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Reduced respiratory neural activity elicits phrenic motor facilitation

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Abstract

We hypothesized that reduced respiratory neural activity elicits compensatory mechanisms of plasticity that enhance respiratory motor output. In urethane-anesthetized and ventilated rats, we reversibly reduced respiratory neural activity for 25–30 min using: hypocapnia (end tidal CO₂ = 30 mmHg), isoflurane (~ 1%) or high frequency ventilation (HFV; ~100 breaths/min). In all cases, increased phrenic burst amplitude was observed following restoration of respiratory neural activity (hypocapnia: 92 ± 22%; isoflurane: 65 ± 22%; HFV: 54 ± 13% baseline), which was significantly greater than time controls receiving the same surgery, but no interruptions in respiratory neural activity (3 ± 5% baseline, p<0.05). Hypocapnia also elicited transient increases in respiratory burst frequency (9 ± 2 versus 1 ± 1 bursts/min, p<0.05). Our results suggest that reduced respiratory neural activity elicits a unique form of plasticity in respiratory motor control which we refer to as inactivity-induced phrenic motor facilitation (iPMF). iPMF may prevent catastrophic decreases in respiratory motor output during ventilatory control disorders associated with abnormal respiratory activity.

Keywords

hypocapnia; isoflurane; activity deprivation; inactivity; respiratory control; phrenic motor facilitation; homeostatic plasticity; control of breathing; phrenic plasticity; reduced neural activity

1. INTRODUCTION

The respiratory control system must produce a regular, rhythmic motor output from birth until death, with only the briefest of pauses. In addition, this motor output must be precisely regulated in order to enable optimal gas exchange, yet maintain sufficient dynamic range to respond to respiratory and metabolic challenges. However, little is known about the neural mechanisms that ensure respiratory neurons continue to function adequately despite changes in neuronal excitability or synaptic inputs that occur throughout life.

Rodent models of spinal injury suggest that reduced respiratory-related inputs elicit compensatory plasticity in the phrenic motor system. For example, while the immediate response to a C₂ hemisection is a complete silencing of ipsilateral phrenic motor output as

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bulbospinal inputs are severed, over time the ipsilateral phrenic nerve will develop modest respiratory-related neural output (Alilain and Goshgarian, 2008; Fuller et al., 2006, 2008, 2009; Golder et al., 2003; Golder and Mitchell, 2005; Nantwi et al., 1999; Vinit et al., 2007), leading to the suggestion that endogenous mechanisms of compensatory plasticity restored partial function to the previously silent phrenic motor pool (Fuller et al., 2006; Goshgarian, 2009; Lane et al., 2009; Mantilla and Sieck, 2009; Mitchell, 2007). Although neurophysiological evidence of this spontaneous partial phrenic recovery typically takes weeks to months following injury to observe, morphological plasticity within the phrenic motor pool can be observed within minutes to hours following injury (Goshgarian et al., 1989; Hadley et al., 1999; Sperry and Goshgarian, 1993). These morphological changes are likely due to disruption of descending respiratory drive to the phrenic motor pool, rather than the injury itself, since reversible C₂ axon conduction block induces similar morphological changes in the phrenic motor pool within 4 hours (Castro-Moure and Goshgarian, 1996, 1997). These morphological changes are accompanied by a functional enhancement of diaphragm EMG activity following restoration of spinal axon conduction (Castro-Moure and Goshgarian, 1996), although it is unknown if enhanced diaphragm activity is due to increased phrenic neural output or increased response at the neuromuscular junction. Nevertheless, these data suggest a robust and rapid response to removal of descending respiratory-related inputs to the phrenic motor pool.

Here, we tested the hypothesis that reduced respiratory-related neural activity elicits a rapid and robust form of plasticity in phrenic motor output. We chose three different methods with different mechanisms of action to induce a reversible, global reduction in respiratory-related activity in separate groups of mechanically ventilated, urethane-anesthetized rats: 1) hyperventilation to lower arterial P_{CO₂} below the apneic threshold (hypocapnia), 2) delivery of a volatile anesthetic to depress respiratory drive (isoflurane) and 3) isocapnic high frequency ventilation (HFV) to induce a vagal-reflex inhibition of breathing. Following 25–30 min of reduced respiratory neural activity, baseline conditions were restored, and changes in phrenic burst amplitude and frequency were measured. We demonstrate for the first time that reduced respiratory neural activity elicits a prolonged phrenic motor facilitation (inactivity-induced PMF; iPMF). An understanding of these novel mechanisms may yield insights concerning ventilatory control disorders associated with abnormal respiratory motor activity.

2. METHODS

2.1 Animals

All experiments were performed on male Sprague-Dawley rats (Harlan colony 217). All protocols conformed to the requirements of the Institutional Animal Care and Use Committee at the University of Wisconsin, Madison.

2.2 Surgical Preparation

Rats were induced with isoflurane (2.5–3.5% in 50% O₂), tracheotomized and pump ventilated (Harvard Apparatus, Rodent Ventilator 683). Rats exposed to hypocapnia or isoflurane were subjected to bilateral vagotomy, whereas the vagi were left intact in rats experiencing high frequency ventilation. The tail vein and femoral artery were catheterized for fluid infusion and blood sampling, respectively. A dorsal approach to the left phrenic nerve was used; the nerve was cut distally, desheathed and submersed in mineral oil. All rats were then converted slowly to urethane anesthesia (1.6–1.8 g/kg, *i.v.*) over 15–25 minutes, and then paralyzed with pancuronium bromide (2.5 g/kg, *i.v.*). A 1.5–2 mL/h fluid infusion of 1:1 lactated ringers and hetastarch (Hespan, 6% hetastarch in 0.9% sodium chloride)

solution was then started, and continued through the experiment. Inspired O₂ was 50% for all experiments.

2.3 Nerve Recordings

The phrenic nerve was placed on a bipolar silver electrode. Phrenic activity was amplified (10kX), band-pass filtered (0.1–5kHz) and integrated (time constant: 50 ms; CWE, MA-821RSP). Raw and integrated traces were passed to a data acquisition system (WinDAQ, DATAQ Instruments or PowerLab, AD Instruments) for digitization.

2.4 Physiological Measurements

End tidal CO₂ was monitored from the expired limb of the ventilator (Novamatrix), and used as an index of arterial P_{CO₂} to enable maintenance of isocapnia. These values were confirmed by blood gas analysis (Radiometer Copenhagen, ABL 500) of 0.2 mL arterial blood samples at key points in the protocol. Blood pressure was monitored and used as an indicator of stable conditions and depth of anesthesia by pressor responses to paw pad pinch.

2.5 Protocols

Baseline—Approximately 1h after conversion to urethane anesthesia, protocols began by determining the apneic and recruitment CO₂ thresholds for phrenic activity. The apneic threshold was determined by monitoring end tidal CO₂ and slowly lowering inspired CO₂ and/or increasing ventilator rate until rhythmic activity ceased. The recruitment threshold was determined by slowly raising end tidal CO₂ and/or lowering ventilator rate and noting the value at which phrenic activity resumed. End tidal CO₂ was raised a further 1–3 mmHg to establish baseline conditions. After nerve signals were steady for >15 minutes (typically 25–40 minutes after threshold determination), an arterial blood sample was drawn to establish baseline arterial P_{CO₂}.

Experimental groups—Six experimental groups were studied. Three groups experienced reduced respiratory neural activity (see below) with: 1) hypocapnia (n = 11); 2) isoflurane (n = 7) or 3) isocapnic high frequency ventilation (HFV; n = 11). A separate group of HFV rats were vagotomized to control for non-vagally mediated changes in phrenic output. Results were compared to “time controls” that received similar surgery and experimental duration, but no neural activity deprivation to control for any time dependent changes in phrenic motor output. One group of time controls were vagotomized (n = 10), while a separate group were vagi-intact (n = 6).

Hypocapnia—Following establishment of baseline, reduced respiratory neural activity was induced by decreasing inspired CO₂ and/or increasing ventilator rate until end tidal CO₂ was 5–10 mmHg below the apneic threshold. Hypocapnia-induced reduced respiratory neural activity was maintained for 25 minutes, and then phrenic neural activity was resumed by returning arterial P_{CO₂} to baseline values.

Isoflurane—Following establishment of baseline in a separate group of rats, isoflurane was slowly delivered through the inspired gas mixture until respiratory-related neural activity was reduced (final isoflurane ~1%). Isocapnia was maintained by increasing inspired CO₂ and/or decreasing ventilator rate; arterial blood samples confirmed that CO₂ remained within ~2mmHg of baseline. Following 30 min of reduced respiratory neural activity, baseline conditions were restored by removing isoflurane from the inspired gas line, while reducing inspired CO₂ and/or increasing ventilator rate to maintain isocapnia.

High Frequency Ventilation (HFV)—In a subgroup of rats the vagi were left intact and allowed to entrain to the ventilator, which was set at 50 breaths/min with a constant tidal volume (2.5mL). Following establishment of baseline, ventilator frequency was increased (~100 breaths/min) to elicit a reflex inhibition of respiratory neural activity. Isocapnia was maintained by increasing inspired CO₂ to maintain end-tidal P_{CO₂} at its baseline value; arterial blood samples confirmed that CO₂ remained within ~2mmHg of baseline. Following 25 min of reduced respiratory neural activity, baseline conditions were restored by returning ventilator frequency to 50 breaths/min, while reducing inspired CO₂ to maintain isocapnia. A separate group of rats were vagotomized prior to HFV to control for non-vagally mediated effects of high ventilation frequencies on phrenic motor output.

Post-stimulus monitoring—Arterial blood samples were analyzed at 15, 30 and 60 minutes after each treatment protocol to ensure adequate maintenance of baseline arterial P_{CO₂}, P_{O₂}, SBE and pH. At the end of each protocol, a maximal CO₂ response (90<PET_{CO₂}<100) was assessed to ensure that observed responses were not influenced by deterioration of the preparation. To be included in the analysis, rats had to meet the following criteria: arterial P_{CO₂} maintained within 1.5 mmHg of baseline, arterial P_{O₂} > 180 mmHg and base excess within 3 mEq/L of baseline values following restoration of respiratory neural activity.

2.6 Statistical Analysis

Integrated phrenic burst amplitude and frequency were analyzed in 30–60 sec bins before (baseline), and 15, 30 and 60 min following reduced respiratory neural activity or an equivalent duration in time controls. Phrenic burst amplitude was expressed as percent change from baseline, which was set at 0%. There were no differences in phrenic burst amplitude at any time point between the two sets of time controls; thus, burst amplitude data from these groups were combined. Phrenic burst frequency (vagotomized rats only) was expressed as an absolute change from baseline. A two-way repeated measures ANOVA was used to detect significant differences (Prism 5, GraphPad Software). Specific group differences were then determined by Bonferroni post-hoc tests at a significance level of 0.05. All data are presented as means ± SEM.

3. RESULTS

3.1 Hypocapnia-induced respiratory neural activity deprivation

To reversibly reduce respiratory neural activity, rats were hyperventilated to lower arterial P_{CO₂} below the apneic threshold. Baseline end-tidal CO₂ was 42.8 ± 1 mmHg, whereas average end-tidal CO₂ during hypocapnia was 30 ± 1 mmHg. Figure 1a depicts a representative compressed phrenic neurogram, illustrating baseline phrenic nerve activity, reduced phrenic activity during hypocapnia and restoration of phrenic bursting following resumption of isocapnia. Figure 2a depicts the average change in phrenic burst amplitude from baseline for one hour following resumption of baseline arterial P_{CO₂}. In all rats, no respiratory-related phrenic activity was detected during hypocapnia. 15 min following restoration of respiratory neural activity, phrenic burst amplitude was significantly elevated from baseline ($73 \pm 17\%$ baseline; $p < 0.005$), which persisted for at least 60 min ($92 \pm 22\%$ baseline; $p < 0.005$), indicating iPMF. Time control rats that remained isocapnic (i.e., no respiratory neural activity deprivation) showed no significant changes in phrenic amplitude relative to baseline at the 15 ($0 \pm 3\%$ baseline), 30 ($-1 \pm 5\%$ baseline) or 60 minute time points ($3 \pm 5\%$ baseline, all $p > 0.05$). At equivalent time points following restoration of respiratory neural activity, hypocapnia-treated rats had significantly higher phrenic burst amplitudes *versus* time controls (all $p < 0.001$).

Burst frequency was increased above baseline at 15, 30 and 60 min following reduced respiratory neural activity with hypocapnia (9 ± 2 , 6 ± 2 , 9 ± 2 bursts/min, respectively; all $p < 0.02$; figure 2b). Burst frequency was also increased in time control rats at a time point equivalent to 60 min (5 ± 1 bursts/min; $p < 0.01$), but not at 15 or 30 min (1 ± 1 , 1 ± 2 bursts/min, respectively; both $p > 0.4$; figure 2b). The change in burst frequency following hypocapnia differed from time controls at 15 ($p < 0.05$), but not 30 or 60 min ($p > 0.05$) following resumption of respiratory neural activity, suggesting that frequency facilitation is only transiently expressed following reduced respiratory neural activity with hypocapnia.

3.2 Isoflurane-induced respiratory neural activity deprivation

The volatile anesthetic, isoflurane, is a profound respiratory depressant (Imai et al., 1999) that has a rapid induction and recovery (Wade and Stevens, 1981; Stachnik, 2006). We took advantage of these properties of isoflurane to induce a reversible reduction in phrenic neural activity, without changes in arterial P_{CO_2} . Following establishment of baseline, isoflurane was slowly added to the inspired gas line. In 4/7 rats, respiratory-related phrenic burst activity was eliminated during isoflurane exposure; in the remaining rats, phrenic output was reduced to $-73 \pm 11\%$ below baseline. After 30 min, isoflurane was removed from the inspired line. Throughout the protocol, end-tidal CO_2 was monitored and adjusted to maintain isocapnia, which was confirmed by periodic blood gas analysis. Figure 1b depicts a representative compressed phrenic neurogram, illustrating baseline phrenic nerve activity, reduced phrenic activity during isoflurane and increased phrenic bursting following isoflurane wash-out. Figure 2a depicts the average change in phrenic burst amplitude from baseline for one hour following removal of isoflurane. Phrenic burst amplitude was significantly increased over baseline at 30 and 60 min following recovery of respiratory activity ($51 \pm 22\%$ and $65 \pm 22\%$ baseline, respectively, $p < 0.05$), but not at 15 min ($21 \pm 20\%$ baseline, $p > 0.3$). Similarly, phrenic burst amplitude at 30 and 60 min following restoration of respiratory neural activity was significantly greater relative to time controls ($p < 0.05$), indicating iPMF.

Phrenic burst frequency was not significantly different from baseline or time controls at any time point following isoflurane ($p > 0.05$; figure 2b), suggesting that frequency facilitation is not apparent in rats following isoflurane-induced reductions in respiratory neural activity.

3.3 Respiratory neural activity deprivation with isocapnic HFV

In a subgroup of rats, we took advantage of vagal reflex inhibition of breathing to induce a reversible reduction in respiratory neural activity. After establishment of baseline, vagi-intact rats were ventilated at high frequencies (> 100 breaths/min), while slowly adding CO_2 to the inspired line to maintain isocapnia. A separate group of rats were vagotomized prior to HFV to control for non-specific effects of HFV on phrenic motor output. Measurements of end tidal and arterial CO_2 levels were used to confirm isocapnia. At the end of the stimulation period, baseline ventilation rate was restored, while maintaining isocapnia. Vagi-intact rats generally exhibited no respiratory-related phrenic activity during HFV, although occasionally short episodes (< 2 min) of bursting were detected; when this occurred, ventilator frequency was increased until bursting stopped. Figure 1c depicts a representative compressed phrenic neurogram, illustrating baseline phrenic nerve activity, reduced phrenic activity during HFV and increased phrenic bursting following resumption of baseline ventilation frequency in vagi-intact rats. Figure 2a depicts the average change in phrenic burst amplitude from baseline for one hour following isocapnic HFV in vagi-intact rats. Phrenic burst amplitude was significantly increased from baseline at 30 and 60 min following restoration of respiratory neural activity ($43 \pm 12\%$, $54 \pm 13\%$ baseline, respectively; both $p < 0.01$), but not at 15 minutes (12 ± 7 , $p > 0.1$). Similarly, the change in phrenic burst amplitude was significantly increased relative to time controls at 30 and 60

min ($p < 0.05$) following HFV in vagi-intact rats, indicating iPMF. As expected, intact vagi were necessary for this effect, since vagotomized rats exposed to isocapnic HFV did not exhibit reduced respiratory neural activity or subsequent iPMF (60 min post-HFV, $13 \pm 4\%$ baseline; $p > 0.05$; data not shown). Since phrenic burst frequency entrains to the ventilator in vagi-intact rats, analysis of burst frequency changes was not relevant in these groups of rats.

3.4 Blood gases and mean arterial pressure

Table 1 lists the average rat body temperature, arterial P_{O_2} , arterial P_{CO_2} , mean arterial blood pressure, apneic threshold and recruitment threshold in all rat groups. Mean arterial pressure was significantly lower than baseline at 60 min following isoflurane; however, since the change in average mean arterial pressure was < 25 mmHg and arterial pressure remained > 100 mmHg, we do not consider this difference to be biologically significant. No other time dependent changes were observed within any other group, indicating that the observed iPMF was not due to unintended changes in blood gases or blood pressure.

4. DISCUSSION

Here, we used three different methods to induce a reversible reduction in respiratory neural activity: hypocapnia, isoflurane or isocapnic high frequency ventilation (HFV). In all three cases, we observed a prolonged increase in phrenic burst amplitude once respiratory neural activity was restored. Since hypocapnia, isoflurane and HFV all have different mechanisms of action in the central nervous system, we argue that the resulting plasticity results, at least in part, from reduced respiratory neural activity *per se* rather than unintended effects of our treatments. While the physiological significance of inactivity-induced phrenic motor facilitation (iPMF) is not yet known, we hypothesize that this form of plasticity may be important in preventing catastrophic decreases in respiratory neural output in situations in which ventilatory control may be compromised.

4.1 Methodological considerations

Hypocapnia—Rats were hyperventilated to lower arterial CO_2 levels below the threshold for breathing (the so-called apneic threshold). This chemoreflex-mediated inhibition of brainstem respiratory activity is easy to implement, readily reversible and the duration/level of respiratory neural activity deprivation can be precisely controlled. However, brain hypocapnia and/or alkalosis elicit other changes in the central nervous system unrelated to chemoreflex control of breathing. For example, brain hypocapnia/alkalosis increases neuronal excitability in the cortex (Balestrino and Somjen, 1988) and spinal cord (King and Rampil, 1994; Zhou and Turndorf, 1998). However, neuronal activity rapidly goes back to baseline levels once normal CO_2 levels are restored (Sparing et al., 2007); thus, residual CO_2 -dependent effects on motor neuron excitability likely does not account for the prolonged increase in phrenic burst amplitude observed here. Hypocapnia also results in decreased cerebral blood flow and reduced oxygen unloading at the tissues (Brian, 1998; Vogel et al., 1996), which could lead to brain hypoxia (Nwaigwe et al., 2000; Schneider et al., 1998), a stimulus known to elicit prolonged increases in respiratory motor output (Bavis and Mitchell, 2003; Blitz and Ramirez, 2002). Thus, it remains possible that increased phrenic motor output following hypocapnia is due to local ischemia, rather than reduced respiratory neural activity *per se*. To control for these effects, we used two other methods of respiratory neural activity deprivation, isoflurane and vagal-inhibition of respiratory neural activity.

Isoflurane—Inhalation anesthetics depress neuronal activity throughout the central nervous system, including in respiratory neurons (Rampil and King, 1996; Stuth et al., 1992;

Takeda and Haji, 1993). In addition to suppression of brainstem respiratory rhythm and pattern formation (Takeda and Haji, 1993), inhalation anesthetics depress motor neuron excitability directly (Antognini et al., 1999; Brandes et al., 2007; Rampil and King, 1996; Sirois et al., 1998). Here, we used isoflurane in urethane-anesthetized rats to induce a reversible reduction in respiratory neural activity without changes in arterial CO₂. In some rats, isoflurane resulted in an incomplete loss of phrenic motor output. Despite residual (but sharply reduced) phrenic activity apparent in some rats, increased phrenic motor output was apparent upon washout of isoflurane, suggesting that complete inactivity is not necessary for iPMF. In contrast to hypocapnia, iPMF was only observed at 30 and 60, but not 15 min post-isoflurane; however, the delay to iPMF expression likely reflects the washout time of isoflurane (Bailey, 1997).

Vagal Stimulation by Lung Stretch Receptors—Pulmonary stretch receptors inhibit breathing and can over-ride the eupnic drive to breathe (Kubin *et al.*, 2006). We took advantage of this reflex inhibition of breathing by subjecting the rats to isocapnic HFV. Similar to isoflurane, HFV elicited increased phrenic burst amplitude within 30 min following resumption of respiratory neural activity. Vagal afferents project throughout the central nervous system, including to the medullary raphe (Evans et al., 1993). Since spinal serotonin has been shown to elicit long-lasting increases in respiratory motor output (Baker-Herman and Mitchell, 2002; Bocchiaro and Feldman, 2004; MacFarlane and Mitchell, 2009), it remains possible that serotonin release near phrenic motor neurons plays a key role in increased phrenic burst amplitude following HFV (see below).

4.2 Burst frequency plasticity

In contrast to phrenic burst amplitude, significant changes in burst frequency following reduced respiratory neural activity were only observed with hypocapnia. Although there was a trend for burst frequency to be elevated at all time points post-hypocapnia, significant increases from time controls were only observed up to 15 min following resumption of neural respiratory activity, suggesting that burst frequency plasticity is more transient than burst amplitude plasticity. It is unknown why transient burst frequency plasticity was only observed in rats following hypocapnia. In isoflurane rats, burst frequency plasticity may have been masked by residual isoflurane, since it takes many minutes for isoflurane to reach 90% elimination in neural tissue (Bailey, 1997; Lockwood, 2010). Alternatively, it is possible that competing inhibitory mechanisms masked changes in burst frequency. For example, vagus nerve stimulation elicits a transient (<1 min) depression of phrenic burst discharge following stimulation (Budzinska and Ilasz, 2006; Eldridge and Millhorn, 1986). It is unknown if a longer duration of increased vagal traffic, such as performed here, would result in a longer lasting phrenic depression. Regardless, these data are consistent with previous studies suggesting that burst frequency plasticity tends to be more variable than phrenic burst amplitude plasticity in anesthetized rats (Baker-Herman and Mitchell, 2008).

4.3 Reduced respiratory neural activity as a stimulus to plasticity

Our findings are consistent with other reports of phrenic motor plasticity following reduced neural activity. For example, phrenic inactivity induced by intense stimulation of the vagus nerve elicits a prolonged increase in phrenic motor output upon recovery (Zhang et al., 2003, 2004). Similarly, phrenic inactivity induced by reversible C₂ axon conduction block elicits profound morphological changes in the phrenic motor pool that may be associated with enhanced motor neuron excitability (Castro-Moure and Goshgarian, 1996, 1997). This rebound facilitation following suppression of neuronal activity may be a general motor neuron property, since prolonged (> 1 hr) increases in evoked lumbar motor neuron responses are observed following recovery from anesthesia with isoflurane (Savola et al.,

1991; Wong et al., 2005), halothane (Wong et al., 2005) or ethanol (Li et al., 2005; Wong et al., 1998).

The mechanisms that give rise to iPMF are almost completely unknown. Evidence suggests that serotonin may play an important role in enabling iPMF. For example, increased phrenic burst amplitude following reduced phrenic activity with high frequency vagal stimulation (Zhang et al., 2003, 2004) or hypocapnia (our unpublished observations) requires activation of non-5-HT₂ receptors. However, we hypothesize that rather than directly inducing iPMF, serotonin receptor activation in this case may be important in enabling plasticity (Gu and Singer, 1995; Kojic et al., 1997, 2000; Maya Vetencourt et al., 2008), perhaps by modifying intracellular Ca⁺⁺ levels (Kirkwood, 2000; Kojic et al., 2000) or NMDA receptor function (Blank et al., 1996; Yuen et al., 2005; Zhong et al., 2008). Indeed, a key role for serotonin in enabling compensatory plasticity following removal of synaptic inputs is illustrated by recent reports suggesting that constitutive 5-HT_{2C} receptor activity is required for spontaneous recovery of motor neuron function following spinal injury (Murray et al., 2010).

4.4 Does iPMF represent “homeostatic plasticity” in respiratory control?

Homeostatic plasticity is emerging as an important principle whereby negative feedback bi-directionally adjusts neuronal activity to maintain neural output in an optimal range (Turrigiano, 2006, 2008). Others have suggested that homeostatic plasticity is a property of neural circuits important for ventilatory control since neurons in the nucleus tractus solitarius receiving peripheral chemoreceptor inputs exhibit synaptic depression following chronic intermittent hypoxia (Kline, 2008; Kline et al., 2007), a stimulus that repetitively activates peripheral chemoreceptors. We do not yet know whether iPMF represents homeostatic plasticity *per se* since enhanced phrenic activity apparent upon reversal of activity deprivation does not spontaneously return to baseline levels, suggesting that this form of plasticity is not bi-directional. The response to respiratory neuron hyperactivity may operate in longer time domains (*versus* hypoactivity); indeed, a bias toward a rapid response to reduced activity may be an important feature of respiratory control since constitutive activity is critical to sustain life. Alternatively, factors necessary for adaptation in response to neuronal hyperactivity (e.g., diaphragm sensory inputs) may be missing in our experimental preparation. Regardless of whether iPMF represents “true” homeostatic plasticity, understanding mechanisms of plasticity induced by reduced activity in respiratory motor neurons is an important area of research due to its clear biological and clinical significance.

4.5 Conclusions

Here, we demonstrate that hypocapnia-, isoflurane- or HFV-induced reductions in respiratory neural activity elicit a long-lasting form of respiratory plasticity, which we refer to as iPMF. Although the physiological role for this form of plasticity is completely unknown, iPMF may compensate for long-lasting changes in synaptic inputs or neuronal excitability, thereby preventing catastrophic decreases in respiratory motor output.

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Figure 1.

Representative integrated phrenic neurograms before, during and 60 min following respiratory neural activity deprivation with 30 min of hypocapnia (A), 30 min of 0.5% isoflurane (B) or 25 min of high frequency ventilation (HFV; C). The white dashed line represents baseline burst amplitude. In all cases, phrenic burst amplitude was increased above baseline within 30 min of returning to respiratory neural activity. Note that in example B, phrenic burst activity did not completely go to zero during isoflurane treatment, suggesting that complete phrenic inactivity is not required for iPMF expression.

Figure 2.

Average iPMF and burst frequency facilitation following reduced respiratory neural activity with hypoxia (■), isoflurane (▼) or high frequency ventilation (HFV; ▲). Results are compared to time controls not receiving neural activity deprivation (●). * indicates significant increase over time controls ($p < 0.05$).

Average rat body temperature, arterial P_{O_2} , arterial P_{CO_2} , mean arterial blood pressure (MAP), apneic threshold and recruitment threshold before and 60 min following reduced respiratory neural activity with hypocapnia, isoflurane or high frequency ventilation (HFV). Values are also included for an equivalent duration in control rats not receiving reduced respiratory neural activity (time controls and vagotomized rats receiving HFV). MAP was significantly lower than baseline at 60 min following isoflurane. No other time-dependent changes were observed in any group.

Table 1

		Temp	P_{aO_2}	P_{aCO_2}	MAP	Apneic threshold	Recruitment threshold
hypocapnia	baseline	36.6 ± 0.1	272 ± 4	43.0 ± 0.7	126 ± 2	37.9 ± 1.0	41.6 ± 0.8
	60 min	36.7 ± 0.1	266 ± 5	43.2 ± 0.7	122 ± 3		
isoflurane	baseline	37.2 ± 0.1	274 ± 7	47.1 ± 1.7	127 ± 4	36.7 ± 2.1	45.2 ± 1.4
	60 min	37.2 ± 0.1	270 ± 5	47.7 ± 1.7	$106 \pm 7^*$		
HFV	baseline	37.2 ± 0.1	234 ± 6	48.6 ± 0.7	120 ± 3	44.3 ± 0.5	46.6 ± 0.5
	60 min	37.3 ± 0.1	238 ± 5	48.3 ± 0.6	124 ± 5		
HFV vagotomized	baseline	37.3 ± 0.2	229 ± 5	45.6 ± 0.8	114 ± 4	39.7 ± 0.4	42.4 ± 0.4
	60 min	37.4 ± 0.2	238 ± 5	45.2 ± 0.6	108 ± 2		
Time control	baseline	37.0 ± 0.2	249 ± 8	44.8 ± 1.4	126 ± 4	41.2 ± 1.3	44.0 ± 1.2
	60 min	36.9 ± 0.2	249 ± 6	44.6 ± 1.3	126 ± 3		

* significantly different than baseline; $p < 0.05$