and synaptic phenotypes in a mouse model of Down syndrome. We therefore examined whether a similar treatment regimen would impact the behavioral and synaptic phenotypes in a mouse model of *MECP2* duplication syndrome. We report that chronic treatment with low doses of PTX ameliorates specific behavioral phenotypes, including motor coordination, episodic memory impairments, and synaptic plasticity deficits. These findings suggest that GABA_A receptor antagonists may offer a possible therapeutic target for the treatment of *MECP2* duplication syndrome.

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INTRODUCTION

Methyl-CpG-binding protein 2 (MeCP2) is widely expressed in neuronal and non-neuronal tissues and was originally purified from the brain as a heterochromatin protein that binds to DNA containing a single methyl-CpG dinucleotide (Lewis *et al*, 1992). Interest in the role of MeCP2 in the brain has been high since the identification of mutations in the *MECP2* gene, predicted to result in the loss of function of the gene, are present in >95% of patients with classic Rett syndrome (Amir *et al*, 1999), an X-linked neurodevelopmental disorder, in which females express hand wringing behavior, autistic behavior, and mental retardation (Hagberg *et al*, 1983). Rather unexpectedly, duplication of chromosome Xq28, which results in increased expression of *MECP2*, has been shown to cause a neurodevelopmental disorder named *MECP2* duplication syndrome, that predominantly affects males (del Gaudio *et al*, 2006; Friez *et al*, 2006; Meins *et al*, 2005; Ramocki *et al*, 2009; Van Esch *et al*, 2005). Individuals diagnosed with *MECP2* duplication syndrome are often characterized with symptoms of

progressive spasticity, difficulty with social interaction, mental retardation, and autistic behavior (Ramocki *et al*, 2010). Although there has been much research focused on the loss of function of MeCP2 in CNS, there have only been a few studies that have investigated the impact of MeCP2 overexpression (Collins *et al*, 2004; Jiang *et al*, 2013; Luikenhuis *et al*, 2004; Na *et al*, 2012).

To study the impact of MeCP2 overexpression in behavior, recent work has focused on the generation of animal models. One line of MeCP2-overexpressing mice was created in which *Mecp2* was targeted into the *Tau* locus (*Tau-Mecp2*), creating a fusion protein with the first exon of *Tau* resulting in MeCP2 overexpression selectively in neurons (Luikenhuis *et al*, 2004). The *Tau-Mecp2* mice have an ~twofold increase in MeCP2 expression in the brain (Luikenhuis *et al*, 2004; Na *et al*, 2012). We recently characterized the *Tau-Mecp2* mice in a wide array of behavioral paradigms and found that they have an elevated anxiety-like phenotype, motor coordination deficits, impaired novel object recognition, and extinction learning, recapitulating key phenotypes observed in *MECP2* duplication syndrome patients. We also observed significant attenuations in long-term potentiation (LTP), a form of neural plasticity that may underlie memory storage (Abel and Lattal, 2001) and alterations in neurotransmission (Na *et al*, 2012). Indeed, recent data have suggested that loss or gain of MeCP2 expression alters specific aspects of synaptic plasticity and neurotransmission that may impact the

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balance of excitation/inhibition in the brain (Asaka *et al*, 2006; Calfa *et al*, 2011; Chao *et al*, 2007; Dani *et al*, 2005; Moretti *et al*, 2006; Nelson *et al*, 2011; Nelson *et al*, 2006; Zhang *et al*, 2008). Imbalances in excitatory and inhibitory neurotransmission have been suggested to be the result of global alterations in neuronal activity or specific antagonism of excitatory or inhibitory neurotransmission (Chubykin *et al*, 2007; Turrigiano, 2007). MeCP2 overexpression has been shown to impact excitation-evoked neurotransmission in the brain resulting in a decrease in probability of neurotransmitter release (Na *et al*, 2012).

Although various putative treatments for Rett syndrome and other forms of mental retardation and autism have been examined by animal studies, no data currently exist on possible therapeutic approaches for *MECP2* duplication syndrome. Given that MeCP2 overexpression selectively impacts evoked excitatory neurotransmission consistent with a decrease in the probability of evoked glutamate release, leading to an excess of inhibition, we wondered whether rebalancing this alteration in neurotransmission would rescue the behavioral phenotypes in the *Tau-Mecp2* mice. In the current study, we tested the impact of the non-competitive GABA_A antagonist picrotoxin (PTX) on behavioral and synaptic plasticity deficits observed in *Tau-Mecp2* mice. We report that a non-epileptic dose of PTX ameliorates some of the behavioral deficits, including cognitive and motor impairments, and also restores normal synaptic function in the *Tau-Mecp2* mice. These data suggest that rebalancing excitation–inhibition using GABA_A antagonists may hold promise as a therapeutic treatment for *MECP2* duplication disorder.

MATERIALS AND METHODS

Animals

Tau-Mecp2 mice were generously provided by Dr Rudolf Jaenisch and have been previously described (Luikenhuis *et al*, 2004). We backcrossed this line for more than 10 generations to C57BL/6 male mice. Mouse genotype was confirmed by PCR using tail samples and the following primers, Tau138 (5'-CTG-GCA-GAT-CTT-CCC-GTC-TA-3'), Tau1078 (5'-TGC-CTG-ACA-GAG-TCC-AGA-TG-3'), and Neo1323 (5'-AGG-GGA-TCC-GTC-CTG-TAA-GT-3'), which amplifies a 941-bp fragment from the wild-type (WT) allele and a 796-bp fragment from the overexpressing allele. Mice were maintained on a 12-h light/dark cycle with *ad libitum* access to food and water. All experiments used male mice aged 10–16 weeks that were *Tau-Mecp2* mice or wild-type littermate controls. All experiments were conducted by an observer who was blind to genotype and treatment. For all testing, mice were allowed to habituate in the behavioral room for 1 h. Mice were tested in the following order: locomotor activity, dark–light cycle, elevated plus maze (EPM), rotarod, fear conditioning, and extinction training. Two separate cohorts of mice were used for cue fear conditioning and novel object recognition. For all experiments, data were presented as mean ± SEM, with significance set as $p < 0.05$. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Drug Treatment

Mice were weighed at the start of experimentation and assigned to one of four groups: (1) WT vehicle (VEH), (2) *Tau-Mecp2* (Tau) VEH, (3) WT PTX, and (4) Tau PTX. Mice were treated chronically for five consecutive days with a daily injection of VEH or PTX (1 mg/kg, i.p.) on the basis of the dose used by Fernandez *et al* (2007) and Ramwell and Shaw (1965). It has previously been shown that systemic doses of 4–8 mg/kg induce epileptic activity in mice; however, lower doses (eg, 0.4–2 mg/kg) do not induce any seizure activity (Ramwell and Shaw, 1965). PTX (Sigma) was dissolved (10 ×) with dimethyl sulfoxide (Sigma) and diluted with isotonic saline to a 1 × working concentration. Mice used for hippocampal slice electrophysiology were given systemic PTX injections chronically based on the timeline outlined by Fernandez *et al* (2007), who demonstrated robust changes in LTP after chronic PTX administration.

Locomotor Activity

Mice were placed in a standard cage with fresh bedding. Locomotor activity was assessed for 2 h under red light by photocell beams linked to computer data acquisition software (San Diego Instruments).

Dark–Light Test

To assess anxiety, mice were examined in the dark–light test. The dark–light test consisted of a box that was partitioned into two compartments, a dark and light side. Mice were placed into the dark side for 2 min before a gate that separates the two compartments was lifted, thereby allowing mice to explore the dark or light sides for 10 min. The dependent variable was the time spent in the light side (Med Associates).

EPM

Mice were placed in the center of an EPM, a plus-shaped maze constructed of two open and two closed arms measuring 33 × 5 cm at 40 lux for 5 min. Time spent in the open arm *versus* time spent in closed arms was quantified by the Ethovision tracking software (Noldus Information Technology).

Rotarod

Each mouse was placed on a rotating rod (IITC Life Science), the speed of which gradually increased over 5 min. The test session ended when the animal fell off the rod or after 5 min. Mice were given four trials across 2 days for a total of eight trials.

Fear Conditioning

Training and testing were conducted under red light as previously described (Na *et al*, 2012). Briefly, mice were placed in individual fear conditioning chambers (Med Associates) and habituated to the chambers for 2 min. Two tone plus shock presentations were administered with an interstimulus interval of 1 min. White noise (90 dB) was

played for 30 s co-terminating with a 1 s footshock (0.5 mA). A minute after the last tone plus shock pairing, mice were returned to their home cages. Chambers were cleaned between each trial. To test context-dependent fear conditioning, mice were placed in the same chamber 24 h later without tone or shock and freezing behavior was recorded for 5 min. Freezing behavior was defined as no movement except for respiration. After 4 h, the cue test was performed. To test cue-dependent fear conditioning, mice were placed in a novel environment scented with vanilla extract and lit with fluorescent lights for 6 min. Freezing behavior was scored every 5 s and assessed during a 3 min baseline period followed by 3 min of tone.

Cue/context Extinction Training

Extinction is considered an active form of learning (Robledo *et al*, 2004) thus, to test if *Tau-Mecp2* mice can learn new contingencies, mice were trained to extinguish conditioned responses to cue and/or context. For cue extinction, mice were exposed to a novel environment (see above), and baseline freezing (3 min) and freezing during presentation of tone (3 min) were assessed. For context extinction training, mice were placed in the same context and freezing behavior was scored for 3 min. Sessions were terminated once WT controls reached baseline levels of freezing during tone and/or context presentation, which occurred after 8–9 days. A cohort of mice was tested for differences in nociception at the conclusion of the fear conditioning experiments. WT and *Tau-Mecp2* mice were exposed to increasing footshock intensity (range 0.05–0.45 mA) to determine the footshock threshold at which animals responded by flinching or vocalizing. For all fear conditioning experiments, an observer blind to genotype and drug treatment scored freezing behavior. Percentage of freezing behavior during tone/context presentation was compared between groups.

Novel Object Recognition (NOR)

On day 1, mice were habituated to a rectangular box (88 × 17 cm) at 40 lux for 10 min. After 4 h, mice were reintroduced to the rectangular box containing two of the same objects (A and A), a metal pipe, for 10 min (familiarization phase). The following day, mice were placed in the rectangular box for 10 min and exposed to two objects: object A and a new object, B, a metal cone. An experimenter who was blind to treatment scored the amount of time that the animal spent investigating the objects and a difference score was calculated. A difference score was calculated by subtracting the time spent with 'A' from the time spent with 'B'.

Hippocampal Slice Electrophysiology

Hippocampal slices were prepared as previously described (Akhtar *et al*, 2012; Kim *et al*, 2012; Na *et al*, 2012; Morris *et al*, 2013). Briefly, following picrotoxin or saline treatment mice were anesthetized with Euthasol (30 mg/ml, 0.2 ml i.p.) and then decapitated. Brains were removed and quickly immersed in ice-cold artificial cerebral spinal fluid (ACSF) that was continuously bubbled with 95% O₂

and 5% CO₂, pH 7.4. A 350- μ m thick transverse section of the hippocampus was sectioned and placed in ice-cold oxygenated ACSF for at least 1 h at 32 °C. The hippocampal slices were then transferred into a recording chamber and superfused with ACSF at a constant rate of 2.5 ml/min at 30 °C. Glass recording electrodes filled with ACSF (resistance, 1–2 M Ω) were used to record field excitatory post synaptic (fEPSP) potentials. Extracellular stimuli were delivered by placing a bipolar platinum–tungsten stimulating electrode to stimulate fibers of the Schaffer collateral pathway while the recording electrode was inserted into the CA1 just beneath the molecular layer. Input–output relationship was determined by providing an ascending series of stimulus input intensities (40–240 μ A) until the maximum fEPSP response was determined. An input stimulus intensity that induced 40–50% of the maximum response was used for measuring paired pulse ratio (PPR) and LTP. An input intensity that induced the maximum response was used for high frequency stimulation-induced LTP. PPR was induced by giving two pulses at decreasing interpulse intervals (500, 400, 200, 100, 50, 30, and 20 ms) and analyzed by dividing the fEPSP slope of pulse 2 by that of pulse 1. Following 20 min of stable baseline fEPSP slope, LTP was induced with two 100 Hz trains of 100 pulses with an intertrain interval of 20 s.

Statistics

Locomotor activity, EPM, DL, and fear conditioning were analyzed using a two-way ANOVA with genotype and drug as between-group factors. LTP and rotarod data were analyzed using two-way repeated measures ANOVA. NOR, PPR, and input–output data were analyzed by one-way ANOVA. Fisher's LSD was used following significant interaction/main effects. A *p*-value of <0.05 was required for statistical significance; however, an adjusted *p*-value of \leq 0.017 was used for Fisher's LSD analyses that contained multiple comparisons.

RESULTS

PTX Treatment in *Tau-Mecp2* Mice Ameliorates Impairments in Episodic Memory

Individuals with *MECP2* duplication often have moderate to severe intellectual disability. The *Tau-Mecp2* mice display specific deficits in some learning and memory paradigms (Na *et al*, 2012). To assess whether PTX treatment has any impact on these learning and memory deficits, we first tested the mice in the novel object recognition paradigm. During familiarization, there was no significant difference in the amount of time that the WT or *Tau-Mecp2* mice spent with the objects (data not shown). In agreement with previous data, *Tau-Mecp2* mice had a significant deficit in performance in the NOR paradigm compared with WT mice (Figure 1a; Na *et al*, 2012). We found that chronic PTX did not significantly alter the performance of the WT mice compared with vehicle treatment (Figure 1a). However, PTX treatment ameliorated the deficits in NOR in *Tau-Mecp2* mice with a substantial increase in the difference score compared with *Tau-Mecp2* mice that were treated with a vehicle (Figure 1a).

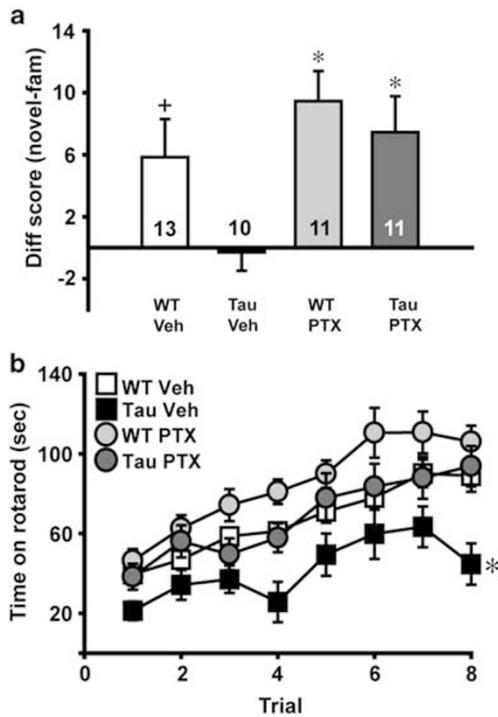


Figure 1 PTX improved deficits in episodic memory and in motor performance. (a) PTX-treated *Tau-Mecp2* mice showed an improved NOR compared with Veh-treated *Tau-Mecp2* mice. Significant interaction effect ($F_{(3,44)} = 3.62$, $p < 0.05$). Fisher's LSD tests revealed significant differences between *Tau-Mecp2* PTX and *Tau-Mecp2* vehicle-treated mice. Difference score = time with novel object – time with familiar object. (b) Motor coordination was improved by PTX treatment in *Tau-Mecp2* mice. Significant main effect of time and drug were seen in WT ($F_{(7,255)} = 19.49$, $p < 0.001$; $F_{(1,255)} = 4.27$, $p = 0.047$), respectively; post-hoc analyses did not reveal significant differences between groups. Significant main effects of drug and time were observed in *Tau-Mecp2* mice ($F_{(1,215)} = 5.65$, $p = 0.03$; $F_{(7,215)} = 10.95$, $p < 0.001$, respectively). Post-hoc analyses indicated a significant difference between *Tau-Mecp2* vehicle-treated mice and *Tau-Mecp2* PTX mice during trial 8 with *Tau-Mecp2* PTX spending more time on the rotarod compared with *Tau-Mecp2* vehicle-treated mice. PTX, picrotoxin; Tau, *Tau-Mecp2*; Veh, vehicle; WT, wild type. Data are means \pm SEM. * $p \leq 0.017$ compared with *Tau-Mecp2* vehicle-treated mice. + $p < 0.05$.

PTX Improves Motor Learning Deficits in *Tau-Mecp2* Mice

Tau-Mecp2 mice have significant deficits in motor learning as assessed by the latency to fall off the rotarod compared with WT controls (Figure 1b). We found that chronic PTX treatment did not impact the performance of WT mice on the rotarod test (Figure 1b). However, PTX treatment improved the motor impairments of *Tau-Mecp2* such that these mice now performed similar to WT mice (Figure 1b).

Chronic PTX Treatment Ameliorated Deficits in LTP and Short-Term Plasticity

Tau-Mecp2 mice have deficits in hippocampal LTP that correlate with some of the learning and memory deficits (Na *et al*, 2012). We therefore examined whether PTX rescues the hippocampal LTP deficits in the *Tau-Mecp2*

mice. We confirmed that *Tau-Mecp2* mice have deficits in LTP compared with WT controls (Figure 2a). We found that chronic PTX treatment had no effect on LTP from WT hippocampal slices (Figure 2b and c), however, it rescued decrements in LTP in *Tau-Mecp2* hippocampal slices with the effect sustained for the full 2 h of the recording (Figure 2d).

We next examined the effect of PTX on baseline transmission and short-term plasticity. We placed stimulating electrodes in the Schaffer collaterals and recorded field potentials in the CA1 region of the hippocampus to generate input-output curves. We found no significant differences as a result of genotype in WT or *Tau-Mecp2* hippocampi or PTX treatment on input/output slopes as determined by the ratio of presynaptic volley amplitude to fEPSP slope (data not shown). We also examined PPR by recording the response to two pulses separated by varying interstimulus intervals and taking the ratio of the second response to first. We observed enhanced PPR at 20, 30, 50, 100, and 200 ms interstimulus intervals in *Tau-Mecp2* hippocampal slices compared with WT controls (Figure 2e). Chronic PTX treatment did not alter any PPF responses in WT animals (Figure 2f and g), however, it normalized the enhanced PPR seen at the 100-ms interstimulus interval in *Tau-Mecp2* mice (Figure 2g and h).

PTX Treatment had no Effect on Anxiety-Like Behavior in *Tau-Mecp2* Mice

Tau-Mecp2 mice have a prevalent anxiety-like phenotype, therefore, we tested the effects of PTX treatment to ameliorate or attenuate the anxiety-related behavior in the dark-light and elevated plus maze tests. In the dark-light test, the *Tau-Mecp2* mice spent significantly less time in the light side than WT control mice (main effect of genotype), suggesting an increase in anxiety-related behavior. We found that PTX treatment had no effect on the behavior of WT or *Tau-Mecp2* mice in this paradigm (Figure 3a). In the elevated plus maze test, *Tau-Mecp2* mice spent significantly less time in the open arm compared with WT mice (main effect of genotype), also suggestive of a heightened anxiety-like phenotype. We found that PTX treatment had no effect on the WT or *Tau-Mecp2* mice in this paradigm (Figure 3b) consistent with the findings in the dark-light test (Figure 3a).

PTX Treatment in *Tau-Mecp2* Mice does not Ameliorate Impairments in Associative Learning

To assess whether PTX treatment has any impact on associative memory, we tested the mice in the fear conditioning paradigm. Baseline freezing between the *Tau-Mecp2* and WT mice was indistinguishable (data not shown). After 24 h of training, the *Tau-Mecp2* had a significant increase in context-dependent (Figure 4a) and in cue-dependent fear conditioning (Figure 4b). We found that chronic PTX treatment had no effect on the WT animals' performance, and that it did not rescue or attenuate the context or cue fear conditioning deficits in the *Tau-Mecp2* mice (Figure 4a and b). The increase in context and cue-dependent fear conditioning displayed by the *Tau-Mecp2* mice indicates an inability to extinguish the conditioned responses (Figure 4c

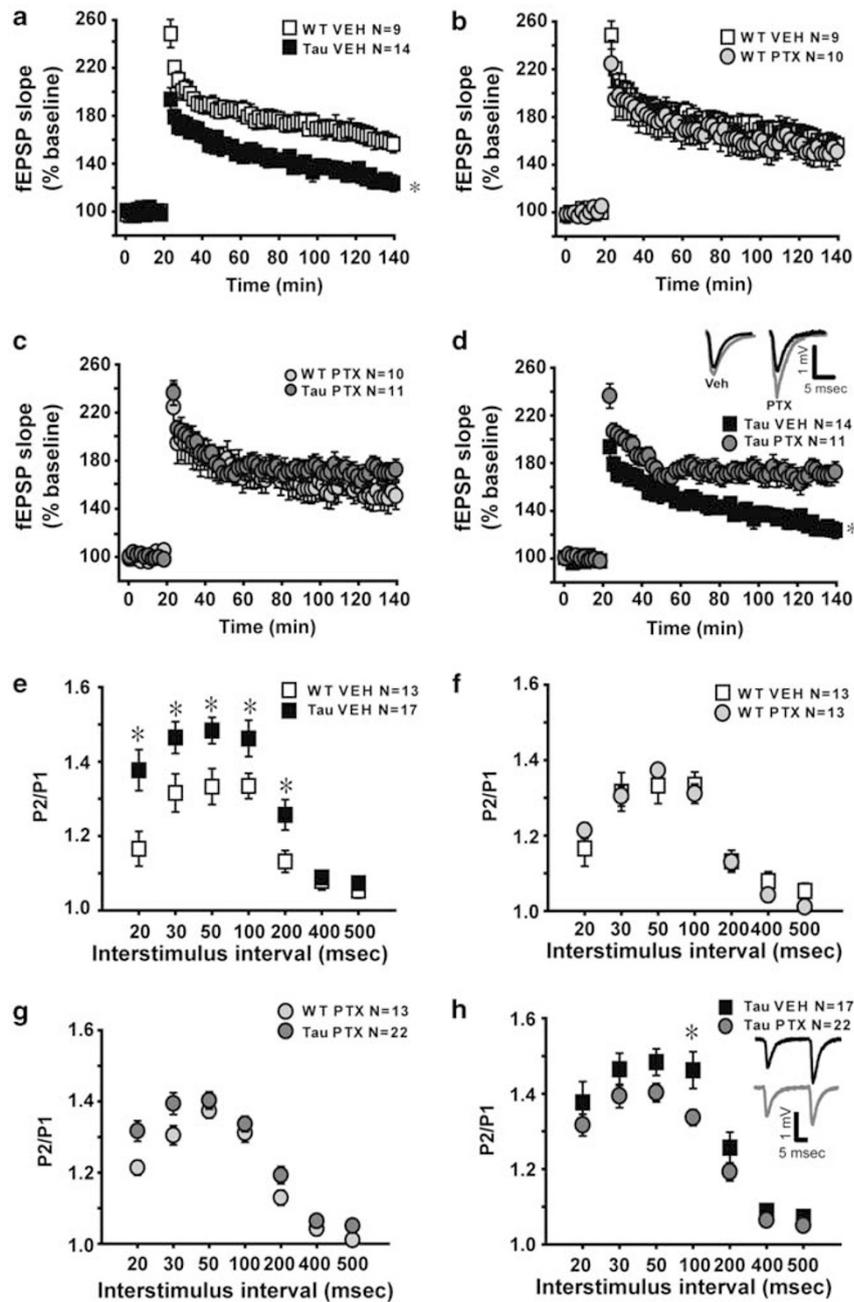


Figure 2 LTP impairments were improved by PTX treatment in *Tau-Mecp2* mice. (a) LTP was attenuated in *Tau-Mecp2* vehicle-treated mice compared with WT vehicle-treated mice; significant interaction effect ($F_{(68,2069)} = 4.57, p < 0.001$). Post-hoc analyses demonstrated significant differences between vehicle-treated groups at almost all time points. (b and c) LTP was not affected by PTX treatment in WT mice nor did it differentially affect LTP between *Tau-Mecp2* and WT mice. (d) LTP deficits were rescued by chronic PTX treatment in *Tau-Mecp2* mice compared with *Tau-Mecp2* vehicle-treated mice. A significant interaction effect was seen ($F_{(68,1931)} = 2.60, p < 0.001$). Post-hoc tests revealed that LTP was higher during the last hour of the test in *Tau-Mecp2* PTX compared with *Tau-Mecp2* vehicle-treated mice. (e) PPR was significantly enhanced at 20, 30, 50, 100, and 200 ms interstimulus intervals in *Tau-Mecp2* hippocampal slices compared with WT slices as demonstrated by Fisher's LSD post-hoc tests. A one-way ANOVA revealed significant differences at 20, 30, 50, 100, and 200 ms time intervals ($F_{(3,64)} = 5.23, p = 0.003$; $F_{(3,64)} = 3.53, p < 0.02$; $F_{(3,64)} = 3.66, p < 0.02$; $F_{(3,64)} = 4.06, p = 0.01$; $F_{(3,64)} = 3.63, p = 0.02$, respectively). (f and g) PTX had no effect on PPR between WT groups and between WT and *Tau-Mecp2* mice. (h) Chronic PTX treatment ameliorated the enhanced PPR at 100 ms in *Tau-Mecp2* mice as revealed by Fisher's LSD tests. PTX, picrotoxin; Tau, *Tau-Mecp2*; Veh, vehicle; WT, wild type. Data are means \pm SEM. * $p < 0.05$. N = 28 mice.

and d; see Na *et al*, 2012). Chronic PTX treatment did not impact the performance of WT mice in extinction learning, and also did not alter the context and cue-dependent extinction deficits in *Tau-Mecp2* mice. The differences in

freezing between genotypes are not due to differences in nociception as *Tau-Mecp2* mice respond similarly to footshock as WT controls and were not altered by drug treatment (Figure 4e).

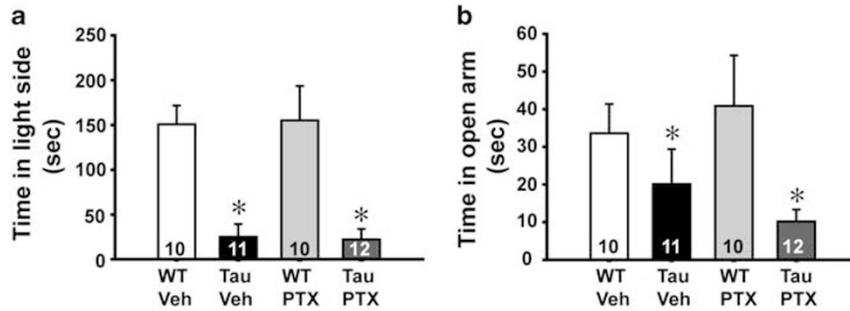


Figure 3 PTX did not impact the heightened anxiety-like phenotype in *Tau-Mecp2* mice. (a and b) PTX did not alter the prevalent anxiety-like phenotype in *Tau-Mecp2* mice in dark–light cycle (a) and elevated plus maze (EPM) (b) tests. Significant main effect of genotype ($F_{(1,38)} = 25.72$, $p = 0.001$; $F_{(1,38)} = 5.79$, $p = 0.02$) for dark–light cycle and EPM, respectively. PTX, picrotoxin; Tau, *Tau-Mecp2*; Veh, vehicle; WT, wild type. Data are means \pm SEM. * $p < 0.05$ compared with *Tau-Mecp2* vehicle-treated mice.

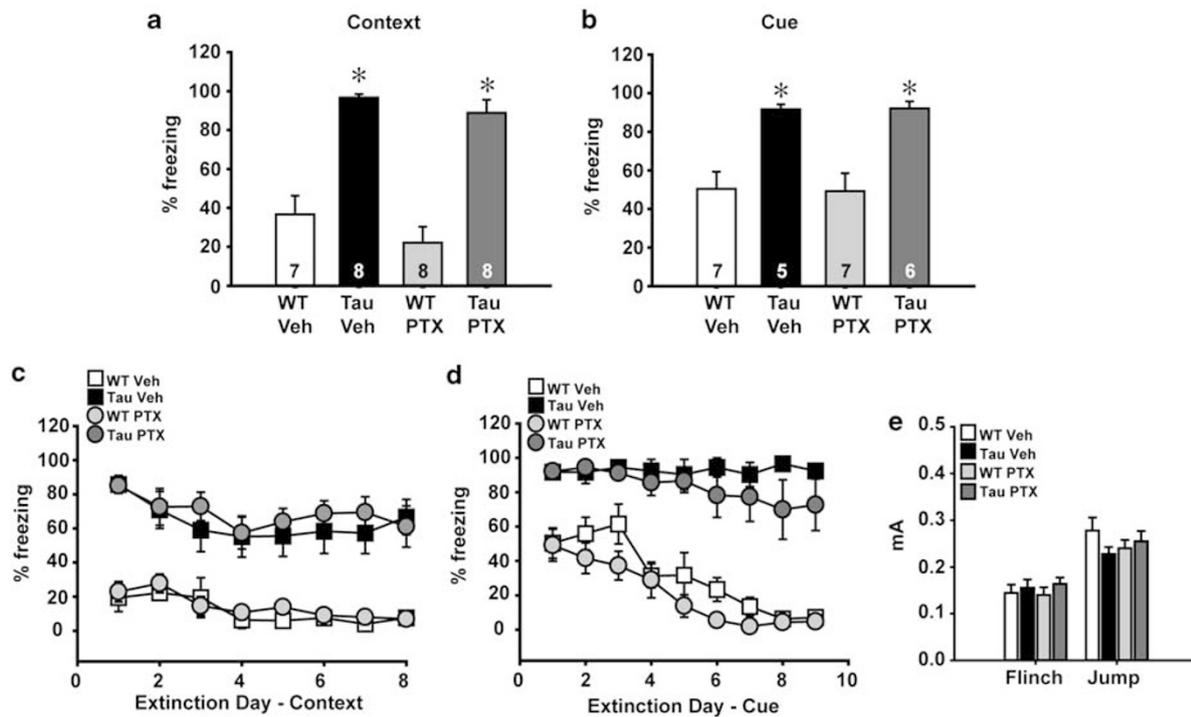


Figure 4 PTX does not rescue context- or cue-dependent fear conditioning deficits. (a and b) PTX did not affect freezing responses in *Tau-Mecp2* or WT mice. *Tau-Mecp2* mice froze significantly more than WT in contextual and cued fear conditioning, 24 h post-training, (significant main effect of genotype $F_{(1,23)} = 56.89$, $p < 0.001$ and significant interaction effect between group and time $F_{(24,215)} = 2.93$, $p < 0.001$, for contextual and cued, respectively). (c and d) PTX did not improve extinction learning in *Tau-Mecp2* mice in cued or contextual fear conditioning. A significant interaction effect indicated that WT mice extinguished freezing responses to cue faster than *Tau-Mecp2* mice ($F_{(24,215)} = 2.93$, $p < 0.001$). Significant main effects for context extinction were found for group and time ($F_{(3,247)} = 21.94$; $F_{(7,247)} = 8.1$), respectively. (e) There are no significant differences in nociception between WT and *Tau-Mecp2* mice in flinching or jumping. There is a main effect of genotype in vocalization between WT and *Tau-Mecp2* mice with *Tau-Mecp2* mice receiving a higher shock intensity before vocalizing ($F_{(1,34)} = 14.94$, $p < 0.01$). PTX, picrotoxin; Tau, *Tau-Mecp2*; Veh, vehicle; WT, wild type. Data are means \pm SEM. * $p < 0.05$ compared with WT vehicle-treated mice.

Body Weight and Locomotor Activity were not Adversely Affected by PTX Treatment

To ensure that PTX did not have any overt detrimental effects, body weight and locomotor activity were assessed in chronically treated mice. There were no significant differences between initial body weight and body weight at the end of experimentation for any of the treatment groups

(Figure 5a). Locomotor activity was similar between *Tau-Mecp2* mice and WT littermates. Chronic treatment with PTX had no significant effect on locomotor activity of either WT or *Tau-Mecp2* mice over a 2-h testing period (Figure 5b, inset) or when plotted as 5-min epochs over the testing period (Figure 5b). Additionally, we did not observe any seizure activity at the dose of PTX used in either WT or *Tau-Mecp2* mice (data not shown), similar to what has

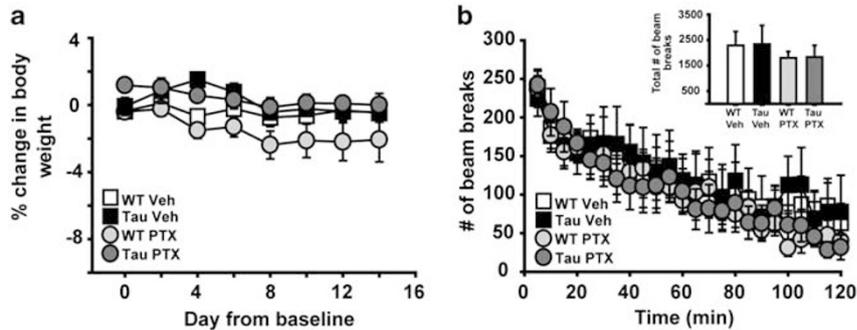


Figure 5 PTX did not impact overt behavior or the heightened anxiety-like phenotype in *Tau-Mecp2* mice. Chronic PTX treatment did not affect (a) body weight or (b) locomotor activity in *Tau-Mecp2* or WT mice. Inset represents average over 2 h of locomotor activity. PTX, picrotoxin; Tau, *Tau-Mecp2*; Veh, vehicle; WT, wild type. Data are means \pm SEM. * $p < 0.05$ compared with *Tau-Mecp2* vehicle-treated mice.

been previously reported (Fernandez *et al*, 2007; McGaugh *et al*, 1990).

DISCUSSION

The present study demonstrates that the GABA_A receptor antagonist, PTX, ameliorates key behavioral and synaptic deficits observed in a mouse model of *MECP2* duplication syndrome. Specifically, we demonstrate that chronic PTX treatment improves performance in the NOR paradigm, as well as motor coordination deficits, but did not impact the heightened anxiety-like phenotype or the associative learning deficits in *Tau-Mecp2* mice. These data suggest that PTX's effects, while beneficial for episodic memory in our mouse model, do not extend to learning processes that involve aversive elements (ie, aversive footshock). PTX treatment also rescued deficits in LTP with a partial rescue of short-term synaptic plasticity. Chronic PTX did not produce any overt detrimental effects and did not impact locomotory activity or body weight during the course of treatment in either the WT or *Tau-Mecp2* mice. These results show that PTX ameliorates particular behavioral and synaptic impairments associated with MeCP2 overexpression and that GABA_A receptors may have promise as a therapeutic target for the treatment of *MECP2* duplication syndrome. Moreover, these data suggest that certain behavioral and synaptic aspects of *MECP2* duplication syndrome stem from different biological alterations induced by MeCP2 overexpression.

Previous work has demonstrated specific behavioral phenotypes in *Tau-Mecp2* mice (Na *et al*, 2012), including deficits in certain forms of learning and memory, thus, we focused on whether PTX could alleviate these impairments. Chronic PTX treatment ameliorated deficits in NOR but did not improve fear conditioning or extinction learning in the *Tau-Mecp2* mice. One explanation for these findings is that PTX does not improve learning and memory processes associated with anxiety or aversion. This hypothesis is supported by the ineffectiveness of PTX on anxiety-like behavior in *Tau-Mecp2* mice. A second possibility is that PTX only improves deficits in non-associative learning and memory tests. NOR is a test of episodic memory (Bevins

and Besheer, 2006), whereas fear conditioning and extinction learning are both associative learning and memory paradigms (Abel and Lattal, 2001). In agreement with this possibility, we observe improved performance in PTX-treated *Tau-Mecp2* mice in rotarod, another non-associative learning task. Taken together, these results support the premise that PTX is beneficial for non-associative or perhaps non-aversive forms of learning and memory in mice that overexpress MeCP2. However, we cannot exclude the possibility that the lack of an effect of PTX in the fear conditioning experiments could be related to the low drug dose used in these experiments. Future work will be necessary to further delineate the effect of PTX on other cognitive tasks.

The PTX-mediated behavioral improvements in the NOR test that were observed with the *Tau-Mecp2* mice were similar to the PTX-mediated improvements in a mouse model of Down syndrome (Fernandez *et al*, 2007; Kleschevnikov *et al*, 2012). In the Down's syndrome study, the impact of PTX on other forms of learning and memory were not assessed. It is conceivable that the improvement we observed with the *Tau-Mecp2* mice in the NOR paradigm may be due to this test being more sensitive to the effects of PTX as outlined above. It is intriguing that animal models of Down syndrome, which is caused by trisomy 21, and *MECP2* duplication syndrome, which is caused by duplication of chromosome Xq28, are responsive to PTX for the alleviation of similar learning and synaptic phenotypes. It is possible that the PTX rescue of hippocampal LTP decrements in these studies could account for the enhanced performance in the novel object recognition test. Past studies have shown the importance of the hippocampus in mediating novel object recognition as electrolytic and ibotenic acid lesions of the hippocampus significantly impair performance in this behavioral task (Broadbent *et al*, 2010). Interestingly, we do not observe an effect of chronic low-dose PTX on LTP in WT mice, a finding that is consistent with previous experiments (Fernandez *et al*, 2007; Pananceau *et al*, 1997). Our current findings support the premise that PTX treatment corrects alterations in excitation/inhibition to ameliorate the LTP deficits in the *Tau-Mecp2* mice, which under normal conditions, are not affected by PTX treatment.

The PTX-mediated improvement of phenotypes in *Tau-Mecp2* mice may arise in part from rebalancing the excitation–inhibition imbalance seen after overexpression of MeCP2 (Asaka *et al*, 2006; Calfa *et al*, 2011; Chao *et al*, 2007; Dani *et al*, 2005; Moretti *et al*, 2006; Nelson *et al*, 2011; Nelson *et al*, 2006; Zhang *et al*, 2008). Prolonged directional change in excitation or inhibition at a synapse results in compensatory alterations in baseline synaptic activity as shown by studies on homeostatic plasticity processes (Turrigiano and Nelson, 2000). One possibility is that chronic suppression of inhibitory neurotransmission corrects the excitation–inhibition imbalance seen in MeCP2 gain-of-function models via a process that is similar to homeostatic synaptic plasticity. Another non-mutually exclusive possibility is that increased activity leads to alterations in aberrant gene expression profiles elicited by MeCP2 overexpression, which could in turn counteract the behavioral and cellular deficits.

Collectively, our results demonstrate that PTX rescues specific learning and memory and synaptic impairments in a mouse model of *MECP2* duplication syndrome. Our findings provide an important proof of principle that GABA_A receptor antagonists may represent a potential treatment approach for patients with *MECP2* duplication syndrome.

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