Effect of inspired oxygen on periodic breathing in methyl-CpG-binding protein 2 (Mecp2) deficient mice

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Abstract:

Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the x-linked gene methyl-CpG-binding protein 2 (Mecp2) that encodes a DNA binding protein involved in gene silencing. Periodic breathing (Cheyne-Stokes respiration) is commonly seen in RTT. Freely moving mice were studied with continuous recording of pleural pressure by telemetry. Episodes of periodic breathing in heterozygous Mecp2 deficient female mice (9.4 ± 2.2 hr⁻¹) exceeded those in wild type animals (2.5 ± 0.4 hr⁻¹) (p=0.010). Exposing Mecp2 +/- animals to 40% oxygen increased the amount of periodic breathing from 118 ± 25 s 30 min⁻¹ in air to 242 ± 57 s 30 min⁻¹ (p=0.001) and 12% oxygen tended to decrease it (67 ± 29 s 30 min⁻¹, p=0.14). Relative hyperoxia and hypoxia did affect the incidence of periodic breathing in Mecp2 +/- animals. The ventilation/apnea ratio (V/A) was less at all levels of oxygen in heterozygous Mecp2 deficient females compare to wild type (P=0.003 to <0.001), indicating that their loop gain is larger. V/A in Mecp2 +/- fell from 2.42 ± 0.18 in normoxia to 1.82 ± 0.17 in hyperoxia (p=0.05) indicating an increase in loop gain with increased oxygen. Hyperoxia did not affect V/A in Mecp2 +/- mice (3.73 ± 0.28 vs 3.5 ± 0.28). These results show that periodic breathing in this mouse model of RTT is
not dependent on enhanced peripheral chemoreceptor oxygen sensitivity. Rather, the breathing instability is of central origin.
Introduction:

Periodic breathing (Cheyne-Stokes respiration) is a common and disturbing feature of Rett syndrome (RTT) that has been reported to occur in 26 to 94% of patients (17, 28). RTT is caused by mutations in the x-linked gene that encodes methyl-CpG-binding protein 2 (Mecp2), a DNA binding protein involved in gene silencing (1). Deletion of exon 3 (8) or exons 3 and 4 (13) has generated mice whose phenotype resemble that of RTT. Heterozygous female (Mecp2+/-) mice developed symptoms at 4-5 months of age (8,13). It has been suggested that Mecp2 deficiency does not affect neuronal development but is essential for maintenance of function. This hypothesis has been confirmed by the recent demonstration that restoration of Mecp2 beginning at 20 weeks of age in Mecp2+/- females reverses their obesity, reduced movement, abnormal gait, hindlimb clasping, tremors and respiratory pauses (12). In an effort to characterize respiratory patterns in Mecp2+/- mice we have instituted studies that continuously record intrathoracic pressure by telemetry (15, 21). After observing that Mecp2+/- animals have a considerable incidence of spontaneous periodic breathing episodes we hypothesized that as in human adults at altitude this respiratory instability was dependent on an increased sensitivity of peripheral
chemoreceptors (3, 7, 20). This initial hypothesis was additionally supported by the reports that Mecp2^{+/−} females and Mecp2^{-/+} males have a somewhat increased ventilatory response to hypoxia compared to wild type (4,5). In the first minute of exposure to hypoxia the increase in minute ventilation for heterozygous females was 18% larger when expressed as relative to baseline and 9% larger if expressed in absolute values (%). In addition we hypothetized that the characteristics of periodic breathing in lower and higher oxygen concentrations would differ between heterozygous Mecp2 deficient females and wild type. Unexpectedly we find that relative hyperoxia increases the amount of periodic breathing in Mecp2^{+/−} mice while hypoxia tends to attenuate it. In contrast changes in oxygen concentration did not affect the amount of periodic breathing in Mecp2^{+/+} animals.
Methods:

Animal Preparation.

The protocols used were approved by the Oregon Health & Sciences Institutional Animal Care and Use Committee and were in agreement with The National Institutes of Health: Guide for the Care and Use of Laboratory Animals. Heterozygous (Mecp2^{+/-}) and wild type (Mecp2^{+/+}) females were obtained by crossing B6.129P2(C)-Mecp2 tm1.1Bird (stock number: 003890; Jackson Laboratory, Bar Harbor ME) heterozygous females with C57BL/6J males. Mice were genotyped using the protocol of the supplier. This strain was originally generated by insertion of \textit{loxP} sites around Mecp2 exons 3 and 4 and crossing homozygous floxed females with CMV Cre males (13).

Under general inhalation anesthesia (1.5% isoflurane in oxygen) a midline abdominal incision was made and the liver gently retracted. A ligature was passed around the esophagus at its entrance to the stomach and the stomach retracted to allow visualization of the lower esophagus. A 25g hypodermic needle was used to make an incision in the longitudinal muscle of the esophagus and with a blunt probe a tunnel was made between the outer and inner layers of the muscle. This tunnel extended beyond the diaphragm into
the thoracic cavity. The tip of a catheter attached to a pressure transducer (model PA-C10, Data Sciences International, St Paul MN) was advanced into the thoracic cavity and secured by gluing it to the entry point in the longitudinal muscle. The suture rib of the pressure transducer was incorporated into the abdominal musculature closure and the skin approximated with a subcuticular suture. This surgical procedure was adapted from published reports (15, 21). Mice were allowed 5 to 7 days to recover before any studies were performed.

Experimental protocols.

Observation: In this protocol mice were studied in their home cages for 2 to 3.5 hours in order to determine their baseline respiratory pattern. The cage was placed on a telemetry receiver (model RBC-1, Data Sciences) and pleural pressure was continuously recorded. These studies were conducted between 18:00 and 21:30h in animals that were on a 12:12 light:dark cycle with lights on from 06:00 to 18:00.

Oxygen or carbon dioxide administration: For these studies mice were placed in a 2L clear plexiglass chamber with access to food and water. The chamber had intake and output ports for flow-through administration of
respiratory gases. Animals were exposed in random order to: Room Air; 12% oxygen/88% nitrogen; 40% oxygen/60% nitrogen or 2% carbon dioxide in air, each for 30 minutes.

Data analysis.

Pleural pressure waveforms were sampled at 2 KHz and breath intervals calculated from the peak negative deflections. Respiratory frequency and breath amplitude were determined using custom written functions in Igor Pro 5.04 B (WaveMetrics Inc., Lake Oswego OR). Periodic breathing was defined as an episode of 3 or more cycles of 3 to 30 breaths, separated by respiratory pauses (Fig. 1). A respiratory pause was defined by a breath interval equal or greater than twice normal intervals. For each episode of periodic breathing the period (time from midpoint of one breath cycle to the next breath cycle), the length of the episode and the ventilation: apnea ratio were calculated. Ventilation:apnea was determined for each episode and averaged for the 30 min trial. The ratio was obtained by dividing the time in which breathing was present by the time occupied by absence of respiratory activity. Animal activity was measured with the telemetry system. As the mouse moves the telemetry signal transmitted to the receiver antennas varies in strength. Changes in signal strength generate an activity count that is
dependent on both distance and speed of movement. Activity was recorded as either inactive (no counts) or active (2-160 counts/min).

Statistical analysis.

Results are given as mean ± S.E.M. Single comparisons such as number of periodic breathing epochs/hr in the observation protocol between Mecp2+/+ and Mecp2+/− mice were made with unpaired Students T test. The effects of oxygen concentration were evaluated with two way repeated measures ANOVA, with strain and oxygen concentration as the two factors. P <0.05 was considered significant.
Results:

Animal characteristics.

Mecp2\(^{+/+}\) (n=10) and Mecp2\(^{+-}\) (n=11) were grossly indistinguishable. Their weights were similar, 23.0 ± 0.7 and 23.5 ± 0.8 g. Brain weights in Mecp2 deficient mice (418 ± 7 mg) were less than wild type (448 ± 6 mg, p=0.013). Ten of eleven Mecp2\(^{+-}\) animals clasped their hind legs when elevated by the tail (13).

Observational study.

Analysis of respiratory frequency during 2-3.5 hour periods showed a bimodal distribution. Activity measurements in 5 Mecp2\(^{+/+}\) and 6 Mecp2\(^{+-}\) mice determined that the rapid breathing occurred when the animal was active and the slower frequency while inactive. Mecp2\(^{+-}\) mice breathed slower than Mecp2\(^{+/+}\) while inactive (2.7 ± 0.1 vs 4.4 ± 0.5 Hz p= 0.019) and during active periods (6.4 ± 0.3 vs 8.5 ± 0.5 Hz, p= 0.008). Periodic breathing was characterized by a waxing and waning pattern in breath amplitude (Figs. 2 and 4). A number the periodic breathing episodes were immediately preceded by a large amplitude breath or sigh: 61.5±4.3% for
Mecp2+/- and 41±7.5 for Mecp2+/+ (eg normoxia in Fig. 4). Episodes of periodic breathing occurred 9.4 ± 2.2 times per hour in heterozygous females compared to 2.5 ± 0.4 in wild type (p=0.01). In addition the epochs were longer in Mecp2 deficient animals than in wild type (15.3 ± 1.7 vs 8.6 ± 0.7 sec, p=0.0003).

The incidence of periodic breathing was not affected by mouse age at the time of study (Fig.1). Mecp2+/- animals comprised two populations. Five of the 11 mice had a frequency of periodic breathing within the range seen in wild type mice while the other six were outside this range (Fig. 1). The two groups of Mecp2+/- mice did not differ with respect to the average length of their periodic breathing episodes (13.4 ± 2.2 vs 14.8 ± 1.7 s) nor to the periodicity of their cycles (2.4 ± 0.2 vs 2.5 ± 0.4 s). The six Mecp2+/- mice with a high incidence of periodic breathing, however, showed a lower ventilation:apnoea ratio compared to the other five (2.06 ± 0.23 vs 2.78 ± 0.18, p=0.042) indicating a higher loop gain (9).

Effect of inspired oxygen.

The effect of breathing varied oxygen concentrations was studied in 6 wild type mice. Oxygen concentrations did not affect the total amount of time in periodic breathing in Mecp2 +/- mice. (Fig. 3A) (p values between 0.508 and
0.613). Exposure to 40% and 12% oxygen did not change the number of periodic breathing episodes: 4.3±1.0 and 0.8±0.40 respectively compared to 4.7±1.80 30 min⁻¹ in air. 40% vs 12% p=0.177; 12% vs air p=0.177%. (Fig 3B). Relative hypoxia eliminated periodic breathing in 3 of 6 wild type mice (Fig 3D). The length of these episodes was also not affected: 10.0±2.4 s in 21%, 7.3±1.1 s in 12% and 7.4±1.1 s in 40% oxygen (p values between 0.209 and 0.878). (Fig. 3C). The ventilation:apnea ratio fell significantly from 5.17±0.50 in hypoxia to 3.73±0.28 and 3.5±0.28 in normoxia and hyperoxia, respectively (p=0.027 and 0.035). (Figs. 2 and 3D)

The effect of breathing 40% oxygen and 12% oxygen for 30 min compared to room air on periodic breathing was examined in 10 heterozygous females. Relative hyperoxia increased the total time spent in periodic breathing from 118 ± 25 s to 242 ± 57 s 30 min⁻¹ (Fig. 5A). 12% oxygen tended to decrease the total periodic breathing to 67 ± 29 s (p=0.14). Oxygen primarily affected the number of periodic episodes: 13.1 ± 2.3 30⁻¹ min in hyperoxia, 8.3 ± 1.4 in air and 4.4 ± 1.5 in hypoxia (Fig. 5B) ((21% vs 12%, p=0.025; 21% vs 40% p=0.007). In two animals hypoxia eliminated periodic breathing (Fig. 5B). 40% oxygen tended to increase the length of each periodic breathing epoch from 14.1 ± 1.4 s in air to 17.7 ± 2.2 s (p=0.069), but hypoxia did not decrease length (12.6 ± 1.9 sec) (p=0.482)(Fig. 5C). Changes in inspired
oxygen did not affect the periodicity of periodic breathing (Data not shown).

There was a significant fall in the ventilation:apnoea ratio when Mecp2+/− mice breathed 40% oxygen to $1.82 \pm 0.17$ from $2.42 \pm 0.18$ in air ($p=0.05$).

The increase in hypoxia to $2.74 \pm 0.44$ was not significant ($p=0.408$) (Fig. 5D).

When the effects of oxygen concentration on periodic breathing in heterozygous Mecp2 deficient females are compared to wild type a number of differences are seen. These are most pronounced when the animals were exposed to 40% oxygen. The total amount of periodic breathing ($p=<0.001$), the number of episodes ($p=0.001$) and the length of each episode ($p=0.002$) were all greater in heterozygotes. The ventilation:apnea ratio was less at all three oxygen concentrations ($p$ between 0.003 and <0.001) for Mecp2+/− compared to Mecp2+/+ (Figs. 3 and 5).

The effects of varied oxygen concentrations on periodic breathing were not secondary to animal activity while breathing the three mixtures. Heterozygous females were active $45\pm9.2$ % of the time in air, $36\pm4.5$% in hypoxia and $42.3\pm6.3$% in hyperoxia ($p=0.51$) Similarly wild type mice were active $38.5\pm3.5$, $63.2\pm10.8$ and $67.5\pm12.9$% in room air, 12% and 40% oxygen respectively ($p=0.25$)
Effect of carbon dioxide.

In separate experiments the incidence of periodic breathing was studied in Mecp2<sup>+/−</sup> mice breathing 2% carbon dioxide 98% air compared to room air. Carbon dioxide reduced the total amount of periodic breathing from 103 ± 15 s to 14 ± 3 s 30 min<sup>−1</sup> (p=0.046). Both the number of episodes (5.7 ± 1.8 vs 1.3 ± 0.2) and the average length of the episodes (13.8 ± 1.2 vs 9.2 ± 0.9 sec) were less when breathing 2% carbon dioxide.
Discussion:

Telemetry recording of continuous pleural pressure for extended time periods has shown that Mecp2 deficient mice have frequent episodes of periodic breathing. As with Rett syndrome patients (17, 28) not all Mecp2+/− animals demonstrated a frequency of periodic breathing that exceeded wild type. As has been reported in human Rett subjects the Mecp2 deficient mice used in these experiments had reduced brain weights (2). Respiratory frequency in the observational protocol, when the mice were active (6.4 and 8.5 Hz) is higher than has been reported in these strains (32). Frequency in heterozygous females when inactive (2.7±0.1 Hz), however, was not different than that found in Mecp2 null males (2.95±0.5) (32). The rate for inactive wild type females in the present study (4.4±0.5) is within 1.1 SD of that found in wild type males (3.25±0.28). These previous studies were conducted using whole body plethysmography, while the animals were without limb, body and head movements (32). Thus comparison to the present frequencies while inactive is the most appropriate.

Strohl and coauthors have shown that periodic breathing in mice is affected by strain (14, 33). C57BL/6J mice demonstrated periodic breathing after short (1 or 5 min) poikilocapnic hypoxia (8% oxygen) when they were
returned to 100% oxygen. In contrast A/J animals did not show this respiratory instability (14). The authors attribute the differences in part to the fact that A/6 mice have short-term potentiation of ventilation after hypoxia that promotes stability, while C57BL/6J animals show post–hypoxic frequency decline that contributes to periodic breathing. On recovery from 5 min of poikilocapnic (8% oxygen) hypoxia heterozygous Mecp2 deficient mice showed a greater decline in ventilation that wild type (5). This may contribute to their greater prevalence of periodic breathing. Previous studies of C57BL/6J animals did not observe spontaneous episodes of periodic breathing (14). The experimental conditions were different from those reported here. Han et al (14) used whole body plethysmography and studied animals between 10:00 and 14:00h while our observations were made with telemetry between 18:00 and 21:30h with the mice in their home cages.

Recently Strohl and associates have extended their studies of periodic breathing in C57BL/6J mice (33). A one min exposure to 8% oxygen followed by recovery in 100% oxygen resulted in periodic breathing in all 9 mice treated with vehicle. In contrast the carbonic anhydrase inhibitor acetazolamide eliminated periodic breathing in all 9 animals. Since acetazolamide caused a decrease in hypercapnic ventilatory response without affecting either poikilocapnic or isocapnic hypoxic responses in
these mice, the authors conclude that respiratory instability under these conditions is caused by mechanisms that impinge on or are within the central respiratory controller. As discussed below this is consistent with the mechanisms we suggest for increased periodic breathing in heterozygous Mecp2 deficient females. It should be noted that periodic breathing in these C57BL/6J animals is always induced during hyperoxic recovery from a brief hypoxia, while the instability seen in Mecp2+/− occurs spontaneously in normoxia.

Previous studies in Mecp2 heterozygous female mice (5) and Mecp2 null males (25, 29, 32) have not reported this pattern of instability. The females were studied with body plethysmography in which they were restrained by a close fitting hole in Parafilm® about their head. These studies lasted less than one hour and were designed to measure acute responses to hypoxia or hypercapnia (5). The stimulation induced by restraint may have precluded observation of periodic breathing. Null males were examined either in whole body plethysmography (25, 32) or using a perfused working heart-brainstem preparation (29). In the whole body studies records were analyzed only when the mice were without limb, body or head movement. Since they may well have been asleep at the time (30) this may explain the difference. In the perfused working heart-brainstem experiments (29) the
lungs are removed thus eliminating the pulmonary afferents that were present in our studies. As periodic breathing episodes are often preceded by an augmented breath (please see results) pulmonary afferents may contribute to this respiratory pattern.

Periodic breathing has for some time been examined in terms of the engineering concept of loop gain (18, 19, 34). Loop gain is the ratio of the response in ventilation to that of the initial change in ventilation that produced a change in alveolar gas tensions. The changes in gas tension are sensed by chemoreceptors (central and peripheral) that in turn result in the ventilatory response. Loop gain has three components. 1) plant factors: those factors that determine the extent that pulmonary blood gas tensions will change for a given change in minute ventilation, such as functional residual capacity. 2) gain imposed by mixing of alveolar capillary blood with that in thoracic vessels and both circulatory delays from lung to peripheral and central chemoreceptors and diffusion delays in reaching central chemoreceptors. Heterozygous Mecp2 deficient females have autonomic cardiovascular regulation that is similar to wild type (6). Cardiac output and circulatory times, however, have not been determined. 3) controller gain: these include sensitivity of peripheral and central chemoreceptors to changes in blood gas tensions and the response of premotor and motoneurons to input
from these chemoreceptors. As mentioned in the introduction the ventilatory response to hypoxia is greater in Mecp2^{+/−} mice compared to Mecp2^{++/−} (4, 5). The failure of relative hyperoxia to correct periodic breathing in the heterozygotes, however, argues against increased peripheral chemoreceptors underlying their respiratory instability.

In man at altitude not all individuals demonstrate periodic breathing during sleep. The ventilatory response to hypoxia was considerably larger in periodic breathers compared to those with stable breathing (20). Similarly in periodic breathing induced by hypoxia coupled with a breathing circuit that allowed augmented inspired oxygen concentrations only a subset of subjects had instability. Periodic breathers had greater responses to both hypoxia and hypercapnia (7). In addition supplemental oxygen suppresses periodic breathing at natural altitude (20) and that seen in an altitude chamber (3). Based on this background and the findings that Mecp2 deficient mice have an augmented hypoxic response (4,5) it was anticipated that relative hyperoxia would relieve, not worsen periodic breathing. Relative hypoxia eliminated periodic breathing in a number of wild type and heterozygous Mecp2 deficient females. This is consistent with the carotid body contributing to respiratory stability in mouse. Addition of 2% carbon dioxide
significantly diminished periodic breathing in heterozygous Mecp2 deficient female mice. This response is similar to that seen in man (3) and animals (9) and suggests that the respiratory pauses in periodic breathing are associated with hypocapnia.

The ventilation:apnoea ratio in Mecp2\textsuperscript{+/−} mice varied inversely with inspired oxygen concentration (Fig. 5D). Increased apnea duration in periodic breathing reflects a greater plant gain. In animals that were ventilated at increased respiratory frequencies and tidal volume to induce periodic breathing, larger tidal volumes resulted in a fall in ventilation:apnoea (9). This mechanical increase in plant gain did not alter the period length during the unstable breathing. This is the same result we have seen with oxygen administration suggesting that relative hyperoxia increases plant gain. This effect of hyperoxia coupled with the increased total amount of periodic breathing and number of episodes compared to normoxia is consistent with the conclusion that peripheral chemoreceptors contribute to respiratory stability in Mecp2 deficient mice.

As opposed to peripheral chemoreceptors underlying periodic breathing in the Mecp2 deficient mice a central mechanism is suggested. Respiratory neurons have inhibitory inputs that occur concurrently with stimulation during inspiratory bursts. Extracellular potentials recorded from medullary
ventral respiratory group and dorsal respiratory group neurons before and after iontophoretic application of bicuculline demonstrated that the GABA_A receptor antagonist produced a marked increase in discharge (11, 27). Similarly in a neonatal rat isolated brainstem-spinal cord preparation bicuculline resulted in an increase in the integrated phrenic motoneuron (C4) burst envelope (23). Immunoblots of whole adult mouse brain showed that Mecp2^+/− mice have only 60-70% of the wild type expression of the β3 subunit of the GABA_A receptor (26). Complete lack of β3 subunit severely impairs inhibition in a number of neuronal circuits. Mice lacking β3 have a 50% reduction in the frequency and amplitude of spontaneous inhibitory postsynaptic currents (16), in the reticular thalmic nucleus. Application of the GABA_A agonist muscimol to granular cells of the olfactory bulb produced much reduced currents in these β3 null animals compared to wild type (22). A reduced GABAergic inhibition to inspiratory bursts in Mecp2^+/− mice would result in an increased controller gain that may underlie their breathing instability. It is not known whether oxygen tension modulates the GABAergic input to phrenic and medullary neurons during inspiration. GABA does increase rapidly during hypoxia (24) and therefore could modulate central drive, resulting in the decrease in periodic breathing observed.
There are, nonetheless, reports that argue against this proposed mechanism. Whole-cell electrophysiological recordings from layer 5 pyramidal neurons in slices from the somatosensory cortex of Mecp2 null male mice (\(-/y\)) found that spontaneous firing was reduced in Mecp2\(^{-/y}\) compared to wild type (10). The difference was not due to the intrinsic excitability of the neurons. Rather, the average excitatory synaptic charge was decreased and the average inhibitory synaptic charge increased in Mecp2 null males. Recent preliminary results from the same authors, however, have shown that noradrenergic neurons in the locus coeruleus of Mecp2\(^{-/y}\) animals have a firing frequency that is 1.7 times that of Mecp2\(^{+/y}\) (31). It may well be that Mecp2 deficiency has opposite effects in the brainstem compared to the cortex.

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**Disclosures:**

The authors have no conflicts of interest to disclose.

**References**


Figure legends

Fig. 1. Incidence of periodic breathing in Mecp2<sup>+/+</sup> and Mecp2<sup>+/−</sup> mice as a function of age.

Fig. 2. Inspired oxygen concentration and periodic breathing in Mecp2<sup>+/+</sup> mice. Representative traces for the three levels of oxygen. In hypoxia cyclic variations of breath amplitude are present but no distinct pauses. Short apneas are seen when breathing room air and 40% oxygen but their duration is similar in both conditions. Time calibration is the same for the three traces.

Fig. 3. Characteristics of periodic breathing in Mecp2<sup>+/+</sup> mice. A. Total amount of time spent in periodic breathing during 30 min exposures. Error bars are ± S.E.M. N = 6. Differences are not significant. B. Number of periodic breathing episodes. 12% vs 21% oxygen p=0.177. 12% vs 40% p=0.177. N = 6 for both comparisons. C. Average length of periodic
breathing episodes. Differences are not significant. D. Effect of oxygen level on the ventilation: apnea ratio in periodic breathing. V/A is greater in 12% compared to 21% (p=0.027) and 40% (p=0.035). N = 3 for 12% and 6 for 21% and 40% oxygen. Some markers and lines are superimposed.

Fig. 4. Inspired oxygen concentration and periodic breathing in Mecp2\(^{+/-}\) mice. During hypoxia the periodicity in breath amplitude is not interrupted by apneas. In normoxia the episode of periodic breathing is preceded by an augmented inspiration and there are apneas between breathing cycles. During hyperoxia the apneas lengthen compared to those in normoxia. Time calibration is the same for the three traces.

Fig. 5. Characteristics of periodic breathing in Mecp2\(^{+/-}\) mice. A. Total amount of time spent in periodic breathing during 30 min exposures. Error bars are ± S.E.M. N = 10. Normoxia vs hyperoxia p=0.001. Hypoxia vs hyperoxia p=<0.001. Normoxia vs hypoxia p=0.14. B. Number of periodic breathing episodes. 12% vs 21% p=0.025. 21% vs 40% p=0.007. C. Average length of periodic breathing episodes. 12% vs 40% p=0.055. 21% vs 40% p=0.069. N = 8 for 12% and 10 for 21% and 40% oxygen.
D. Effect of oxygen level on the ventilation: apnea ratio in periodic breathing. V/A is greater in 12% and 21% compared to 40% oxygen. 12% vs 40% p=0.046. 21% vs 40% p=0.05. N = 8 for 12% and 10 for 21% and 40% oxygen.