Current Literature

In Basic Science

Mutations in methyl–CpG-binding protein-2 (MeCP2, a transcription factor binding methylated DNA) most often cause Rett Syndrome (RTT). The incidence of RTT is approximately 1:10,000 female births. Clinically, female patients with RTT have apparently normal infant development followed by a plateau and then regression; males succumb in utero or develop a severe, lethal epileptic encephalopathy. During this regression, autism is often diagnosed and epilepsy may begin (1, 2). Diagnosis of epilepsy is often difficult, as many patients have a combination of nonepileptic stereotypic spells, epileptic seizures (complex partial, tonic, tonic–clonic, and myoclonic) and electrographic-only seizures. The electrographic phenotype can also include abnormalities associated with severe epileptic encephalopathy (3–5). Epilepsy in RTT is often medically refractory. The incidence of epilepsy and the overall severity of the disease correlates with specific MeCP2 mutations (6–8). Epilepsy severity correlates with behavioral impairments (4, 9). This has driven research to understand the mechanisms of the RTT phenotype, including epilepsy, utilizing genetically altered mouse models. This is important, as translational studies have demonstrated that some aspects of the RTT phenotype can be ameliorated, though none have addressed epilepsy (10, 11).

Initial studies in genetically altered mice targeted alterations in MeCP2 throughout the brain. These studies have led to the question of whether distinct region-autonomous, cell-autonomous, glial, or neuronal cell types may individually contribute to the different aspects of the phenotype. Specificity of MeCP2 alterations can be driven by cell- or region-specific expression of Cre recombinase acting on engineered floxed MeCP2 alleles. Mutant male mice are typically used in these studies as they often develop symptoms earlier (weeks), whereas floxed female (even without Cre-driven recombination) display delayed onset of phenotype (months), skewed X-chromosome inactivation and reduced expression. Consideration of the specific mutants used is important when making comparisons between studies. Specifically, cortical neurons using the floxed MeCP2 mutants (conditional Cre-driven floxed deletion of exon 3 or 3 and 4, MeCP2-flox) exhibit reduced dendritic complexity and spine density while mutations with a premature stop codon (MeCP2-308) do not (reviewed by [2]). Electrographic and behavioral seizures have been reported in male MeCP2-308 (12) and electrographic seizures have been reported in MeCP2-flox(13). Behavioral seizures have been reported in 4 percent of female mice engineered with a common human mutation (MeCP2-R168X) (14). No study has monitored continuous video-electroencephalograms (vEEG) over days or weeks in a sufficient population to fully characterize the likelihood, onset, and semiology of epilepsy in any mutant. In prior work by others, cortical hyperexcitability had been isolated to MeCP2 function in GABAergic interneurons (IN) where MeCP2 regulates the expression of glutamic acid

Commentary

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Loss of MeCP2 From Forebrain Excitatory Neurons Leads to Cortical Hyperexcitation and Seizures.

Mutations of MeCP2 cause Rett syndrome (RTT), a neurodevelopmental disorder leading to loss of motor and cognitive functions, impaired social interactions, and seizure at young ages. Defects of neuronal circuit development and function are thought to be responsible for the symptoms of RTT. The majority of RTT patients show recurrent seizures, indicating that neuronal hyperexcitation is a common feature of RTT. However, mechanisms underlying hyperexcitation in RTT are poorly understood. Here we show that deletion of MeCP2 from cortical excitatory neurons but not forebrain inhibitory neurons in the mouse leads to spontaneous seizures. Selective deletion of MeCP2 from excitatory but not inhibitory neurons in the forebrain reduces GABAergic transmission in layer 5 pyramidal neurons in the prefrontal and somatosensory cortices. Loss of MeCP2 from cortical excitatory neurons reduces the number of GABAergic synapses in the cortex, and enhances the excitability of layer 5 pyramidal neurons. Using single-cell deletion of MeCP2 in layer 2/3 pyramidal neurons, we show that GABAergic transmission is reduced in neurons without MeCP2, but is normal in neighboring neurons with MeCP2. Together, these results suggest that MeCP2 in cortical excitatory neurons plays a critical role in the regulation of GABAergic transmission and cortical excitability.

What You Seize Is What You Get:
Do We Yet Understand Epilepsy in Rett Syndrome?
PN MeCP2 expression is necessary to maintain GABAergic synapses. They used a conditional floxed MeCP2 allele (Jaenisch [15]). Mice with floxed MeCP2 conditional alleles were bred with mice expressing Cre-recombinase in brain-specific regions with cellular specificity: EMX1-Cre (deletes MeCP2 from forebrain PN and glia but not IN, EMX1-MeCP2), Dlx5/6-Cre (deletes MeCP2 from forebrain IN but not PN and glia, DLX6-MeCP2), and SERT-Cre (deletes MeCP2 from a sparse subset of forebrain PN but not IN or glia, SERT-MeCP2); all with a fluorescent reporter to identify recombination. Only male progeny were used; comparisons were made to wildtypes bred to Cre-recombinase expressing mice with some comparisons to mice with the floxed (non-recombinant) MeCP2 allele.

The authors implanted 6- to 8-week-old EMX1-MeCP2 (n = 5), DLX6-MeCP2 (n = not reported), and controls (n = 4) for EEG recording. Recordings were performed for only approximately 2 hours per day for 2 days. Only EMX1-MeCP2 demonstrated, on average, 1-second bursts of approximately 7 Hz generalized spike-wave discharges that were simultaneously associated with behavioral arrest. No other seizure types or discharges were described. Male MeCP2 mutants at this age would likely demonstrate other aspects of RTT, though not described.

To investigate this further, the authors used whole-cell patch-clamp measurements of synaptic currents of layer 5 PN in medial prefrontal cortex (mPFC) and somatosensory cortex in acute brain slices from immature (2–3 week) and mature (6–7 week) mice. The authors found a selective reduction in the frequency of spontaneous and miniature inhibitory postsynaptic currents (IPSCs) in EMX1-MeCP2; IPSC amplitude and kinetics were not affected. Miniature excitatory postsynaptic currents (EPSCs) were unaffected. Alterations of spontaneous IPSC frequency were not found in immature DLX6-MeCP2 mice (but mature mice were not investigated). Further, the authors then used the SERT-MeCP2 mutants to show a selective reduction of spontaneous IPSC frequency in layer 2/3 somatosensory cortex PN that expressed Cre-recombinase; neighboring PN not expressing Cre-recombinase were unaffected. The authors concluded that MeCP2 expression in PN, not IN, is necessary for the function of GABAAergic synapses. Importantly, this was not dependent upon MeCP2 function in glia or dependent upon expression in the entire region. Evoked IPSCs in EMX1-MeCP2 were smaller compared to controls at increasing stimulus intensities; however, paired pulses were unaltered. This supported normal presynaptic function but reduced postsynaptic effectiveness. Given normal quantal size found initially, this suggested a reduction in the number of GABAAergic synapses. This was confirmed in mPFC using immunocytochemistry, which demonstrated a reduced number of VGAT-positive puncta, representing a reduced number of GABAAergic synapses.

The authors concluded that while IN MeCP2 expression may regulate GABAAergic quantal size observed by others (13), PN MeCP2 expression is necessary to maintain GABAAergic synaptic number, and the latter is more influential on cortical excitability and seizures. This finding supports the concept that RTT represents an imbalance between excitation and inhibition and suggests that targeting inhibition to improve this balance may be important for seizures and other aspects of the RTT phenotype.

Several limitations prevent the full acceptance of these conclusions. It would have been useful to know the status of GABAAergic synaptic puncta (size, number, and function) in DLX6-MeCP2 at all developmental stages. This information would have supported a cell-autonomous role of MeCP2 isolated to PN to mediate GABAAergic synaptic function. Dendritic complexity was not investigated nor was excitatory drive onto IN, as this could also influence the output of IN. The relationship of the hyperexcitable phenotype seen with vEEG to the well-characterized underlying abnormalities in GABAAergic synaptic function is only correlative. Most importantly, it is not conclusive that the generalized-absence epileptic phenotype described is truly epileptic or representative of RTT.

The average duration of generalized discharges determined (1 second) is too short to be considered a true seizure. In most studies of genetic epilepsy characterized in mouse mutant models, electrographic seizures are defined as at least 5 seconds (16, 17). In animal models of absence epilepsy, the briefest seizures are considered to be at least 1 to 2 seconds (18–20), as it is difficult to ascribe a clinical correlate to anything briefer. Indeed, the discharges considered here allowed a lower limit of 0.4 seconds, which, considering mouse behavior, would be impossible to reliably call as a behavioral arrest (20). It is more plausible that the discharges concerned represent an abnormality in cortical processing of attention, rather than epileptic discharges or true seizures (20). Regardless, generalized or even absence seizures are not the only or predominant form of the epileptic phenotype seen in RTT.

Overall, there is a general lack of quantification of the underlying EEG abnormalities in this and other works. While there is ample correlation between the other aspects of the RTT phenotype and underlying pathophysiology, it is surprising that the epileptic phenotype has been so undercharacterized in a developmental epileptic syndrome. Epileptic phenotypes of given mutations must be fully characterized and compared to subsequent cell/region specific mutants, to all appropriate controls across development and the human phenotype (see 21). Since human RTT involves both epileptic without behavioral correlate and nonepileptic events, vEEG in rodents must be the gold standard for any conclusions. Spike-counts, frequency domain analysis, or other quantitative measures must be employed to assess hyperexcitability with appropriate statistical comparisons. Comparisons in vitro must match the age of findings in vivo. Long-term recordings must be pursued, which would provide a basis for consideration of interventions before the onset of epilepsy, a strategy that would address both translational and mechanistic questions on the role of seizures themselves. Such studies are not easy, as these are typically fragile animals. However, without such information, the cellular mechanisms underlying epilepsy in RTT will remain unclear.

by Tim A. Benke, MD, PhD


Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

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4. Manuscript/Article Title: What you seize is what you get: do we yet understand epilepsy in Rett syndrome?

5. Journal Issue you are submitting for: 14.5

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