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***Mecp2* disruption in rats causes reshaping in firing activity and patterns of brainstem respiratory neurons**

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Abstract

People with Rett Syndrome (RTT), a neurodevelopmental disorder caused by mutations in the *MECP2* gene, have breathing abnormalities manifested as periodical hypoventilation with compensatory hyperventilation, which are attributable to a high incidence of sudden death. Similar breathing abnormalities have been found in animal models with *Mecp2* disruptions. Although RTT-type hypoventilation is believed to be due to depressed central inspiratory activity, whether this is true remains unknown. Here we show evidence for reshaping in firing activity and patterns of medullary respiratory neurons in RTT-type hypoventilation. Experiments were performed in decerebrate rats *in vivo*. In *Mecp2*-null rats, abnormalities in breathing patterns were apparent in both decerebrate rats and awake animals, suggesting that RTT-type breathing abnormalities take place in the brainstem without forebrain input. In comparison to their wild-type counterparts, both inspiratory and expiratory neurons in *Mecp2*-null rats extended their firing duration, and fired more action potentials during each burst. No changes in inspiratory or expiratory neuronal distributions were found. Most inspiratory neurons started firing in the middle of expiration and changed their firing pattern to a phase-spanning type. The proportion of post-inspiratory neurons was reduced in the *Mecp2*-null rats. With the increased firing activity of both inspiratory and expiratory neurons in null rats, phrenic discharges shifted to a slow and deep breathing pattern. Thus, the RTT-type hypoventilation appears to result from reshaping of firing activity of both inspiratory and expiratory neurons without evident depression in central inspiratory activity.

Keywords

Rett Syndrome; medullary respiratory neurons; phrenic activity; electrophysiology

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Author contributions

YW contributed to the study design, data collection, data analysis and manuscript preparation. NC contributed to the data collection. HX contributed to the data analysis. WZ, CA and CMJ participated in the data analysis. CJ contributed to the study design and manuscript preparation. All authors read and approved the final manuscript.

Availability of data and materials

Data are available from the corresponding author on a reasonable request.

Competing interests

The authors declare that they have no competing financial interests.

Introduction

Rett Syndrome (RTT) is a neurodevelopmental disease caused mainly by mutations in the *MECP2* gene. People with RTT show irregular breathing characterized by periodical hypoventilation with compensatory hyperventilation. The key event in the RTT breathing is hypoventilation as it may contribute to the high incidence of sudden death and developmental defects of the central nervous system [1–4]. The RTT-type hypoventilation, including hypopnea, apnea and breath hold, is seen in both human [4, 5] and animal models [6–9], which becomes more severe as the disease progresses.

The mechanisms underlying RTT-type hypoventilation are unclear, although dysfunction in brainstem respiratory neuronal activity seems critical. The hypoventilation could result from insufficient central inspiratory activity leading to a decrease in inspiratory premotor output [10, 11]. The insufficient central inspiratory activity could involve a shortening of inspiratory bursts and/or a decrease of inspiratory spike frequency combined with increased expiratory activity. However, certain alternative cellular events may play a role as well, including reshaping firing patterns of respiratory neurons, unexpected firing activity, and altered cell grouping in some major brainstem respiratory neuronal groups.

Unveiling the cellular processes underlying RTT-type hypoventilation may be helpful in developing novel therapeutic strategies. Therefore, we carried out *in vivo* electrophysiological recordings from brainstem respiratory neurons and the phrenic nerve in *Mecp2*-null rats, a RTT model that recapitulates most human RTT phenotypes and allows for *in vivo* experimental approaches [6]. Surprisingly, we found that inspiratory (I) and expiratory (E) neurons did not show reduced firing activity in hypoventilating *Mecp2*-null rats. Instead, both of them extended their firing duration, and fired more action potentials in each burst. With increased firing activity, most I neurons changed firing pattern to the phase-spanning type.

Experimental procedures

Animal

The experiments were approved by Georgia State University Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals. *Mecp2*^{+/-} female rats (SD-*Mecp2*^{tm1sage}, strain code: TGRA6090) were purchased from Horizon Discovery Group (Boyertown, PA) and crossbred with the male Sprague-Dawley wild-type rats (WT) to get male *Mecp2*-null rats (*Mecp2*^{-/-}) as previously described. Animals used in the experiments were 1.5-2.5 month male rats.

Plethysmograph recording

Breathing activity was recorded from awake animals with a plethysmograph chamber as we described previously [6]. After 20 min stabilization, respiratory activity was recorded with AxoScope 10.3 software and analyzed with Clampfit 10.3 software (Molecular Devices, Sunnyvale, CA). The awake status of the animals was ensured during recording.

In vivo Electrophysiology

Rats were anaesthetized with urethane (1.5 g/kg, i.p.) followed by intercollicular decerebration in a stereotactic device. The animals were allowed to breathe spontaneously. The phrenic nerve was exposed at cervical cord C3-C6 level from the dorsal side, kept intact, and placed on a bipolar silver electrodes for recording. Mineral oil was applied to the nerve to prevent drying during recording. The phrenic signal was amplified and filtered (100 Hz to 5 kHz) with an A-M system (Model 1700 Differential AC Amplifier, A-M SYSTEMS™). The phrenic discharge was recorded with Clampex 8.2 software and analyzed with Clampfit 10.3 software. The integrated phrenic signal was obtained through a Paynter filter (50 ms time constant, BAK Electronics Inc., Rockville, MD.).

The cerebellum was partially removed using vacuum suction to expose the brainstem. Single units of action potential/spikes were recorded with tungsten microelectrodes covered with glass. The signals were amplified (Instrumentation Amplifier, Model 210 A, Brownlee BP precision) (Axoclamp-2B Current and Voltage Clamp Amplifier, Axon Instruments. Inc.), digitized (Digidata 1322A, Axon Instruments. Inc.), recorded (Clampex 8.2 software, Molecular Devices), and analyzed offline (Clampfit 10.3 software, Molecular Devices). After completion of the surgery, rats were allowed to stabilize for 20-30 min. The obex on the dorsal surface of the medulla oblongata was used as the landmark to locate the recording sites. Body temperature and heart rate were closely monitored during surgical and recording procedures. Fluid lost during the procedure was estimated and replaced with warm saline via i.p. injection.

Data analysis

Neuronal activities and phrenic activity were assessed by evaluating at least 40 consequent bursts of eupnea. Data analyzed with Student's *t* test were presented as means $\pm$ SE. Data analyzed with either Student's *t* test or χ^2 test were considered to have significant difference when $P < 0.05$.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Breathing similarity in decerebrate and awake *Mecp2*-null rats

Experiments were performed on both WT and *Mecp2*-null rats that underwent intercollicular decerebration following initial anesthesia. Without forebrain control, phrenic nerve discharges in *Mecp2*-null rats (Fig. 1A₁,B₁,C₁) showed breathing rhythmic abnormalities that are similar to plethysmograph recordings from awake null rats (Fig. 1A₂,B₂,C₂) [6, 7]. These breathing abnormalities such as high apnea rate and breathing frequency variation in *Mecp2*-null rats were detected as early as postnatal 4 weeks, and became severer at age 2 months. When the *Mecp2*-null animals reached humane endpoints, apneas occurred more frequently, and the irregular breathing became more severe. These breathing changes were accompanied by overgrown incisors, poor body conditions, and low mobility.

Firing patterns of E and I neurons

We performed *in vivo* recordings from neurons in the ventral respiratory column (VRC), the region known to contain most respiratory neurons in the brainstem. In the decerebrate rats, the VRC resembles the ventral respiratory groups (VRGs) in cats. In the most rostral end of the VRC most cells showed expiratory firing pattern, which is known as the Bötzinger Complex (BötC) in cats [12–14]. Immediately next to this group of E neurons, most cells were inspiratory, corresponding to the rostral VRG (rVRG). At the caudal end of the VRC, most cells were expiratory, identical to the caudal VRG (cVRG) in cats [13, 15]. Because of these similarities, we adopted the same terminologies as used in cats for each subgroup of neurons. Note that I and E cells were classified according to when they firing relation to phrenic discharge. Although respiratory neurons were still retained in the three major subgroups in *Mecp2*-null rats, their firing patterns were clearly altered (Fig. 2). In comparison to the WT, the average firing frequency of I neurons in the rVRG (86.1 ± 5.8 Hz, $n=48$ in WT vs 53.1 ± 3.7 Hz, $n=63$ in *Mecp2*^{-/-}, $P<0.001$, student's *t*-test) and E cells in the cVRG (59.5 ± 6.9 Hz, $n=29$ in WT vs 30.8 ± 3.3 Hz, $n=29$ in *Mecp2*^{-/-}, $P<0.001$) was significantly reduced in null rats, while no difference was found in BötC neurons (35.6 ± 3.1 Hz, $n=43$ in WT vs 28.8 ± 2.0 Hz, $n=65$ in *Mecp2*^{-/-}, $P=0.069$) (Fig. 3A). However, the peak frequency of all three groups of neurons remained the same compared to their WT counterparts (rVRG: 203.3 ± 15.5 Hz, $n=48$ in WT vs 169.1 ± 13.0 Hz, $n=63$ in *Mecp2*^{-/-}, $P=0.067$; cVRG: 125.8 ± 20.3 Hz, $n=29$ in WT vs 92.5 ± 17.7 Hz, $n=29$ in *Mecp2*^{-/-}, $P=0.221$; BötC: 82.3 ± 12.0 Hz, $n=43$ in WT vs 96.4 ± 11.8 Hz, $n=65$ in *Mecp2*^{-/-}, $P=0.403$) (Fig. 3B). One obvious and consistent change in null rats was the extension of burst duration of all three types of neurons (rVRG: 311.5 ± 14.2 ms, $n=48$ in WT vs 826.8 ± 78.8 ms, $n=63$ in *Mecp2*^{-/-}, $P<0.001$; cVRG: 453.1 ± 27.0 ms, $n=29$ in WT vs 1212.9 ± 76.5 ms, $n=29$ in *Mecp2*^{-/-}, $P<0.001$; BötC: 374.2 ± 18.9 ms, $n=43$ in WT vs 1340.9 ± 84.1 ms, $n=65$ in *Mecp2*^{-/-}, $P<0.001$) (Fig. 3C). The extended firing duration tend to lead to an underestimation of neuronal firing activity, as the burst duration is the denominator for calculating the average frequency of all neurons. Thus, we analyzed the overall neuronal firing activity using spikes number per burst (Fig. 3D), as well as the firing frequency (in Hz) multiplied by firing duration (in sec) (Fig. 3E). Both I and E neurons in the ventral column showed higher overall firing activity in *Mecp2*-null rats than in the WT. Spike number per breath was 21.3 ± 1.5 , $n=48$ in WT vs 25.6 ± 1.9 , $n=63$ in *Mecp2*^{-/-} ($P=0.033$) in the rVRG neurons, 16.8 ± 1.9 , $n=29$ in WT vs 31.2 ± 3.0 , $n=29$ in *Mecp2*^{-/-} ($P<0.001$) in the cVRG cells, and 12.6 ± 0.9 , $n=43$ in WT vs 31.2 ± 2.6 , $n=65$ in *Mecp2*^{-/-} ($P<0.001$) in the BötC cells. Similarly, the calculated activity for all these three groups of neurons were significantly higher in *Mecp2*-null rats than in the WT rats (rVRG: 27.4 ± 2.3 , $n=48$ in WT vs 38.9 ± 3.8 , $n=63$ in *Mecp2*^{-/-}, $P<0.05$; cVRG: 26.0 ± 3.5 , $n=29$ in WT vs 37.6 ± 4.3 , $n=29$ in *Mecp2*^{-/-}, $P<0.05$; BötC: 13.8 ± 1.7 , $n=43$ in WT vs 38.4 ± 3.7 , $n=65$ in *Mecp2*^{-/-}, $P<0.001$).

Cell grouping

As mentioned above, E neurons were mostly recorded in the caudal and far rostral parts of the VRC, corresponding to the cVRG and BötC in cats, respectively. I neurons were found in between, corresponding to the rVRG, although there were some overlaps in the cell type

distributions (Fig. 4). As far as these three subgroups were concerned, there were no evident differences in cell type groups between *Mecp2*-null and WT rats.

E-I phase-spanning neurons

In the rVRG, some neurons started firing in the middle of E phase, and stopped at the end of I phase with incrementing firing frequency. Such firing pattern resembles the expiratory-inspiratory (E-I) phase-spanning or pre-inspiratory (pre-I) neurons reported previously [17–19]. Neurons with this firing pattern were seen in both WT and *Mecp2*-null rats (Fig. 3A₂, Fig. 5A,B). Strikingly, we found that most neurons (85 out of 120 cells) in the rVRG of *Mecp2*-null rats had the E-I phase-spanning firing pattern, in comparison to only 18% (18 out of 100 phase-spanning neurons) in WT rats ($P<0.001$, χ^2 test) (Fig. 5C). Like other respiratory neurons in *Mecp2*-null rats, the burst duration of the phase-spanning neurons was much longer than that of the WT phase-spanning cells, especially in the E phase (217.3 ± 20.9 ms, $n=14$ in WT vs 1031.5 ± 125.1 ms, $n=43$ in *Mecp2*^{-/-}, $P<0.001$, student's *t*-test) (Fig. 5D). This findings suggest that I cells in the rVRG extend firing duration consistent with the finding in Fig. 3, and switch their firing to an early starting phase-spanning pattern in *Mecp2*-null rats.

Post-I neurons

The termination of inspiration is followed by the post-inspiratory (Post-I) phase. During this phase, the crural diaphragm and laryngeal adductor muscles remain active, while the intercostal expiratory motoneurons are not yet activated [16, 17]. A group of neurons fired action potentials in the Post-I phase of the phrenic discharge [18, 19]. The Post-I cells exhibited a decrementing firing pattern with initial maximum and gradual decline in firing activity (Fig. A). These post-I neurons have previously been shown to exist in the BötC area. We found 49 Post-I neurons in 105 recorded BötC cells in the WT, but only 15 out of 101 BötC neurons in *Mecp2*-null rats ($P<0.001$, χ^2 test) (Fig. 6B). The location of these Post-I cells around BötC area is indicated in Fig. 6C-F, and their distribution was not obviously different between *Mecp2*-null and WT rats. What causes the reduction in the number of Post-I cells is unclear, although there is a possibility that the increased firing activity of these cells may extend their firing burst length making their firing pattern similar to that of other E cells.

Changes in phrenic discharge patterns

As previously reported, only 50% of *Mecp2*-null rats survive up to two months of postnatal age [6, 7, 20]. At this age their breathing rate was much lower than that of the WT (Fig. 7A,B). Analysis of the eupneic phrenic activity revealed that the inspiration time (T_I) was not significantly different between null and WT animals (294.1 ± 18.0 ms in 21 WT rats vs 291.4 ± 28.2 ms in 18 *Mecp2*^{-/-} rats, $P=0.936$, student's *t*-test) (Fig. 7C). The expiration time (T_E), which represents the interval of phrenic burst, was significantly elongated in *Mecp2*-null rats (512.4 ± 42.1 ms in 21 WT rats vs 1183.3 ± 143.1 ms in 18 *Mecp2*^{-/-} rats, $P<0.001$, student's *t*-test) (Fig. 7D). The elongated T_E was consistent with the increased burst length of medullary E neurons and the increased overall activity of the E cells (Fig. 3). The increase in T_E causes a significant reduction of the T_E to T_I ratio in *Mecp2*-null rats,

which may explain the slower breathing in the *Mecp2*-null animals (0.63 ± 0.05 in 21 WT rats vs 0.31 ± 0.04 in 18 *Mecp2*^{-Y} rats, $P < 0.001$, student's *t*-test) (Fig. 7E). In contrast, the frequency per burst of phrenic discharges (as measured by the amplitude of integrated phrenic activity) was increased in null rats, and was about 70% higher than in the WT (0.071 ± 0.007 in 30 WT rats vs 0.120 ± 0.015 in 21 *Mecp2*^{-Y} rats, $P < 0.01$, student's *t*-test) (Fig. 7F). This suggests that the *Mecp2*-null rats switched their phrenic output to a slow and deep respiratory pattern to compensate for a lower breathing frequency. Similar changes were observed in plethysmograph breathing recording in conscious rats [6].

Discussion

This is the first demonstration of central respiratory neuronal activity in RTT models *in vivo*, in which we have systematically studied all major types of respiratory neurons in the ventral respiratory column of the medulla oblongata using the decerebrate preparation with spontaneous breathing, which is known to generate a relatively normal breathing pattern [21].

Whether breathing abnormalities in RTT patients occur during wakefulness and sleep is under debate. Several previous studies in RTT patients suggest no or few breathing abnormalities during sleep. Thus, some authors suggest that the appearance of disordered breathing is due to the abnormalities of the voluntary respiratory control caused by the impaired function of the limbic cortex and/or hypothalamus [1, 3, 22]. However, cumulating evidence suggests the RTT breathing phenotypes are also found during sleep, implicating the involvement of autonomic breathing [5, 23]. Breathing activities in RTT animals recorded by plethysmography are not enough to clarify the role of autonomic regulation in disordered RTT breathing. Therefore, preparations excluding the interference of voluntary control are needed. In our current study, severe irregular breathing patterns and hypoventilation were observed in decerebrate *Mecp2*-null rats without voluntary control by forebrain areas, indicating impairment of autonomic breathing control caused by *Mecp2* disruption. These autonomic breathing disorders may explain why RTT patients have increased risk of sudden death, which usually happens during sleep.

Our results have shown that the high incidence of hypopnea and breathing variations in the RTT model is not associated with insufficient central inspiratory activity. Instead, I neurons, as well as the E neurons, show excessive firing activity with extended firing duration. How the increased firing activity of both I and E neurons leads to hypopnea and breathing variations is unknown. Several hypothetical scenarios may help to explain our data: The VRC contains the neuronal networks critical for rhythmic breathing activity depending on coordinated synaptic interactions between the E and I neurons [24]. E-I phase-spanning neurons are abundant in the *Mecp2*-null rats. The E-I phase-spanning neurons, also known as pre-I neurons [25], in the rVRG and PBC are thought to function in transitioning from expiration to inspiration in normal animals [26]. The extended firing of these neurons does not lead to an extension of inspiration as seen in phrenic discharges in the null rats. Their firing during expiration without phrenic activity suggests that the I motor output of the brainstem is not fully established, or the E neuronal activity is not terminated properly. The latter is consistent with our finding that E neurons also raise their firing activity and extend

firing duration. Such overall excitation of both I and E neurons may be related to insufficient GABAergic synaptic inhibition, as synaptic inhibitions by both GABA_A receptors and GABA_B receptors are deficient in *Mecp2*-null mice [27]. As a result, respiratory phase transitions may not take place reliably in a timely manner, causing destabilization of the rhythmic respiratory oscillation and variation in breathing rate. Some Post-I cells are involved in terminating the inspiratory phase [16]. The reduction in their numbers may also contribute to inadequate I-E switch as well.

The excessive firing activities of respiratory neurons may be attributed to defects in inhibitory synaptic transmission. Decreases in GABAergic synaptic inhibition are found in several brain regions, including the ventrolateral medulla in *Mecp2* knockout mice [27]. Selective deletion of *Mecp2* in mouse cortical excitatory neurons leads to a reduced number of GABAergic synapses and neuronal hyperexcitation [28]. This suggests that inadequate GABAergic inhibition, and perhaps other inhibitory inputs, may underlie the respiratory neuronal hyperexcitation. Without sufficient synaptic inhibition, respiratory neurons may not be able to fulfill timely phase transitions, resulting in extended firing durations. If this hypothesis is verified, inhibitory intervention of respiratory neurons may help correct their firing abnormalities and the RTT-type hypoventilation.

Our phrenic recordings indicate a reduced breathing frequency at ~2 months of age, consistent with previous reports in awake *Mecp2*-null mice [29]. The slow breathing in null rats is characterized by increased expiration interval without significant change in inspiration duration. With comparison to the WT, the T_E is increased by ~130%, and phrenic discharge (measured as the amplitude of integrated phrenic activity) is ~70% higher in null rats (Fig. 7D,F). The increased T_E is consistent with our finding that E neuronal firing duration is extended. The increased phrenic discharge resembles the augmented I neuronal firing frequency. Because the changes in expiratory duration and phrenic discharge are not proportional, hypoventilation ensues in *Mecp2*-null rats, which can lead to systemic hypoxia and hypercapnia. Although lower body weight and reduced motility of the null rats may play a role in the RTT hypoventilation, they do not seem to be the primary cause. Periodic hyperventilation may be able to correct systemic hypoxia and hypercapnia, allowing the null rats to maintain sustainable O₂ and CO₂ levels. However, the impaired CO₂ chemosensitivity identified in mouse models of RTT appears to compromise such a compensatory response, contributing to the severity of the hypoventilation [9, 30]. Therefore, abnormalities in respiratory neuronal firing patterns and activity are likely to contribute to the breathing disturbances in *Mecp2*-null rats, and vulnerability to sudden death.

Conclusion

Our results suggest that *Mecp2* disruption causes extension of firing durations in both I and E neurons and changes their firing patterns. The changes in medullary respiratory neuronal firing patterns and activity appear to disrupt the balance of alternating activation of these neurons, delaying the onset of inspiration and destabilizing the rhythmicity required for normal ventilation. Elucidating the cellular basis of hypoventilation in RTT may inform the rational design of potential therapeutic interventions to reduce breathing abnormalities and sudden death.

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Abbreviations

RTT:	Rett Syndrome
I:	inspiratory
E:	expiratory
WT:	wild-type
VRC:	ventral respiratory column
VRG:	ventral respiratory group
BötC:	Bötzinger Complex
PBC:	Pre-Bötzinger Complex

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Figure 1. Breathing abnormalities in *Mecp2*-null rats.

Plethysmograph recording (Pleth, **A₁**, **B₁**, **C₁**) and phrenic activity (PhN, **A₂**, **B₂**, **C₂**) recorded the breathing activities of WT and *Mecp2*-null rats. The *Mecp2*-null rats exhibited variations in breathing frequency and amplitude as well as apnea starting from postnatal 4 weeks. The respiratory abnormalities were especially severe when the animals reached humane endpoints.

Figure 2. Firing activity and patterns of respiratory neurons.

Neurons with inspiratory (I) rhythms were found in the rVRG (A), and cells with expiratory (E) rhythms were recorded from the cVRG (B) and the BötC (C). The firing patterns of all clear changes including firing duration (D) and firing frequency (F) but not peak frequency (E). The spike number of each burst (G) in all groups of neurons was significantly higher in null rats than in WT rats as well as the neuronal activity calculated as frequency $\times$ duration (H). Traces: phrenic nerve activity (PhN), integrated phrenic nerve discharge ($\int \text{PhN}$). Note the presence of ECG activity in PhN.

Figure 3. Analysis of the firing properties of respiratory neurons.

(A) Firing frequency of rVRG I neurons and cVRG E neurons was significantly lower in null rats than in WT rats, while the firing frequency of BötC E neurons was not significantly different between WT and null rats (I neurons in rVRG: $n=48$ in WT and $n=63$ in *Mecp2*^{-/-} null rats, respectively; cVRG E neurons: $n=29$ and $n=29$; BötC E neurons: $n=43$ and $n=65$).

(B) Peak frequency of all three groups of neurons showed no difference between WT and null rats. (C) Neuronal burst duration of all types of cells showed significant increase in null rats.

(D) Spike number per burst was measured in all groups of neurons, which was significantly higher in null rats than in WT rats. (E) Neuronal activity calculated as frequency (Hz) $\times$ duration (sec) was significantly higher in null rats than in the WT. *,

$P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Student's *t*-test); NS, no significance.

Figure 4. The spatial distribution of inspiratory (I) and expiratory (E) neurons in ventral respiratory column.

Location was measured from the obex on the sides of brainstem in a transverse plane. Cells with different firing patterns were separated into three areas with some overlap. Point of expiratory neuron, Ex N (blue); overlapping point of inspiratory and expiratory neuron, In +Ex N (green); point of inspiratory neuron, In N (yellow).

Figure 5. Phase-spanning activities in WT and *Mecp2*-null rats.

(A and B) Neurons with E-I phase-spanning activity were recorded from the rVRG in WT and *Mecp2*-null rats. Black bar, phase-spanning duration in expiration. Line, phase-spanning duration in inspiration. (C) The number of E-I phase-spanning cells in the rVRG was significantly greater in *Mecp2*-null rats than in the WT (***) $P < 0.001$, χ^2 test). (D) The phase-spanning duration in expiration (T_E) was significantly longer in null rats as well ($P < 0.001$, $n = 43$, Student's *t*-test).

Figure 6. The ratio and distribution of post-inspiratory neurons in WT and *Mecp2*-null rats.

(A) Neurons with Post-I activity were detected in the BötC area in both WT and *Mecp2*-null rats. (B) The ratio of Post-I neurons within all other E cells in the BötC area was compared between WT and *Mecp2*-null rats (** $P < 0.001$, χ^2 test). (C-F) The localization of Post-I neurons in WT and *Mecp2*-null rats is indicated with black hollow and red dots respectively. The axes indicate the distances from obex in a transverse plane (C and D) and the distances from the dorsal surface (E and F).

Figure 7. Phrenic nerve discharges in WT and *Mecp2*-null rats

(A) Phrenic discharge (PhN) and integrated phrenic activity ($\int \text{PhN}$) recorded from a WT rat show relatively stable rhythmicity and amplitude. (B) In *Mecp2*-null rats, phrenic activity was slow and irregular with large amplitude. (C) The inspiratory duration (T_I) of phrenic activity was not significantly different between WT and null rats ($P=0.936$, measured in 21 WT and 18 *Mecp2*-null rats, with at least 40 phrenic bursts from each animal). (D) Expiratory duration (T_E) was significantly longer in null than WT rats ($P<0.001$). (E) The ratio of inspiration vs expiration duration (T_I/T_E) was significantly smaller in null rats than the WT ($P<0.001$). (F) The amplitude of integrated phrenic activity was larger in null rats than the WT at age 1.5 to 2.5 months (30 WT rats vs 21 *Mecp2*-null rats with $P<0.001$). Arbitrary unit (a.u.). Student's *t*-test.