Breathing Disorders in Rett Syndrome: Progressive Neurochemical Dysfunction in the Respiratory Network after Birth

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Abstract

Disorders of respiratory control are a prominent feature of Rett syndrome (RTT), a severely debilitating condition caused by mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*). RTT patients present with a complex respiratory phenotype that can include periods of hyperventilation, apnea, breath holds terminated by Valsalva maneuvers, forced and deep breathing and apneustic breathing, as well as abnormalities of heart rate control and cardiorespiratory integration. Recent studies of mouse models of RTT have begun to shed light on neurologic deficits that likely contribute to respiratory dysfunction including, in particular, defects in neurochemical signaling resulting from abnormal patterns of neurotransmitter and neuromodulator expression. The authors hypothesize that breathing dysregulation in RTT results from disturbances in mechanisms that modulate the respiratory rhythm, acting either alone or in combination with more subtle disturbances in rhythm and pattern generation. This article reviews the evidence underlying this hypothesis as well as recent efforts to translate our emerging understanding of neurochemical defects in mouse models of RTT into preclinical trials of potential treatments for respiratory dysfunction in this disease.

1. INTRODUCTION

Rett syndrome (RTT) is a complex neurodevelopmental disorder whose underlying pathogenic mechanisms remain poorly understood. RTT affects approximately 1 in 10,000 live female births and is characterized by apparently normal early postnatal development followed by neurological decline around 6 – 18 months of age. The disorder has a highly variable course and affected individuals exhibit a broad array of symptoms that generally includes loss of acquired speech, head growth deceleration, autistic features such as emotional withdrawal and diminished eye contact, motor stereotypies, early hypotonia followed by rigidity, epileptiform seizures, exaggerated responses to stress and severe respiratory and autonomic (cardiac and

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gastrointestinal) dysfunction (Hagberg et al., 1983; Shahbazian et al., 2002; Vorsanova et al., 2004; Chahrour and Zoghbi 2007, Chahrour, et al., 2008; Ogier and Katz, 2008; Weese-Mayer et al. 2006, 2008). Approximately 25% of RTT patients may die prematurely of cardiorespiratory failure (Kerr et al., 1997).

At least 95% of typical RTT cases result from loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2; Amir et al., 1999; Shahbazian et al., 2002), one of a number of methyl-binding proteins (Klose and Bird, 2006) that regulate gene expression by repressing transcription at methylated promoters. Over 200 different *MECP2* mutations have been found in RTT patients and tend to cluster within two functional domains of the protein; a methyl-binding domain that recognizes methylated CpG dinucleotides with particular flanking sequences (Klose and Bird, 2006), and a transcription repression domain. The *MECP2* gene is X-linked, and homozygous mutation in females (or hemizygous mutation in males) is invariably lethal. Thus, affected females are heterozygotes and somatic mosaics for MeCP2, i.e., cells in which the mutated allele occurs on the inactive X are phenotypically normal, whereas cells in which the mutated allele occurs on the active X are mutant. Disease phenotype is therefore affected not only by the specific *MECP2* mutation but by the skewing of X chromosome inactivation; individuals in which inactivation is skewed towards the mutant allele are less severely affected, and *vice versa*. For a more detailed discussion of molecular genetic aspects of RTT the reader is referred to an excellent recent review by Chahrour and Zoghbi (2007).

2. CARDIO-RESPIRATORY PHENOTYPES IN RTT

Breathing abnormalities are among the clinical diagnostic criteria for RTT, and include alternating periods of hyperventilation and apneas, breath holds terminated by Valsalva's maneuvers, forced and deep breathing as well as apneustic breathing (Fig. 1) (cf. Elian and Rudolf, 1991;Julu et al. 2001;Julu and Witt-Engerström, 2005;Marcus et al. 1994,Weese-Mayer et al., 2006,2008). Perhaps the most striking breathing abnormalities are the frequent breath holds and apneas as they reveal abnormal cardiorespiratory coupling. In particular, each breath hold is associated with an instantaneous subtle decrement in heart rate, followed by an exaggerated increase that is not seen in controls. We hypothesize that the frequent reoccurrence of breath holds with an overshoot in the heart rate response likely results in an alternating exposure to hypoxic and oxidative stress, which may in turn have detrimental long-term consequences on cardiorespiratory homeostasis (Nanduri et al., 2009). Recurring breath holds may have another important consequence for cardiovascular function as they are thought to lead to prolonged QT syndrome (Guideri et al. 1999,2001,2004;Sekul et al. 1994). Indeed, decreased heart rate variability and prolonged QTc are characteristic features of RTT patients (Weese-Mayer et al. 2006). A long QT syndrome is significant as it has been linked with sudden death in non-RTT children and could therefore also explain the sudden and unexpected deaths in RTT patients (Weese-Mayer et al. 2006,2008;Kerr et al. 1997).

Another key feature of the RTT breathing phenotype is its state-dependency. Respiratory disturbances in RTT are significantly more severe during wakefulness compared to sleep (Weese-Mayer et al., 2008) and are exacerbated by behavioral arousal. However, it must be emphasized that breathing during sleep is not normal (Weese-Mayer et al. 2008). Indeed, the abnormalities in breathing are reminiscent of those observed during the day, but are less frequent and severe. As during the day, nocturnal breathing is more irregular, and breathing frequency, mean airflow and heart rate are significantly increased in RTT patients compared with controls. Similarly, heart rate variability is decreased during the night. Thus, cardiorespiratory dysregulation in RTT occurs during wakefulness and sleep, but the degree of dysregulation is quantitatively much less dramatic during night time compared to wakefulness. The state-dependence of breathing abnormalities in RTT suggests that problems

with modulation of the respiratory rhythm and motor pattern play a critical role in shaping the ventilatory phenotype in these patients. It is therefore tempting to speculate that breathing dysregulation in RTT results from disturbances in mechanisms that modulate the respiratory rhythm, acting either alone or in combination with more subtle disturbances in rhythm and pattern generation. Disturbances in neuromodulatory mechanisms could be responsible in particular for the significant worsening of breathing irregularities during the day, as well as with behavioral arousal and postnatal maturation. These disturbances could primarily reflect dysregulation of aminergic, peptidergic and neurotrophin-mediated inputs to the rhythm generating network that are critical for adaptive responses to sensory feedback and changing behavioral demands as well as postnatal maturation of the respiratory motor pattern. The persistence of breathing irregularities during both day and night could, in addition, reflect disruption of the normal balance between fast excitatory and inhibitory synaptic mechanisms within the rhythm generating network itself. These two possible types of disturbances are discussed in more detail below.

3. RESPIRATORY PHENOTYPES IN MOUSE MODELS OF RTT

The discovery that mutations in *MECP2* are responsible for most cases of RTT led to the development of mouse models in which *Mecp2* is either deleted, mutated or overexpressed, including 1) *Mecp2*$^{-/y}$ mice with extended exonic deletion of the *Mecp2* gene (Chen et al., 2001; Guy et al., 2001; Pelka et al., 2006), 2) *Mecp2*$^{308/y}$ mice with truncation of MeCP2 protein at amino acid 308, a human RTT mutation (Shahbazian et al., 2002), 3) *Mecp2*$^{Flox/y}$ mice expressing a hypomorphic *Mecp2* allele (Samaco et al., 2008) and 4) *Mecp2*^{Tg1} mice that overexpress MeCP2 protein (Collins et al., 2004). As with RTT patients, homozygous female *Mecp2* mutant mice are not viable and heterozygous females are phenotypically heterogeneous due to variable patterns of X-chromosome inactivation. Therefore, most laboratories study hemizygous males (*Mecp2*$^{-/y}$), which are completely null for *Mecp2* and therefore tend to be more phenotypically homogenous than female heterozygotes (see however Bissonnette and Knopp, 2006, 2008; Bissonnette et al., 2007). In addition, male hemizygotes typically become symptomatic within several weeks after birth, whereas female heterozygotes tend to develop symptoms only after several months.

The different loss-of-function mouse models exhibit varying degrees of RTT-like pathophysiology, including, in most cases, disturbances of breathing that closely resemble respiratory dysfunction in human RTT patients (Fig. 2). All strains of MeCP2 deficient mice studied thus far exhibit erratic breathing, characterized in particular by pronounced apneic spells that appear after an initial period of apparently normal postnatal development, similar to human patients (Viemari et al., 2005;Ogier et al., 2007; for details see below). The developmental course of breathing disorders in RTT is mimicked in male *Mecp2* knockout mice (*Mecp2*$^{tm1.1Bird\ -/y}$;Viemari et al. 2005), as the onset of arrhythmic breathing at rest occurs in these animals around postnatal day (P) 30 (Viemari et al. 2005), an age that correlates, at least in some major aspects, with approximately 1 to 1.5 years in humans (Watson et al., 2006). In addition to apneas, *Mecp2*$^{tm1.1Jae}$ mice (Chen et al., 2001) exhibit significant periods of abnormally fast breathing that result in a 20% increase in mean respiratory frequency (Ogier et al., 2007), as in RTT patients (Weese-Mayer et al., 2008).

Analysis of the respiratory motor-pattern in *Mecp2*$^{tm1.1Bird}$ mice (Guy et al., 2001) with an *in situ* perfused brainstem preparation showed that erratic breathing and apneas are related to spontaneous fluctuation of postinspiratory motor output to laryngeal adductor muscles during the early expiratory phase (Stettner et al. 2007). The disturbed postinspiratory motor activity accounts in particular for apneas with active glottal closure and could therefore underlie the loss of speech and impaired swallowing observed in RTT patients (Stettner et al., 2008). Postinspiratory activity is indeed essential for airflow modulation during speech, while glottal

closure is needed to protect the lungs from invasion of noxious substances (Dutschmann et al., 2008) or food during swallowing (Gestreau et al., 2005). Therefore, we speculate that disturbed respiratory related functions in RTT, such as vocalization and swallowing, are also associated to some degree with brainstem dysfunction (Stettner et al., 2007, 2008). Moreover, since neural regulation of the respiratory and cardiovascular systems are closely linked, it is not surprising that RTT patients also suffer from cardiac sympathetic imbalance, peripheral vasomotor disturbances (Guideri et al. 1999; Julu et al. 1997) and exaggerated heart rate responses during breath-holds (Weese-Mayer et al., 2006, 2008). However, abnormal cardiovascular function has not yet been described in mouse models of RTT (cf. Bissonnette et al., 2007).

The phenotypic complexity of RTT is undoubtedly related to the fact that *Mecp2* mutations result in the dysregulation of very large numbers of genes, some of which increase in expression whereas others decrease (cf. Chahrour et al., 2008). Despite this complexity, growing evidence from mouse models of RTT points to abnormal synaptic function, particularly related to defects in neurochemical signaling, as a key factor contributing to neuropathophysiology caused by loss of function mutations in *Mecp2* (cf Wang et al., 2006; Monteggia and Kavalali, 2009).

4. ABNORMAL NEUROCHEMICAL SIGNALING IN RTT MICE

4.1. Disturbed GABAergic and glutamatergic neurotransmission

Growing evidence suggests that at least some of the neurological endophenotypes in RTT, including breathing abnormalities, are associated with changes in MeCP2-mediated regulation of excitatory glutamatergic or inhibitory GABAergic synaptic transmission (Blue et al., 1999; Samaco et al., 2005; Chao et al., 2007; Stettner et al., 2007). Moreover, significant metabolic anomalies, including altered glutamate metabolism, have been described in *Mecp2* null mice (Viola et al., 2007). It is well established that proper function of the respiratory network, i.e. elaboration and adaptation of the rhythmic central drive to behavioral and environmental changes, requires both glutamatergic excitatory and GABAergic inhibitory synapses. Thus, disturbed function of these synapses in the absence of MeCP2 would be expected to have a direct impact on neural control of breathing.

Developmental alterations in the composition of glutamate and GABA receptor subunits are normally associated with postnatal maturation of respiratory cell groups in the medulla and pons (Wong-Riley and Liu, 2005, 2008). As early as postnatal day (P) 7, $Mecp2^{-/y}$ mice exhibit reduced expression of GABA-A2 and GABA-A4 receptor subunits in the rostral ventrolateral medulla oblongata (Medrihan et al., 2008), a region that contains diverse neuronal populations important for cardio-respiratory control, compared to wild type (WT) animals. In addition, GABAergic, but not glycinergic, synaptic transmission is strongly depressed at P7 in $Mecp2^{-/y}$ mice, leading to an imbalance in excitatory and inhibitory neurotransmission. However, whether or not these early changes in GABAergic signaling contribute to respiratory dysfunction in *Mecp2* null mice remains unclear, as plethysmographic recordings did not reveal abnormalities in resting ventilation at P7 (Medrihan et al., 2008). Moreover, even in the isolated rhythmic brainstem slice preparation, respiratory motor output appears normal before P14 (Viemari et al., 2005). Further studies are required, however, to determine whether or not GABAergic signaling deficits in *Mecp2* null neonates affect respiratory adaptation to behavioral challenges, despite normal resting ventilation in these animals.

Although changes in glutamatergic neurotransmission in brainstem circuits of $Mecp2^{-/y}$ mice have not yet been investigated in detail, pathophysiological changes in the NMDA receptor subunits NR2A and NR2B have been reported in forebrain structures (Asaka et al., 2006; Lee et al., 2008). At the level of the brainstem, exaggerated respiratory responses to glutamate microinjections into the Kölliker-Fuse nucleus in $Mecp2^{-/y}$ mice at P40 provide indirect

evidence for altered glutamatergic neurotransmission at an age at which the RTT respiratory phenotype is fully expressed (Stettner et al., 2007).

In addition to neurons, brain glia may be another target at which loss of MeCP2 affects fast excitatory and/or inhibitory transmission. Glial cells normally play a major role in the elimination of glutamate in the extracellular mileu and thereby help to prevent excitotoxic neuronal damage and synaptopathy resulting from glutamate excess (Tilleux and Hermans, 2007; Schousboe et al., 2004; Danbolt, 2001). Recent studies indicate that *Mecp2* null astrocytes are abnormal in some respects, particularly regarding their ability to support neurite outgrowth from hippocampal neurons in culture (Ballas et al., 2009). Although defects in glial regulation of brain glutamate metabolism in *Mecp2* null mice have not yet been described, these findings raise the possibility that astrocyte dysfunction could contribute to disruption of glutamatergic signaling in RTT.

4.2. Deficits in BDNF expression and synaptic modulation

Brain-Derived Neurotrophic Factor (BDNF) regulates neuronal survival and maturation during early development, and synaptic function and plasticity throughout life, and is required for normal breathing behavior (for review see Katz, 2005). The synaptic modulatory role of BDNF is related to the fact that BDNF is expressed and secreted by many neurons in an activity–dependent manner throughout life (Balkowiec and Katz, 2000, 2002). Indeed, BDNF null mice exhibit abnormal central respiratory output at birth (Balkowiec and Katz, 1998) and die shortly thereafter of apparent respiratory failure (Erickson et al., 1996). Recent findings indicate that the BDNF gene may be a transcriptional target of MeCP2 (Chen et al., 2001; Martinowich et al., 2003) and $Mecp2^{-/y}$ mice exhibit marked deficits in brain content of BDNF mRNA and protein after birth (Chang et al., 2006; Wang et al., 2006; Ogier et al., 2007). Some of the earliest and most dramatic deficits occur in the nodose ganglion (NG) and the nucleus tractus solitarii (nTS) of the dorsal brainstem, structures that play pivotal roles in reflex control of cardiorespiratory output. Specifically, $Mecp2^{tm1.1Jae-/y}$ mice exhibit 40–50% WT levels of BDNF in the NG and brainstem by 5 weeks after birth (Wang et al., 2006). These declines are not due to cell death, because BDNF deficits in $Mecp2^{-/y}$ mice occur after neurons are no longer dependent on BDNF for survival (Wang et al., 2006). Rather, BDNF-expressing neurons have less BDNF available for release (Wang et al., 2006), adversely affecting BDNF-dependent synaptic signaling. Normally, BDNF regulates glutamatergic synaptic function in newborn and adult animals at multiple sites within the respiratory network, including the nTS (Balkowiec et al., 2000), preBötzinger complex (Thoby-Brisson et al., 2003, Bouvier et al., 2008), Kolliker-Fuse nucleus (Kron et al., 2007a, 2007b) and spinal phrenic motoneurons (Baker-Herman et al., 2004). For example, previous studies demonstrated that BDNF can modulate activity of second order neurons in nTS by inhibiting postsynaptic glutamatergic AMPA receptors (Balkowiec et al., 2000). We hypothesize, therefore, that in *Mecp2* null mice, one consequence of decreased BDNF expression by NG sensory neurons is a deficit in modulation of fast glutamatergic transmission at primary afferent synapses in nTS. This hypothesis is supported by our recent finding that primary afferent stimulation elicits significantly larger excitatory postsynaptic responses in nTS relay neurons in *Mecp2* null mice compared to WT controls (Kline et al., 2008). This functional synaptopathy may in turn explain the finding of Stettner et al. (2007) that *Mecp2* null mice exhibit a loss of habituation in the Breuer-Hering reflex, an nTS-mediated response that plays an essential role in regulating the post-inspiratory phase of the respiratory cycle. Similarly, $Mecp2^{-/y}$ mice exhibit exaggerated ventilatory responses to hypoxia at P30 (Voituron et al., 2009), consistent with a deficit in modulation of afferent inputs to nTS.

4.3. Disturbed monoaminergic modulation

There is compelling evidence that monoaminergic neuromodulatory systems show pathophysiological changes in RTT patients and mouse models. In their initial description of RTT, A. Rett and colleagues reported abnormally low values of noradrenaline (NA) and serotonin (5-HT) in the brain of single RTT patients and suggested that a defect in central monoaminergic systems could be an underlying cause of the disease (Riederer et al., 1985, 1986; Brücke et al., 1987). The characteristic symptoms and the polysomnography records of RTT patients are indeed consistent with central defects in NA and 5HT (Nomura et al., 1985). Further, postmortem examination confirmed a 50% reduction of NA and 5HT contents in the substantia nigra in 2/4 patients with typical features of RTT syndrome (Lekman et al., 1989). Zoghbi et al. (1985) reported a significant decrease in NA but not 5HT metabolites in the CSF of six RTT patients. However, other studies of NA and 5HT metabolites in the CSF or urine from larger samples of RTT patients did not confirm significant differences from controls (Lekman et al., 1990; Temudo et al., 2009). Recently, analysis of 5HT binding in postmortem brain samples from eleven girls with RTT revealed an altered 5HT innervation and/or uptake in the dorsal motor nucleus of the vagus, suggesting abnormal 5HT modulation of this major autonomic nucleus (Paterson et al., 2005).

The neurons of the NA and 5-HT arousal systems have been closely studied for their relationship to behavioral state. Neurons in the locus coeruleus and the raphé nuclei, for example, have relatively characteristic, state dependent firing rates; both groups fire fastest during wakefulness and slow down or even stop during NREM and REM sleep, respectively (for an excellent review see Saper et al., 2001). The NA and 5HT neurons are not only involved in the control of the sleep-wake cycle but also have a major role in the neural control of breathing (Hilaire et al., 2004; Richter et al., 2003). NA and 5HT first appear at embryonic stages and are important for perinatal maturation of the respiratory network and its postnatal function (Hilaire, 2006; Bissonnette and Hilaire, 2008; Gargaglioni et al., 2008; see also Viemari and Tryba, 2009, this issue). Genetic alterations of the NA system alter maturation of respiratory control, with severe consequences for postnatal survival. In neonatal rodents, NA released from the brainstem A6, A5, A2 and A1 groups exerts a complex modulation on the activity of the medullary respiratory network. NE neurons from the pontine A5 and A6 groups are synaptically connected to the medullary respiratory network and either reduce (A5) or facilitate (A6) its rhythmic activity (Hilaire et al., 2004), whereas the medullary A1/C1 and A2/C2 neurons either facilitate (A1/C1) or stabilize (A2/C2) the respiratory rhythm (Hilaire, 2006; Zanella et al., 2006). Pharmacological and genetic alterations of the 5HT system also disrupt the formation of central networks (somatosensory, visual and locomotor networks: Cases et al., 1996; Upton et al., 1999; Cazalets et al., 2000, respectively), including the brainstem-cervical respiratory network (Bou-Flores et al., 2000, Burnet et al., 2001; Bras et al, 2008).

In *Mecp2* null mice, biogenic amine systems exhibit abnormal postnatal developmental profiles. Ide et al. (2005) demonstrated that whole brain levels of NA and 5HT in *Mecp2* null mice are normal at birth and then decline from P14 onwards. Viemari et al (2005) compared the NA and 5HT contents in the forebrain, pons and medulla of WT and *Mecp2* null mice and found a significant postnatal decline in NA by P30 that was specific to the medulla. Thereafter, the NA deficit increased, and 5HT content in the medulla was significantly reduced by P60. Interestingly, the reduction of NA contents in the medulla preceded the appearance of overt breathing deficits. Comparison of the number of NA and 5HT neurons in the medulla of WT and *Mecp2* null mice revealed an initial reduction of NA neurons in the A2/C2 group of mutants by P30; by P60, the reduction was even more severe and also affected NA neurons in the A1/C1 group without any effect on 5HT cell counts (Viemari et al., 2005). In contrast, comparison of mRNAs encoding 5HT metabolic enzymes, 5-HT receptor subtypes and the 5-HT

transporter in the preBötzinger complex of WT and *Mecp2* null mice has revealed significant decreases in mutant animals by P40 (Manzke et al., 2008). In addition, it has been recently shown that MeCP2 deficiency in mice results in dysregulation of the gene encoding L-dopa decarboxylase, an enzyme required for monoamine biosynthesis (Urdinguio et al., 2008).

It will be important to understand the potential impact of monoaminergic deficits on progressive respiratory dysfunction in *Mecp2* null mice and RTT patients in relationship to the role of these systems in regulation of the sleep-wake cycle. However, the current body of data may not suffice to fully explain how respiratory dysfunction in RTT relates to behavioral arousal. The erratic breathing in RTT occurs predominantly during wakefulness when both NA and 5-HT neurons exhibit their highest firing rates (Saper et al. 2001). Hence, if an aminergic deficit contributes to breathing irregularities, one would expect that breathing stabilizes during the day when monoaminergic drive is enhanced and conversely becomes irregular during the night. However, the opposite is the case. Such considerations might apply to controls with a normal physiological state, but not to RTT patients in whom cardiorespiratory physiology and various aspects of neuromodulation are perturbed. Abnormalities could include, for example, changes in the responsiveness to neuromodulators, in modulatory release mechanisms or in the balance between inhibitory and excitatory aminergic drive. The state-dependency of the breathing abnormalities needs to be considered also in a developmental context. In all mammals a progressive increase in waking time with decreased REM sleep takes place during postnatal maturation (for review see Garcia-Rill et al., 2008). Therefore, the contribution of NA and 5HT systems to the modulation of breathing may be rather minimal at the neonatal stage. As these systems become more active with age and increased waking time, the impact of NA and 5HT deficits on respiratory behavior may become more pronounced. Hence, the appearance of breathing abnormalities later in postnatal development in *Mecp2* null mice would be consistent with an increased dependency on monoaminergic drive.

5. EXPERIMENTAL STRATEGIES TO TREAT BREATHING DISORDERS IN *Mecp2* NULL MICE

Recent studies in conditional *Mecp2* null mice have demonstrated that reactivation of the *Mecp2* gene, even in severely symptomatic animals, can rescue neurologic function to a remarkable degree (Guy et al., 2007; see also Giacometti et al., 2007). These findings indicate that deficits caused by loss of MeCP2 function reflect defects in neuronal signaling, rather than neurodegeneration, and, therefore, are potentially reversible. In addition, genetic overexpression of the BDNF gene in $Mecp2^{tm1-1Jae}$ null mice improves somatomotor function and prolongs lifespan (Chang et al., 2006). However, given current technical barriers to effective gene therapy, several laboratories are investigating the possibility of rescuing neurologic function in *Mecp2* null mice and, eventually, RTT patients, by pharmacologic means.

A recent study by Ogier et al. (2007) explored the possibility that pharmacologic elevation of endogenous BDNF expression with ampakine drugs could improve respiratory function in $Mecp2^{tm1-1Jae}$ null mice. Ampakines are benzamide derivatives that acutely facilitate the activity of glutamatergic AMPA receptors (Lynch, 2006). In addition, repeated administration of ampakines in rats and mice increases expression of BDNF mRNA and protein in the forebrain for several days (Lauterborn et al., 2003; Rex et al., 2006) and augments BDNF dependent synaptic plasticity (Ingvar et al., 1997; Porrino et al., 2005; Rex et al., 2006; Wezenberg et al., 2007). Indeed, treatment of $Mecp2^{tm1-1Jae-/y}$ mice with the ampakine CX546 for 3 days restores normal respiratory frequency and ventilation (Ogier et al., 2007). These effects on ventilation were accompanied by a significant increase in expression of BDNF protein in vagal afferent neurons in the nodose ganglion. Although further studies are required to elucidate the mechanism of ampakine action in this model, these data are consistent with

the hypothesis that decreased BDNF levels contribute to the respiratory phenotype of *Mecp2* null mice and that BDNF may be a pharmacological target for improving respiratory function in RTT. It is not yet known whether ampakine treatment improves other aspects of the RTT phenotype.

Other pharmacological approaches are targeting the NA deficiency in *Mecp2* null mice. Treatment with desipramine, a clinically approved NA re-uptake inhibitor, yields some improvement in breathing and prolongs the life span of *Mecp2* null mice up to P90 (Roux et al., 2007; Zanella et al., 2008). This is consistent with MeCP2 deficiency dysregulating the gene encoding L-dopa decarboxylase, one of the enzymes in the NE biosynthetic pathway (Urdinguio et al., 2008). However, although desipramine treatment delayed the onset of fatal breathing disorders in a subset of $Mecp2^{-/y}$ mice, ultimately, it did not rescue the mouse RTT phenotype.

In RTT girls, pharmacological treatments with bioamine precursors that significantly increased catecholamine metabolites in CSF did not result in clinical improvement (Nielsen et al., 1990). On the other hand, pharmacological treatment of one RTT girl with the 5HT1A receptor agonist buspirone improved her breathing and oxygen saturation (Andaku et al., 2005) and one of three RTT patients treated with the 5HT reuptake blocker fluoxetine showed clinical improvement (Temudo et al., 2009). However, in the absence of large scale, well controlled trials of NA or 5HT active drugs in RTT patients, the potential for such interventions to improve respiratory function in this population remains speculative at this point.

As illustrated in the present review, at least five different neurochemical signaling systems -- glutamate, GABA, BDNF, NA and 5HT – exhibit abnormal expression and/or function in mouse models of RTT and most appear to contribute to breathing abnormalities in these animals. The fact that these are parallel interacting systems suggests that poly-pharmaceutical approaches will be required to effectively treat breathing disorders in RTT. Such approaches may also be synergistic. For example, treatment with a dual NA/5HT re-uptake inhibitor has been shown to significantly elevate BDNF levels in rodent cortex (Mannari et al., 2008). Conversely, elevation of BDNF levels, which are important for development of NA neurons (Akbarian et al., 2002; Copray et al., 1999; Guo et al., 2005) and NA turnover (Jönsson et al., 2008), may help to address depressed monoamine expression in the absence of MeCP2.

6. CONCLUSIONS

Our current understanding of the respiratory phenotype in RTT leads us to the following conclusions:

1. The RTT breathing phenotype is extremely complex (hyperventilation, breath holds, forced and deep breathing and apneustic breathing) and includes disturbances in cardiorespiratory coupling. This complexity suggests that cardiorespiratory dysfunction in RTT is unlikely to result from a single underlying mechanism.

2. The RTT breathing phenotype is abnormal during sleep and wakefulness; however, the abnormalities are exaggerated during wakefulness and can be worsened by behavioral arousal. Thus the RTT breathing phenotype may involve two distinct types of disturbances: (a) the strong state-dependence suggests defects in modulation of the respiratory rhythm and motor pattern that may, in addition, be superimposed on (b) more subtle alterations in rhythm and pattern generation.

3. The RTT breathing phenotype develops relatively late during postnatal development, implying either that processes involved in stabilization of mature respiratory behavior are affected or alternatively that the breathing phenotype is the consequence of a cascade of changes at different levels that result in a cascade of compensatory

mechanisms and instabilities as the RTT patient matures. The repeated breath holds could for example lead to a cascade of biochemical changes that would in turn exaggerate the breathing phenotype.

6.1. A Neurochemical Hypothesis

Loss of MeCP2 alters multiple neurochemical signaling mechanisms, and in some cases, these alterations arise from a failure of neurons to maintain their differentiated phenotypes; e.g., BDNF and TH expressions are normal at birth but subsequently decline. The neurochemical systems affected by loss of MeCP2 are important both for acute synaptic signaling and, in some cases, activity-dependent maturation of neuronal circuits (e.g., BDNF). We hypothesize, therefore, that the *Mecp2* null respiratory phenotype is the net result of 1) acute synaptopathies (neurons don't release or respond to their normal complement of transmitters/modulators) and 2) perturbed circuit development and/or loss of plasticity. For example, the loss of BDNF-dependent modulation of glutamatergic transmission may result in an inability of the network to undergo long-term modifications in synaptic strength that underlie emergence of a stable respiratory pattern after birth.

Respiratory network dysfunction in RTT may result in particular from progressive synaptopathies in pathways associated with the processing of, and adaptive responses to, afferent information as well as adaptive responses to modulatory dysfunction. This scenario would reflect the fact that the behavioral repertoire of mammals is naturally increasing in complexity with age. *Mecp2* null mice exhibit progressive neurochemical deficits in BDNF, NE and 5-HT systems that play key roles in behavioral adaptation of respiratory motor output. Specific tasks such as exercise, food foraging, vocalization, etc., are associated with multi-modal and multi-synaptic integration of afferent inputs that are required for adaptive responses of the respiratory system to changing behavioral demands. Thus, the effects of synaptic dysfunction coupled with dysregulated modulatory inputs to the respiratory network would be expected to increase progressively as these systems play increasingly important roles in shaping mature respiratory behavior. Therefore, the complexity, developmental progression and state-dependence of respiratory dysfunction in RTT might be explained by defects in neurochemical signaling mechanisms important for acute and adaptive modulation of respiratory motor output.

7. SUMMARY

The complexity of the RTT breathing phenotype may well be related to the diversity of neurochemical changes caused by loss of MeCP2 function, as all of the affected neurotransmitter and neuromodulatory systems described thus far are important for expression, modulation and/or adaptation of respiratory motor output. Further advances in understanding cardiorespiratory pathophysiology in RTT will benefit from integrative experimental strategies that are able to link specific neurochemical deficits at the molecular and cellular level to specific features of respiratory network dysfunction. Such an approach will also facilitate further development of therapeutic modalities for the treatment of breathing disorders in RTT.

Acknowledgments

ABBREVIATIONS

The following abbreviations are used for the gene encoding methyl-CpG-binding protein 2 and its respective protein product:

MECP2

human gene

Mecp2

mouse gene

MeCP2

protein

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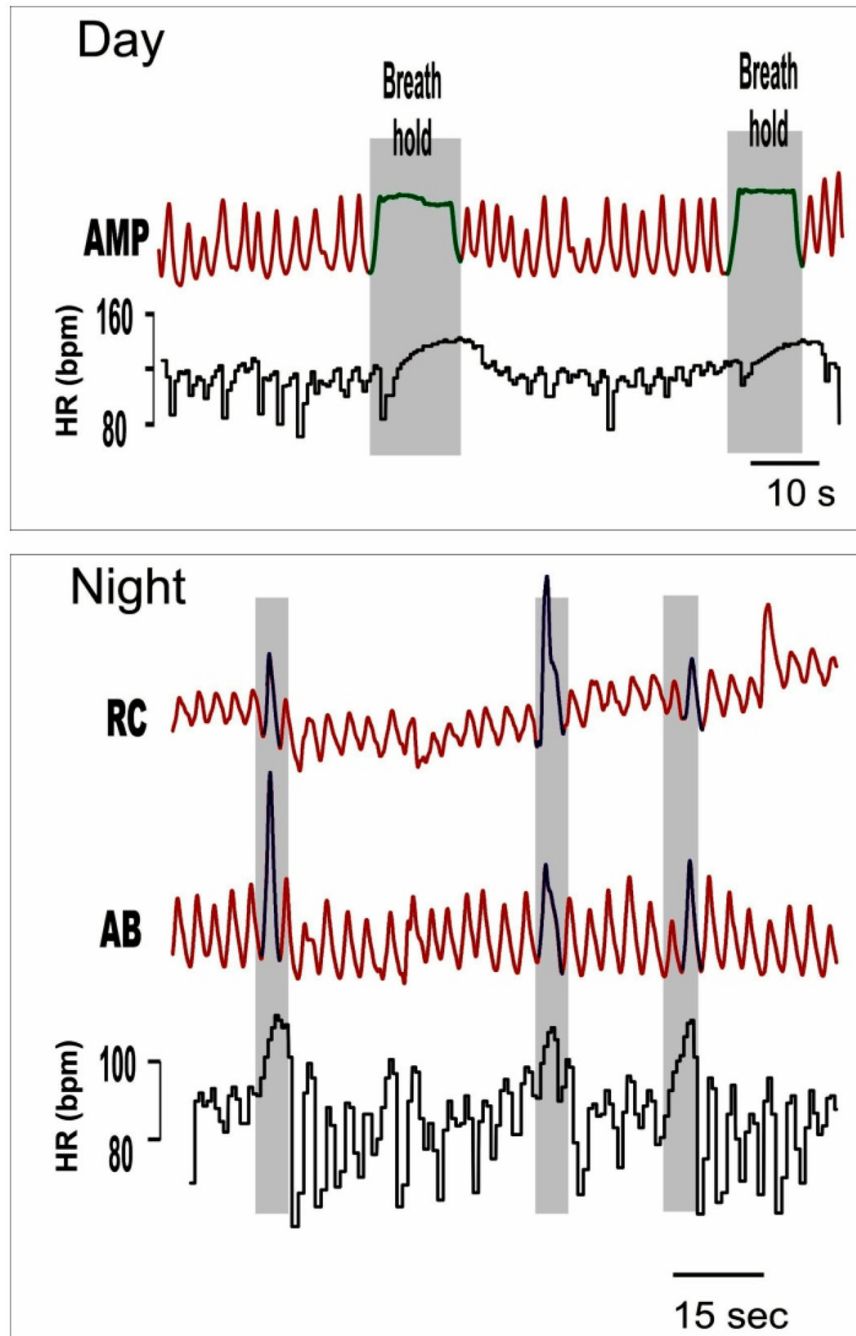
Figure 1.
Breaths and breath-holds in a representative child with Rett Syndrome obtained during the day and night using inductance plethysmography waveforms: sum amplitude (AMP), rib cage (RC), abdomen (AB), and heart rate (HR) are shown. Note that respiratory irregularities and characteristic heart rate changes occur during both the day and night. Adapted from Weese-Mayer et al. 2006, 2008.
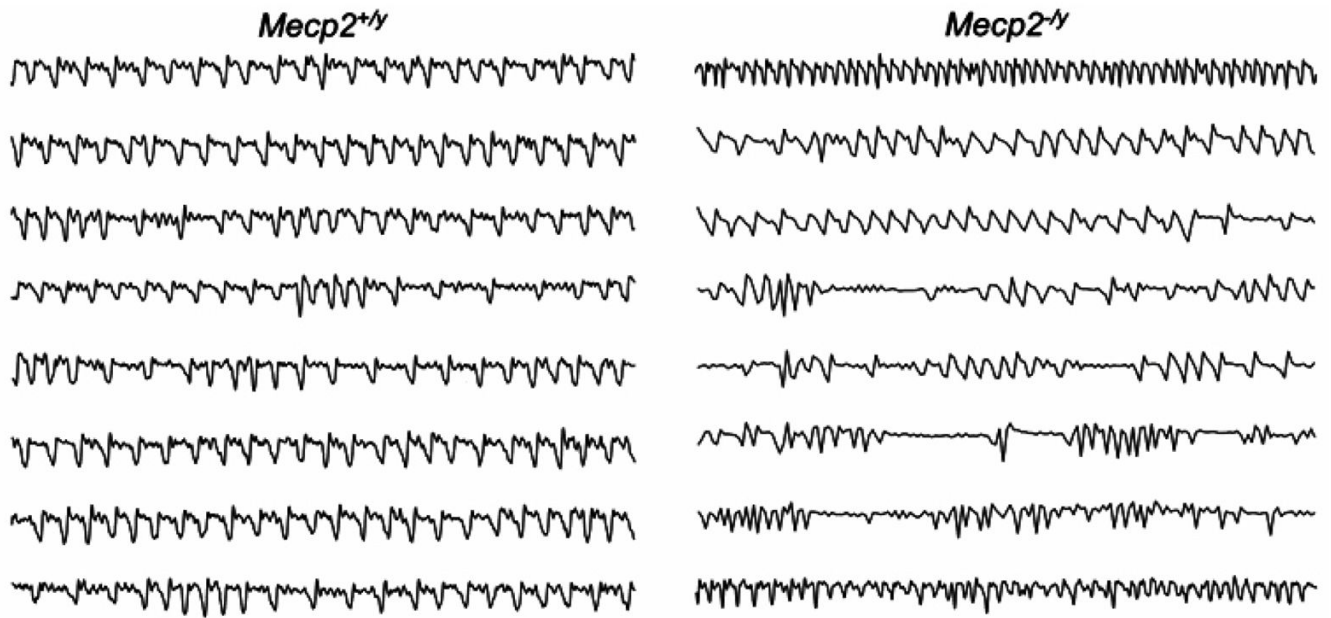
Figure 2.
Plethysmographic recordings from P35 WT (*Mecp2*$^{+/y}$) and *Mecp2* null (*Mecp2*$^{-/y}$) mice (*Mecp2*$^{tm1.1Jae}$ strain). Each horizontal trace = 10s quiet breathing in room air. Adapted from Ogier et al., (2007).