Published in final edited form as:

Exp Neurol. 2017 January ; 287(Pt 2): 235–242. doi:10.1016/j.expneurol.2016.07.020.

Reduced respiratory neural activity elicits a long-lasting decrease in the CO₂ threshold for apnea in anesthetized rats

N.A. Baertsch^a and T.L. Baker^a

^aDepartment of Comparative Biosciences, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53706, USA

Abstract

Two critical parameters that influence breathing stability are the levels of arterial pCO₂ at which breathing ceases and subsequently resumes – termed the apneic and recruitment thresholds (AT and RT, respectively). Reduced respiratory neural activity elicits a chemoreflex-independent, long-lasting increase in phrenic burst amplitude, a form of plasticity known as inactivity-induced phrenic motor facilitation (iPMF). The physiological significance of iPMF is unknown. To determine if iPMF and neural apnea have long-lasting physiological effects on breathing, we tested the hypothesis that patterns of neural apnea that induce iPMF also elicit changes in the AT and RT. Phrenic nerve activity and end-tidal CO₂ were recorded in urethane-anesthetized, ventilated rats to quantify phrenic nerve burst amplitude and the AT and RT before and after three patterns of neural apnea that differed in their duration and ability to elicit iPMF: brief intermittent neural apneas, a single brief “massed” neural apnea, or a prolonged neural apnea. Consistent with our hypothesis, we found that patterns of neural apnea that elicited iPMF also resulted in changes in the AT and RT. Specifically, intermittent neural apneas progressively decreased the AT with each subsequent neural apnea, which persisted for at least 60 min. Similarly, a prolonged neural apnea elicited a long-lasting decrease in the AT. In both cases, the magnitude of the AT decrease was proportional to iPMF. In contrast, the RT was transiently decreased following prolonged neural apnea, and was not proportional to iPMF. No changes in the AT or RT were observed following a single brief neural apnea. Our results indicate that the AT and RT are differentially altered by neural apnea and suggest that specific patterns of neural apnea that elicit plasticity may stabilize breathing via a decrease in the AT.

Keywords

plasticity; apnea; apneic threshold; iPMF; phrenic; chemoreflex; central sleep apnea; CSA; control of breathing; reduced respiratory neural activity; respiratory motor neurons

Corresponding author: Tracy Baker, PhD, Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive, Madison, WI, bakert@svm.vetmed.wisc.edu.

Author contributions: NAB and TLB: designed research, performed research, analyzed data and wrote the paper

Conflict of Interest: The authors declare no conflict of interest.

INTRODUCTION

There is considerable interest in the neural regulation of breathing stability, both under normal conditions, such as during sleep, and in clinical patients with ventilatory control disorders such as apnea of prematurity, central sleep apnea (CSA), chronic heart failure (CHF), spinal cord injury (SCI), and difficulty weaning from mechanical ventilation (Deak and Kirsch, 2014; Hagen, 2015; Javaheri and Dempsey, 2013; MacIntyre, 2013; Martin et al., 2012; Mateika and Syed, 2013; Strey et al., 2013; Sowho et al., 2014; Yue and Guilleminault, 2010). During steady-state conditions, changes in breathing are primarily under chemical control and are dominated by fluctuations in arterial $p\text{CO}_2$. Acting through central and peripheral chemoreceptors, CO_2 drives breathing via a negative feedback loop, and the linear relationship between the ventilatory response to changes in PaCO_2 is a major determinant of breathing stability (Dempsey and Skatrud, 1986; Javaheri and Dempsey, 2013; Khoo et al., 1982). Two critical parameters of this relationship are the point at which PaCO_2 has been sufficiently reduced for ventilation to cease and the subsequent point at which PaCO_2 has been sufficiently elevated for breathing to resume, designated as the apneic (AT) and recruitment (RT) thresholds, respectively. The difference between the level of PaCO_2 during eupnea and the PaCO_2 at the AT (i.e., the CO_2 reserve) is predictive of breathing stability, with a larger CO_2 reserve promoting stability by increasing the amount by which PaCO_2 needs to fall to cause apnea (Javaheri and Dempsey, 2013; Figure 1).

A fundamental property of the respiratory neural network that can alter ventilatory responses is plasticity, which is defined as a change in future behavior based on a prior experience (Mitchell and Johnson, 2003; Morris et al., 2003). Importantly, respiratory *plasticity* is distinct from respiratory *modulation* since plasticity does not require the presence of an ongoing stimulus, whereas modulation does. For example, following a period of reduced phrenic motor neuron activity, a long-lasting increase in phrenic motor output to the diaphragm is observed, a behavior that represents a form of respiratory plasticity called inactivity-induced phrenic motor facilitation (iPMF) (Figure 1). iPMF is pattern sensitive since it is more efficiently induced by intermittent versus sustained episodes of neural apnea (Baertsch and Baker-Herman 2013; 2015). Although iPMF has been most commonly studied following hypocapnia-induced central neural apnea (Baertsch and Baker-Herman, 2013, 2015; Broytman et al., 2013; Streeter and Baker-Herman, 2014b; Strey et al., 2012), iPMF does not require changes in chemoreceptor feedback (O_2 or CO_2 ; Mahamed et al., 2011). Instead, the primary stimulus for iPMF is reduced synaptic inputs to the phrenic motor pool since C2 axon conduction blockade in descending fiber tracts to phrenic motor neurons is sufficient to elicit iPMF (Streeter and Baker-Herman, 2014a).

Despite our growing understanding of the mechanistic characteristics of inactivity-induced respiratory plasticity, there have been few insights into the long-lasting physiological effects of neural apnea and iPMF on breathing stability. Moreover, although modulation of the AT and RT is well recognized (Altose et al., 1986; Boden et al., 1998; Chowdhuri et al., 2010b; Duffin et al., 2005; Nakayama et al., 2002; Pleschka et al., 1965; Tanaka et al., 1993), the ability of these important respiratory parameters to undergo plasticity is relatively unknown. Here, we tested the hypothesis that patterns of neural apnea associated with iPMF elicit long-lasting changes in the CO_2 -dependent AT and RT for phrenic inspiratory activity. Our

results provide the first evidence that plasticity induced by reduced respiratory neural activity (i.e., iPMF) is associated with a long-lasting decrease in the AT, without altering the RT. These findings may have important implications for our understanding of how breathing is stabilized in healthy individuals, as well as inspire novel therapeutic strategies for patients with ventilatory control disorders characterized by breathing instability.

METHODS

Animals

Data were collected from 2.5–3.5 month old male rats (n=50) from Harlan (colony 217; n=22; HSD) and Charles River (colony P09; n=28; CRSD) Sprague Dawley rat substrains. Animals were housed 2 per cage with 12 hr light/dark cycles and food and water ad libitum. All procedures and experimental protocols were approved by the Animal Care and Use Committee at the University of Wisconsin, Madison.

Surgical Procedures

All rats underwent similar anesthesia and surgical preparation as described by Baertsch and Baker-Herman (2013, 2015). Briefly, isoflurane anesthesia was induced in a closed container and then continued via a nose cone with 2.5–3.5% isoflurane (50% O₂, N₂ balance) flowing from a vaporizer. A custom heated surgery table was used to maintain body temperature near 37.0°C, which was measured via a rectal thermometer (physitemp, model 700 1H). The tail vein was catheterized for delivery of fluids (1–3 ml/hr; lactated Ringers solution, 0–20% sodium bicarbonate as necessary) to maintain blood pressure and pH throughout experimental protocols. A tracheostomy was performed to enable mechanical ventilation (Harvard Apparatus, Model 683; ~70 br/min, TV: 1 ml/100 g body weight; 50% O₂, N₂ balance). To prevent entrainment of respiratory neural activity with the ventilator, the vagus nerves were cut bilaterally at the cervical level. End-tidal CO₂ (ETCO₂) was measured continuously from the expired line immediately adjacent to the bifurcation of the ventilator circuit as an index of arterial pCO₂ with a flow-through capnograph (Capnogard, Respirationics). Tracheal pressure was monitored and the fraction of inspired CO₂ was adjusted to ensure that respiratory efforts were present throughout the surgery and to prevent unintended neural apnea. A catheter was placed in the femoral artery to monitor blood pressure and draw blood samples (~0.3 ml) for pH and blood-gas analysis (ABL800; Radiometer, Copenhagen, Denmark). Rats were transferred to urethane anesthesia (1 ml/100 g of 0.175 g/ml urethane infused at 6 ml/hr *i.v.*) and isoflurane was gradually withdrawn. Pressor responses to toe-pinch and/or corneal reflex were tested to assess depth of anesthesia, and supplemental urethane was administered *i.v.* if a response was observed. The left phrenic nerve was isolated, cut distally, de-sheathed, submerged in mineral oil, and placed on bipolar silver electrodes for electrophysiological recording. Once respiratory neural output could be monitored to ensure continued respiratory effort, pancuronium bromide was infused (1 mg/kg, *i.v.*) to induce neuromuscular paralysis.

Experimental Protocols

HSD and CRSD rats underwent the same experimental protocols. Rats were slightly hyperventilated to enable rapid induction of a neural apnea without changing ventilator

settings (see below), so a small amount of inspired CO_2 was added to the inspired gas mixture to obtain an ETCO_2 of ~ 45 mmHg (Baertsch and Baker-Herman, 2015). 15–20 min of “baseline” phrenic nerve burst amplitude and frequency was established at least one hour following transfer to urethane anesthesia to allow sufficient washout of isoflurane. An arterial blood sample was drawn to obtain baseline pCO_2 , pO_2 , and pH measurements (temperature corrected). Neural apnea was then induced in one of three different patterns: five brief intermittent neural apneas (~ 1.25 min each, separated by 5 min), a single brief massed neural apnea of an equal cumulative duration (~ 6.25 min), or a single prolonged neural apnea (30 min). To induce neural apnea, the fraction of CO_2 in the inspired gas mixture was reduced by clamping the CO_2 line upstream of the rotameter and ventilator. The ETCO_2 at the point where all rhythmic phrenic activity ceased was noted and designated as the apneic threshold (AT) for phrenic inspiratory activity. In rats receiving brief intermittent neural apnea, the flow of CO_2 was then immediately restored by removing the clamp, and the ETCO_2 at which respiratory neural activity resumed was noted and designated as the recruitment threshold (RT) for phrenic inspiratory activity. In rats exposed to brief massed or prolonged neural apnea, neural apnea was induced as stated above; however, once apneic, the flow of CO_2 was only moderately increased to maintain $\text{ETCO}_2 \sim 2$ mmHg below the AT for the duration of the apnea. Since neural apneas were elicited by manipulating the fraction of inspired CO_2 , lung volume and breathing frequency remained consistent during experimental protocols. In all rats, PaO_2 was maintained with the ventilator during neural apnea, and baseline ETCO_2 was restored following the neural apnea. Arterial blood was drawn at 5, 15, 30, and 60 min time-points following neural apnea to confirm PaCO_2 was maintained within 1.5 mmHg of baseline. At the end of each protocol another brief (~ 1.25 min) neural apnea was induced using the same method to identify any persistent effects of our treatments on the AT and RT.

Data Analysis

ETCO_2 was digitized and analyzed using PowerLab (AD Instruments; Lab Chart 7.0 software). ETCO_2 values were obtained at the onset of each neural apnea and at resumption of respiratory neural activity, indicating the AT and RT, respectively. Because we have previously demonstrated that HSD and CRSD rat substrains have similar responses to both intermittent and brief massed neural apnea (Baertsch and Baker-Herman, 2013, 2015), data from both substrains was pooled for these groups. When these substrains were analyzed independently, similar results following intermittent and brief massed neural apnea were obtained (data not shown). In rats exposed to brief intermittent neural apnea, changes in the AT and RT over the course of five consecutive neural apneas were calculated as an absolute change from the initial AT and RT. Progressive changes in the AT and RT during brief intermittent neural apnea were analyzed using a one-way repeated measures ANOVA with Tukey’s post-hoc test (Prism 6, Graph Pad software). In all protocols, differences between the AT of the first neural apnea and the AT of the neural apnea at the end of each protocol (60 min post-neural apnea) were calculated using “normalized” ETCO_2 measurements since direct measurements of PaCO_2 (not ETCO_2) were used to maintain isocapnia during electrophysiological protocols. Therefore, to account for occasional small changes in ventilation/perfusion efficiency (resulting in changes in ETCO_2 relative to PaCO_2) that can occur over time in these in vivo preparations, the AT ETCO_2 values during the first apnea

were subtracted from the ETCO_2 at baseline, and the AT values during the neural apnea 60 min post treatment were subtracted from the ETCO_2 immediately prior to the 60 min blood sample. The resulting values were then compared to determine the change in the AT and RT (see Figure 3E). Statistical differences between treatment groups were determined using one-way ANOVA and Tukey's post-hoc test (Prism 6, Graph Pad software); data are shown as mean $\pm$ SE. To examine changes in phrenic amplitude, nerve burst activity was amplified (X10k), band-pass filtered (0.3–10 kHz; AM Systems), integrated (time constant 50 ms), and rectified. The resulting signal was digitized and peak nerve burst amplitude was analyzed in 60-breath bins taken at baseline and 60 min post-neural apnea using PowerLab (AD Instruments; Lab Chart 7.0 software). Changes in phrenic amplitude at 60 min were expressed as a percent change from baseline (% baseline). Linear regression analysis was used to compare changes in the RT and AT to the magnitude of iPMF expressed following neural apnea. A significance level of $p < 0.05$ was used for all comparisons.

RESULTS

Repetitive neural apnea elicits a progressive decrease in the apneic, but not recruitment, threshold

To determine if repetitive neural apnea alters the AT and RT, five successive neural apneas were induced during concurrent ETCO_2 and phrenic recordings (Figure 2). With each subsequent neural apnea, the ETCO_2 level at which phrenic inspiratory activity ceased became progressively lower, indicating a decrease in the AT (AT 2: -0.60 ± 0.16 , AT 3: -1.3 ± 0.23 , AT 4: -1.86 ± 0.26 , AT 5: -2.34 ± 0.30). Indeed, AT 3, 4, and 5 were significantly different from zero ($p < 0.001$). By contrast, changes in the RT for phrenic inspiratory activity were relatively small and inconsistent (RT 2: 0.17 ± 0.18 , RT 3: -0.55 ± 0.35 , RT 4: -0.95 ± 0.39 , RT 5: -0.78 ± 0.41), and not significantly different from each other or zero ($p > 0.05$). Collectively, these data demonstrate that repetitive neural apnea lowers the CO_2 apneic threshold for phrenic inspiratory activity without a matching decrease in the recruitment threshold.

Changes in the apneic threshold are persistent and proportional to the magnitude of iPMF

To determine if neural apnea-induced decreases in the AT were long-lasting, the AT was re-tested 60 min following the last episode of neural apnea (Figure 3). The AT was significantly lower 60 min following 5 brief episodes of neural apnea (-2.86 ± 0.30) than during the first AT test ($p < 0.01$), indicating that intermittent neural apneas elicit a long-lasting decrease in the CO_2 apneic threshold for phrenic inspiratory activity (Figure 3 A,F). By contrast, intermittent neural apneas did not elicit a long-lasting change in the RT (0.74 ± 0.57 , $p > 0.05$; data not shown).

To determine if long-lasting decreases in the AT are a general consequence of neural apnea exposure, the AT was tested 60 min following a single "brief massed" neural apnea of an equivalent cumulative duration (~ 6.25 min) (Figure 3 B,F). In contrast to brief intermittent neural apnea, the AT 60 min following a brief massed neural apnea was not decreased (1.05 ± 0.37), indicating that a brief massed neural apnea is ineffective in producing a similar long-lasting effect on the AT.

Since brief intermittent and brief massed neural apnea differentially elicit iPMF, we tested if expression of iPMF is associated with changes in the AT. In order to address this question, we examined long-lasting changes in the AT in two substrains of Sprague Dawley rats (HSD and CRSD) that differentially express iPMF following exposure to a prolonged (i.e., 30 min) neural apnea (Baertsch and Baker-Herman, 2015; Streeter and Baker-Herman, 2014) (Figure 3 C,D). As expected HSD, but not CRSD, rats expressed long-lasting iPMF (HSD: 72 ± 10 , CRSD: 16 ± 4 , %baseline; data not shown). Similarly, the AT was differentially affected in HSD and CRSD rats following prolonged neural apnea since the AT was significantly decreased in HSD rats (-2.46 ± 0.51 ; $p < 0.0001$), but not in CRSD rats (-0.37 ± 0.29 ; $p > 0.05$), and the response in both substrains was significantly different from the other ($p < 0.05$). Because patterns of neural apnea that elicit long-lasting iPMF in HSD and CRSD rat substrains (shaded bars in Figures 3F) elicited a long-lasting decrease in the AT, we compared persistent changes in the AT with the magnitude of iPMF observed 60 min following brief intermittent, brief massed, and prolonged neural apneas for both HSD and CRSD rats (Figure 3F) and found a significant negative relationship ($R^2 = 0.345$; $p < 0.001$). If analyzed independently, HSD and CRSD rats both showed a similar negative relationship ($R^2 = 0.424$; $p = 0.001$ and $R^2 = 0.300$; $p = 0.002$, respectively; data not shown). These data indicate that the decrease in the AT is proportional to the magnitude of iPMF elicited by neural apnea.

The recruitment threshold is transiently decreased following longer durations of neural apnea

In contrast to the AT, there were no persistent changes in the RT since there were no significant differences in the RT at 60 min between treatment groups ($p > 0.05$; data not shown). However, we observed that there were differences in the RT, but not the AT, immediately after the first neural apnea between treatment groups (Figure 4; i.e., upon resumption of respiratory neural activity after the first apnea). No differences were observed in the first AT measured for each treatment ($p > 0.05$), indicating that all rats started out with a similar AT. However, the RT following prolonged neural apnea in both HSD and CRSD was significantly lower than that measured following brief massed neural apnea ($p < 0.05$), whereas brief intermittent and brief massed neural apnea were not different ($p > 0.05$; figure 4B). Thus, in both HSD and CRSD rats, only a prolonged duration of neural apnea reduced the RT. In contrast to the AT, The RT was not correlated with the magnitude of iPMF ($R^2 = 0.04$, $p = 0.16$; Figure 4B). Collectively, these data indicate that changes in the RT are not related to the expression of iPMF but are associated with prolonged exposure to neural apnea.

Blood gases

Mean PaCO_2 , PaO_2 , MAP, pH, and body temperature were monitored and analyzed during critical time-points in all experimental protocols; these variables are presented for baseline and 60 min following neural apnea in Table 1. 60 min following brief intermittent neural apneas, there was a small decrease in PaO_2 ; however, oxygen was maintained well above (> 180 mmHg) levels that would be expected to impact phrenic motor activity in this preparation. In HSD rats, there was a small (< 0.03) but significant decrease in pH following prolonged neural apnea, however this decrease was not correlated with changes in the AT,

RT, or the expression of iPMF ($p>0.05$), and is therefore unlikely to have influenced our results. There were no statistically significant differences in PaCO_2 , PaO_2 , or body temperature between substrains or experimental groups at any time point ($p>0.05$). However, HSD rats had a higher baseline mean arterial pressure (MAP) than CRSD rats ($p<0.05$), as previously reported (Fuller et al., 2001). There was a small but significant decrease in MAP following intermittent neural apnea and CRSD prolonged neural apnea ($p>0.05$), which is typical of this surgical preparation (Baertsch and Baker-Herman, 2013; Streeter and Baker-Herman, 2014a). Since small changes in MAP (~ 20 mmHg) do not significantly alter phrenic motor output in rats (Walker and Jennings, 1998) and these changes were not related to changes in the AT, RT, or the expression of iPMF, these changes are not considered to have contributed to our results. Similar to values previously reported (Boden et al., 1998; 2000), the mean AT (during the first neural apnea) for all rats was 30.8 ± 0.5 mmHg, and there were no significant differences between rat substrains or treatment group ($p>0.05$). Therefore, similar baseline conditions were achieved for all groups and critical physiological variables were tightly regulated throughout all experimental protocols.

DISCUSSION

Here, we present data that support our overarching hypothesis that CO_2 thresholds for breathing can be remarkably adaptable in response to central neural apnea. Our data reveal that the apneic threshold (AT) progressively decreases during recurring central neural apneas, suggesting that reductions in respiratory neural activity transform the respiratory control network in a way that minimizes the likelihood of a subsequent apnea. This plasticity in the AT is long-lasting and requires specific patterns of neural apnea for it to be induced. Strikingly, changes in the AT in response to central neural apnea are proportional to the magnitude of a form of locally-mediated plasticity in phrenic motor output induced by a reduction in phrenic synaptic inputs (i.e., iPMF), suggesting that these mechanisms may be linked. By contrast, the recruitment threshold (RT) is only transiently decreased following long durations of neural apnea, and this decrease is not associated with long-lasting phrenic motor plasticity. Thus, CO_2 -dependent breathing thresholds are differentially affected by neural apnea and the induction of phrenic plasticity induced by reduced respiratory related synaptic inputs.

Modulation and plasticity of CO_2 thresholds

The specific levels of arterial CO_2 necessary to maintain breathing as well as to initiate breathing are not static. Indeed, the AT and RT are highly state dependent. During wakefulness, the presence of the so called “wakefulness drive” to breathe provides the respiratory control centers with excitatory input, stabilizing breathing by lowering the AT and increasing the CO_2 reserve. As a result, central apnea is extremely rare during wakefulness. However, during sleep the “wakefulness drive” to breathe is lost and the level of PaCO_2 sufficient to induce central apnea becomes much closer to the eupneic PaCO_2 (i.e., reduced CO_2 reserve), which increases the propensity for apnea (Javaheri and Dempsey, 2013). Due to the inherent difficulty of measuring the AT and RT in rats with highly fragmented natural sleep, we studied these parameters under steady-state urethane anesthesia since it is widely accepted that the wakefulness drive to breathe is lost during general

anesthesia (Fink et al., 1963), and urethane has effects on breathing similar to natural sleep (Pagliardini et al., 2012; 2013).

In addition to sleep, other factors that change respiratory drive can influence the stability of breathing through modulation of the ventilatory thresholds to CO₂ (Nakayama et al., 2002). “Modulation” refers to a change that is observed during the continued presence of a stimulus, but once that stimulus is gone, the system reverts back to its previous state. Generally, stimuli that result in hyperventilation stabilize breathing (Chowdhuri et al., 2010b; Fiamma et al., 2013; White et al., 1982), despite lowering eupneic PaCO₂, by decreasing the AT and thereby widening the CO₂ reserve. Conversely, stimuli that result in hypoventilation tend to destabilize breathing (Dempsey et al., 2004, Nakayama et al., 2002) by increasing the AT and narrowing the CO₂ reserve. The AT may also be modulated by variables such as body temperature (Pleschka et al., 1965), pH (Duffin, 2005), changes in mechanical feedback (Altose et al., 1986), postnatal maturity (Canet et al., 1993), and sex hormones (Rowley et al., 2006; Zhou et al., 2003). To minimize unintended modulation of the AT and RT, care was taken to maintain relevant variables constant throughout experimental protocols (Table 1), adult male rats were used in all cases, and rats were vagotomized, paralyzed, and ventilator tidal volume and frequency were maintained constant before and after neural apnea to minimize changes in mechanical feedback. Therefore, modulation of the AT through these mechanisms is unlikely to have contributed to our results.

Plasticity of CO₂ ventilatory thresholds is less clearly understood. Unlike modulation, plasticity implies a persistent change in a response, which continues in the absence of the stimulus. There are mixed reports in the literature suggesting that intermittent episodes of hypoxia may elicit plasticity of the AT (Ling et al., 2001; Katayama et al., 2007; Mahamed and Mitchell, 2008a; 2008b; but see Chowdhuri et al., 2010a; Xie et al., 2001), which has been speculated to result in improved breathing stability. In this study, we directly examined the effect of central neural apnea (without hypoxia) on the AT and found that only specific patterns of neural apnea result in a long-lasting decrease in the AT, an effect that is predicted to promote breathing stability by increasing the amount PaCO₂ must change to induce a subsequent apnea. However, direct measurements of the “true” CO₂ reserve were not possible since rats were mechanically ventilated, creating an artificial “eupneic” PaCO₂ that was held constant before and after neural apnea. Indeed, in a spontaneously breathing rat, the associated increase in phrenic motor output (i.e., iPMF) following neural apnea is likely to lower eupneic PaCO₂, which may limit any gains in CO₂ reserve created by the decreased AT. However, we hypothesize that similar to other stimuli that increase ventilatory drive (Chowdhuri et al., 2010b; Fiamma et al., 2013; White et al., 1982), increased phrenic motor output and a decreased AT following neural apnea stabilizes breathing through a net increase in CO₂ reserve.

Does phrenic motor plasticity alter the apneic threshold?

Traditionally, the AT is thought to be “set” by peripheral and central chemoreceptor interactions with brainstem respiratory rhythm generating neurons (Javahari and Dempsey, 2013; Smith et al., 2007). However, here we provide evidence that changes in the AT are

associated with a form of spinal motor plasticity, iPMF. This observation is striking since available evidence suggests that mechanisms local to the phrenic motor pool give rise to iPMF (Strey et al., 2012; Streeter and Baker-Herman, 2014a). Thus, a central question is: where does plasticity in the AT occur and how is it related to iPMF induction/expression? Since the effects of neural apnea on the respiratory neural network are widespread, and plasticity has been shown to occur at multiple levels within this system (Brocciaro and Feldman, 2004; Dahan et al., 2007; Dutschmann and Dick, 2012; Forester et al., 2010; Johnson and Mitchell, 2013; Kumar and Prabhakar, 2012; Streeter and Baker-Herman, 2014a), understanding how and where neural apnea elicits plasticity in the AT is not straightforward. Indeed, there are multiple possible explanations for how changes in the AT and expression of iPMF may be linked: 1) Spinal iPMF may be only one of multiple forms of plasticity induced by neural apnea that are expressed both spinally and supra-spinally. Thus, it is possible that mechanisms occurring elsewhere in the respiratory circuit (e.g., respiratory rhythm generator) that communicate with the phrenic motor pool give rise to neural apnea-induced plasticity in the AT. 2) Neural apnea-induced changes in the AT may be the result of the same spinal mechanisms that give rise to iPMF. Indeed, the amount that the AT is changed following neural apnea is proportional to iPMF magnitude. Moreover, a distinct serotonin-dependent form of spinal phrenic motor plasticity known as phrenic long-term facilitation (pLTF) (Baker-Herman and Mitchell, 2002) is associated with an increase in the AT to RT difference, which is no longer observed when pLTF is blocked with spinal methysergide (serotonin receptor antagonist) (Mahamed and Mitchell, 2008a; 2008b). Mechanisms whereby the AT is altered by phrenic motor neuron plasticity are unknown; however, it is possible that some brainstem inspiratory neurons may remain rhythmically active near the AT, but fail to transmit this activity to motor pools with high thresholds for activation (Batsel, 1967; Ezure et al., 2003; Garcia et al., 2016; Kam et al., 2013; St. John, 1998). iPMF may augment phrenic motor neuron excitability sufficiently to enable expression of this otherwise sub-threshold descending drive. Finally, 3) Spinal plasticity could induce medullary plasticity indirectly via spinal interneurons with spinobulbar projections (Fuller et al. 2013, Golder et al., 2001; Lane et al., 2009). Indeed, C2 hemisection elicits spinal and brainstem respiratory plasticity (Golder et al., 2001; Zimmer and Goshgarian, 2007), possibly via alterations in spinal interneuronal connectivity (Lane, 2011). Of interest, some reports suggest a lower AT after spinal injury in rats (Golder et al., 2011) and humans (Simon et al., 1995; but see Sankari et al., 2014). Further studies are warranted to differentiate between these diverging hypotheses.

Interactions between the recruitment threshold and neural apnea duration

In contrast to the AT, long-lasting plasticity in the RT was not elicited by central neural apnea. Instead, a transient decrease in the RT was only observed following a prolonged duration of hypocapnia-induced neural apnea, which recovered to baseline levels within 60 min. This observation is consistent with previous reports in spontaneously breathing humans and dogs (Edelist and Osorio, 1969; Neumark et al., 1975). It has been hypothesized that during hyperventilation, HCO_3^- is transported out of the CSF, preventing a sustained alkalosis during hypocapnia. Once hyperventilation is reversed, the rise in pCO_2 causes a decrease in the pH of the cerebrospinal fluid (CSF) to more acidotic levels, providing a higher drive to breathe at a lower PaCO_2 (Edelist and Osorio, 1969). Longer durations of

hypocapnia result in a greater reduction in CSF HCO_3^- , therefore enhancing this effect and lowering the RT (Edelist and Osorio, 1969). Since rats were mechanically ventilated in our studies allowing PaCO_2 to be restored to baseline levels following neural apnea, one might expect an even greater initial decrease in CSF pH following reversal of neural apnea that gradually equilibrates as CSF HCO_3^- is restored. Consistent with this possibility, CSF acidification can drive breathing through an increase in respiratory frequency (Krause et al., 2009), and a transient increase in respiratory frequency is observed following neural apnea that is much greater following prolonged *versus* brief intermittent or brief massed neural apnea (Baertsch and Baker-Herman, 2013; 2015).

Significance of neural apnea, iPMF, and the apneic threshold

Breathing instability, particularly during sleep, is a major clinical challenge that often results in pathological reductions in respiratory neural activity (Strey et al., 2013). For example, central sleep apnea (CSA), which is characterized by recurrent episodes of absent or reduced (hypopnea) respiratory motor output, is common in the clinical population (De Backer, 1995; Javaheri and Dempsey, 2013). Central apnea may also occur in healthy individuals during sleep; however, the frequency of apneic events is typically minimal and not considered clinically significant (Javaheri, 2010). Repetitive central neural apnea is also a hallmark of Cheyne-Stokes respiration typically observed in patients with chronic heart failure (CHF; Traversi et al., 1997), and is common in infants, with a higher prevalence in preterm infants (“apnea of prematurity”; Eichenwald et al., 1997; Khan et al., 2005). Even in otherwise healthy individuals, periodic breathing and central apnea become common during sleep upon ascent to altitude (Pack, 2011).

A shared feature in the majority of individuals with these conditions is a narrow CO_2 reserve (Javaheri and Dempsey, 2013). Indeed, elevating eupneic PaCO_2 by a small amount ($\sim 2\text{--}3$ mmHg) to increase the CO_2 reserve often minimizes central neural apnea and stabilizes breathing in CHF (Javaheri and Dempsey, 2013), CSA (Xie et al., 1997), during apnea of prematurity (Alvaro et al., 2012), and at altitude (Lahiri et al., 1983). In infants, the high incidence of periodic breathing commonly observed from 2–4 weeks of age is also likely due, at least in part, to a narrow CO_2 reserve (Khan et al., 2005). However, this seems to improve with development since breathing stabilizes by ~ 6 months of age, and by adolescence, children are more resistant to periodic breathing during sleep at altitude than adults due to a lower AT (Edwards et al., 2013; Kohler et al., 2008). We speculate that mechanisms of plasticity induced by repetitive episodes of central neural apnea may represent a homeostatic response that prevents such events from occurring again by increasing phrenic motor output and lowering the PaCO_2 at which apnea occurs, thereby stabilizing breathing. We further speculate that a failure of these mechanisms may contribute to pathological respiratory instability in patients with ventilatory control disorders characterized by central apneas.

Acknowledgments

This work was supported by a grant from the National Heart, Lung and Blood Institute (NHLBI), grant number HL105511.

Abbreviations

aCSF	artificial cerebrospinal fluid
AT	apneic threshold
CHF	chronic heart failure
CRSD	Charles River Sprague Dawley
CSA	central sleep apnea
ETCO₂	end-tidal CO ₂
HSD	Harlan Sprague Dawley
iPMF	inactivity-induced phrenic motor facilitation
OSA	obstructive sleep apnea
pLTF	phrenic long-term facilitation
RT	recruitment threshold

References

- Altose MD, Castele RJ, Connors AF Jr, Dimarco AF. Effects of volume and frequency of mechanical ventilation on respiratory activity in humans. *Respir Physiol.* 1986; 66(2):171–80. [PubMed: 3101155]
- Alvaro RE, Khalil M, Qurashi M, Al-Saif S, Al-Matary A, Chiu A, Minski J, Manfreda J, Kwiatkowski K, Cates D, Rigatto H. CO₂ inhalation as a treatment for apnea of prematurity: a randomized double-blind controlled trial. *Journal of Pediatrics.* 2012; 160:252–257. [PubMed: 21907349]
- Baertsch NA, Baker-Herman TL. Inactivity-induced phrenic and hypoglossal motor facilitation are differentially expressed following intermittent vs. sustained neural apnea. *J Appl Physiol.* 2013; 114(10):1388–95. [PubMed: 23493368]
- Baertsch NA, Baker-Herman TL. Intermittent reductions in respiratory neural activity elicit spinal TNF α -independent, atypical PKC-dependent phrenic motor facilitation. *Am J Physiol Regul Integr Comp Physiol.* 2015; 308(8):R700–7. [PubMed: 25673781]
- Baker-Herman TL, Mitchell GS. Phrenic long-term facilitation requires spinal serotonin receptor activation and protein synthesis. *J Neurosci.* 2002; 22(14):6239–46. [PubMed: 12122082]
- Batsel HL. Activity of bulbar respiratory neurons during passive hyperventilation. *Exp Neurol.* 1967; 19(3):357–74. [PubMed: 6053660]
- Bocchiaro CM, Feldman JL. Synaptic activity-independent persistent plasticity in endogenously active mammalian motoneurons. *Proc Natl Acad Sci USA.* 2004; 101(12):4292–5. [PubMed: 15024116]
- Boden AG, Harris MC, Parkes MJ. apneic threshold for CO₂ in the anesthetized rat: fundamental properties under steady-state conditions. *J Appl Physiol.* 1998; 85(3):898–907. [PubMed: 9729563]
- Boden AG, Harris MC, Parkes MJ. A respiratory drive in addition to the increase in CO₂ production at raised body temperature in rats. *Exp Physiol.* 2000; 85(3):309–19. [PubMed: 10825418]
- Broytman O, Baertsch NA, Baker-Herman TL. Spinal TNF is necessary for inactivity-induced phrenic motor facilitation. *J Physiol.* 2013; 591(Pt 22):5585–98. [PubMed: 23878370]
- Canet E, Praud JP, Laberge JM, Blanchard PW, Bureau MA. Apnea threshold and breathing rhythmicity in newborn lambs. *J Appl Physiol.* 1993; 74(6):3013–9. [PubMed: 8366001]

- Chowdhuri S, Shanidze I, Pierchala L, Belen D, Mateika JH, Badr MS. Effect of episodic hypoxia on the susceptibility to hypocapnic central apnea during NREM sleep. *J Appl Physiol*. 2010a; 108(2): 369–77. [PubMed: 19940101]
- Chowdhuri S, Sinha P, Pranathiageswaran S, Badr MS. Sustained hyperoxia stabilizes breathing in healthy individuals during NREM sleep. *J Appl Physiol*. 2010b; 109(5):1378–83. [PubMed: 20724559]
- Dahan A, Nieuwenhuijs D, Teppema L. Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO₂ sensitivity. *PLoS Med*. 2007; 4(7):e239. [PubMed: 17676946]
- Deak MC, Kirsch DB. Sleep-disordered breathing in neurologic conditions. *Clin Chest Med*. 2014; 35(3):547–56. [PubMed: 25156770]
- De Backer WA. Central sleep apnea, pathogenesis and treatment: an overview and perspective. *Eur Respir J*. 1995; 8(8):1372–83. [PubMed: 7489805]
- Dempsey JA. Crossing the apneic threshold: causes and consequences. *Exp Physiol*. 2004; 90(1):13–24. [PubMed: 15572458]
- Dempsey JA, Skatrud JB. A sleep-induced apneic threshold and its consequences. *Am Rev Respir Dis*. 1986; 133(6):1163–70. [PubMed: 3717765]
- Duffin J. Role of acid-base balance in the chemoreflex control of breathing. *J Appl Physiol*. 2005; 99(6):2255–65. [PubMed: 16109829]
- Dutschmann M, Dick TE. Pontine mechanisms of respiratory control. *Compr Physiol*. 2012; 2(4): 2443–69. [PubMed: 23720253]
- Edelist G, Osorio A. Postanesthetic initiation of spontaneous ventilation after passive hyperventilation. *Anesthesiology*. 1969; 31(3):222–7. [PubMed: 5801263]
- Edwards BA, Sands SA, Berger PJ. Postnatal maturation of breathing stability and loop gain: the role of carotid chemoreceptor development. *Respir Physiol Neurobiol*. 2013; 185(1):144–55. [PubMed: 22705011]
- Eichenwald EC, Aina A, Stark AR. Apnea frequently persists beyond term gestation in infants delivered at 24 to 28 weeks. *Pediatrics*. 1997; 100:354–359. [PubMed: 9282705]
- Ezure K, Tanaka I, Saito Y. Activity of brainstem respiratory neurones just before the expiration-inspiration transition in the rat. *J Physiol*. 2003; 547(Pt 2):629–40. [PubMed: 12562954]
- Fiamma MN, O'Connor ET, Roy A, Zuna I, Wilson RJ. The essential role of peripheral respiratory chemoreceptor inputs in maintaining breathing revealed when CO₂ stimulation of central chemoreceptors is diminished. *J Physiol*. 2013; 591(Pt 6):1507–21. [PubMed: 23359670]
- Fink BR, Hanks EC, Ngai SH, Papper EM. Central regulation of respiration during anaesthesia and wakefulness. *Ann NY Acad Sci*. 1963; 109:892–899.
- Forster HV, Krause KL, Kiner T, Neumueller SE, Bonis JM, Qian B, Pan LG. Plasticity of respiratory rhythm-generating mechanisms in adult goats. *Adv Exp Med Biol*. 2010; 669:151–5. [PubMed: 20217339]
- Fuller DD, Baker TL, Behan M, Mitchell GS. Expression of hypoglossal long-term facilitation differs between substrains of Sprague-Dawley rat. *Physiol Genomics*. 2001; 4(3):175–81. [PubMed: 11160996]
- Fuller DD, Lee KZ, Tester NJ. The impact of spinal cord injury on breathing during sleep. *Respir Physiol Neurobiol*. 2013; 188(3):344–54. [PubMed: 23791824]
- Garcia AJ 3rd, Zanella S, Dashevskiy T, Khan SA, Khuu MA, Prabhakar NR, Ramirez JM. Chronic Intermittent Hypoxia Alters Local Respiratory Circuit Function at the Level of the preBötzinger Complex. *Front Neurosci*. 2016; 10:4.doi: 10.3389/fnins.2016.00004 [PubMed: 26869872]
- Golder FJ, Fuller DD, Lovett-Barr MR, Vinit S, Resnick DK, Mitchell GS. Breathing patterns after mid-cervical spinal contusion in rats. *Exp Neurol*. 2011; 231(1):97–103. [PubMed: 21683697]
- Golder FJ, Reier PJ, Davenport PW, Bolser DC. Cervical spinal cord injury alters the pattern of breathing in anesthetized rats. *J Appl Physiol*. 2001; 91(6):2451–8. [PubMed: 11717204]
- Hagen EM. Acute complications of spinal cord injuries. *World J Orthop*. 2015; 6(1):17–23. [PubMed: 25621207]
- Javaheri S. Central sleep apnea. *Clin Chest Med*. 2010; 31(2):235–48. [PubMed: 20488284]

- Javaheri S, Dempsey JA. Central sleep apnea. *Compr Physiol*. 2013; 3(1):141–63. [PubMed: 23720283]
- Johnson RA, Mitchell GS. Common mechanisms of compensatory respiratory plasticity in spinal neurological disorders. *Respir Physiol Neurobiol*. 2013; 189(2):419–28. [PubMed: 23727226]
- Kam K, Worrell JW, Janczewski WA, Cui Y, Feldman JL. Distinct inspiratory rhythm and pattern generating mechanisms in the preBötzinger complex. *J Neurosci*. 2013; 33(22):9235–45. [PubMed: 23719793]
- Katayama K, Smith CA, Henderson KS, Dempsey JA. Chronic intermittent hypoxia increases the CO₂ reserve in sleeping dogs. *J Appl Physiol*. 2007; 103(6):1942–9. [PubMed: 17932301]
- Khan A, Qurashi M, Kwiatkowski K, Cates D, Rigatto H. Measurement of the CO₂ apneic threshold in newborn infants: possible relevance for periodic breathing and apnea. *J Appl Physiol*. 2005; 98(4):1171–6. [PubMed: 15772056]
- Khoo MC, Kronauer RE, Strohl KP, Slutsky AS. Factors inducing periodic breathing in humans: a general model. *J Appl Physiol Respir Environ Exerc Physiol*. 1982; 53(3):644–59. [PubMed: 7129986]
- Kohler M, Kriemler S, Wilhelm EM, Brunner-LaRocca H, Zehnder M, Bloch KE. Children at high altitude have less nocturnal periodic breathing than adults. *Eur Respir J*. 2008; 32(1):189–97. [PubMed: 18287125]
- Kumar P, Prabhakar NR. Peripheral chemoreceptors: function and plasticity of the carotid body. *Compr Physiol*. 2012; 2(1):141–219. [PubMed: 23728973]
- Krause KL, Forster HV, Davis SE, Kiner T, Bonis JM, Pan LG, Qian B. Focal acidosis in the pre-Bötzinger complex area of awake goats induces a mild tachypnea. *J Appl Physiol*. 2009; 106(1):241–50. [PubMed: 19008492]
- Lahiri S, Maret K, Sherpa MG. Dependence of high altitude sleep apnea on ventilatory sensitivity to hypoxia. *Respir Physiol*. 1983; 52(3):281–301. [PubMed: 6412339]
- Lane MA. Spinal respiratory motoneurons and interneurons. *Respir Physiol Neurobiol*. 2011; 179(1):3–13. [PubMed: 21782981]
- Lane MA, Lee KZ, Fuller DD, Reier PJ. Spinal circuitry and respiratory recovery following spinal cord injury. *Respir Physiol Neurobiol*. 2009; 169(2):123–32. [PubMed: 19698805]
- Ling L, Fuller DD, Bach KB, Kinkead R, Olson EB Jr, Mitchell GS. Chronic intermittent hypoxia elicits serotonin-dependent plasticity in the central neural control of breathing. *J Neurosci*. 2001; 21(14):5381–8. [PubMed: 11438615]
- MacIntyre NR. The ventilator discontinuation process: an expanding evidence base. *Respir Care*. 2013; 58(6):1074–86. [PubMed: 23709201]
- Mahamed S, Mitchell GS. Respiratory long-term facilitation: too much or too little of a good thing? *Adv Exp Med Biol*. 2008a; 605:224–7. [PubMed: 18085276]
- Mahamed S, Mitchell GS. Simulated apneas induce serotonin-dependent respiratory long-term facilitation in rats. *J Physiol*. 2008b; 586(8):2171–81. [PubMed: 18292130]
- Mahamed S, Strey KA, Mitchell GS, Baker-Herman TL. Reduced respiratory neural activity elicits phrenic motor facilitation. *Respir Physiol Neurobiol*. 2011; 175(3):303–9. [PubMed: 21167322]
- Martin RJ, Di Fiore JM, Macfarlane PM, Wilson CG. Physiologic basis for intermittent hypoxic episodes in preterm infants. *Adv Exp Med Biol*. 2012; 758:351–8. [PubMed: 23080182]
- Mateika JH, Syed Z. Intermittent hypoxia, respiratory plasticity and sleep apnea in humans: present knowledge and future investigations. *Respir Physiol Neurobiol*. 2013; 188(3):289–300. [PubMed: 23587570]
- Mitchell GS, Johnson SM. Neuroplasticity in respiratory motor control. *J Appl Physiol*. 2003; 94(1):358–74. [PubMed: 12486024]
- Morris KF, Baekey DM, Nuding SC, Dick TE, Shannon R, Lindsey BG. Invited review: Neural network plasticity in respiratory control. *J Appl Physiol*. 2003; 94(3):1242–52. [PubMed: 12571145]
- Nakayama H, Smith CA, Rodman JR, Skatrud JB, Dempsey JA. Effect of ventilatory drive on carbon dioxide sensitivity below eupnea during sleep. *Am J Respir Crit Care Med*. 2002; 165(9):1251–60. [PubMed: 11991874]

- Neumark J, Bardeen A, Sulzer E, Kampine JP. Miniature intravascular pCO₂ sensors in neurosurgery. *J Neurosurg.* 1975; 43(2):172–6. [PubMed: 1185248]
- Pack AI. Central sleep apnea. *Handbook Clin Neurol.* 2011; 98:411L9.
- Pagliardini S, Funk GD, Dickson CT. Breathing and brain state: urethane anesthesia as a model for natural sleep. *Respir Physiol Neurobiol.* 2013; 188(3):324–32. [PubMed: 23751523]
- Pagliardini S, Greer JJ, Funk GD, Dickson CT. State-dependent modulation of breathing in urethane-anesthetized rats. *J Neurosci.* 2012; 32(33):11259–70. [PubMed: 22895710]
- Pleschka K, Albers C, Heerd E. Effect of body temperature on the CO₂ response threshold of the respiratory system. *Pflugers Arch Gesamte Physiol Menschen Tiere.* 1965; 286(2):142–58.
- Rowley JA, Zhou XS, Diamond MP, Badr MS. The determinants of the apnea threshold during NREM sleep in normal subjects. *Sleep.* 2006; 29(1):95–103. [PubMed: 16453986]
- Sankari A, Bascom AT, Chowdhuri S, Badr MS. Tetraplegia is a risk factor for central sleep apnea. *J Appl Physiol.* 2014; 116(3):345–53. [PubMed: 24114704]
- Simon PM, Leever AM, Murty JL, Skatrud JB, Dempsey JA. Neuromechanical regulation of respiratory motor output in ventilator-dependent C1–C3 quadriplegics. *J Appl Physiol.* 1995; 79(1):312–23. [PubMed: 7559237]
- Smith CA, Chenuel BJ, Henderson KS, Dempsey JA. The apneic threshold during non-REM sleep in dogs: sensitivity of carotid body vs. central chemoreceptors. *J Appl Physiol.* 2007; 103(2):578–86. [PubMed: 17495123]
- St-John WM. Alterations in respiratory neuronal activities in the medullary ‘pre-Bötzinger’ region in hypocapnia. *Respir Physiol.* 1998; 114(2):119–31. [PubMed: 9865586]
- Streeter KA, Baker-Herman TL. Decreased spinal synaptic inputs to phrenic motor neurons elicit localized inactivity-induced phrenic motor facilitation. *Exp Neurol.* 2014a; 256:46–56. [PubMed: 24681155]
- Streeter KA, Baker-Herman TL. Spinal NMDA receptor activation constrains inactivity-induced phrenic motor facilitation in Charles River Sprague-Dawley rats. *J Appl Physiol.* 2014b; 117(7): 682–93. [PubMed: 25103979]
- Strey KA, Baertsch NA, Baker-Herman TL. Inactivity-induced respiratory plasticity: protecting the drive to breathe in disorders that reduce respiratory neural activity. *Respir Physiol Neurobiol.* 2013; 189(2):384–94. [PubMed: 23816599]
- Strey KA, Nichols NL, Baertsch NA, Brodyman O, Baker-Herman TL. Spinal atypical protein kinase C activity is necessary to stabilize inactivity-induced phrenic motor facilitation. *J Neurosci.* 2012; 32(46):16510–20. [PubMed: 23152633]
- Sowho M, Amatoory J, Kirkness JP, Patil SP. Sleep and respiratory physiology in adults. *Clin Chest Med.* 2014; 35(3):469–81. [PubMed: 25156763]
- Tanaka A, Nishino T, Hiraga K. The effect of PaO₂ on apneic threshold PCO₂ (PATCO₂). *Masui.* 1993; 42(12):1783–7. [PubMed: 8301825]
- Traversi E, Callegari G, Pozzoli M, Opasich C, Tavazzi L. Sleep disorders and breathing alterations in patients with chronic heart failure. *G Ital Cardiol.* 1997; 27(5):423–9. [PubMed: 9199954]
- Walker JK, Jennings DB. Respiratory effects of pressor and depressor agents in conscious rats. *Can J Physiol Pharmacol.* 1998; 76:707–714. [PubMed: 10030450]
- White DP, Zwillich CW, Pickett CK, Douglas NJ, Findley LJ, Weil JV. Central sleep apnea. Improvement with acetazolamide therapy. *Arch Intern Med.* 1982; 142(10):1816–9. [PubMed: 6812522]
- Xie A, Rankin F, Rutherford R, Bradley TD. Effects of inhaled CO₂ and added dead space on idiopathic central sleep apnea. *J Appl Physiol.* 1997; 82(3):918–26. [PubMed: 9074983]
- Xie A, Skatrud JB, Dempsey JA. Effect of hypoxia on the hypopnoeic and apnoeic threshold for CO₂ in sleeping humans. *J Physiol.* 2001; 535(Pt 1):269–78. [PubMed: 11507176]
- Yue HJ, Guillemineault C. Opioid medication and sleep-disordered breathing. *Med Clin North Am.* 2010; 94(3):435–46. [PubMed: 20451025]
- Zhou XS, Rowley JA, Demirovic F, Diamond MP, Badr MS. Effect of testosterone on the apneic threshold in women during NREM sleep. *J Appl Physiol.* 2003; 94(1):101–7. [PubMed: 12391093]

Zimmer MB, Goshgarian HG. Spinal cord injury in neonates alters respiratory motor output via supraspinal mechanisms. *Exp Neurol*. 2007; 206(1):137–45. [PubMed: 17559837]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HIGHLIGHTS

- Repeated central neural apneas progressively decrease the apneic threshold
- Intermittent and prolonged neural apneas elicit iPMF, a form of spinal plasticity
- Long-lasting decreases in the apneic threshold are associated with iPMF
- Prolonged neural apneas transiently decrease the recruitment threshold
- Decreases in the recruitment threshold are not associated with iPMF

Figure 1.

Schematic defining iPMF and respiratory parameters pertaining to breathing stability.

Reduced phrenic neural activity during central neural apnea elicits a rebound increase in drive to the diaphragm via augmentation of phrenic nerve burst amplitude (iPMF). A neural apnea can occur if CO_2 is reduced below the apneic threshold (AT), and breathing will resume once CO_2 has been subsequently elevated above the recruitment threshold (RT). The difference between CO_2 during normal breathing (eupnea) and the level of CO_2 at which the neural drive to breathe is lost (apneic threshold) is defined as the CO_2 reserve.

Figure 2.

Intermittent neural apnea causes a progressive decrease in the apneic threshold, but not the recruitment threshold. **A.** Representative compressed phrenic neurogram (*top*) and concurrent end-tidal CO₂ trace (*bottom*) during five brief neural apneas. Colored arrows indicate the initial AT and RT to which all subsequent apneic and recruitment thresholds (black arrows) are compared. **B.** Mean changes in the AT (*left*) and the RT (*right*) during intermittent neural apnea. Diagonal shading indicates significantly different from zero. *significantly different according to Tukey's post-hoc test; p<0.05. Data are presented as means±SE.

Figure 3.

iPMF is associated with a persistent and proportional decrease in the apneic threshold. **A–D.** Representative compressed phrenic neurograms and concurrent end-tidal CO₂ traces during brief intermittent (**A**), brief massed (**B**), and prolonged (**C**, **D**) neural apnea and during a subsequent neural apnea 60 min later. **E.** Schematic depicting quantification of long-lasting changes in the AT. **F.** Average long-lasting changes in the AT and scatter plot showing the negative linear relationship between changes in the AT and the magnitude of iPMF. Shaded bars denote treatments that elicit long-lasting iPMF. *significantly different according to Tukey's post-hoc test; $p<0.05$. Data are presented as means $\pm$ SE.

Figure 4.

The recruitment threshold is decreased following prolonged durations of neural apnea. **A.** Representative compressed phrenic neurograms and concurrent end-tidal CO₂ traces (same as shown in Figure 3 A–C) during brief intermittent (*top*), brief massed (*middle*), and prolonged (*bottom*) neural apneas. Red and blue stars indicate the AT and RT during the first neural apnea, respectively. **B.** Average AT and RT of the first neural apnea relative to baseline ETCO₂ and scatter plot comparing changes in the RT and phrenic amplitude (iPMF). Shaded bars indicate treatments that elicit long-lasting iPMF. *significantly different according to Tukey's post-hoc test; $p<0.05$. Data are presented as means $\pm$ SE.

Table 1

Physiological variables at baseline and 60 min following neural apnea. Values are means $\pm$ SE. PaCO₂, partial pressure of arterial CO₂; PaO₂, partial pressure of O₂; MAP, mean arterial pressure.

Neural apnea pattern	Time	PaCO ₂ (mmHg)	PaO ₂ (mmHg)	MAP (mmHg)	pH	Temp (°C)
Brief Intermittent	Baseline	45.9 $\pm$ 0.7	284 $\pm$ 3	120 $\pm$ 6	7.37 $\pm$ 0.01	37.15 $\pm$ 0.03
	60 min	46.4 $\pm$ 0.8	271 $\pm$ 7*	101 $\pm$ 6*	7.36 $\pm$ 0.01	37.08 $\pm$ 0.03
Brief Massed	Baseline	45.2 $\pm$ 0.7	283 $\pm$ 7	118 $\pm$ 7	7.36 $\pm$ 0.01	37.07 $\pm$ 0.06
	60 min	45.3 $\pm$ 0.8	285 $\pm$ 5	119 $\pm$ 6	7.36 $\pm$ 0.01	37.14 $\pm$ 0.04
HSD Prolonged	Baseline	46.9 $\pm$ 0.8	286 $\pm$ 7	150 $\pm$ 3 ^a	7.35 $\pm$ 0.01	37.12 $\pm$ 0.04
	60 min	47.4 $\pm$ 0.8	286 $\pm$ 8	150 $\pm$ 4 ^a	7.32 $\pm$ 0.01* ^b	37.06 $\pm$ 0.03
CRSD Prolonged	Baseline	46.4 $\pm$ 1.7	280 $\pm$ 4	113 $\pm$ 6	7.36 $\pm$ 0.01	37.18 $\pm$ 0.05
	60 min	46.0 $\pm$ 1.6	268 $\pm$ 7	99 $\pm$ 3*	7.35 $\pm$ 0.01	37.18 $\pm$ 0.04

^aMAP significantly different than brief intermittent, brief massed, and CRSD prolonged neural apnea at equivalent time point;

^bpH significantly different than brief intermittent, brief massed, and CRSD prolonged neural apnea at equivalent time point;

* significantly different than baseline, (p<0.05).