COVER SHEET

TITLE: Inactivity-induced phrenic motor facilitation is associated with decreased phrenic burst-to-burst variability

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ABSTRACT

Inactivity-induced phrenic motor facilitation is associated with decreased phrenic burst-to-burst variability

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Reduced respiratory neural activity in ventilated rats elicits rebound increases in phrenic discharge upon neural activity restoration, a plasticity called inactivity-induced phrenic motor facilitation (iPMF). We hypothesized that iPMF is associated with reduced phrenic burst-to-burst variability. Phrenic discharge was measured in Harlan (H) and Charles River (CR) Sprague-Dawley rats exposed to a neural apnea (NA), compared to time controls. Inspiratory (Ti) and expiratory time (Te) was assessed using Poincare plot analyses at baseline, 5 and 60 min post-apnea. In H+NA rats, standard deviations (SD1 and SD2) for Ti and Te were significantly decreased at 5 and 60 min post-apnea ($p< 0.01$) when iPMF was expressed, while CR+NA rats experienced significant decreases in SD1 and SD2 for Ti and Te at 5 ($p<0.01$), but not 60 min when iPMF was not expressed. These data suggest that iPMF is associated with decreased variability in Ti and Te. UW Regent Scholars Fund.

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Abstract:

Reduced respiratory neural activity in ventilated rats elicits rebound increases in phrenic discharge once neural activity has been restored, a form of plasticity called inactivity-induced phrenic motor facilitation (iPMF). We hypothesized that iPMF is associated with reduced phrenic burst-to-burst variability. Phrenic discharge was measured in anesthetized, vagotomized and ventilated Harlan and Charles River (CR) Sprague Dawley rats exposed to a 30 min neural apnea or an equivalent duration of baseline conditions (time control). As expected, Harlan rats expressed iPMF at 5 and 60 min ($p<0.05$) post-apnea, whereas CR rats expressed iPMF only at 5 ($p<0.05$), but not 60 ($p>0.05$) min. Inspiratory time (Ti), expiratory time (Te) and peak area variability was assessed using Poincaré plot analyses for burst n *vs.* burst n+1 before, and 5 and 60 min post-apnea (400 consecutive bursts). In Harlan rats exposed to a neural apnea, standard deviations of the Poincaré plot (SD1 and SD2) for Ti and Te were significantly decreased at 5 and 60 min post-apnea ($p< 0.01$). In CR rats exposed to a neural apnea, significant decreases in SD1 and SD2 for Ti and Te were observed at 5 ($p<0.01$), but not 60 ($p>0.05$) min post-apnea. No changes in SD1 or SD2 for Ti or Te were observed in time controls ($p>0.05$), and no changes in SD1 or SD2 for peak area were observed in any rat group ($p>0.05$). These data suggest that iPMF is associated with decreased variability in Ti and Te.

Introduction:

Intrinsic variability in systems regulating homeostasis is essential for an organism's survival. Biological systems are balanced so that the body can physiologically respond to changes in external and internal environments. A change in respiratory or cardiovascular variability during times of stress or fatigue can be fatal. Scientists have investigated several clinical applications of homeostatic malfunction. They have found a correlation between the poor survival rates of congestive heart failure (CHF) patients and a decrease in heart beat-to-beat variability (Frenneaux 2004). The hypothesis is that there is an imbalance between the sympathetic and parasympathetic nervous systems, and this imbalance leads to patient mortality.

There have also been studies that investigate the ability of a patient to successfully wean from mechanical ventilation, and whether it correlates to high or low respiratory variability. Patients were categorized on whether they were able to successfully wean from mechanical ventilation or if continual ventilator support was needed. In this study, patients that were unable to wean from ventilation were found to have decreased breath-to-breath variability, in comparison to patients who successfully weaned (Bien *et al.* 2004). This could be another pathophysiological example of an imbalance of homeostasis.

The respiratory system, in particular, uses endogenous plasticity mechanisms to confer long-lasting adjustments in the brain networks controlling rhythmic respiration. A specific form of respiratory plasticity is a concept known as inactivity induced phrenic motor facilitation (iPMF). iPMF is induced by disrupting synaptic inputs to phrenic motor neurons controlling diaphragm contraction. It is manifested as a "rebound" increase in phrenic motor inspiratory output, which is apparent once phrenic synaptic inputs have been restored. (Strey *et al.* 2011). Little is known about the pathways behind the phenomenon, but current research is being done to better understand the mechanisms involved in iPMF.

Differences in iPMF expression have been found to occur in different laboratory lab strains. The Harlan Sprague-Dawley rat has been shown to exhibit a sustained iPMF, that persists up to 60 minutes post neural apnea. However, a Charles River (CR) Sprague-Dawley rat, which diverged from the Harlan-Sprague Dawley rat line 30 years ago, exhibits a transient iPMF that does not persist beyond 15 minutes post neural apnea. However, CR rats may be induced to express a prolonged iPMF if NMDA receptors are inhibited (Strey and Baker-Herman, 2010). These differences may demonstrate that there are epigenetic differences between the rat strains (Fuller *et al.* 2001), but further research must investigate the differences in physiological mechanisms.

Here I investigated changes in burst-to-burst variability of the phrenic nerve, associated with iPMF. I compared these changes in Harlan and Charles River Sprague-Dawley rats, which express iPMF for different durations. I hypothesized that when iPMF was being expressed, there would be a decrease in variability of the phrenic neurogram.

Materials and Methods:

All male Harlan Sprague-Dawley and Charles River rats were used., under RARC approved protocols. Rats were urethane-anesthetized, paralyzed, vagotomized, and mechanically ventilated. The phrenic nerve

was located and bilateral electrophysiological data was recorded via an electrode. The protocol included 15 minutes of baseline, 30 minutes of neural apnea created by hyperventilation, and 60 minutes returned at baseline levels. Separate subgroups of rats, exposed to neural apnea, received an NMDA receptor antagonist, APV, via an intrathecal catheter at the phrenic motor pool near the 4th cervical vertebrae (C4). Time control rats received similar surgical preparations, but no neural apnea. The different rat treatment groups included time controls (n=14), Harlan/neural apnea (Harlan + NA, n=17), CR/neural apnea (CR NA, n=16), Harlan/neural apnea/APV (Harlan NA + APV, n=6), and CR/neural apnea/APV (CR NA + APV, n=8). Vitals were monitored through blood gas sampling and blood pressure screenings at baseline, and 5, 15, 30, and 60 minutes post neural apnea, to ensure that rats were kept in a stable physiological state.

Data from the electrophysiology protocols was then exported into the program Lab Chart. Approximately 400 consecutive breaths were sampled from the time points at baseline, 5 minutes post hypocapnia, and 60 minutes post hypocapnia. Four parameters were analyzed, including inspiratory time (Ti, ms), expiratory time (Te, ms), peak area (mV), and height (mV). These data points were then compiled into files and analyzed through the "R" statistical software. This program provided each time point and parameter with a Poincaré plot and standard deviations 1 and 2 (SD1 and SD2). The SD1 (line of identity, y=x) and SD2 (the line normal to y=x) data was then statistically analyzed using a two-way ANOVA with repeated measures.

Results:

The following data summarizes the results of this project. There was no statistical significance found in the raw phrenic nerve bursts, for any parameters ($p > 0.05$). Tables 1 and 2 represent the results of SD1 and SD2, as a percentage of the rats' phrenic output at baseline. Figure 1 serves as a graph of similar results, demonstrating the decrease in variability at different time points. Figure 2 is a visual representation of the Poincaré plots, from all four treatment groups at different time points, along with the SD 1 and SD 2 lines of deviation.

Discussion:

We found that a neural apnea elicits sustained iPMF in Harlan rats, but only a transient iPMF in CR rats. A transient iPMF in CR rats can be converted to a sustained iPMF if spinal NMDA receptors are inhibited. Our data suggests that iPMF is associated with a decrease in phrenic burst-to-burst variability. Sustained

iPMF is associated with a decreased phrenic Ti and Te variability at 5 and 60 minutes post neural apnea. Transient iPMF in CR rats is associated with decreased phrenic Ti and Te variability at 5 minutes when, but not at 60 minutes. This is illustrated in Tables 1 and 2, as well as the graphs in Figures 1, 2, and 3.

While the underlying causes that contribute to alterations in breathing variability are not completely understood, decreased in variability in other systems (like cardiovascular and heart rate variability) is associated with pathological conditions. While the physiological significance of iPMF remains unknown, we hypothesize that iPMF-like mechanisms may protect against catastrophic decreases in phrenic motor output, and a consequential loss of respiration. However, further research needs to be performed to investigate this phenomenon. This variability analysis should be employed to examine other mechanisms of respiratory plasticity, like long-term facilitation. Understanding how breath-to-breath variability changes during iPMF expression is essential in order to understand the role of respiratory plasticity in the control of breathing. The promise is this research lies in its relevant clinical applications. Harnessing these endogenous plasticity mechanisms and utilizing them in a mechanical ventilation situation may assist patients from weaning from respiratory ventilators. This, overall, will improve their quality and longevity of life.

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Appendix:

Table 1: Inspiratory time (Ti), as a percentage of baseline

Treatment Group	SD 1		SD 2	
	5 min.	60 min	5 min	60 min.
Time Control	N.S.	N.S.	N.S.	N.S.
Harlan NA	$p < 0.01$	$p < 0.01$	$p < 0.0001$	$p < 0.0001$
CR NA	$p < 0.05$	N.S.	$p < 0.0001$	$p < 0.05$
Harlan NA + APV	$p < 0.01$	$p < 0.01$	$p < 0.0001$	$p < 0.0001$
CR NA + APV	N.S.	$p < 0.01$	$p < 0.001$	$p < 0.0001$

Table 2: Expiratory time (Te), as a percentage of baseline

Treatment Group	SD 1		SD 2	
	5 min.	60 min	5 min	60 min.
Time Control	N.S.	N.S.	N.S.	N.S.
Harlan Hypo	N.S.	$p < 0.01$	$p < 0.01$	$p < 0.01$
CR Hypo	N.S.	N.S.	$p < 0.05$	N.S.
Harlan APV	N.S.	N.S.	$p < 0.01$	$p < 0.01$
CR APV	N.S.	$p < 0.01$	$p < 0.05$	$p < 0.01$

Figure 1: Standard deviation 1 for inspiratory time (Ti), as a percent change from baseline (*$p < 0.05$). SD 1 data for expiratory time shows the same trends.

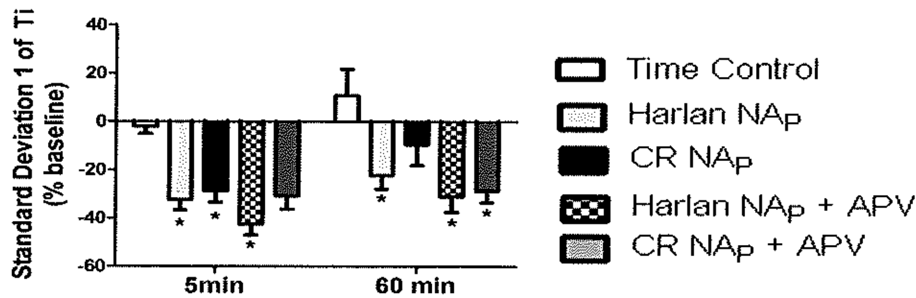


Figure 2: Standard deviation 2 for inspiratory time (Te), as a percent change from baseline (*$p < 0.05$). SD 2 data for expiratory time shows the same trends.

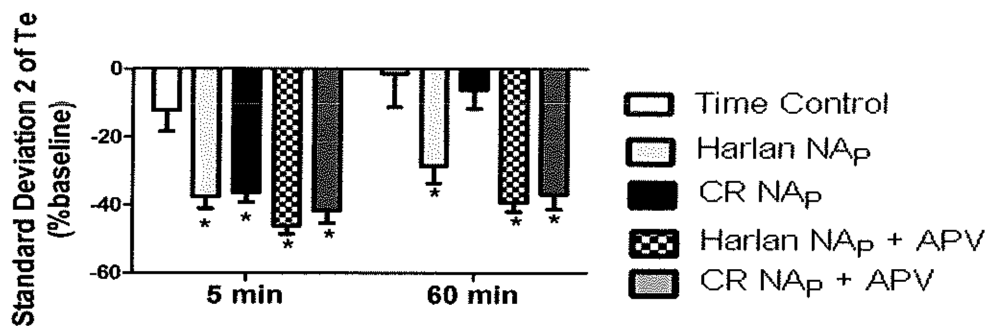
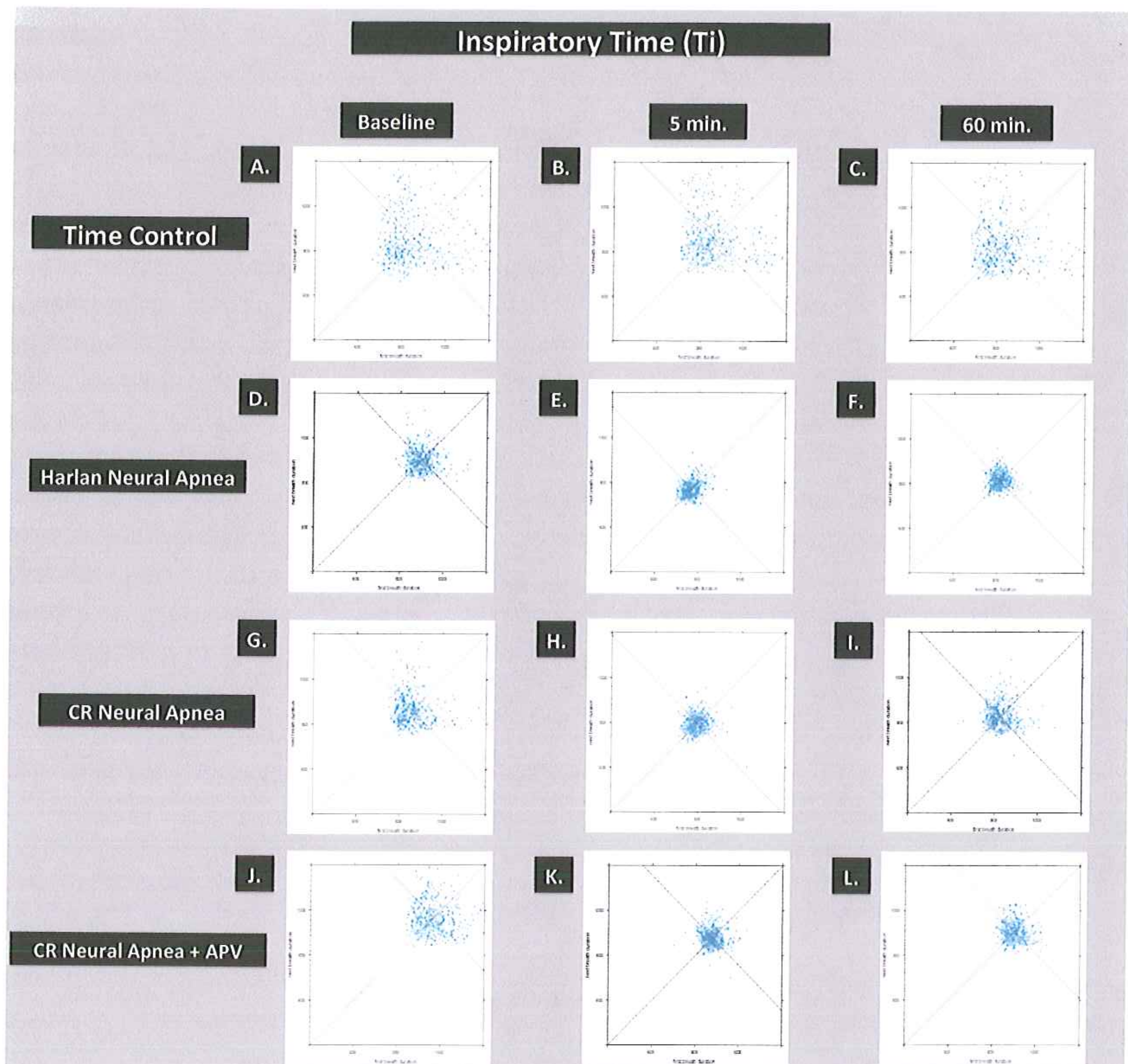


Figure 3: Representative Poincaré plots of inspiratory time (Ti) before (baseline) and 5 and 60 min post - neural apnea. Ti at baseline and 5 and 60 min post-neural apnea in a time control rat (A,B,C), HSD rat exposed to a neural apnea (D,E,F), CRSD exposed to a neural apnea (G,H,I) and CRSD with APV, exposed to a neural apnea (J,K,L). Representative Poincaré plots of expiratory time (Te), at all time points, showed similar trends



Bibliography:

- Bien, MY *et al. Breathing pattern variability: a weaning predictor in post-operative patients recovering from systemic inflammatory response syndrome.* Intensive Med Care 2004
- Frenneaux, M. *Autonomic changes in patients with heart failure and in post-myocardial infarction patients,* Heart, 2004
- Fuller DD, Baker TL, Behan M, Mitchell GS. *Expression of hypoglossal long-term facilitation differs between substrains of Sprague-Dawley.* Physiol Genomics. 2001, 4: 175-181.
- Strey KA, Baker-Herman TL. *Expression of inactivity-induced phrenic motor facilitation differs between substrains of Sprague-Dawley rats.* Society for Neuroscience Abstracts. 298.19. 2010
- Strey KA and Baker-Herman TL. *Local reductions in synaptic inputs to the phrenic motor pool elicits inactivity-induced phrenic motor facilitation (iPMF).* 2011