Published in final edited form as:

Curr Opin Neurobiol. 2011 December ; 21(6): 827–833. doi:10.1016/j.conb.2011.06.006.

Synaptic microcircuit dysfunction in genetic models of neurodevelopmental disorders: Focus on *Mecp2* and *Met*

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Abstract

Recent findings in the genetics of neurodevelopmental syndromes have ushered in an exciting era of discovery in which substrates of neurologic dysfunction are being identified at the synaptic and microcircuit levels in mouse models of these disorders. We review recent progress in this area, focusing on two examples of mouse models of autism spectrum disorders (ASDs): *Mecp2* models of Rett syndrome, and a *Met*-knockout model of non-syndromic forms of autism. In both cases, a dominant theme is changes in synaptic strength, associated with hyper- or hypoconnectivity in specific microcircuits. Alterations in intrinsic neuronal excitability are also found, but do not appear to be as common. The microcircuit-specific nature of synaptic changes observed in these ASD models indicates that it will be necessary to define mechanisms of circuit dysfunction on a case-by-case basis, not only in neocortex but also in brainstem and other subcortical areas. Thus, functional microcircuit analysis is emerging as an important line of investigation, highly complementary to neurogenetic and molecular strategies, and holds promise for generating models of the underlying pathophysiology and for guiding development of novel therapeutic strategies.

Introduction

Autism spectrum disorders (ASDs) are a cluster of behavioral syndromes characterized by early childhood onset of neurodevelopmental abnormalities including impairments in social interactions and communication, and a restricted range of interests, often associated with repetitive and stereotyped behaviors [1–3]. ASDs are common, diagnosed in ~1% of children. A 4:1 male gender bias and high heritability indicate strong genetic components. The well known clinical heterogeneity of ASDs is thought to represent interactions between genetic and environmental factors [4].

A number of genes associated with ASDs have been identified, ranging from those, such as methyl-CpG binding protein 2 (*MECP2*), that are involved in distinct monogenic neurodevelopmental disorders with autistic features, e.g., Rett syndrome (RTT), to more recently discovered genes such as the receptor tyrosine kinase *MET* involved in polygenic non-syndromic forms of autism [5]. Here, we review recent progress in identifying synaptic microcircuit-level pathophysiology in mouse models of ASDs, focusing on *Mecp2* models

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of RTT, and highlighting recent progress on a *Met*-knockout model of non-syndromic autism. We chose these two particular models because (1) they represent distinct yet overlapping entities on the spectrum of ASDs and may therefore help illuminate both unique and shared mechanisms of microcircuit pathophysiology in these disorders, and (2) in both cases high-resolution methods such as pair recordings and laser scanning photostimulation have been applied to functionally characterize specific microcircuits, offering a basis for meaningful comparisons of microcircuit pathophysiology between the two models. The term 'microcircuit' is used here to mean stereotypic patterns of synaptic connections between individual members of identified cell classes in particular brain regions; for example, excitatory connections from layer 2/3 pyramidal neurons to layer 5B corticostriatal neurons in neocortex, or from primary afferent inputs to second-order sensory relay neurons in brainstem.

Neocortical microcircuits in MeCP2 deficiency models of Rett syndrome (RTT)

RTT is a progressive neurodevelopmental disorder caused by loss-of-function mutations arising in the male germ line in *MECP2*, a gene on the X chromosome that encodes a transcriptional regulatory protein (reviewed in Chahrour and Zoghbi, 2007). Because of its X-linked genetics, RTT affects primarily girls, who are somatic mosaics for normal and mutant *MECP2*, respectively. Boys carrying *MECP2* mutations on their single X chromosome have no normal *MECP2* allele and most die soon after birth from a severe encephalopathy. Early in childhood, RTT girls typically lose acquired speech and motor skills and develop severe abnormalities in cognitive, motor, respiratory, and autonomic function and behavior. Stereotypic motor behaviors are common, particularly hand-wringing, and severely interfere with motor control. Clinically, RTT is unique, but the fact that it includes autistic features has added to its appeal as a model system for neuroscientists interested in understanding the neurobiology of ASDs and neurodevelopmental disorders more generally (Neul and Zoghbi, 2004).

The discovery that *MECP2* mutations cause RTT [6] led to development of mouse models of the disease (reviewed in [7, 8]), which enabled electrophysiological analysis of neocortical neurons and circuits. Dani and colleagues [9] recorded from layer 5 pyramidal neurons in acute brain slices of somatosensory cortex prepared from *Mecp2*-null mice, and made several key observations: (1) intrinsic excitability was unchanged; (2) excitatory postsynaptic currents (EPSCs) were markedly reduced; (3) inhibitory inputs (IPSCs) were slightly increased; (4) synaptic changes were evident already at week 2, prior to overt symptoms, and grew stronger by weeks 3–4. The overall interpretation was of a pro-inhibitory shift in the balance of synaptic excitation and inhibition, leading to reduced cortical activity. A surprise, at least in light of ideas about 'hyperconnectivity' in intracortical circuits in ASDs (discussed below), was the finding of reduced rather than increased excitatory connectivity. A major implication of these studies was that cortical changes in RTT primarily involve synapses and microcircuits, with relative sparing of the intrinsic properties of neurons. However, caution is required in generalizing these findings to other regions of the brain. In the pontine nucleus locus coeruleus (LC), for example, neurons lacking MeCP2, in either null or heterozygous mutants, are intrinsically hyperexcitable compared to wild-type cells, and this is associated, at least in part, with reductions in passive membrane conductance and the amplitude of the slow afterhyperpolarization [10].

Subsequent studies of *Mecp2*-null models have confirmed and extended these initial discoveries. Dani and Nelson (2009) used paired recordings to analyze unitary connectivity and synaptic plasticity in cortical circuits and found intact long-term potentiation but

reduced connectivity between layer 5 pyramidal neurons. A reduction in specific intracortical excitatory synaptic pathways, with no change in inhibitory inputs or intrinsic properties, was also observed in a study of local synaptic connections onto layer 2/3 pyramidal neurons in acute brain slices from *Mecp2*-null mice [11]. An electrophysiological study of monocultured hippocampal neurons prepared from either *Mecp2*-null mice or mice with a genetic duplication of *Mecp2* found that the number of excitatory synapses was reduced in the nulls and increased with duplication; interestingly, quantal properties and short-term plasticity were otherwise unaffected [12]. Decreased synapse number was also observed in the null hippocampus *in vivo* [12].

Genetically restricted manipulations of *Mecp2* have begun to illuminate how different brain regions and/or cell types contribute to the disease process in RTT. Zhou and colleagues [13] developed a strategy based on RNA interference (RNAi) for *Mecp2* knockdown in transfected neurons, illuminating the role of phosphorylation- and activity-dependent regulation of *Mecp2* in dendritic spine morphogenesis and synaptic maturation. Following up on this, a study in which the same knockdown constructs were transfected into layer 2/3 neurons showed a pathwayspecific reduction in excitatory input to layer 2/3 neurons in acute brain slices, with no change in either inhibitory inputs or intrinsic properties [14].

The specific role of inhibitory interneurons was recently investigated in mice in which MeCP2 deficiency was genetically restricted to γ -amino butyric acid (GABA) expressing cells either throughout the nervous system (*Viaat-Mecp2* mice) or regionally restricted to forebrain areas [15]. These mice displayed reduced GABA content and features of RTT, including repetitive motor behaviors. Consistent with the reduction in GABA, layer 2/3 pyramidal neurons showed a reduction in mIPSCs, with no effect on mEPSCs, and the cortical EEG exhibited hyperexcitability. Interestingly, these mice displayed reduced cortical inhibition, whereas mice with global MeCP2 deficiency exhibit reduced cortical excitation (see above) – yet both display RTT-like features. Clearly, further studies are required to unravel how specific RTT endophenotypes relate to either hypo- or hyperconnectivity resulting from *Mecp2* deficiency in particular cortical cells and microcircuits.

Major questions remain about the precise role of MeCP2 in synaptic maturation in wild-type animals, how *MECP2* mutations lead to synaptic and microcircuit dysfunction in RTT, and what can be done about it. It is intriguing that much is normal and intact in the cortex in various models of MeCP2 deficiency, and also that the specific patterns of changes observed in different models are somewhat complementary. For example, in models in which excitatory inputs are preferentially affected, inhibitory inputs are not and LTP is spared [9, 11, 14, 16], whereas in a model in which inhibitory inputs are preferentially affected, the excitatory inputs are normal but LTP is affected [15].

The reversibility of symptoms and functional abnormalities in mouse models of ASDs [17–22], together with the absence of gross macroscopic neuroanatomic abnormalities in RTT and other ASDs [23, 24], implies that dysfunction occurs at the microcircuit level, affecting the functional connectivity of axonal boutons and dendritic spines and shafts (i.e., microscopic structural plasticity) and synaptic transmission, rather than at the level of larger scale rearrangements of entire axonal and/or dendritic branches and arbors. This view is consistent with findings of dendritic spine abnormalities and dendritic hypoplasia in *Mecp2*-null mice [8, 13, 25–27] and RTT patients [27–30]. Indeed, microstructural changes at the level of dendritic spines are a common finding in a wide variety of neuropsychiatric disorders [31].

Under normal circumstances, connections between neocortical neurons are highly selective, forming only at a fraction of possible sites where axons and dendrites intersect [32]. Cortical circuits thus have enormous ‘plasticity potential’; that is, the geometry of cortical neurons presents a rich structural substrate for increasing or decreasing the functional connectivity between specific subsets of cells [33, 34]. Thus it is likely that functional cortical changes in ASDs involve microcircuit-level rearrangements within this anatomical hyperspace of potential connectivity.

Synaptic dysfunction in respiratory networks in RTT

The brainstem respiratory network provides particularly good models for examining the relationship between synaptic dysfunction and disease pathophysiology in RTT. Brainstem circuitry underlying the generation and patterning of normal respiratory motor output has been defined in considerable detail, and much is known about mechanisms regulating synaptic strength and plasticity in the respiratory network. RTT patients suffer from highly irregular breathing patterns characterized by hyperventilation and tachypnea (rapid breathing), breathholds and apneas, and complications arising from respiratory dysfunction can be severely debilitating and life-threatening (reviewed in [35]). Thus, considerable effort is being made to identify mechanisms that underlie respiratory circuit dysfunction in mouse models of the disease.

As in cortex, excitatory-inhibitory imbalance is a hallmark of synaptic dysfunction in the brainstem respiratory network in *Mecp2*-null mice. However, in contrast to cortex, loss of *Mecp2* is associated with enhanced excitation in brainstem cell groups, including cranial nerve motor nuclei [18], the medullary nucleus tractus solitarius (nTS; [36]), the ventrolateral medulla (VLM; [37]), the pontine nucleus Kölliker-Fuse (KF; [38]) and the LC [10]. Synaptic contributions to excitatory-inhibitory imbalance in the brainstem of *Mecp2*-null mice have thus far only been analyzed in detail in the nTS [36] and VLM [37]. The nTS is the site of the first synaptic relay in visceral sensory reflex pathways in the brainstem and thereby plays a critical role in afferent regulation of autonomic and respiratory homeostasis. The VLM, on the other hand, contains motor and pre-motor cell groups involved in the generation and patterning of respiratory, sympathetic, and parasympathetic motor outflow, including the pre-Bötzinger complex, a key site for respiratory rhythm generation.

In the absence of MeCP2, nTS neurons exhibit increased amplitude of spontaneous and evoked EPSCs and an increased probability of action potential firing in response to presynaptic input, without any change in bicuculline-sensitive inhibitory transmission or intrinsic neuronal excitability [36]. Given the role of nTS in gating sensory input to brainstem reflex pathways, synaptic hyperexcitability in *Mecp2*-null mice would be expected to result in exaggerated reflex function. Indeed, several recent studies have shown that the hypoxic chemoreflex, mediated by primary afferent inputs to nTS from the carotid body, is markedly exaggerated in *Mecp2*-null mice compared to wild-type controls [39, 40], supporting a role for excitatory-inhibitory imbalance in nTS in respiratory circuit abnormalities. Exaggerated chemoreflex function, in turn, may underlie the generation of respiratory tachypnea in RTT.

Recent studies have identified deficits in brain-derived neurotrophic factor (BDNF) as a likely cause of synaptic hyperexcitability in nTS in *Mecp2*-null mice [36]. BDNF is normally released in an activity dependent manner from visceral sensory neurons [41] and modulates excitatory glutamatergic transmission at primary afferent synapses in nTS [42]. BDNF expression in visceral sensory neurons declines markedly after birth in *Mecp2* null mice [43], and exogenous BDNF can restore wild-type levels of synaptic excitability at primary afferent synapses in nTS [36]. On the basis of these findings, efforts are currently

underway to rescue autonomic reflex function in *Mecp2* null mice using therapies designed to restore normal BDNF signaling in nTS [17] (Schmid et al., submitted).

Synaptic hyperexcitability has also been found in the VLM of *Mecp2*-null mice, in association with deficits in inhibitory signaling by GABA [37]. Specifically, mutant VLM neurons exhibit decreased presynaptic GABA release, reduced expression of the vesicular inhibitory transmitter transporter and reduced levels of postsynaptic $\alpha 2$ and $\alpha 4$ subunits of the GABA(A)-receptor. Accordingly, VLM neurons in *Mecp2*-null mice exhibit significant increases in the amplitude and frequency of spontaneous EPSCs [37], raising the possibility that this may be a site at which synaptic hyperexcitability contributes to enhanced excitatory drive in cranial motor outflow pathways.

A clear lesson emerging from studies of synaptic dysfunction in *Mecp2*-null mice is that the effects of MeCP2 loss are diverse and highly context dependent. In light of this heterogeneity, it is therefore striking that some broad patterns are evident in studies of *Mecp2*-null mice, such as enhanced excitation throughout brainstem circuits (at least those examined thus far) *versus* reduced excitation in cortical circuits. These patterns suggest that there may be rules governing regional patterns of MeCP2 function that remain to be discovered.

Microcircuit changes in non-syndromic autism: Met as a model system

The receptor tyrosine kinase MET has recently emerged as a gene with a compelling association with non-syndromic, sporadic forms of autism. Multiple genetic studies have shown a strong link between *MET* gene changes (polymorphisms, copy number variants) and autism [44–48]. MET expression in the brain occurs at the right time (at the beginning of and during a time of peak synaptogenesis) and place (select forebrain neurons) to play a critical role in the development and maintenance of neocortical circuits [49]. Moreover, cortical MET expression is reduced in autism cases [50]. In mouse neocortex, Met expression is restricted to a subset of excitatory pyramidal neurons in layers 2/3, 5, and 6 [51]. In primates, MET expression shows the same laminar distribution and timing but is furthermore areally restricted to temporal, ventral occipital, and posterior cingulate regions of the developing human and monkey brain [52]. These are regions associated with the processing of complex visual information used for social behavior (such as faces).

Recent studies have demonstrated a role for Met in synapse formation and clustering of synaptic proteins [53]. Conditional deletion of *Met* from cortical neurons induces morphological changes in their dendritic arbors and spines [52]. The functional topography of cortical microcircuits in anterior frontal cortex was recently examined [54]. An increase in excitatory synaptic connectivity from layer 2/3 to layer 5 neurons was found that was specific for layer 5B corticostriatal neurons (which project intracerebrally), and not observed in corticopontine neurons (which project to the brainstem). Short-term synaptic plasticity was unaffected, as was the intrinsic excitability of neurons. Thus, local-circuit ‘hyperconnectivity’ appeared to accrue from increases in both the probability and amplitude of unitary connections.

In rats treated *in utero* with valproic acid (VPA), a toxicological model of ASD [55], local-circuit hyperconnectivity has also been found [56, 57]. In this case, however, hyperconnectivity is attributable to an increase in connection probability only; unitary connection amplitudes are instead reduced.

Findings of cortical hyperconnectivity in mouse models of ASD may have parallels to recent findings of cortical hyperactivity in ASD patients (reviewed in [58]). Local hyperconnectivity (or ‘overconnectivity’) in the cortex has been proposed as a pathological

state in autism, (e.g. [4, 59, 60] and as a mechanism to explain hyper-reactivity to sensory stimulation in autistic patients (dubbed “Intense World Syndrome”) [58].

The changes in functional connectivity observed in *Mecp2* and *Met* mouse models of ASDs imply that ASD genes and their downstream targets are especially important for regulating proteins critical for synapse development and/or synaptic transmission. *Mecp2* mutations alter expression of numerous genes involved in expression or synthesis of synaptic transmitters, modulators, transporters, and receptors, including BDNF [61–63], tyrosine hydroxylase [39], dopamine β -hydroxylase [64], VIATT and GABA-R subunits [37], UBE3A [65], Dlx5 [66], GABRB3 [65], and dendritic growth (FXYP1; [67]). Moreover, loss of *Mecp2* alters regulation of dense core vesicle fusion and release [68], synaptic vesicle density, release probability, and recycling [69], and glutamatergic receptor number [12]. Similarly, *Met* signaling feeds into PI3 kinase and ERK biochemical pathways involved in neurodevelopmental events such as axon guidance and synaptogenesis, and many of its downstream targets (e.g. *Pten*, *Nf1*, *Tsc1*) are directly implicated in ASDs [70, 71]. The possibility of direct interactions between *Mecp2* and *Met* remains to be explored.

Microcircuit dysfunction in *Mecp2*, *Met*, and ASDs in general

Is it possible to unify this rapidly growing body of findings from different models, brain regions, and cell types? Perhaps not yet, but some themes do appear to be emerging.

Synaptic dysfunction is common, altered neuronal excitability is not

Neural networks exhibit a remarkable ability to scale their activity in response to perturbations in order to maintain network stability, a phenomenon referred to as homeostatic plasticity (reviewed in [72, 73]). The principal substrates of network homeostasis are compensatory adaptations in synaptic strength and intrinsic excitability (reviewed in [72–76]). To a large extent, both for *Mecp2* and *Met* models, pathophysiology appears to affect functional synaptic connectivity more often than intrinsic neuronal excitability. This is somewhat surprising given that ASDs are often associated with changes in dendritic morphology (e.g. shrunken dendritic arbors and reduced cell size) that could potentially alter neuronal excitability [8, 23]. A notable exception is *Mecp2* null neurons in the LC, which are both small and hyperexcitable compared to wild-type cells [10]. It may be that many – but not all – neurons in ASD brains can compensate physiologically for morphological changes to maintain intrinsic excitability within the homeostatic range.

Synaptic changes are microcircuit-specific

For both *Mecp2* and *Met*, the effects of gene manipulations vary in important ways depending on neural ‘context’. This is exemplified by the distinct effects of *Mecp2* deficiency on synaptic function in cortical *versus* brainstem circuits (hypo- *versus* hyperconnectivity, respectively) and of *Met* mutation on corticostriatal *versus* corticopontine microcircuits (hyperconnectivity *versus* no effect). We expect that microcircuit specificity will continue to be a major theme as more ASD models are investigated. An ongoing challenge, therefore, will be to determine the degree to which abnormal function in any one circuit reflects compensatory adaptations to changes elsewhere in the brain, rather than (or in addition to) intrinsic defects. Progress in this area has the potential to guide pharmacotherapeutic strategies for selectively targeting the core deficits leading to neurological dysfunction.

Developmental timing matters

For both *Mecp2* and *Met*, cortical expression peaks in the early postnatal/juvenile phase of development, a period when synaptic microcircuits are undergoing development,

refinement, and maturation [49, 77]. This fits with findings at the microcircuit level, where the changes are in the form of altered synaptic strength rather than in circuit topography. These new findings further support the view that ASDs are disorders affecting refinement and maintenance of circuits during development, rather than initial specification [23, 78].

Synaptic changes involve hyper- and/or hypoconnectivity

In diverse mouse models of ASDs, we see evidence for some kind of synaptic hyperconnectivity at the microcircuit level, including reduced synaptic inhibition leading to greater probability of synaptically evoked action potential firing in nTS neurons in *Mecp2*-null mice [36], increased excitatory input to corticostriatal neurons in *Met*-knockout mice [54], and increased connectivity rates between layer 5 pyramidal neurons in the VPA model of ASD [56, 57]. Thus far, hypoconnectivity, as in the *Mecp2* deficient cortex, is a less frequent finding, though this may change as more brain regions are sampled across multiple ASD models. Indeed, transient hypoconnectivity in cortical circuits has also been observed in FMR1-knockout mice, a model of Fragile X syndrome which, like RTT, is a severe monogenic form of ASD [79]. Conceivably, the common observation of connectivity changes in ASD models reflects involvement of cell adhesion molecules, which are integrally tied to synaptic connectivity and neurodevelopment, and extensively linked genetically with ASDs [5, 80–82].

Brainstem microcircuits are crucial pieces of the puzzle

Examination of brainstem circuits in *Mecp2*/RTT models has been highly informative, revealing among other things diverse mechanisms underlying synaptopathic changes in microcircuits, including reduced BDNF and GABA signaling. Brainstem microcircuits have yet to be scrutinized in experimental models for non-syndromic ASDs, but should be; although there has been a tendency to focus on cortical circuits in autism models, we suggest that it will be equally important to identify changes in synaptic microcircuits at subcortical levels, which after all are critical for the expression and modulation of cortically-mediated behaviors. Moreover, physiological behaviors such as respiration that are mediated by subcortical microcircuits provide robust and readily quantifiable outcome measures for preclinical evaluation of potential therapeutics (reviewed in [35]; see also [18]).

Highlights

Identifies emerging themes of microcircuit dysfunction in autism spectrum disorders
Compares and contrasts *Mecp2* and *Met* mouse models of ASDs
Changes occur more often in synaptic strength than in intrinsic neuronal excitability
Microcircuit changes, e.g., hyper- vs hypo-connectivity, are cell-context specific
Distinguishing core defects vs compensatory changes is a high priority

Acknowledgments

We thank Charles Anderson, Pat Levitt, Roberto Galan, and Shenfeng Qiu for useful comments and suggestions. Grant support: NIH (GS: NS061963; DMK: NS057398); Simons Foundation (GS); International Rett Syndrome Foundation (GS and DMK).

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