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REVIEW

The Impact of MeCP2 Loss- or Gain-of-Function on Synaptic Plasticity

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Methyl-CpG-binding protein 2 (MeCP2) is a transcriptional regulator of gene expression that is an important epigenetic factor in the maintenance and development of the central nervous system. The neurodevelopmental disorders Rett syndrome and *MECP2* duplication syndrome arise from loss-of-function and gain-of-function alterations in MeCP2 expression, respectively. Several animal models have been developed to recapitulate the symptoms of Rett syndrome and *MECP2* duplication syndrome. Cell morphology, neurotransmission, and cellular processes that support learning and memory are compromised as a result of MeCP2 loss- or gain-of-function. Interestingly, loss-of-MeCP2 function and MeCP2 overexpression trigger diametrically opposite changes in synaptic transmission. These findings indicate that the precise regulation of MeCP2 expression is a key requirement for the maintenance of synaptic and neuronal homeostasis and underscore its importance in central nervous system function. This review highlights the functional role of MeCP2 in the brain as a regulator of synaptic and neuronal plasticity as well as its etiological role in the development of Rett syndrome and *MECP2* duplication syndrome.
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INTRODUCTION

Epigenetic modifications of chromatin structure can lead to rapid and enduring changes in gene expression that are important in central nervous system development. A number of studies have demonstrated that alterations in epigenetic processes are also important mediators of gene expression in postmitotic neurons suggesting that these key regulatory mechanisms are important in the brain throughout life. Recent work has shown that dysregulation of epigenetic mechanisms underlie several neurodevelopmental disorders including Rubinstein–Taybi syndrome, autism, Rett syndrome (RTT), and *MECP2* duplication syndrome among others. This review will focus on the role of the epigenetic factor methyl-CpG-binding protein 2 (*MECP2*) that is located on the X-chromosome, the dysfunction of which underlies RTT and *MECP2* duplication syndrome.

In 1999, *MECP2* was identified as the gene that causes RTT (Amir *et al*, 1999). Patients with RTT are typically females who appear to develop normally up to 6–18 months of age and then begin to regress with the loss of social and communication skills and development of severe motor and autonomic abnormalities. Although there are hundreds of identified disease-causing *MECP2* mutations, it is the loss-of-function of the MECP2 protein that is associated with RTT. Gain-of-function has also been identified in humans, typically through duplication or triplication of the *MECP2* gene, and is associated with the more recently defined neurodevelopmental disorder *MECP2* duplication syndrome. These bidirectional associations point to a fundamental role for MECP2 in the brain and demonstrate the importance of precisely controlled MECP2 expression for normal development and neuronal function (Chao and Zoghbi, 2012). The monogenic determinant of RTT and *MECP2* duplication syndrome has allowed for successful generation of mouse models for these disorders, both of which are the subject of this review.

MeCP2 LOSS-OF-FUNCTION IS ASSOCIATED WITH RETT SYNDROME

MECP2 is a member of the methyl-binding domain family of proteins. MECP2 is well characterized as a transcription

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factor important for controlling gene expression through the interpretation and regulation of epigenetic markers. Initial studies identified two key functional domains—a methyl-DNA-binding domain important for MECP2's interaction with methylated cytosine residues located near target genes and a transcriptional repression domain that governs the formation of co-repressor protein complexes such as histone deactylases (Nan *et al*, 1998). The discovery of MECP2 target genes, however, has proven a rather daunting task. Early-on large-scale expression arrays revealed only subtle changes in gene expression (Traynor *et al*, 2002; Tudor *et al*, 2002). Additional research into alternative functions of MECP2 suggests that the protein can also influence RNA splicing as well as activate gene expression (Chahrour *et al*, 2008; Lasalle and Yasui, 2009; Young *et al*, 2005). This range of MECP2 functions indicates a complex assortment of possible mechanisms leading to neurological dysfunction in RTT and *MECP2* duplication syndrome.

Significant insight into the functional consequences of MeCP2 in the brain has come from the study of transgenic mice. A number of previously written comprehensive review articles have helped consolidate the past 10 + years of research performed on *Mecp2*-deficient mice, the conventional animal model of RTT (Calfa *et al*, 2011; Moretti *et al*, 2006; Na and Monteggia, 2011). Studies of mice with various temporal and spatial deletions of *Mecp2* have revealed numerous morphological changes and alterations in synaptic transmission and plasticity that likely underlie the observed cognitive and behavioral deficits reminiscent of human RTT. As more discoveries are made, questions are being asked as to whether the loss of *Mecp2* results in neurological malfunction during development and maturity, or if perhaps MeCP2 has a more fundamental role in the maintenance of synapse function and neuronal connectivity (Guy *et al*, 2011).

MeCP2 KNOCKOUT MOUSE MODELS

First attempts at generating constitutive *Mecp2* knockout (KO) mice resulted in early lethality, with hemizygous males dying at around 7–10 weeks (Chen *et al*, 2001; Guy *et al*, 2001). Overt phenotypes included motor deficits and respiratory abnormalities along with reduced brain weight and neuronal size, indicators that loss of MeCP2 was primarily affecting the brain. Mice as young as 2–4 weeks of age displayed reduced cortical thickness, increased neuronal density, and immature synapse formation (Fukuda *et al*, 2005). The severity of these neurological phenotypes promoted the generation of brain-specific *Mecp2* KOs. Deletion of *Mecp2* using the loxP system of recombination in which floxed MeCP2 were crossed with a *Nestin-Cre* mouse line specifically reduced expression of the gene in neurons and glia as early as embryonic day (E)11 (Chen *et al*, 2001; Guy *et al*, 2001). These mice developed normally for the first few weeks and then displayed similar features as constitutive KO mice, including abnormal gait, hindlimb clasping, and shortened lifespan. A Ca^{2+}/calmodulin-dependent protein kinase II promoter driving Cre recombination (*CamK-Cre93*)-mediated conditional *Mecp2* KO mouse, in which deletion occurs in forebrain neurons during early postnatal development, showed similar yet delayed and less severe symptoms. Subsequent behavioral characterization of *Camk-Cre* conditional *Mecp2* KO mice revealed additional impairments in motor coordination, increased anxiety, abnormal social behavior, and deficits in learning and memory (Gemelli *et al*, 2006). Together, these mice demonstrate the necessity of MeCP2 in neurons, particularly during postnatal phases of development, indicating a possible role in neuronal maturation.

Another *Mecp2* transgenic mouse was created in which the allele was truncated at amino-acid residue 308 ($Mecp2^{308/y}$), similar to particular mutations observed in RTT patients. These mice appear normal up to about 6 weeks and then begin to develop many of the same neurological deficits as *Mecp2* null mice (Shahbazian *et al*, 2002). Symptomatic $Mecp2^{308/y}$ phenotypes include hypoactivity, forepaw rubbing, hindlimb clasping, tremors, seizures, ataxia, and motor dysfunction. Abnormal diurnal activity, nesting, and social behavior were also observed in these mice (Moretti *et al*, 2006). Additional behavioral tests of learning and memory as well as electrophysiological measurements of synaptic function have been performed on $Mecp2^{308/y}$ mice. Contextual fear conditioning, Morris water maze, and social recognition tests revealed deficits in various hippocampal-dependent memory behaviors (Moretti *et al*, 2006). These data recapitulate the learning and memory deficits that are consistently seen in RTT patients and underscore the importance of MeCP2 in learning and memory processes. Thus, it logically follows that neuronal processes that underlie learning and memory may be affected by MeCP2 dysfunction.

A variety of studies have identified and explored a role for MeCP2 in specific brain areas. The anxiety and impaired motor coordination phenotypes observed in *Mecp2* mutant mice point to the amygdala and cerebellum as particular regions of interest (Gemelli *et al*, 2006; Pelka *et al*, 2006). *Mecp2* knockdown using viral-mediated recombination targeted specifically at the basolateral amygdala was sufficient to induce a heightened anxiety response and impair amygdala-dependent learning and memory when compared with control mice (Adachi *et al*, 2009). Utilizing various cell-type-specific gene promoters, several conditional *Mecp2* KO mice have been generated. *Sim1-Mecp2* KO mice with targeted deletion of *Mecp2* in the hypothalamus displayed obesity, increased aggressive behavior, and elevated corticosterone levels in response to stress (Fyffe *et al*, 2008). *Pet1-Mecp2* mice with gene knockdown targeted to serotonergic neurons also demonstrated increased aggression, whereas tyrosine hydroxylase-*Mecp2* KO mice that have reduced *Mecp2* expression in dopaminergic and noradrenergic neurons showed impaired motor abilities (Samaco *et al*, 2009). Finally, mice lacking MeCP2 in GABAergic neurons presented motor dysfunction, altered

social behavior, and spatial memory deficits (Chao *et al*, 2010).

The phenotypic effects of *Mecp2* mutations resemble that observed in mouse models in which MeCP2 is knocked down, indicating that general loss-of-function, regardless of how it is induced, is sufficient to recapitulate the behavioral symptoms that are characteristic of RTT: anxiety, impaired learning, and memory as well as impaired motor coordination. Although there have been many studies examining MeCP2 loss-of-function in animal models, the impact of MeCP2 overexpression in animal models is only beginning to be elucidated.

MeCP2 GAIN-OF-FUNCTION IS ASSOCIATED WITH *MECP2* DUPLICATION SYNDROME

MECP2 duplication syndrome is inherited in an X-linked manner with 100% penetrance in males with carrier mothers. Females are typically asymptomatic carriers although a recent report has shown that heterozygous females display some core features of *MECP2* duplication syndrome including anxiety, depression, and an autistic-like phenotype (Ramocki *et al*, 2009). *MECP2* duplication syndrome has been associated with duplications of Xq28, which includes the *MECP2* gene (Smyk *et al*, 2008). Clinical cases of *MECP2* triplication have been documented and it is noteworthy that these patients have far more severe symptoms compared with those diagnosed with *MECP2* duplication syndrome (Tang *et al*, 2012) indicating that symptom severity does not necessarily plateau with *MECP2* duplication syndrome.

This debilitating neurodevelopmental disorder is marked by severe mental retardation, stunted motor development, early onset hypotonia, epileptic seizures, as well as progressive spasticity (Van Esch, 2012). A majority of patients diagnosed with *MECP2* duplication syndrome are susceptible to severe recurrent respiratory infections, which contributes to a significantly reduced lifespan of 25 years in 50% of affected individuals (Ramocki *et al*, 2010; Van Esch, 2012). Other hallmark symptoms include autistic-like features, anxiety, stereotypic hand movements, and spontaneous and intermittent writhing of the arms, hands or head (Ramocki *et al*, 2010). Motor dysfunction is a significant core symptom of *MECP2* duplication syndrome; affected patients show delays in basic developmental milestones such as sitting, crawling, and walking (Van Esch, 2012). *MECP2* duplication syndrome patients also show general hypoactivity although one case of *MECP2* duplication syndrome has reported hyperkinesis (Budisteanu *et al*, 2011). Speech development is substantially impaired in affected individuals with many patients speaking first words between the ages of 18 months and 4 years of age. Moreover, 80% of male patients regress in speech development and eventually lose all ability to communicate verbally (Ramocki *et al*, 2010). These cognitive and motor deficits may be the result of epileptic seizures that afflict many *MECP2* duplication syndrome patients (Van Esch, 2012). The severity and early onset of these symptoms present a particular challenge to affected individuals as well as family members and thus basic research is imperative to understand the etiology of this disorder.

ANIMAL MODELS OF *MECP2* DUPLICATION SYNDROME

To generate MeCP2 overexpression mice, a research study used the approach of using a large genomic clone that contained the entire human *MECP2* locus (Collins *et al*, 2004). Four viable lines of MeCP2 overexpressing mice were created: *MeCP2-TG1*, *MeCP2-TG3*, *MeCP2-TG11*, and *MeCP2-TG 22* all with varying levels of protein expression. The *MeCP2-TG1*, *MeCP2-TG3*, *MeCP2-TG11*, and *MeCP2-TG22* lines expressed 2-, 7-, 1-, and 2-fold higher levels of endogenous MeCP2 protein, respectively. Interestingly, phenotype severity corresponded with protein level; mouse lines with high levels of MeCP2 displayed a more exacerbated phenotype compared with mice from lower expressing lines. Of the phenotypes reported from these mice, forepaw clasping, aggressiveness, hypoactivity, as well as kyphosis were observed. It was also found that the *MeCP2-TG1* mice developed seizures that worsened with age and that corresponded to abnormal EEG patterns. The earliest lethality was observed in the highest expression line of *MeCP2-TG* mice with *MeCP2-TG3* mice dying by 3 weeks of age while *MeCP2-TG1* mice died between 20 weeks of age and 1 year. Additional experiments were conducted on *MeCP2-TG1* mice because of their viability and because the MeCP2 protein levels mirrored that seen in clinical populations of *MECP2* duplication syndrome. In depth behavioral characterization of *MeCP2-TG1* mice demonstrated accelerated motor learning and enhanced contextual fear conditioning, with no obvious anxiety-like phenotype (Collins *et al*, 2004).

An exciting area of research has been examining whether restoring MeCP2 expression in the *Mecp2* null animals would also rescue behavioral phenotypes. In an elegant set of experiments, it was shown that activation of MeCP2 expression reverses some of the neurological phenotypes in a MeCP2 null mouse (Guy *et al*, 2007). In a separate study, it was demonstrated that crossing a mouse line overexpressing MECP2 in the *tau* locus (*Tau-Mecp2*), which results in specific overexpression of MeCP2 in postmitotic neurons, with a *Mecp2* null mice was able to rescue specific phenotypes (Luikenhuis *et al*, 2004). Collectively, these two studies indicate that the neurological deficits/phenotypes are not simply due to neurodevelopmental abnormalities but rather to a specific impairment of *Mecp2* function that, when corrected, may provide a viable treatment option (Luikenhuis *et al*, 2004). Of note, the *Tau-Mecp2* mice recapitulated aspects of *MECP2* duplication syndrome, including profound motor dysfunction characterized by

side-to-side swaying, tremors, and gait ataxia (Luikenhuis *et al*, 2004).

Our laboratory recently examined the *Tau-Mecp2* mice in an array of behavioral paradigms (Na *et al*, 2012). A heightened anxiety-like phenotype in *Tau-Mecp2* mice was demonstrated by both elevated plus maze and dark–light tests suggesting that MeCP2 overexpression is sufficient to recapitulate the anxiety phenotype observed in *MECP2* duplication syndrome patients. *Tau-Mecp2* mice also had deficits in motor learning reminiscent of motor impairments commonly observed in afflicted individuals. Learning and memory was also assessed in these mice and using fear conditioning paradigms it was discovered that *Tau-Mecp2* mice displayed a significant increase in freezing 24h after training. Further analysis, however, revealed that these animals are not necessarily better at associative learning compared with controls, but actually have significant deficits in extinction learning. Additional studies using novel object recognition confirmed that this mouse line does indeed have impairments in multiple forms of learning and memory (Na *et al*, 2012). These results show that neuronal overexpression of MeCP2 has detrimental effects on learning and memory processes and produces an increased anxiety-like phenotype. Therefore, the *Tau-Mecp2* mouse model may be useful for developing treatments for *MECP2* duplication syndrome and could yield insight into downstream mechanisms affected by MeCP2 overexpression. Further experiments will be necessary to resolve the differences observed between the *Tau-Mecp2* and the *MeCP2-TG* mouse lines.

LOSS- OR GAIN-OF-MeCP2 FUNCTION HAS DELETERIOUS EFFECTS ON DENDRITIC MORPHOLOGY

Alterations in MECP2 expression have been shown to impact dendritic plasticity. Post-mortem studies of RTT patients revealed lower hippocampal spine density and reductions in dendritic branching of CA1 pyramidal cells and decreased spine density in the frontal, parietal, temporal, and occipital cortices (Armstrong *et al*, 1995; Armstrong, 2001; Belichenko and Dahlstrom, 1995). In addition, neuronal cell size (Chen *et al*, 2001; Hagberg *et al*, 2001) and white matter volume are reduced, whereas there is also evidence of cerebellar degeneration in RTT patients (Reardon *et al*, 2010). Mouse models of RTT have demonstrated similar reductions in dendritic complexity and cell size (Fukuda *et al*, 2005; Kishi and Macklis, 2004; Kriaucionis and Bird, 2003; Zoghbi, 2003) with smaller cell size and increased density of neurons (Chen *et al*, 2001) and reduced brain volume in the amygdala, hippocampus, and striatum in loss-of-function models (Stearns *et al*, 2007).

The rare occurrence of *MECP2* duplication syndrome patients has yielded limited post-mortem anatomical and morphological analysis. Nevertheless, *in vitro* experiments have shown that overexpression or elimination of MeCP2 levels in hippocampal and cortical cultures decreases dendritic arbor complexity and spine density (Chapleau *et al*, 2009; Kishi and Macklis, 2010; Zhou *et al*, 2006) although overexpression of MeCP2 has been shown to increase glutamateric synapse number (Chao *et al*, 2007). These abnormalities in dendritic branching and spine number may suggest a mechanism whereby MeCP2 dysfunction ultimately compromises CNS plasticity and as such may be a contributing factor to the learning and memory deficits that are a hallmark symptom of disorders related to *Mecp2* mutations.

LOSS- OR GAIN-OF-MeCP2 FUNCTION INFLUENCES SYNAPTIC PLASTICITY

Changes in presynaptic function can be electrophysiologically assessed by paired pulse stimulation, a form of short-term plasticity that is indicative of the probability of neurotransmitter release (Citri and Malenka, 2008). In this paradigm, two stimulations are given at varying interstimulus intervals and the slope of the second response is compared with the first. A depression in the second response relative to the first response is due to either inactivation of calcium or voltage-dependent sodium channels and is indicative of an increased probability of neurotransmitter release. Conversely, a facilitation in the second response would suggest a decreased probability of neurotransmitter release therefore residual calcium from the first stimulation presumably would contribute to the magnitude of the second response (Citri and Malenka, 2008). Loss-of-*Mecp2* function has been associated with reduced paired pulse ratios and faster excitatory postsynaptic response depression, measures of short-term synaptic plasticity (Asaka *et al*, 2006; Moretti *et al*, 2006; Nelson *et al*, 2006). Alternatively, gain-of-*Mecp2* function has been shown to augment paired pulse responses (Collins *et al*, 2004; Na *et al*, 2012) suggesting a bidirectional relationship between short-term plasticity and MeCP2 levels in which MeCP2 levels directly affect either calcium concentration in the presynaptic terminal or perhaps may alter proteins involved in neurotransmitter release. Levels of synaptophysin-1, synaptotagmin-1, and synaptobrevin-2, proteins that mediate presynaptic function, are not differentially affected by MeCP2 loss-of-function (Asaka *et al*, 2006). These data, however, do not rule out the possibility that other presynaptic proteins are influenced by MeCP2 expression. It is clear that in general MeCP2 loss- or gain-of-function mutations alter mechanisms associated with presynaptic function.

Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity that are believed to underlie long-term memory formation. Impairments in LTP and LTD induction and/or maintenance have been correlated with general learning and memory deficits and point to enduring alterations in synaptic plasticity/connectivity. For these reasons, the study of LTP and LTD has become a powerful tool in understanding neurobiological

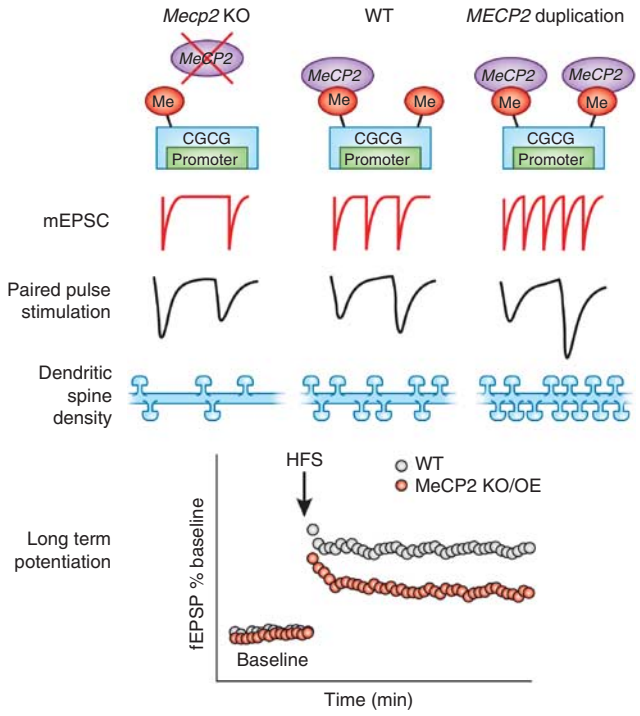

Figure 1. Schematic representation of the cellular effects of MeCP2 loss- or gain-of-function. Miniature excitatory postsynaptic current (mEPSC) frequency is bidirectionally affected by decreased or increased MeCP2 expression with a positive correlation between levels of MeCP2 expression and spontaneous excitatory transmission. Short-term plasticity as measured by paired pulse stimulation is also bidirectionally regulated by MeCP2 expression with decreased expression and increased expression resulting in increased and decreased neurotransmitter release probability, respectively. Dendritic spine density is significantly altered by MeCP2 levels with decreased expression associated with lower spine density and increased expression associated with greater spine density (although lower spine density has also been reported following MeCP2 overexpression (Chapleau *et al*, 2009)). Long-term potentiation (LTP) does not appear bidirectionally influenced by MeCP2 expression as both knockout and overexpressing mice show similar deficits in LTP magnitude and maintenance (but see also Collins *et al*, 2004).

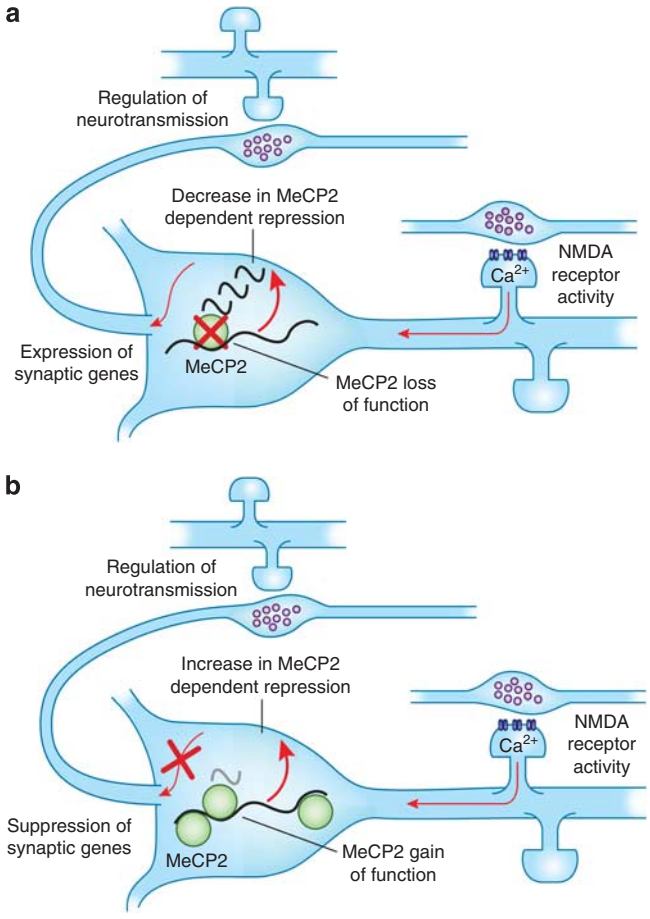

Figure 2. The role of MeCP2 in the regulation of neurotransmission. One hypothesis is that the level of MeCP2 expression titrates synaptic gene expression, which in turn alters neurotransmission in a proportional manner. (a) Our findings suggest that loss of MeCP2 increases gene expression of synaptic genes and alters neurotransmission, specifically a decrease in spontaneous excitatory neurotransmission and an increase in evoked excitatory neurotransmission. (b) The overexpression of MeCP2 suppresses the expression of synaptic genes, which augments spontaneous excitatory transmission but decreases evoked release probability.

mechanisms affected by disruptions in MeCP2 function. Schaffer-collateral LTP and LTD are significantly attenuated in symptomatic *Mecp2* null mice as well as in mice expressing disease-causing *Mecp2* mutations (Asaka *et al*, 2006; Moretti *et al*, 2006; Weng *et al*, 2011). Impaired synaptic plasticity has also been observed in hippocampal slices from *Mecp2* null mice and in cortical slices from *Mecp2*$^{308/y}$ mice (Asaka *et al*, 2006; Moretti *et al*, 2006), suggesting that these changes in synaptic plasticity consistently result from the loss of MeCP2.

Hippocampal LTP has also been examined in the MeCP2 overexpression mouse lines. The *MECP2-TG1* and *TG3* lines showed enhanced hippocampal LTP responses compared with littermate controls (Collins *et al*, 2004). In contrast, the *Tau-Mecp2* mice displayed attenuated hippocampal LTP responses (Na *et al*, 2012). A number of likely factors may explain the phenotypic differences between the two mouse lines, including the expression pattern of MeCP2. Although there is still much work needed to explain these differences, it is evident that alterations in MeCP2 expression influence long-term synaptic plasticity Figure 1.

MeCP2 PHOSPHORYLATION HAS A ROLE IN NEURONAL PLASTICITY

External cues can activate specific intracellular signaling cascades and thereby impact gene expression. Recent work has shown that membrane depolarization induces *de novo* phosphorylation of MeCP2 at serine amino-acid residue 421 (S421) that may regulate Bdnf transcription (Chen *et al*, 2003; Zhou *et al*, 2006) although activity-dependent DNA methylation involving dissociation of the MeCP2 repression complex may also regulate Bdnf transcription (Martinowich *et al*, 2003). Neuronal activity induces differing phosphorylation states of MeCP2 and may be an important mechanism through which MeCP2 regulates neuronal plasticity through activity-dependent gene transcription. Tao *et al* (2009) have proposed that MeCP2 phosphorylation

may provide a regulatory 'switch' such that at rest, S80 phosphorylation binds MeCP2 to chromatin but during depolarization S421 phosphorylation allows MeCP2 to dissociate from chromatin thereby providing a transcriptionally permissive state. This activity-dependent phosphorylation of MeCP2 appears to have widespread effects on synaptic plasticity (Li *et al*, 2011) as mice in which phosphorylation at S421 are lacking display enhancements in LTP, increased excitatory synaptogenesis, as well as improvements in hippocampal-related learning and memory tests (Li *et al*, 2011). Neuronal activity also triggers dephosphorylation of MeCP2 at serine amino-acid 80 (S80), which alters transcription of various genes (Tao *et al*, 2009). Although phosphorylation of MeCP2 is implicated as a key regulator of activity-dependent gene expression, there is still much work to do to identify the target genes involved in these critical processes. Moreover, it would not be surprising if other key phosphorylation sites on MeCP2 were identified and shown to have important roles in impacting MeCP2 activity and ultimately gene expression that mediates effects on short- and long-term synaptic plasticity as well as behavioral processes.

LOSS- OR GAIN-OF-MeCP2 EXPRESSION BIDIRECTIONALLY AFFECTS EXCITATORY NEUROTRANSMISSION

Recordings from cortical and hippocampal slices and primary hippocampal cultures prepared from *Mecp2*-deficient mice indicate a fundamental imbalance between excitation and inhibition (Kavalali *et al*, 2011). Evaluation of spontaneous and miniature postsynaptic currents and field potentials suggest decreased excitatory and increased inhibitory neurotransmission in MeCP2-deficient hippocampal cultures (Chao *et al*, 2007; Dani *et al*, 2005; Nelson *et al*, 2006; Tropea *et al*, 2009), whereas analysis of evoked excitatory postsynaptic currents and short-term synaptic plasticity indicate enhanced excitatory drive that may manifest itself through the seizures and tremors observed in *Mecp2* KO mice (Asaka *et al*, 2006; Moretti *et al*, 2006; Nelson *et al*, 2011). These disparate findings between excitatory balance in spontaneous and evoked transmission are surprising but not without precedent (Nelson *et al*, 2011). An important implication of this work is that MeCP2's effect on hippocampal spontaneous transmission appears to be due to its role as a transcriptional repressor (Nelson *et al*, 2006). Moreover, we found that the impact of MeCP2 on the dynamics of evoked excitatory neurotransmission was similar to what is observed after loss of key histone deacetylases (histone deacetylase 1 and 2), enzymes that form a co-repressor complex with MeCP2 suggesting that alterations in transcriptional repression mediate the deficits in evoked synaptic activity (Akhtar *et al*, 2009).

MeCP2 overexpression also impacts excitatory neurotransmission. Overexpression of MeCP2 leads to a significant increase in excitatory miniature synaptic transmission in hippocampal cell cultures (Chao *et al*, 2007; Na *et al*, 2012). Studies have not yet examined the impact of MeCP2 overexpression on evoked neurotransmission. Collectively, the studies to date suggest that loss- or gain-of-MeCP2 function exerts bidirectional control in neurotransmission in cortical and hippocampal regions of the brain. Ongoing work is seeking to determine whether restoring the balance between excitatory/inhibitory neurotransmission may reverse some phenotypes observed in these mouse models of RTT and *MECP2* duplication syndrome (ie, seizures).

FUTURE RESEARCH DIRECTIONS

Epigenetic mechanisms have important roles in brain development, synaptic plasticity, and in behavior including learning and memory and have been shown to underlie certain neurodevelopmental disorders. A wealth of evidence now substantiates the functional importance of MeCP2, an epigenetic factor, in the regulation of synaptic and neuronal plasticity. A relative lack or excess of MeCP2 levels lead to surprisingly similar behavioral and neurological phenotypes: anxiety, cognitive impairments, and motor coordination deficits. Similarly, loss and gain of MeCP2 function result in significant effects on neuronal plasticity, dendritic morphology, processes associated with short- and long-term plasticity, and in excitatory/inhibitory balance in neurotransmission. It is striking that levels of MeCP2 appear to have a bidirectional effect on excitatory neurotransmission indicating that mechanisms that regulate neurotransmission are sensitive to MeCP2 levels. The putative role of MeCP2 as a transcriptional factor suggests that dysregulation of downstream gene targets may underlie RTT and *MECP2* duplication syndrome. However, possible targets of MeCP2 that contribute to the behavioral deficits observed in these disorders have been rather elusive. Potential confounds in identifying targets include the regional specificity for MeCP2's effects on specific downstream targets as well as differences between particular neuronal populations. One of the current challenges is to understand molecular and cellular downstream targets that are affected by MeCP2 dysfunction. Identifying downstream mechanisms associated with alterations in MeCP2 expression may lead to the development of promising therapeutics in the treatment of RTT and *MECP2* duplication syndrome (Figure 2).

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DISCLOSURE

Dr Monteggia has been in the Speaker Bureau for Sepracor and Roche. The remaining authors declare no conflict of interest.

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