Enhancement of postsynaptic GABA$_A$ and extrasynaptic NMDA receptor-mediated responses in the barrel cortex of Mecp2-null mice

Fu-Sun Lo,1 Mary E. Blue,2 and Reha S. Erzurumlu1
1Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland; and 2Hugo Moser Research Institute at Kennedy Krieger, Inc. and Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted 14 October 2015; accepted in final form 15 December 2015

Lo FS, Blue ME, Erzurumlu RS. Enhancement of postsynaptic GABA$_A$, and extrasynaptic NMDA receptor-mediated responses in the barrel cortex of Mecp2-null mice. J Neurophysiol 115: 1298–1306, 2016. First published December 16, 2015; doi:10.1152/jn.00944.2015.—Rett syndrome (RTT) is a neurodevelopmental disorder that results from mutations in the X-linked gene for methyl-CpG-binding protein 2 (MECP2). The underlying cellular mechanism for the sensory deficits in patients with RTT is largely unknown. This study used the Bird mouse model of RTT to investigate sensory thalamocortical synaptic transmission in the barrel cortex of Mecp2-null mice. Electrophysiological results showed an excitation/inhibition imbalance, biased toward inhibition, due to an increase in efficacy of postsynaptic GABA$_A$ receptors rather than alterations in inhibitory network and presynaptic release properties. Enhanced inhibition impaired the transmission of tonic sensory signals from the thalamus to the somatosensory cortex. Previous morphological studies showed an upregulation of NMDA receptors in the neocortex of both RTT patients and Mecp2-null mice at early ages [Blue ME, Naidu S, Johnston MV. Ann Neurol 45: 541–545, 1999; Blue ME, Kaufmann WE, Bressler J, Eyring C, O’Driscoll C, Naidu S, Johnston MV. Anat Rec (Hoboken) 294:1624–1634, 2011]. Although AMPA and NMDA receptor-mediated excitatory synaptic transmission was not altered in the barrel cortex of Mecp2-null mice, extrasynaptic NMDA receptor-mediated responses increased markedly. These responses were blocked by memantine, suggesting that extrasynaptic NMDA receptors play an important role in the pathogenesis of RTT. The results suggest that enhancement of postsynaptic GABA$_A$ and extrasynaptic NMDA receptor-mediated responses may underlie impaired somatosensation and that pharmacological blockade of extrasynaptic NMDA receptors may have therapeutic value for RTT.

Rett syndrome; Mecp2 gene; excitation/inhibition balance; barrel cortex

Rett syndrome (RTT) (Rett 1966) is a developmental disorder predominantly occurring in females. It is caused by mutations in the methyl-CpG-binding protein 2 (MECP2) gene on the X chromosome (Amir et al. 1999). MeCP2 acts as a transcription repressor of many genes throughout the genome (Calfa et al. 2011; Na et al. 2013). RTT manifests itself as developmental retardation and regression beginning between ages 6 and 18 mo. Characteristics include slowed brain and head growth, stereotypical hand movements, seizures, intellectual disability, osteoporosis, and respiratory and autonomic dysfunction (Hagberg 2002; Naidu et al. 1990; Neul et al. 2010). Postmortem brains of RTT patients show a disruption of the growth of axodendritic synapses (Johnston et al. 1995) and age-related biphase changes in NMDA, AMPA, kainic acid, and metabotropic glutamate and GABA receptors in various brain regions (Blue et al. 1999b; Johnston et al. 2001, 2005).

Sensory impairments in RTT patients have been reported with somatosensory evoked potential techniques (Badr et al. 1987; Bader et al. 1989; Glaze 2005; Kalmanchey 1990; Kimura et al. 1992; Yamanouchi et al. 1993). However, the cellular mechanisms underlying sensory deficits are poorly understood.

Mouse models provide powerful approaches in revealing cellular mechanisms of developmental brain disorders. Currently there are several Mecp2 mutant mouse lines. The initial constitutive Mecp2 deletion resulted in embryonic lethality (Tate et al. 1996). Guy et al. (2001) circumvented embryonic lethality by a conditional gene deletion approach (Bird model) and yielded Mecp2-null (Mecp2$^{-/-}$) males. The brain of Mecp2-null mice showed age-related changes in expression of NMDA receptors (NDMArs) (Blue et al. 2011) that were similar to those observed in patients with RTT (Blue et al. 1999a, 1999b). RTT mouse models show synaptic transmission deficits that are region specific (for reviews see Boggio et al. 2010; Della Sala and Pizzorusso 2014; Monteggia and Kavalali 2009; Na et al. 2013; Na and Monteggia 2011). For example, deletion of the Mecp2 gene leads to decreased excitatory responses in hippocampal neurons (Chao et al. 2007), layer 5 pyramidal cells in the motor cortex (Tropea et al. 2009) and in the somatosensory cortex (Danci et al. 2005), and layer 2/3 pyramidal cells in the motor-frontal cortex (Wood et al. 2009; Wood and Shepherd 2010), in the visual cortex (Castro et al. 2014), and in the lateral geniculate nucleus (Noutel et al. 2011). In contrast, increased excitatory responses have been noted in the nucleus of the solitary tract (Kline et al. 2010) and the hippocampus CA3 region (Cafta et al. 2015). Loss of function of Mecp2 gene results in reduction of GABAergic inhibition in hippocampal CA1 pyramidal neurons (Ma et al. 2014), thalamic ventrobasal complex (Zhang et al. 2010), ventrolateral medulla (Cafla et al. 2011; Medrihan et al. 2008), and locus coeruleus (Jin et al. 2013b). However, GABAergic inhibition is enhanced in the thalamic reticular nucleus (Zhang et al. 2010), the visual cortex (Durand et al. 2012), and the hippocampus CA3 region (Cafla et al. 2015). These results imply that the effect of Mecp2 gene on synaptic transmission shows region specificity.

We studied synaptic transmission in trigeminal thalamocortical slices (Agmon and Connors 1992; Lee et al. 2005) from Mecp2-null mice. We show that the excitation/inhibition (E/I) balance in layer 4 excitatory neurons of Mecp2-null mice is

Address for reprint requests and other correspondence: R. S. Erzurumlu, 20 Penn St., HSF II, S251, Dept. of Anatomy and Neurobiology, UMB School of Medicine, Baltimore, MD 21201 (e-mail: rerzurumlu@som.ummmed.edu).
biased toward inhibition, which results from an increase of postsynaptic inhibition due to enhanced postsynaptic GABA_\text{A} receptor (GABA_\text{A}R) efficacy. While the postsynaptic excitation mediated by both AMPA receptors (AMPARs) and NMDARs is not altered, the excitatory response mediated by extrasynaptic NMDARs is increased remarkably. Thus deletion of Mecp2 gene leads to an increase in postsynaptic GABA_\text{A}R efficacy and extrasynaptic NMDAR-mediated response in the layer IV excitatory neurons.

**MATERIALS AND METHODS**

**Animals.** Breeding Mecp2 heterozygous (Mecp2^{+/y}) females with C57BL/6 males yields heterozygous (HET) females and hemizygous (Mecp2^{y/y}) (Mecp2-null) males (Guy et al. 2001). Since the Mecp2 gene is located in the X chromosome, hemizygous males (Mecp2^{y/y}) are considered “null” mutations and they do not breed. All surgical procedures followed National Institutes of Health guidelines and were approved by the University of Maryland Institutional Animal Care and Use Committee (IACUC). 

**Brain slice preparation.** Three- to five-week-old wild-type (WT, n = 25) and Mecp2-null (n = 22) male mice were anesthetized with isoflurane and decapitated. The brain was rapidly removed and immersed in cold (<4°C) artificial cerebrospinal fluid (ACSF, in mM: 125 NaCl, 2.5 KCl, 1.25 NaHPO_4, 1 MgSO_4, 25 NaHCO_3, 10 glucose, 2 CaCl_2, pH = 7.4) bubbled with 95% O_2-5% CO_2. Thalamocortical slices were cut (350 μm) with a vibratome (Campden 7000msz) at an angle of 50° from the midsagittal plane and 10° from the coronal (Agmon and Connors 1999; Lee et al. 2005). After 1-h incubation in ACSF at 33°C, the slices were kept at room temperature for at least 1 h. The slice containing the thalamocortical pathway was transferred into a submerged-type recording chamber (27L, Warner Instruments) and continuously perfused (>2 ml/min) with normal ACSF at room temperature.

**Electrophysiology.** Whole cell patch micropipettes were pulled horizontally in three stages from borosilicate glass (WPI, K150F-4) with a P-87 puller (Sutter Instrument). The patch electrodes were backfilled with a Cs-based intracellular solution (in mM: 115 CsMeSO_3, 10 NaCl, 1 KCl, 4 MgCl_2, 1 CaCl_2, 11 EGTA, 20 HEPES, 3 Na_2-ATP, 0.5 Na_2-GTP, pH = 7.25, >290 mosM) with a tip resistance of 5–9 MΩ. Layer IV excitatory neurons in the barrels not in the septa (×4/0.13 objective) were visualized with infrared light (IR)-differential interference contrast (DIC) optics of an Olympus BX51WI upright microscope (long-working-distance water immersion objective ×400/0.80 W). After whole cell configuration was formed, depolarizing current pulses were passed through the patch pipette to identify the firing pattern of excitatory neurons (Agmon and Connors 1992; Beierlein et al. 2003; Feldmeyer et al. 1999) in current-clamp mode. A concentric stimulating electrode (WPI, TM33CCINS) was inserted into the thalamic ventrobasal complex (VB). Electrical monophasic pulses (0.3-ms duration, 0.2 Hz, 0–500 μA) were passed through the electrode (core negative) to evoke excitatory and inhibitory postsynaptic responses in both current- and voltage-clamp modes. Paired pulses at an interval of 200 ms were delivered to test the paired-pulse ratio (PPR) of excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents. All biological data were acquired by an InstruTECH ITC-16 interface unit and stored on a Dell DM061 computer with a PULSE (HEKA) software program.

**Multiple input index analysis.** For excitatory connections, layer IV excitatory neurons were voltage-clamped at +60 mV. In the presence of 50 μM picrotoxin (PTX), a GABA\text{A}R antagonist, EPSCs were induced by stimulation of VB at 0.2 Hz. The stimulus intensity was gradually increased from 0 to 500 μA in steps of 10 μA as described previously (Lo et al. 2011). The peak amplitudes of EPSCs were measured and plotted against stimulus intensity. The amplitude of EPSCs enhanced in a stepwise manner following the increase in stimulus intensity. We first measured the baseline noise of recordings and calculated the standard deviation (SD) of the noise. The variation in amplitude of EPSCs was analyzed. If the amplitude of an EPSC was larger than the prior EPSC by >3 × SD, a “jumping step” was defined, because the fluctuation of EPSCs induced by the same stimulus intensity was always <3 × noise SD. The number of “jumping steps” [multiple input index (MII)] provided an estimate of the lower-limit number of VB neurons that innervate the recorded cortical neuron.

For inhibitory connections, layer IV excitatory neurons were voltage-clamped at 0 mV. IPSCs were induced by stimulation of VB with increasing stimulating intensity. In the same way, the MII of IPSCs was obtained.

**Spontaneous EPSC and spontaneous and miniature IPSC recordings.** AMPAR-mediated spontaneous EPSCs (AMPAR sEPSCs) were recorded at a holding potential of −70 mV. GABA\text{A}R-mediated spontaneous IPSCs (sIPSCs) were recorded at 0 mV. GABA\text{A}R-mediated miniature IPSCs (mIPSCs) were recorded at 0 mV in the presence of tetrodotoxin (TTX, 1 μM). NMDAR-mediated sEPSCs (NMDAR sEPSCs) were recorded at −70 mV in Mg_2⁺-free ACSF containing 10 μM DNQX, an AMPAR antagonist. The amplitude of sEPSCs, mIPSCs, and mIPSCs was measured with Mini Analysis software.

**Extrasynaptic NMDAR-mediated responses.** In Mg_2⁺-free ACSF with DNQX (10 μM, an AMPAR antagonist), PTX (50 μM, a GABA\text{A}R antagonist), and MK-801 (5 μM, an open-channel NMDAR blocker), maximal stimulation with a single pulse (0.3-ms duration, 500 μA, core negative) at 0.1 Hz of VB induced postsynaptic NMDAR-mediated EPSCs at −70 mV in layer IV excitatory neurons, which were gradually blocked. Then stimulation with 5–10 pulses (same as single pulse) at 0.1 Hz made glutamate spillout to extrasynaptic sites and induce extrasynaptic NMDAR-mediated EPSC (eNMDA-EPSC; Harris and Pettit 2008). Memantine (100 μM), a specific extrasynaptic NMDAR antagonist (Wu and Johnson 2015; Xia et al. 2010), blocked the eNMDA-EPSC. The ratio of synaptic and extrasynaptic NMDARs was calculated by the maximal amplitude of single pulse-induced EPSC/eNMDA-EPSC.

In the present study, we did not differentiate between the spiny stellate and star pyramid classes of excitatory neurons in layer IV because the main functional difference between the two types is reportedly in their intracortical connections (Feldmeyer 2012; Schubert et al. 2003). Furthermore, in analysis of our data, we pooled responses from both classes randomly for the two genotypes of mice and obtained significant differences.

Here it is also important to note that RTT is more prevalent in human females than in males. We used Mecp2^{y/y} mice, namely males, because although girls with RTT are heterozygous for the MECP2 mutation, for mice the hemizygous males (KO-Mecp2-null) have a phenotype more similar to the human condition, especially for mechanistic studies of developmental plasticity. HET female mice eventually become symptomatic but not until they are 3–6 mo of age, and thus they are not a very useful animal model for studying developmental mechanisms. In contrast, Mecp2-null mice (i.e., Mecp2^{y/y} males) become symptomatic as early as 3 wk of age, the age of mice that were examined in this study.

**RESULTS**

We performed whole cell patch recordings from layer IV excitatory neurons in an in vitro thalamocortical slice preparation from 3- to 5-wk-old male and female WT and Mecp2-null mice. The properties of thalamocortical synaptic transmission were compared between WT and Mecp2-null mice.

**Impaired temporal summation of thalamocortical excitatory transmission in Mecp2-null mice.** Layer IV excitatory neurons from WT and Mecp2-null mice were identified by their adapt-
ing train of spikes (regular spiking; Agmon and Connors 1992; Beierlein et al. 2003; Feldmeyer et al. 1999) during membrane depolarization (Fig. 1, A and B). Stimulation of VB induced a monosynaptic excitatory postsynaptic potential (EPSP) followed by a disynaptic feedforward inhibitory postsynaptic potential (IPSP) (Feldmeyer 2012). The IPSP was reversed between −60 mV and −80 mV (approximately −70 mV), indicating that it was mediated by GABA_A Rs (Fig. 1C and D, top vs. bottom). At −60 mV in the WT mice, the IPSP shortened the EPSP but did not hyperpolarize the membrane potential below −60 mV, i.e., the amplitude of EPSP ≥ IPSP (Fig. 1C, top). However, in MeCP2-null mice the IPSP at −60 mV always hyperpolarized the membrane potential below baseline (Fig. 1D, top), indicating that the amplitude of the EPSP was less than that of the IPSP. Thus in layer IV excitatory neurons from MeCP2-null mice, inhibitory responses were relatively larger than excitatory responses.

To investigate functional processing in the barrel cortex of MeCP2-null mice, we applied a train of 50-Hz stimuli to the VB (subthreshold for action potentials) to mimic thalamic, slowly adapting (tonic) afferents induced by whisker deflection (Simons and Carvel 1989). In WT mice, temporal summation of postsynaptic responses at resting potential (−60 mV) built up a depolarizing plateau (Fig. 1E). The curve of averaged temporal summation of EPSPs (Fig. 1G; n = 16 from 8 mice) indicated that the peaks of subsequent EPSPs (measured from peak to −60 mV) were higher than the peak of the first EPSP. When thalamic afferents are stronger, each EPSP may trigger a sodium spike, suggesting that thalamic tonic inputs are faithfully transmitted to barrel cortex. However, in MeCP2-null mice, temporal summation led to membrane hyperpolarization (Fig. 1F). The curve of averaged peaks of subsequent EPSPs (measured from peak to −60 mV, n = 7 from 4 mice; Fig. 1G) declined toward hyperpolarization. Thus subsequent thalamic inputs failed to induce postsynaptic spikes. The outcome of temporal summation of postsynaptic excitatory responses depends upon both presynaptic transmitter release probability (Pr) and postsynaptic response properties (Lo et al. 2013). We used a paired-pulse protocol to test the Pr of thalamic afferent terminals from WT and MeCP2-null mice. EPSCs were recorded at a holding potential of +60 mV in the presence of PTX (50 μM), a GABA_A R antagonist (Fig. 1, H and I). The amplitude of the second EPSC was smaller than that of the first one [paired-pulse depression (PPD)] in both cases. The averaged PPR of EPSCs for WT mice (0.63 ± 0.03; n = 34 from 17 mice) was similar to that for MeCP2-null mice (0.58 ± 0.04; n = 15 from 8 mice, P > 0.37; Fig. 1J). Therefore, the Pr of thalamocortical afferent terminals did not change in MeCP2-null mice. Thus the failure of temporal summation of thalamic excitatory inputs in MeCP2-null mice results exclusively from the change in the E/I ratio of postsynaptic responses.

E/I ratio in thalamocortical pathway is decreased in MeCP2-null mice. To quantify the change in E/I ratio, we voltage-clamped layer IV excitatory neurons to the reversal potentials of GABA_A R (approximately −70 mV) and glutamate receptors (approximately 0 mV). As shown in Fig. 2A, between 0 mV and −70 mV the outward (upward) current had a linear correlation (R = 0.99, P = 0.001) with holding potentials and closed to zero at −70 mV (Fig. 2B). The outward current at 0 mV was completely blocked by 50 μM PTX, a GABA_A R antagonist (Fig. 2C, trace 1 before and trace 2 after application of 50 μM PTX, a GABA_A R antagonist).
Thus the outward current at 0 mV was a GABAAR-mediated IPSC and the inward (downward) current at -70 mV was an AMPAR-mediated EPSC because it was completely blocked by DNQX (10 μM), an AMPAR antagonist, as shown in Fig. 2D. These were true for both WT and Mecp2-null mice.

We then recorded AMPAR-mediated EPSC and GABAAR-mediated IPSC induced by maximal stimulus in each of the neurons from both types of mice (Fig. 2, E and F) and calculated the ratio of AMPA to GABA. The averaged ratio of AMPA to GABA in Mecp2-null mice (0.79 ± 0.07, n = 7 from 4 mice) was significantly lower than that in WT mice (1.67 ± 0.22, n = 11 from 6 mice, P < 0.001; Fig. 2G).

Excitatory and inhibitory inputs to layer IV of barrel cortex do not change in Mecp2-null mice. There are two possibilities for the decrease in E/I ratio in Mecp2-null mice: either changing neural network and/or changing expression of postsynaptic receptors. To test whether layer IV neurons of Mecp2-null mice receive fewer excitatory synaptic connections and/or more inhibitory connections, we used the MII (Lo et al. 2011) to estimate excitatory and inhibitory connections to single cortical layer IV neurons. In the presence of 50 μM PTX, the EPSCs at +60 mV were induced by stimulation of VB with increasing intensity from 0 to 500 μA. The amplitude of EPSCs increased in a stepwise way (representative records in Fig. 3, A and B). The number of steps gave an estimate of the minimal number of innervating VB neurons (Lo et al. 2011). The averaged MII for EPSCs in WT mice (5.05 ± 0.28, n = 20...
from 10 mice) was similar to that in Mecp2-null mice (5.13 ± 0.21, n = 8 from 4 mice, P > 0.85; Fig. 3C). Thus layer IV neurons in Mecp2-null mice do not receive fewer thalamic excitatory connections than neurons in WT mice. We also estimated inhibitory connections in WT and Mecp2-null mice (representative records of IPSCs at 0 mV in Fig. 3, D and E). Similar to EPSCs, the mean MII of IPSCs in WT mice (4.10 ± 0.18, n = 9 from 5 mice) was not significantly different (P > 0.82) from that in MeCP2-null mice (4.20 ± 0.33, n = 5 from 3 mice; Fig. 3F). Thus the layer IV neural network is unaltered in Mecp2-null mice.

We also found that PPRs of IPSCs were similar (P > 0.26) in the two types of mice. Figure 3, G and H, are representative records. Mean PPRs for IPSCs (0.94 ± 0.02, n = 9 from 5 mice) in WT mice were not significantly different from those in Mecp2-null mice (0.92 ± 0.01, n = 13 from 7 mice; Fig. 3I). On the basis of these negative results, we hypothesized that the decrease in E/I ratio in the barrel cortex of Mecp2-null mice results from changes in postsynaptic receptor efficacy.

There are no changes in postsynaptic AMPAR- and NMDAR-mediated responses in barrel cortex of Mecp2-null mice. AMPAR-mediated spontaneous EPSCs (AMPAR-sEPSCs) were recorded at −70 mV (Fig. 4, A and B). The mean amplitude of AMPAR-sEPSCs in WT mice (12.5 ± 0.4 pA, n = 386 from 5 neurons of 3 mice) was not different from that in Mecp2-null mice (13.3 ± 0.2 pA, n = 268 from 5 neurons of 3 mice, P > 0.10; Fig. 4C). NMDAR sEPSCs were recorded at −70 mV in Mg2+-free ACSF with the AMPAR antagonist DNQX (Fig. 4, D and E). Amplitude of NMDAR sEPSCs in WT mice (8.05 ± 0.10 pA, n = 274 from 4 neurons of 2 mice) was similar to that in Mecp2-null mice (8.15 ± 0.12 pA, n = 306 from 5 neurons of 3 mice, P > 0.55; Fig. 4F). These results indicate that the density of glutamate receptors in layer IV excitatory neurons is unaltered in Mecp2-null mice.

Enhanced response of postsynaptic GABA_A receptors in barrel cortex of Mecp2-null mice. We tested GABA_A,R-mediated sIPSCs and mIPSCs. GABA_A,R-mediated sIPSCs were recorded at 0 mV (Fig. 5, A and B). The amplitude of sIPSCs in Mecp2-null mice (31.1 ± 2.4 pA, n = 147 from 4 neurons of 2 mice) was significantly higher (P < 0.001) than in WT mice (16.1 ± 0.5 pA, n = 238 from 6 neurons of 3 mice; Fig. 5C). The shift in the cumulative probability curve of Mecp2-null mice to a higher amplitude (Fig. 5D) also illustrated the difference in the sIPSC amplitude. To avoid action potential-dependent IPSCs, we applied TTX to block sodium spikes and recorded mIPSCs (Fig. 5, E and F). The amplitude of mIPSCs in Mecp2-null mice (23.8 ± 0.5 pA, n = 249 from 5 neurons of 3 mice) was significantly larger (P < 0.001) than that in WT mice (12.5 ± 0.5 pA, n = 207 from 5 neurons of 3 mice; Fig. 5, G and H). Note that the averaged amplitude of mIPSCs for both types of mice was smaller than those of sIPSCs. We conclude that the decrease in E/I ratio in the thalamocortical pathway in Mecp2-null mice is caused by an increase in postsynaptic GABA_A,R efficacy without changes in postsynaptic AMPAR and NMDAR expression.

Autoradiographic studies (Blue et al. 1999b, 2011) showed increased NMDAR expression in the cerebral cortex of young girls with RTT and in 2-wk old Mecp2-null mice. In light of our findings, we reasoned that the increased NMDARs might be located outside the postsynaptic density, i.e., in extrasynaptic membranes.

J Neurophysiol • doi:10.1152/jn.00944.2015 • www.jn.org
malized eNMDA-EPSC in MeCP2-null mice (72.8 ± 6.4, n = 6 from 3 mice) was significantly (P < 0.001) higher than in WT mice (34.8 ± 1.7, n = 6 from 3 mice). Therefore, in the barrel cortex of MeCP2-null mice, expression of eNMDARs is also upregulated.

DISCUSSION

We chose the trigeminal thalamocortical pathway to investigate the underlying mechanisms of somatosensory impairment in RTT for two major reasons. First, children with RTT show prominent somatosensory cortical processing problems and associated behaviors, such as unique oral and facial sensitivities and aberrant responses to affective touch. Second, this pathway is well characterized both anatomically and physiologically in mice, and several groups including ours have used it to assess synaptic defects in a variety of mutant mouse models (Albright et al. 2007; Iwasato et al. 2008; Li et al. 2013; Lo et al. 2013; Lu et al. 2001, 2003, 2006). The deficits in the function of this pathway caused by deletion of the MeCP2 gene are unknown. Our results show that trigeminal thalamocortical synapses in MeCP2-null mice have a dramatically lower E/I ratio than in WT mice.

A proper E/I balance is critical for keeping normal neural network activity. Imbalance of the E/I ratio is proposed to be a cellular mechanism underlying many neurological and psychiatric disorders, such as autism spectrum disorder, epilepsy, schizophrenia, and RTT (for reviews see Gatto and Broadie 2010; Zhang and Sun 2011). Our findings are mostly similar to the studies in other brain regions in MeCP2-null mice. The difference is that the change in E/I ratio in other brain regions results from decreased excitatory responses (Castro et al. 2014; Chao et al. 2007; Dani et al. 2005; Noutel et al. 2011; Tropea et al. 2009; Wood et al. 2009; Wood and Shepherd 2010). However, in the barrel cortex of MeCP2-null mice, AMPAR-

Fig. 5. Upregulated response of postsynaptic GABA<sub>A</sub> receptors in barrel cortex of MeCP2-null mice. A and B: representative recordings of GABA<sub>A</sub> receptor-mediated spontaneous IPSCs (sIPSCs). C and D: averaged amplitude of sIPSCs is larger in MeCP2-null mice than in WT mice. E and F: representative recordings of GABA<sub>A</sub> receptor-mediated miniature IPSCs (mIPSCs). G and H: averaged amplitude of mIPSCs in MeCP2-null mice is also larger than that for WT mice.

Fig. 6. Extrasynaptic NMDAR-mediated response is upregulated in the barrel cortex of MeCP2-null mice. A and B: representative recordings show extrasynaptic NMDAR-mediated responses. In Mg<sup>2+</sup>-free ACSF with AMPA and GABA receptor antagonists and MK-801, an open-channel NMDAR blocker, VB stimulation with single pulses at 0.1 Hz induces postsynaptic NMDAR-mediated EPSCs (circles) that are gradually blocked. After that, stimulation with 5–10 pulses at 100 Hz evokes extrasynaptic NMDAR (eNMDAR)-mediated EPSCs (stars, indicated by arrows). Note that in MeCP2-null mice (B), the amplitude of eNMDAR-mediated EPSCs is higher than in WT mice (A). The eNMDAR antagonist memantine blocks eNMDAR-mediated EPSCs. C: the normalized eNMDAR-mediated EPSC is larger in MeCP2-null mice than in WT mice.
and NMDAR-mediated excitation is not different from that in WT mice. The decrease in E/I ratio is mainly, if not completely, caused by an increase in postsynaptic inhibition that impairs synaptic transmission of tonic sensory signals. Our results provide another example of region specificity of the Mecp2 gene’s function. The enhanced postsynaptic inhibition was demonstrated by an increase in the amplitude of GABAAR-mediated sIPSCs and mIPSCs, suggesting that the efficacy of GABAARs increases in Mecp2-null mice. GABAARs mediate most of the fast inhibitory neurotransmission in the brain. They exhibit extensive structural heterogeneity, as indicated by the 21 different subunit genes that encode various GABAAR subtypes. Because of cell type-specific gene expression and differential assembly of subunits into receptor complexes, various GABAAR subtypes are expressed in different neuronal cell types. Structurally distinct receptor subtypes then exhibit different GABA sensitivity and channel functions (Fritschy and Brunig 2003; Jacob et al. 2008; Luscher et al. 2011; Luscher and Keller 2004; Michels and Moss 2007; Vithlani et al. 2011). Because synaptically released neurotransmitters (including GABA) saturate their receptors (Clements 1996), the strength of inhibitory synaptic currents is directly correlated with the number of synaptic GABAARs (Nusser et al. 1997, 1998; Otis et al. 1994). Most likely, postsynaptic GABAAR density is increased in the barrel cortex of Mecp2-null mice. Recently, it was reported that Mecp2 gene may modulate GABAAR trafficking in the locus coeruleus (Jin et al. 2013); we do not know whether a similar situation exists for the thalamocortical system.

The following possibilities for the increased GABAAR efficacy in Mecp2-null mice are worth noting: 1) It results from a rearrangement of the inhibitory inputs, such that a cell would have the same total number but they are now electronically closer to the soma. This possibility needs ultrastructural evidence for confirmation. 2) It is caused by changes in the receptor phenotype via subunit shifts. Subunit composition of postsynaptic GABAARs in the barrel cortex of WT mice is still unknown, although GABAARs contain α5-subunit in the prefrontal and visual cortices (Jang et al. 2013; Redrobe et al. 2012). Without such information, it is not possible to exclude this possibility. 3) In the barrel cortex of Mecp2-null mice, some endogenous GABAAR positive allosteric modulators (PAMs) that bind to benzodiazepine (BZ) site may increase GABAAR-mediated current by inducing a conformational change in GABAAR structure. An endogenous GABAAR PAM has been reported for α3-subunit-containing GABAARs of thalamic reticular nucleus (Christian et al. 2013) but not for barrel cortex.

Another finding of the present study was enhancement of eNMDARs in the barrel cortex of Mecp2-null mice. NMDARs are found both on postsynaptic sites and along the surface of neurons (i.e., extrasynaptic). eNMDARs are often associated with contacts along axons and glia. eNMDARs contain NR1, NR2A, or, most commonly, NR2B subunits and form clusters in association with scaffolding proteins such as PSD-95 and SAP102 or adhesion proteins such as cadherins (Petralia et al. 2010). eNMDARs are activated by glutamate spillover from synapses or from ectopic glutamate release from astrocytes (Hamilton et al. 2010; Jourdain et al. 2007; Matsui et al. 2005; Petralia et al. 2005). In general, there are two separate roles for differently located NMDARs: synaptic NMDA receptors (sNMDARs) activate signaling pathways leading to long-term plasticity (LTP/LTD) and gene expression changes that are mediated by cAMP-response element-binding-protein (CREB) and induce prosurvival events. On the other hand, Ca²⁺ signaling through eNMDARs shuts off CREB activity and leads to mitochondrial dysfunction and cell death (Frasca et al. 2011; Hardingham et al. 2002; Hardingham and Bading 2010). The localization hypothesis of NMDARs suggests that activation of eNMDARs promotes cell survival while activation of eNMDARs promotes cell death (Hardingham et al. 2002). Despite some notable recent exceptions, the localization hypothesis is strongly supported by recent observations of elevated eNMDAR expression in various neurological disorders such as Alzheimer disease, Huntington disease, ischemia/hypoxia, traumatic brain injury, and epilepsy (for review see Parsons and Raymond 2014). There is also some evidence indicating increased concentration of glutamate in the brains and CSF of children with RTT (Hamberger et al. 1992; Horska et al. 2009; Wenk 1997). Increased eNMDARs in Mecp2-null mice suggest that eNMDARs may contribute to synaptic dysfunction in RTT.

Our results showed that the eNMDAR-mediated responses were blocked by memantine in both WT and Mecp2-null mice. Memantine is a relatively low-affinity, open-channel blocker with a fast off-rate. Its uncompetitive nature results in an effective blockade of chronic extrasynaptic NMDAR activity and displays minimal adverse effects (Chen and Lipton 2006; Hardingham and Bading 2010; Lipton 2006). Memantine at therapeutic concentrations preferentially blocks extrasynaptic rather than synaptic currents mediated by NMDARs (Milnerwood et al. 2010; Okamoto et al. 2009; Wu and Johnson 2015; Xia et al. 2010); thus it is able to inhibit the prolonged influx of Ca²⁺ ions from extrasynaptic receptors (Lipton 2006) as a neuroprotective agent for treating Alzheimer disease (for review see Matsunaga et al. 2015), Huntington disease (Dau et al. 2014; Levine et al. 2010; Milnerwood et al. 2010; Okamoto et al. 2009; Olivares et al. 2012), and autism spectrum disorder (Rossignol and Frye 2014). It has not been used to treat RTT clinically. In vitro experiments showed that memantine was capable of partially reversing the deficient synaptic plasticity caused by the loss of Mecp2 in hippocampal slices (Bello et al. 2013; Weng et al. 2011). Our results provide further evidence for memantine blockade of enhanced eNMDAR-mediated responses in the barrel cortex of Mecp2-null mice and suggest that memantine could have a potential therapeutic value for Rett syndrome. Future studies are aimed to test whether memantine can ameliorate behavioral deficits observed in Mecp2-null mice.

ACKNOWLEDGMENTS

We thank K. Arakawa and Dr. M. Chen for mouse breeding and genotyping.

GRANTS

This work was supported by National Institutes of Health Grants NS-092216 (R. S. Erzurumlu) and U54 HD-079123 (M. E. Blue).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: F.-S.L. conception and design of research; F.-S.L. performed experiments; F.-S.L. analyzed data; F.-S.L. interpreted results of experiments; F.-S.L. prepared figures; F.-S.L. drafted manuscript; M.E.B. and R.S.E. edited and revised manuscript; R.S.E. approved final version of manuscript.

REFERENCES


