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Breathing challenges in Rett Syndrome: Lessons learned from humans and animal models☆,,☆☆

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Abstract

Breathing disturbances are a major challenge in Rett Syndrome (RTT). These disturbances are more pronounced during wakefulness; but irregular breathing occurs also during sleep. During the day patients can exhibit alternating bouts of hypoventilation and irregular hyperventilation. But there is significant individual variability in severity, onset, duration and type of breathing disturbances. Research in mouse models of RTT suggests that different areas in the ventrolateral medulla and pons give rise to different aspects of this breathing disorder. Pre-clinical experiments in mouse models that target different neuromodulatory and neurotransmitter receptors and MeCP2 function within glia cells can partly reverse breathing abnormalities. The success in animal models raises optimism that one day it will be possible to control or potentially cure the devastating symptoms also in human patients with RTT.

Keywords

Pre-Boetzinger Complex; Mecp2; dysautonomia

1. Introduction

Rett Syndrome (RTT) affects approximately 1 in 10,000 female births (Laurvick et al., 2006; Neul et al., 2010). Children with RTT develop apparently normal until the age of 18 months. At this age, most patients with RTT achieve the normal milestones with regards to motor functions and communication skills. But subsequently girls enter a stagnation phase (Hagberg, 2005) that is followed by a developmental regression (Neul, 2012). This regression is characterized by a loss of hand skills, mobility skills, and speech, and the girls typically show stereotypic hand movements, develop ataxia, gait apraxia and often seizures. Microcephaly, growth deficits, scoliosis are also characteristic features (Weng et al., 2011a).

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The loss of communication skills is one of the reasons why RTT is categorized as an autism spectrum disorder (Castro et al., 2013; Neul, 2012).

Among the core symptoms of RTT, severe disturbances in breathing are particularly devastating (Glaze, 2005; Katz et al., 2009; Kerr, 1992; Ogier and Katz, 2008; Rohdin et al., 2007; Weese-Mayer et al., 2008, 2006). 65-93% of RTT patients display bouts of hypoventilation that alternate with irregular breathing or hyperventilation (Amir et al., 2000; Julu et al., 2001). But the breathing disturbances are variable and a large catalog of disturbances has been reported (Kerr, 1992). The breathing abnormalities have been categorized by various authors as periods of forced breathing, deep breathing, hyperventilation (rapid shallow breathing), hypoventilation, central and obstructive apneas, apneustic breathing, Valsalva's maneuvers, Biot's breathing, periodic breathing and breath holds (Julu et al., 2001; Weese-Mayer et al., 2008). Moreover, many of these breathing disturbances are associated with a significant dysregulation in cardio-respiratory coupling (Julu et al., 2001; Weese-Mayer et al., 2008). The complexity of the breathing phenotype in Rett Syndrome is in part explained by differences in the genotype/phenotype relationships, specifically the types of mutation and degree of X-chromosome inactivation (Amir et al., 2000).

2. The genetic basis of Rett Syndrome

Rett Syndrome is caused by a mutation in the methyl-CpG binding protein 2 (MECP2) gene (Amir et al., 1999). The genomic locus of *MECP2* in humans is approximately 80 kb and consists of 4 exons from which two different isoforms of MeCP2 may be transcribed, differing in their inclusion of the second exon. The basic structure of the *MECP2* locus and protein is conserved across species. Absence of the second intron allows for translation from the first exon and is referred to as the MeCP2e1 isoform, inclusion of the second exon stops translation products originating from the first exon and utilizes its own separate translation start site to generate the MeCP2e2 isoform (Fig. 1) (Adkins and Georgel, 2011). Thus, the two isoforms differ in their N-terminal sequences and in their expression patterns; MeCP2e1 is the predominant isoform expressed in the brain, MeCP2e2 is expressed in peripheral tissues as well as in the brain during early postnatal development before being restricted to subregions such as the dorsal hypothalamus and cortical layer V (Dragich et al., 2007).

At the protein level, MeCP2 contains two distinct functional domains, originally defined by deletion mapping and in vitro methyl-CpG-binding and transcription assays, a methyl-CpG binding domain (MBD), and a transcriptional repression domain (TRD) (Fig. 1) (Nan et al., 1993). The putative nuclear localization sequence (NLS) for MeCP2 was deletion mapped to the region containing amino acids 255–286 (Nan et al., 1996). However, despite the occurrence of these conserved domains within the protein, the structure of MeCP2 is believed to be highly disordered, a feature that may contribute to its functional interactions with chromatin (Adkins and Georgel, 2011). MeCP2 has been shown to bind methylated CpG sites and repress transcription by the recruitment of histone deacetylase complexes; alternatively, MeCP2 also is capable of activating transcription through interactions with CREB (Chahrour et al., 2008). Several genes are misexpressed by loss of MeCP2 including reduction of factors that promote general neuronal survival and plasticity such as BDNF, and genes that define neuronal subpopulations such as GAD1, GAD2, TPH2, or TH (Chahrour et al., 2008; Chao et al., 2010; Samaco et al., 2009). The gene expression changes within neuronal subpopulations often result in biosynthetic deficiencies, reducing the ability to synthesize and release normal quantities of their neurotrasnmitters such as GABA, serotonin, dopamine, or norepinephrine contributing to neurological dysfunction. Additionally, loss of MeCP2 function has been tied to increased expression of FXYD1 a modulator of Na^+/K^+ ATPase activity tied to dendritic morphology (Deng et al., 2007).

3. Breath-holds and apneic events in Rett Syndrome

Breath holds or apneic events are consistently observed in RTT (Figs. 2 and 3) (Weese-Mayer et al., 2008). These events have a periodic nature (Fig. 2A) and they are interspersed by bouts of hyperventilation and irregular breaths (Julu et al., 2001; Southall et al., 1988; Weese-Mayer et al., 2008).

Yet, defining these breathing cessations has been the source of considerable confusion. Indeed, the same type of events may have been described by different authors as “breath-hold”, “apnea” or “Valsalva maneuver” (Julu et al., 2001; Weese-Mayer et al., 2008). The existing confusion may be surprising, because breath holds, apneas and Valsalva maneuvers are sufficiently defined so that they shouldn’t be mixed-up, unless in RTT syndrome the integration of breathing with the cardiovascular system is significantly altered so that the defining features of breath holds, apneas, or Valsalva are different from healthy subject. For this reason we would like to briefly review the features that differentiate these different forms of breathing events.

“Apneas” are generally defined as “cessation of breathing” and are usually associated with the absence of inspiratory activity (Ramirez et al., 2013, in this issue), as opposed to “apneusis” during which respiratory activity is centrally maintained in inspiration (Cohen, 1979). As will be described in more detail in the section on mouse models, respiratory activity recorded in situ, suggests that the apneas in the mouse models of RTT are indeed apneas as there is a cessation of inspiratory activity (Fig. 3B; Abdala et al., 2010). However, typically the lungs become deflated during a central apnea, which is not the case in RTT girls. In these children, the lungs remain inflated during these events, which is typical for “breath holds” as is apparent in Fig. 3 (Southall et al., 1988; Weese-Mayer et al., 2008). The in situ preparation provides a possible explanation: As inspiratory activity ceases there is an overexcitation of expiratory muscles that pushes the air against the closed glottis (Fig. 3B; Abdala et al., 2010) – this would keep the lungs inflated in the absence of inspiratory activity. Interestingly, a similar breathing behavior has been described for reptiles during the non-ventilatory period. In this situation inspiratory activity ceases and air is trapped due to a closed glottis. It is suggested that this mechanism retains air with minimal muscular effort (Naifeh et al., 1971).

In healthy human subjects, a defining feature of breath holds is a sustained drop in heart rate. By contrast the “breath holds” in RTT girls are characterized by a biphasic heart rate change: an initial heart rate drop is followed by a significant heart rate increase (Weese-Mayer et al., 2008). This biphasic response is somewhat reminiscent to the biphasic heart rate change of “Valsalva maneuvers” (Elisberg, 1963). But there are differences. Valsalva maneuvers are voluntarily generated in healthy subjects and reflex mechanisms seem to be primarily responsible for the heart rate changes. It is thought that pressure changes in thorax and abdomen cause biphasic changes in blood pressure, which via the baroreflex result in biphasic changes in the heart rate. Upon resumption of normal breathing, the heart rate exhibits a reflex bradycardia and returns to baseline shortly after cessation of the Valsalva maneuver (Elisberg, 1963). By contrast, the heart rates remain elevated upon resumption of breathing in RTT patients (Weese-Mayer et al., 2008). The elevated heart rate is strikingly regular and remains elevated even as breathing resumes. We therefore propose that the biphasic heart rate changes in RTT are not primarily reflexive in nature, but instead caused by central dysautonomia. This doesn’t mean that the heart rate change is exclusively caused centrally. The progressive hypoxemia may activate peripheral chemoreceptors that could contribute to the heart rate increase.

In conclusion the breathing cessation in RTT cannot simply be classified as “breath hold”, “central apnea”, or “Valsalva maneuver”, but instead constitutes a breathing disturbance that is specific to RTT patients. The confusion that exists in the field may be partly explained by the fact that these RTT-specific disturbances have features that resemble those of “breath holds” (=pressing air against the closed glottis), “apneas” (=cessation of inspiratory activity) and “Valsalva maneuvers” (=increase in heart rate).

4. Hyperventilation and breath-to-breath irregularities

Hyperventilation associated with breath-to-breath irregularities is another characteristic disturbance in RTT patients (Southall et al., 1988; Weese-Mayer et al., 2008). The breaths in these patients are on average deeper, faster and more irregular in amplitude and timing (Weese-Mayer et al., 2008). However, the degree of these disturbances varies from individual to individual (Julu et al., 2001; Southall et al., 1988). Hyperventilation seems to be generated centrally as it is neither indirectly caused by hypercapnia or hypoxia, nor is hyperventilation caused by preceding apneas and irregular breathing (Southall et al., 1988). In RTT patients exhalation is reduced by 25%, while inhalation by only 10% (Weese-Mayer et al., 2008). The significant reduction in exhalation is inconsistent with findings in the so-called in situ preparation in which post-inspiration seems to be prolonged in mutant mice (Stettner et al., 2007). However, this discrepancy may be explained by this particular experimental approach. The respiratory frequency generated in the in situ preparation is significantly slower compared to the real breathing in in vivo mice. This slowing is primarily due to an increase in the duration of expiratory activity, which may exaggerate the duration of post-inspiration

In patients with RTT hyperventilation is most pronounced during active wakefulness, i.e. during the day. But, breathing in RTT patients is also disturbed during the night (Southall et al., 1988; Weese-Mayer et al., 2008), as breathing is significantly faster and more irregular when compared with age-matched controls (Weese-Mayer et al., 2008). Further mechanistic studies will be needed to explain the characteristic state-dependency of breathing in RTT.

5. Breathing disturbances in mouse models of RTT syndrome

The discovery that RTT is caused by a mutation in *Mecp2* (Amir et al., 1999) facilitated animal studies aimed at mechanistically explaining the devastating symptoms of this disorder. Two mouse lines were developed in the laboratories of Rudolph Jaenisch and Adrian Bird in which *Mecp2* was conditionally knocked-out: *Mecp2*$^{\mathrm{TM}1Jae}$ and *Mecp2*$^{\mathrm{TM}1Bird}$ (Chen et al., 2001; Guy et al., 2001). Initial characterization of breathing abnormalities was performed using null alleles of *Mecp2* derived from the aforementioned conditional alleles: *Mecp2*$^{\mathrm{TM}1.1Jae}$ and *Mecp2*$^{\mathrm{TM}1.1Bird}$. More recently, Adrian Bird’s lab developed an allele allowing conditional rescue of *Mecp2*: *Mecp2*$^{\mathrm{TM}2Bird}$. These mouse lines have been used to test both neuroanatomic origins of MeCP2 dependent phenotypes as well as pre-clinical therapies to treat them. Like in humans, mice show hyperventilation, apnea, and increased variability in breath frequency (Fig. 2A) (Ogier et al., 2007; Voituron et al., 2010, 2009). But, there is uncertainty as to whether breath-holds or apneas are similar in mouse models when compared to humans. In mice these events seem to occur with the lungs deflated and are akin to either central or obstructive apneas (Ogier et al., 2007; Voituron et al., 2010, 2009). However, as discussed in the comparison of the human and mouse disturbances (Fig. 3) these long-lasting events could have common underlying mechanisms.

Male and female mice that possess either of the null alleles of *Mecp2* consistently display apneas. Hyperventilation has been most often attributed to mice possessing the *Mecp2*$^{\mathrm{TM}1Jae}$ allele. However, newer studies suggest that the previously observed difference between the

alleles was likely due to differences in methodology and the genetic background. *Mecp2*$^{TM1.1Jae}$ mice were often studied on a mixed 129S6, C57BL/6, and BALBC background while *Mecp2*$^{TM1.1Bird}$ mice were typically studied backcrossed to C57BL/6 (Voituron et al., 2010, 2009; Ward et al., 2011). Male *Mecp2*$^{TM1.1Bird/Y}$ mice show relatively normal breathing until they approach adulthood and begin to display apneic and bradypnic breathing patterns that progressively worsen until death (Viemari et al., 2005; Voituron et al., 2009). By challenging young mice with hypoxia or hypercapnia, breathing irregularities can be evoked several weeks before they typically become symptomatic as adults. Similarly, anxiety-evoking conditions can contribute to irregular breathing in particular in younger mice, at a time when adults are not symptomatic (Ren et al., 2012). Allowing mice to mature to adulthood before removing MeCP2 function still causes the progression of breathing dysfunction (Cheval et al., 2012), while restoring MeCP2 function in symptomatic adults can still ameliorate breathing disturbances (Robinson et al., 2012). Mechanistically, these manipulations indicate that formation of the respiratory network is not permanently disturbed by the lack of MeCP2. Instead, MeCP2 seems to be critical for maintaining stable respiratory rhythmic activity.

Like in humans breathing disturbances are worse during wakefulness and conditions that increase anxiety (Ren et al., 2012). Similar to the improvement in breathing regularity during sleep observed in patients, light anesthesia of *Mecp2*$^{TM1.1Bird}$ mice improves their breathing regularity (Viemari et al., 2005). The responses to hypercapnia seems to be affected by mutations in MeCP2. *Mecp2*$^{TM1.1Bird/Y}$ mice exhibit a selective loss in the response to mild hypercapnia (1–3%), and show more regular breathing in response to 6–9% (Zhang et al., 2011). This abnormal chemosensory response seems to be due to the overexpression of a particular potassium current (Kir4.1.) (Zhang et al., 2011). As hypothesized by Zhang et al. (2011), RTT patients may not detect mild hypercapnia, and they begin to hyperventilate only when hypercapnia becomes severe. As a consequence, the insensitivity to mild hypercapnia could lead to the periodic alternation of hypo- and hyperventilation, which is a hallmark of the breathing disturbances in human RTT. *Mecp2*$^{TM1.1Bird/Y}$ mice also possess an increased and persistent response to acute moderate hypoxia (8–10% Oxygen). Hypoxia evokes an increased respiratory rate, which fails to transition into a hypoxic ventilatory decline, which is normally observed in adult mice (Voituron et al., 2009; Ward et al., 2011). These alterations are consistent with a progressive loss of respiratory network functions that include early changes in respiratory rhythm generation and significant alterations in the normal homeostatic responses to changes in levels of O_2 and CO_2.

6. Neuronal basis of breathing disturbances in RTT

Different aspects of breathing are controlled by different components of the respiratory network (Ramirez et al., 2011). By using conditional alleles of Mecp2 and monitoring the consequences in in vitro experiments it is possible to dissect which areas contribute to what aspects of RTT disturbances. Restoring MeCP2 to regions in the medulla and caudal pons restores a normal hypoxic breathing response (Ward et al., 2011). This is consistent with the role of the ventrolateral medulla, in particular the preBoetzinger Complex (preBoetC) in the generation of the hypoxic response (Garcia et al., 2011; Hill et al., 2011; Quintana et al., 2012). The pre-Boetzinger complex (preBoetC) is a well defined neuronal network that is essential for breathing, and that continues to generate respiratory rhythmic activity when isolated in brainstem slice preparations (Carroll and Ramirez, 2013; Ramirez et al., 1998; Smith et al., 1991; Tan et al., 2008). But, restoring MeCP2 in these regions is insufficient to prevent the characteristic hyperventilation, which suggests that contributions from rostral brain regions may influence this phenotype. This finding is consistent with the human

observation, and the strong association between behavioral and emotional state and hyperventilation (Ren et al., 2012; Ward et al., 2011).

The use of the in situ preparation has yielded additional evidence for the instability of the central respiratory network. Respiratory cycle times and intra-cycle phases, including inspiration, post-inspiration, and late-expiration, from *Mecp2*$^{TM1.1Bird/Y}$ samples showed greater irregularity in duration, and a propensity for central apnea. Surprisingly, this preparation shows an increased post-inspiratory duration, which was linked to excitatory inputs from the Kolliker-Fuse (Stettner et al., 2007). As already mentioned in human patients an increase in postinspiratory duration is not evident during ongoing breathing and unlikely contributes to the breathing irregularities and hyperventilation (Weese-Mayer et al., 2006). But a dysregulation of postinspiratory activity may contribute to the generation of the central apnea or breath-holds as described by Weese-Mayer et al. (2006) (Fig. 3A). Indeed glutamate micro-injections into the rostral brainstem of the in situ preparation of *Mecp2*$^{TM1.1Bird/Y}$ mice induced central apnea, suggesting that the Kolliker-Fuse nucleus may contribute to the apneas or breath holds that are characteristic for RTT (Stettner et al., 2007). Plethysmography measurements of nasal/oral airflow and the characterization of chest movements identified two types of apnea in Mecp2-Bird mice: (1) central apneas that are caused by the failure to initiate inspiration, and (2) obstructive apneas that are caused by a transient upper airway obstruction identified by chest movements in the absence of airflow through the nose or mouth (Voituron et al., 2010). The Kolliker-Fuse nucleus could be responsible for an obstruction caused by a poor coordination of glottal closure, which together with a central apnea could result in the breath-hold or apnea in RTT patients (Fig. 3B; Abdala et al., 2010).

Absence of MeCP2 has major effects on various neurotransmitter and neuromodulatory systems. It decreases norepinephrine (NE), dopamine (DA), serotonin (5-HT), GABA, and BDNF levels. Moreover, this reduction relates to the specific role of MeCP2 in the neurons that release these substances (Chao et al., 2010; Viemari et al., 2005). Tyrosine Hydroxylase (TH) containing neurons show an age-dependent decline in TH expression, which was associated with decreased NE and DA levels in *Mecp2*$^{TM1.1Bird}$ mice (Viemari et al., 2005). This may have direct consequences on respiratory rhythm generation (Viemari et al., 2011; Viemari and Ramirez, 2006; Viemari et al., 2005). Noradrenergic nuclei can be isolated in transverse slice preparations from *Mecp2*$^{TM1.1Bird}$ (Viemari et al., 2005), and an early loss in TH expressing medullary neurons could partly explain the irregularities observed in the isolated medullary network (Figs. 2B and 4A1). By exogenously applying norepinephrine regular rhythmic activity can be restored (Fig. 4A2).

Although, the onset of breathing abnormalities in mice with null alleles of *Mecp2* becomes only obvious at a later postnatal state, cellular and network level abnormalities have been identified prior to the onset of overt breathing symptoms. Viemari et al. (2005) identified significant irregularities in the respiratory network located within the preBoetC (Fig. 2B). At these earlier stages such irregularities were not observed in the in situ preparation, a preparation which contains additional neuronal circuitry and modulatory systems that are located rostral to the level of the preBoetC (Ren et al., 2012). Thus, the larger central respiratory network may be able to compensate for the irregularities that can be seen early on in the isolated preBoetC. An impairment of GABAergic neurons in the ventrolateral medulla could also contribute to the early disturbances. As early as post-natal day 7, amplitude and frequency of spontaneous inhibitory synaptic currents (sIPSC) is decreased, while amplitude and frequency of spontaneous excitatory synaptic currents (sEPSC) is increased in *Mecp2*$^{TM1.1Bird/Y}$ mice (Medrihan et al., 2008). Furthermore, organotypic slice cultures from the medulla of 3 day old *Mecp2*$^{TM1.1Bird/Y}$ display several deficits in respiratory pacemaker neurons of the PreBoetC. These neurons show decreased resting

cAMP levels, diminished cAMP production in response to stimulation, and slow arrhythmic bursting that can be characterized by Ca^{+2} imaging and whole cell recordings (Mironov et al., 2011). Moreover, in these preBoetC cultures, neurons from mice lacking MeCP2 were less inter-connected compared to wild type littermates. Decreased network connectivity within the preBoetC is predictive of instability at the network level (Carroll and Ramirez, 2013; Carroll et al., 2013). Consistent with the overall phenotype, connectivity within the organotypic slices progressively worsened from p3 to p20, and supplementation of the slices with BDNF ameliorated this deficit (Mironov et al., 2009). A critical role for BDNF in the neuronal control of breathing is also suggested by changes in the nucleus of the tractus solitarius (NTS). The NTS is a key relay and processing area that receives important inputs from the peripheral nervous system, including O_2 and lung inflation status. BDNF expression within the NTS is reduced in MeCP2 mutant mice, which correlates with excitatory/inhibitory synaptic imbalance as defined by an increase in amplitude and frequency of sEPSC's recorded from medial NTS neurons (Kline et al., 2010). The dysregulation of BDNF in RTT includes not only decreased BDNF expression, but also BDNF release (Wang et al., 2006).

7. Disturbances in different interacting mechanisms may give rise to the RTT phenotype

The mouse models revealed a wide range of neuronal mechanisms that likely play a role also in human RTT patients (Fig. 5). Particularly important for the pathogenesis of breathing as well as other clinical phenotypes of RTT are (a) imbalances in synaptic transmission (Chao et al., 2010; Gatto and Broadie, 2010; Kron et al., 2012; Medrihan et al., 2008; Nelson et al., 2011; Shepherd and Katz, 2011; Zoghbi, 2003), and (b) alterations in a variety of neuromodulatory systems (Ladas et al., 2009; Panayotis et al., 2011; Samaco et al., 2009; Taneja et al., 2009; Toward et al., 2013; Viemari et al., 2005; Zhang et al., 2011; Zoghbi et al., 1985) (Fig. 5). Alterations in the processes controlling synaptic scaling may be partly responsible for the synaptic imbalances (Blackman et al., 2012; Qiu et al., 2012; Zhong et al., 2012). This mechanism may lead to different forms of synaptic dysregulation that could result in breathing problems, seizures, and disturbances in neurocognitive and motor functions. Disturbances in the modulatory milieu, on the other hand, can have equally far-reaching consequences on numerous neuronal networks. These disturbances could also explain the characteristic state-dependency of the breathing disorders in RTT syndrome (Viemari et al., 2005).

Moreover, these synaptic and modulatory disturbances may be highly intertwined (Fig. 5). Thus, assigning distinct disturbances to distinct mechanisms may be very difficult, since short term- and long term changes will contribute to a given clinical phenotype. Neuronal networks will respond to modulatory changes and synaptic imbalances with complex compensatory mechanisms resulting in secondary consequences that are difficult to separate from the direct causes of the Mecp2 mutation itself. From this mechanistic perspective it is not surprising that breathing irregularities display inter-individual variability, and that these disturbances can come and go during the development of a child. To understand the causes of RTT breathing disturbances future research will need to address the complex issues of compensation and secondary consequences, and begin to discriminate between direct and indirect mechanisms. Specifically we need to unravel the mechanisms that directly cause the breathing irregularities and discriminate those from the mechanisms that are indirectly affected by these breathing abnormalities. Breathing disturbances occurring early during the development may trigger compensatory responses that could initially be adaptive and improve breathing in some children, while in other children these disturbances may lead to a vicious cycle of detrimental responses that may in fact worsen the breathing irregularities. The phenomenon that breathing disturbances have long-term consequences is well described

for the case of obstructive sleep apnea (e.g. Ramirez et al., 2013, in this issue). In cases of RTT where a child exhibits recurrent apneas and breath-holds, there is significant hypoxic and oxidative stress (Fig. 5; De Felice et al., 2009), which is known to be detrimental to many neuronal and cardiovascular functions. The effect of oxidative stress can be exaggerated if the responsiveness to intermittent hypoxia is altered in RTT (Vermehren-Schmaedick et al., 2012).

Thus, the clinical phenotype of RTT is not only defined by the already complex genetic causes, but also by the numerous interacting mechanisms involving a variety of compensatory mechanisms, synaptic and neuromodulatory alterations, disturbed homeostatic mechanisms and oxidative stress associated with the breath-holds (Fig. 5).

8. Translational considerations

The complexity of the clinical phenotypes in RTT and the underlying mechanisms can be very discouraging to those trying to manage this devastating neurological disorder in human patients. Although, some improvement in the breathing disturbances have been reported in small trials and case reports (Andaku et al., 2005), large systematic clinical trials have not delivered reliable treatment avenues. Thus, there is a desperate need for novel therapies for the breathing disorder in RTT.

Several pharmacological therapies have been tested in animal models for efficacy at improving breathing disturbances. A variety of clinical phenotypes, including breathing disturbances and shortened lifespan were fully or partly reversed in animal models (Abdala et al., 2010; Guy et al., 2007; Katz et al., 2012; Kline et al., 2010; Ogier et al., 2007; Robinson et al., 2012; Schmid et al., 2012; Toward et al., 2013; Tropea et al., 2009; Weng et al., 2011b). Interestingly, such improvements can be achieved by drugs that act on a variety of different pharmacological targets. The findings that the BDNF gene is a transcriptional target of MeCP2 (Chen et al., 2001; Martinowich et al., 2003) and that mutant mice exhibit major deficits in BDNF mRNA and protein for example in the nodose ganglion and brainstem including the NTS (Chang et al., 2006; Ogier et al., 2007; Wang et al., 2006) inspired treatment with an ampakine (CX546), which increased BDNF protein expression and restored respiratory frequency and ventilation (Kline et al., 2010; Ogier et al., 2007). Similarly, targeting specifically the TrkB receptor (a high-affinity BDNF receptor) attenuates disease progression, increased the life span and partly improved breathing irregularities and returned tidal volumes to control levels (Johnson et al., 2012; Schmid et al., 2012). Furthermore, treatment with the N-terminal (1-3)IGF-1 tripeptide in an attempt to stimulate PI3K and MAPK pathways similar to BDNF, resulted in a modest improvement in breathing rate variability (Tropea et al., 2009). Derecki and colleagues suggest that using microglial cells derived from transplanted wild type bone marrow are capable of rescuing several abnormalities in RTT, including breathing irregularity and apnea (Derecki et al., 2012; Lioy et al., 2011). The finding that Mecp2 null mice are deficient for norepinephrine (Viemari et al., 2005) inspired treatment with the norepinephrine re-uptake blocker desipramine, which improved breathing by reducing the number of apneas and breathing irregularities (Fig. 4B and C). Although, these disturbances were only temporarily improved, the life span of Mecp2 null mice was significantly enhanced (Roux et al., 2007; Zanella et al., 2008). Substances that enhance GABA-A mechanisms and those that act on the 5-HT1A receptor can reduce apneas, correct breathing irregularities, restore chemosensitivity and increase life span (Abdala et al., 2010; Toward et al., 2013).

9. Conclusions

Breathing abnormalities belong to the most detrimental clinical phenotype in RTT. These problems are associated with severe central dysautonomia that are worse during wakefulness. Other symptoms associated with RTT, such as increased anxiety further exacerbate the breathing disturbances. Although, disturbances in neuronal network functions can be observed early on, many symptoms become overt only later during postnatal development. Modern genetic and neurophysiological tools have led to a better understanding of the various deficits caused by the loss of MECP2. These deficits include altered function across several neuromodulatory and neurotransmitter systems that are associated with significant excitatory/inhibitory synaptic imbalances and neuromodulatory deficits across several neuronal networks. Our increased understanding of the underlying cellular mechanisms has led to promising pre-clinical data in mouse models. Targeting GABAergic, noradrenergic, serotonergic systems and BDNF signaling are all potential avenues for rationale therapies. However, it will be necessary to design preclinical trials that fulfill the rigor that is necessary for conducting future clinical trials.

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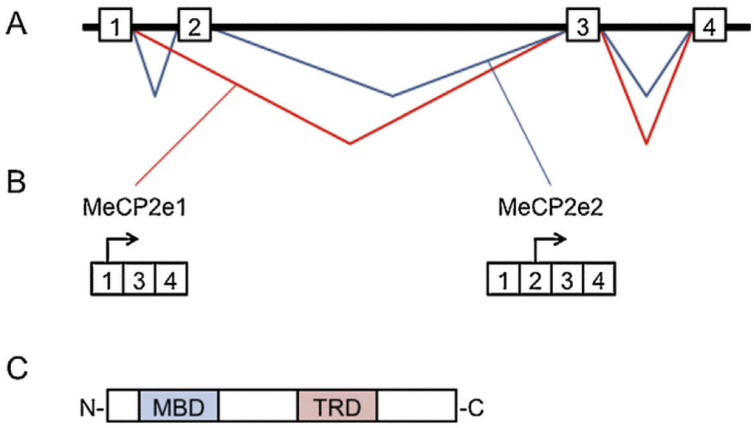

Fig. 1.

Structure and organization of *MECP2*. The genomic locus of *MECP2* is spread across 76 kb and is composed of 4 exons (A). Alternative splicing generates two different isofoms of *MECP2* (B). Both isoforms possess the main functional domains of MeCP2 (C). Arrows indicate translation start sites. MBD methyl CpG binding domain. TRD transcription repression domain.

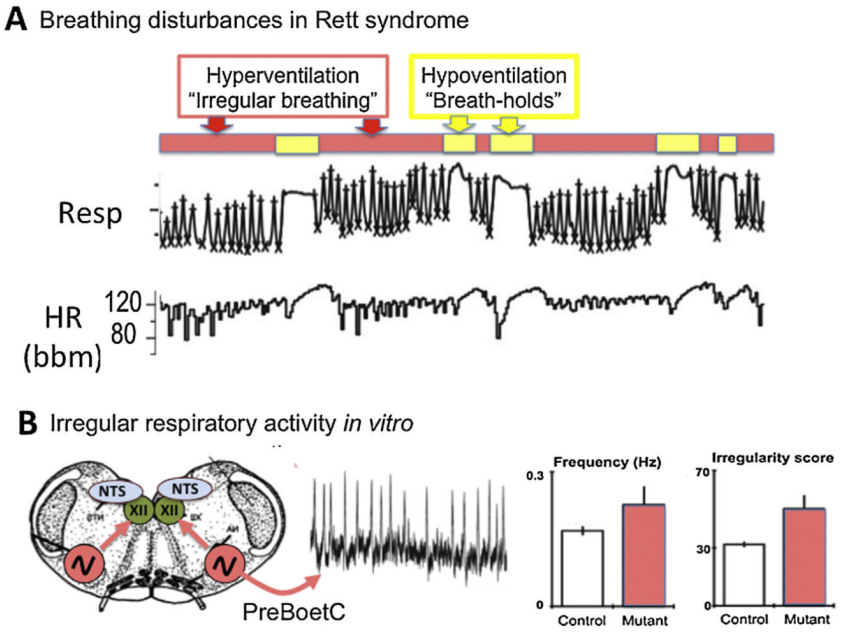

Fig. 2.

Respiratory disturbances in Rett Syndrome patient and animal model. (A) Breathing disturbances in Rett Syndrome typically consist of alternating periods of hyperventilation and hypoventilation. During hyperventilation, respiratory activity is typically irregular with regards to frequency and amplitude. Hypoventilation is characterized by long-lasting events that are referred to as breath-holds; for more explanation see text. RESP (upper trace) indicates the respiratory trace – upwards deflections represent inspiration. HR (lower trace) represents the simultaneously recorded heart rate. *Panel A: modified from* Weese-Mayer et al. (2006). (B) The respiratory network isolated in a transverse slice preparation (see schematic) from MECP2 mutant mice exhibits irregular respiratory rhythmic activity that can be recorded as integrated population activity (trace). The slice preparation contains the pre-Boetzinger complex (preBoetC, in this figure marked in red with a curve to symbolize an oscillator), the nucleus tractus solitarius (NTS, marked in blue) and the hypoglossal nucleus (XII, marked in green). The respiratory rhythmic activity in the slice tends to be faster and is significantly more irregular as indicated in the graphs. *Panel B: modified from* Viemari et al. (2005). More details regarding the recording conditions can be found in Viemari et al. (2005).

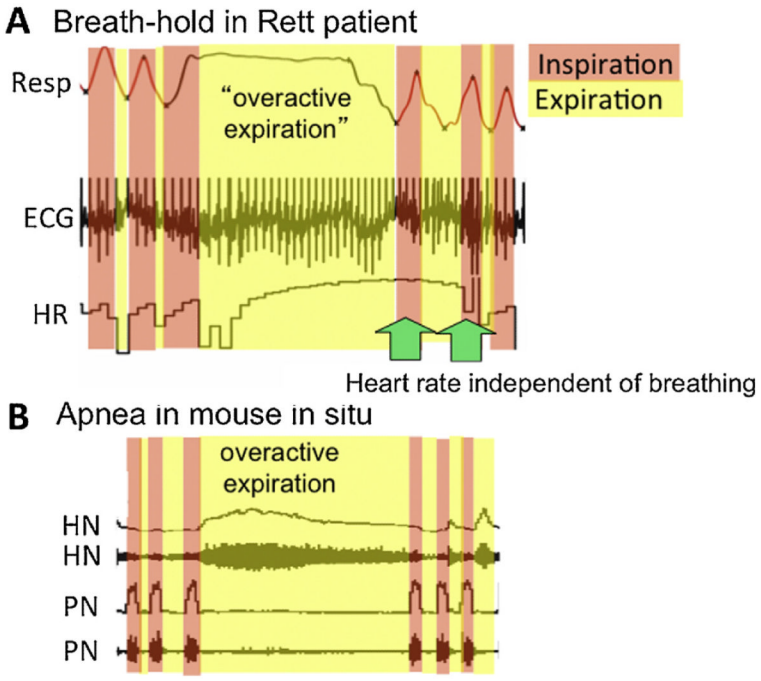

Fig. 3.

Breath hold event in Rett Syndrome patient and animal model. (A) As shown in the respiratory trace (upper trace, RESP) breath holds in Rett Syndrome patients typically begin with an inspiration that is followed by the continued lung inflation. The continued lung inflation is likely due to an overexcited expiratory activation pressing air against a closed glottis. Note each normal inspiration (red) is marked by a heart rate (HR, ECG) increase, while expiration is marked by a heart rate decrease (yellow). Note following the initial heart rate increase at the onset of the breath hold, there is a marked heart rate decrease, which likely marks the onset of the overexcited expiratory activity. But as the breath hold continues, the heart rate rises and this heart rate increase outlasts the breath hold itself, and continues as normal breathing resumes. Indeed the second inspiratory effort after the breath hold is associated with a heart rate decrease. This disturbed coupling between the respiratory and cardiovascular system is indicative for the marked dysautonomia in Rett Syndrome patients. *Panel A*: modified from Weese-Mayer et al. (2006). (B) The respiratory network isolated in a so-called in situ preparation from a MECP2 mutant mouse shows similarities with the breath hold in the Rett patients. An increased hypoglossal activity (HN) and overexcited expiratory activity (not shown) occur during a prolonged apnea which is characterized by the absence of inspiratory activity as illustrated by the recording from the phrenic nerve (PN). *Panel B*: modified from Abdala et al. (2010).

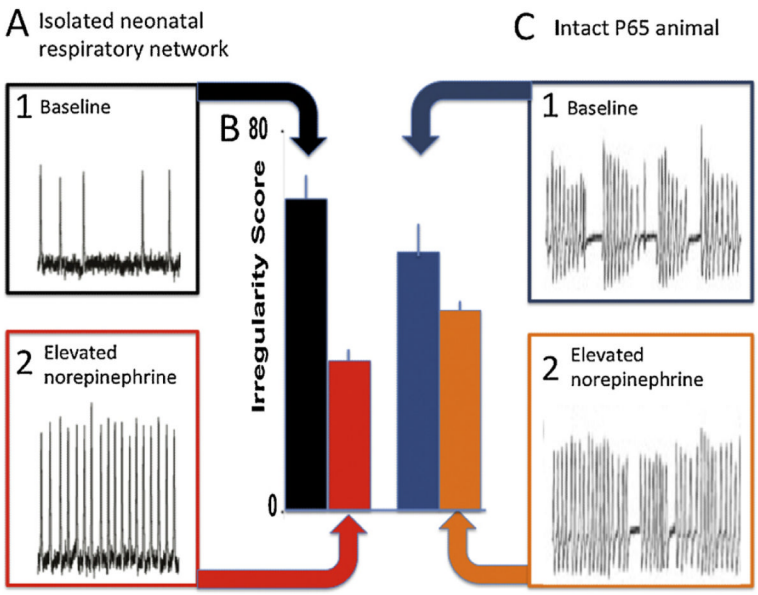

Fig. 4.

Norepinephrine can decrease respiratory irregularities (B) in the isolated respiratory network (A) and intact animal (C). (A) Extracellular population recordings reveal under baseline conditions significant irregularity in the respiratory network isolated from a Mecp2 mutant mouse (A1). This activity becomes regular in the presence of norepinephrine (A2). (B) The irregularity score in the isolated respiratory network obtained from Mecp2 mutant mice (black bar) is significantly decreased in the presence of norepinephrine (red bar). The high irregularity score (blue bar) obtained from an intact adult Mecp2 mutant mouse (P65) is significantly reduced after giving the mice desipramine, a norepinephrine uptake blocker (orange bar). (C) Breathing is highly irregular and shows frequent apneas in intact adult Mecp2 mutant mouse (C1). Breathing is more regular following exposure to desipramine (C2). Data from isolated respiratory network – modified from Viemari et al. (2005); data from intact Mecp2 mutant mouse modified from Zanella et al. (2008).

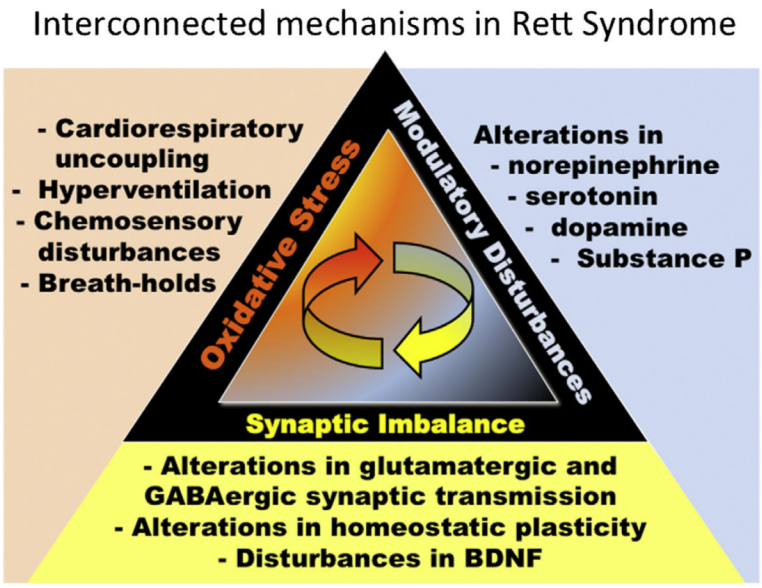

Fig. 5Schematic illustrating the interaction between synaptic mechanisms (yellow), neuromodulatory mechanisms (blue) and oxidative stress (orange) in the etiology of Rett Syndrome. For more details see text.