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Impact of Unilateral Denervation on Transdiaphragmatic Pressure

Luther C. Gill¹, Carlos B. Mantilla^{1,2}, and Gary C. Sieck^{1,2}

¹Department of Physiology and Biomedical Engineering, Mayo Clinic, 200 First Street SW, Rochester, Minnesota, USA

²Department of Anesthesiology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA

1. INTRODUCTION

Mechanical activation of the diaphragm muscle (DIAM) mediates airflow into the lungs by generating a pressure difference across the muscle—transdiaphragmatic pressure (Pdi). Previous work from our lab in cats (Fournier and Sieck, 1988b; Sieck and Fournier, 1989), hamsters (Sieck, 1994), rats (Mantilla et al., 2010) and more recently in mice (Greising et al., 2013) used Pdi measurements to estimate DIAM force generation across a range of ventilatory (rhythmic gas exchange) and higher force, non-ventilatory behaviors. In these studies we found that Pdi generated during ventilatory behaviors is consistently less than 50% of maximal Pdi ($P_{di_{max}}$) elicited by bilateral phrenic nerve stimulation. For example, Pdi generated during quiet rhythmic breathing (eupnea) ranged from 10–27% of $P_{di_{max}}$ depending on species (Greising et al., 2013; Mantilla et al., 2010; Sieck and Fournier, 1989; Watchko et al., 1986). Stimulating breathing by exposure to a hypoxic-hypercapnic (10% O_2 –5% CO_2) gas mixture increased Pdi generated during ventilatory behaviors; however, Pdi never exceeded 36% of $P_{di_{max}}$ across several species (Greising et al., 2013; Mantilla et al., 2010; Sieck and Fournier, 1989; Watchko et al., 1986).

Although lung inflation may result, the goal of higher force, non-ventilatory behaviors of the DIAM is not gas exchange. Often DIAM activation during these behaviors is preparatory for expulsive airway clearance, e.g., coughing or sneezing. The Pdi generated during higher force, non-ventilatory behaviors is substantially greater than that generated during ventilatory behaviors (Greising et al., 2013; Mantilla et al., 2010; Sieck and Fournier, 1989; Watchko et al., 1986). For example in cats, mechanical stimulation of the oropharynx induces a gagging/coughing behavior in which Pdi approximates $P_{di_{max}}$ (Sieck and Fournier, 1989). Also in cats, the Pdi generated during sneezing induced by mechanical stimulation of the nasopharynx was found to be maximal (comparable to $P_{di_{max}}$). Similarly in rats, a sneezing behavior induced by intranasal injection of capsaicin is associated with generation of near maximal Pdi (94% of $P_{di_{max}}$) (Mantilla et al., 2010). The Pdi's generated during other higher force, non-ventilatory behaviors of the DIAM are also greater than

during ventilatory behaviors. For example, during sustained airway occlusion, Pdi ranges from 43–70% of Pdi_{max} across species (Greising et al., 2013; Mantilla et al., 2010; Sieck and Fournier, 1989; Watchko et al., 1986).

The DIAM comprises separate left and right hemidiaphragms, each with its own phrenic nerve innervation, with no crossover of innervation (Fournier and Sieck, 1988a). Accordingly, unilateral denervation (DNV) (Argadine et al., 2009; Geiger et al., 2001; Gosselin et al., 1994; Sieck, 1994; Sieck and Zhan, 2000) induces DIAM hemi-paralysis, reducing maximum force generating capacity of the DIAM by ~50%. We hypothesized that following unilateral DNV, the ability of the contralateral DIAM to generate sufficient Pdi to accomplish ventilatory behaviors will not be compromised and normal ventilation (as determined by arterial blood gas measurements) will not be impacted, although neural drive to the DIAM increases. In contrast, we hypothesized that those higher force, non-ventilatory behaviors requiring Pdi generation greater than 50% of Pdi_{max} will be compromised following DIAM hemiparalysis, i.e., increased neural drive cannot compensate for lack of force generating capacity.

2. METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. A total of 18 adult, male Sprague-Dawley rats (300–350 g) from Harlan Laboratories (Indianapolis, IN) were used for this study. Rats were anesthetized via intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg) injections for all experimental procedures. Animals were randomly assigned to either the sham control (n=6) or DNV (n=6) groups. Additional animals (n=6) were used to measure changes in ventilatory parameters and blood gases before and after DNV.

2.1. Denervation

The right phrenic nerve was isolated in the lower neck and sectioned. A 10–20 mm length of the nerve was removed to ensure complete DIAM DNV. In all animals, DIAM hemiparalysis was verified by the absence of EMG activity in the ipsilateral (right) DIAM. The sham group underwent a similar surgical procedure as the animals in the DNV group, but the right phrenic nerve remained intact.

2.2. Transdiaphragmatic Pressure Measurements

Measurements of Pdi were performed based on the difference between esophageal and gastric pressures as previously described (Fournier and Sieck, 1988a; Greising et al., 2013; Mantilla et al., 2010; Sassoon et al., 1996; Sieck and Fournier, 1989; Watchko et al., 1986). In anesthetized animals, two 3.5 French Millar solid-state pressure catheters (SPR-524; Millar Instruments, Houston, TX) were inserted through the mouth into the esophagus and stomach, spanning the thoracic and abdominal borders of the DIAM, respectively. Correct catheter position was determined based on the direction of signal deflection and postmortem analysis. Measurements were collected during the following conditions and sequence: (1) breathing of room air (eupnea) for 5 min, (2) exposure to a hypoxia (10% O₂)–hypercapnia (5% CO₂) gas mixture for 5 min, (3) sustained airway occlusion (by covering nose and

mouth for ~40 s), (4) maximum Pdi ($P_{di_{max}}$) obtained by supramaximal bilateral phrenic nerve stimulation (using a stimulus isolation unit to control the current pulse) at 75 Hz (0.5 ms duration pulses in 300 ms trains repeated each s) using bipolar electrodes (FHC, Bowdoin, ME); and (5) sneezing, induced by intranasal infusion of 10 μ l of 30 μ M capsaicin. Measurements were also obtained when animals took deep breaths (“sighs”) defined as spontaneously occurring inspiratory events that were >2 times eupneic Pdi amplitude. Rats were given 5–10 min intervals between behaviors to allow for re-acclimatization to a tidal breathing condition and for Pdi amplitude to return to eupneic values.

Intra-thoracic and abdominal pressures were measured independently and recorded with a PowerLab 8/35 data acquisition system with an integrated amplifier following the manufacturer recommended calibration procedure. Pressure data were sampled at 100 Hz using LabChart (Millar Instrumentation) and band-pass filtered (0.3–30 Hz). Data from LabChart was exported to MATLAB for custom-designed automated analyses of peak pressure amplitudes and corresponding baselines on an event-by-event (e.g., breath-by-breath) basis. Baseline pressure values were determined systematically from the average of all inflection points in the segment preceding each peak. Data were analyzed and averaged across behaviors for 2 min of eupnea, 2 min of hypoxia–hypercapnia, and 5 maximal breaths during occlusion, all spontaneous deep breaths, all sneezes, and the maximal value obtained during bilateral phrenic nerve stimulation at 75 Hz. Pdi measurements across all conditions were obtained before and after unilateral DIAM DNV. Across all behaviors, movement of the abdomen was constrained using a custom-made binder to approximate isometric conditions (determined by Pdi response) and to minimize changes in functional residual capacity (FRC) of the lung.

2.3. Diaphragm Electromyography (EMG)

In all animals, DIAM EMG was measured using methods similar to those previously described (Dow et al., 2006; Mantilla et al., 2011; Mantilla et al., 2010; Sieck and Fournier, 1990; Trelease et al., 1982). Briefly, pairs of multistranded fine wire, insulated (stripped to expose ~2 mm segment) stainless steel electrodes (0.28 mm diameter – model AS631, Cooner Wire Inc., Chatsworth, CA) were implanted (~3 mm apart) into the mid-costal regions of both right and left sides of the DIAM following laparotomy. The compound EMG signal was differentially amplified (2000 $\times$), bandpass filtered (20–1000 Hz) using an analog amplifier (Model 2124, DATA Inc.) and digitally sampled at a frequency of 2 kHz using a data acquisition board (National Instruments, Austin, TX) controlled by a custom-made program (LabView 8.2; National Instruments). The root-mean squared (RMS) of the EMG signal was computed using a 100-ms window. Respiratory rate, inspiratory burst duration, duty cycle, the RMS value at 75 ms after the onset of DIAM activity (RMS_{75} – an estimate of neural drive (Seven et al., 2014), and peak RMS (RMS_{peak}) were determined from the EMG signal. The maximum DIAM RMS EMG value was estimated based on the positive linear relationship between DIAM RMS EMG and Pdi measurements that we previously observed (Mantilla et al., 2010). Accordingly, in each animal, the maximum DIAM RMS EMG value was extrapolated from RMS EMG and Pdi measurements obtained in other behaviors.

2.4. Plethysmography

A commercially available whole-body plethysmography system (Buxco Inc., Wilmington, NC, USA) was used to quantify ventilation in additional anesthetized animals. The plethysmograph was calibrated by injecting known volumes of gas into a Plexiglas recording chamber using a 5-mL syringe. The chamber pressure, temperature and humidity, and atmospheric pressure as well as the rectal temperature of the rat were used in an equation first described by (Drorbaugh and Fenn, 1955) to calculate respiratory volumes, and peak airflow rates before and after DNV. Ventilatory parameters were calculated from the airflow traces, which were continuously sampled at 500 Hz. During the experiments, gas mixtures flowed through the chamber at a rate of 2 L/min to enable control of inspired gases.

2.5. Arterial Blood Gas Measurements

Arterial PO₂, PCO₂ and pH (i-Stat, Heska, Fort Collins, CO) were measured during eupnea in a subset of animals (n=6) that did not undergo the sequence of ventilatory and non-ventilatory behaviors utilized during Pdi measurements. After animals were anesthetized, the right femoral artery was cannulated with a catheter (PE-10 tubing, OD 0.61 mm, ID 0.28mm) filled with heparinized saline. Arterial blood samples were drawn before (0.25 ml) and after (0.25 ml) unilateral DNV.

2.6. Statistical Analyses

Differences in Pdi across motor behaviors were evaluated using a two-way repeated measures ANOVA (condition: pre- and post-DNV $\times$ behavior) with Tukey–Kramer honestly significant difference post hoc tests when appropriate (Tukey, 1949). Differences in arterial blood gas values, respiratory rate, burst duration, duty cycle, DIAM RMS_{peak} and RMS₇₅ EMG were determined using ANOVA and post hoc paired-sample t-tests. All statistical evaluations were performed using standard statistical software (JMP 8.0, SAS Institute Inc., Cary, NC). Statistical significance was established at the 0.05 level. All experimental data are presented as mean $\pm$ standard error (SE) across animals, unless otherwise specified.

3. RESULTS

In all animals, unilateral DNV and DIAM hemiparalysis was verified by the absence of EMG activity in the ipsilateral (right) DIAM (Fig. 1). Based on DIAM EMG recordings from the contralateral (left) DIAM during eupnea, respiratory rate increased from 63 ± 3 breaths/min to 85 ± 4 breaths/min ($p < 0.0002$) after unilateral DNV. Inspiratory burst duration also increased after DNV from 281 ± 3 ms to 349 ± 18 ms ($p < 0.005$). The duty cycle (inspiratory burst duration divided by total respiratory cycle duration) of DIAM activity also increased from $30 \pm 3\%$ to $49 \pm 4\%$ after DNV ($p < 0.0001$).

3.1. Transdiaphragmatic Pressure

Representative tracings of Pdi generated during ventilatory and higher force, non-ventilatory motor behaviors are shown in Figure 1. The maximum Pdi generated by bilateral phrenic nerve stimulation at 75 Hz was 36.6 ± 1.4 cm H₂O. Figure 2 summarizes the impact of sham surgery and unilateral DNV on the Pdi's generated during different ventilatory and higher force, non-ventilatory behaviors. There were no differences in Pdi's generated during

different ventilatory and higher force, non-ventilatory behaviors after sham surgery. After unilateral DNV, the Pdi generated by stimulation of the intact phrenic nerve was 23.1 ± 1.7 cm H₂O (63% of Pdi_{max} before DNV) ($p < 0.001$). Before DNV, the average Pdi generated during eupnea was 8.6 ± 0.6 cm H₂O (24% of Pdi_{max}) and 9.8 ± 0.7 cm H₂O (27% of Pdi_{max}) during hypoxia-hypercapnia. After DNV, the Pdi generated during these ventilatory behaviors was unaffected; during eupnea, Pdi was 8.5 ± 0.6 cm H₂O (23% of Pdi_{max}) and 9.5 ± 0.5 cm H₂O (26% of Pdi_{max}) during hypoxia-hypercapnia. The Pdi's generated by the DIAm during higher force, non-ventilatory behaviors were significantly reduced after DNV. The average Pdi generated during airway occlusion was reduced from 23.5 ± 1.6 cm H₂O (64% of Pdi_{max}) before DNV to 17.0 ± 0.9 cm H₂O (49% of Pdi_{max}) after DNV ($p < 0.01$). The Pdi generated during deep breaths (sighs) was 19.6 ± 0.9 cm H₂O (53% of Pdi_{max}) before DNV to 16.8 ± 0.9 cm H₂O (46% of Pdi_{max}) after DNV ($p < 0.01$). During sneezing, Pdi was 22.7 ± 0.6 cm H₂O (62% of Pdi_{max}) before DNV and this decreased to 15.6 ± 0.5 cm H₂O (43% of Pdi_{max}) after DNV ($p < 0.01$).

3.2. Diaphragm Electromyography (EMG)

Representative traces for compound DIAm EMG and RMS EMG generated during ventilatory and higher force, non-ventilatory DIAm motor behaviors are shown in Figure 1. Figure 2 summarizes the impact of sham surgery and unilateral DNV on DIAm EMG RMS_{peak} (normalized to the estimated maximum RMS EMG - RMS_{max}) generated during different ventilatory and higher force, non-ventilatory behaviors. There were no differences in DIAm RMS_{peak} EMG generated during different ventilatory and higher force, non-ventilatory behaviors after sham surgery. Although the Pdi's generated during ventilatory behaviors were unaffected by unilateral DNV, DIAm RMS_{peak} EMG increased significantly during these motor behaviors after DNV ($p < 0.01$). In contrast, although the Pdi's generated during higher force, non-ventilatory behaviors were reduced after unilateral DNV, DIAm RMS_{peak} EMG values during these motor behaviors were unaffected by DNV.

As previously observed (Mantilla et al., 2010), there was a strong correlation between Pdi and RMS EMG measures across motor behaviors (Fig. 3; $r^2 = 0.78$; $p < 0.0001$). After unilateral DNV, this correlation persisted but was shifted downward and to the right, i.e., greater DIAm RMS_{peak} EMG was observed for a given level of Pdi ($p < 0.01$).

As previously described (Seven et al., 2014), the RMS₇₅ EMG value was used to estimate neural drive to the contralateral DIAm. Representative traces for RMS_{peak} and RMS₇₅ EMG generated during ventilatory and higher force, non-ventilatory DIAm motor behaviors are shown in Figure 4. In the control group, there were no differences in RMS_{peak} and RMS₇₅ EMG during different ventilatory and higher force, non-ventilatory behaviors before and after sham surgery. In contrast, both RMS_{peak} and RMS₇₅ EMG values increased significantly after DNV during ventilatory behaviors (Fig. 2 C&D; Fig. 4 A&B). During eupnea, RMS₇₅ EMG increased from $11 \pm 1\%$ before DNV to $19 \pm 2\%$ after DNV. During hypoxia-hypercapnia, RMS₇₅ EMG increased from $12 \pm 1\%$ before to $21 \pm 2\%$ after DNV. During airway occlusion, DIAm RMS_{peak} and RMS₇₅ EMG values did not change after DNV (Fig. 2 C&D; Fig. 4 E&F). Before DNV, RMS₇₅ EMG was $25 \pm 3\%$ during airway occlusion and $6 \pm 2\%$ after DNV.

3.3. Plethysmography and Arterial Blood Gases

Ventilatory parameters before and after DNV are shown in Table 1. Although these measures were obtained in a separate group of animals, respiratory rate and inspiratory time were comparable when measured using plethysmography to measurements obtained from the DIAM EMG. As a result of the increase in respiratory rate (by 48%; $p < 0.01$) and despite a small decrease in tidal volume (17% reduction; $p < 0.01$), minute ventilation increased by 24% ($p < 0.01$) after DNV. However, this was not reflected by changes in blood gases (Table 2), suggesting a change in dead-space ventilation without a change in alveolar ventilation. Comparing before and after DNV, peak inspiratory flow during eupnea decreased by 12% ($p < 0.01$), expiratory time decreased by 34% ($p < 0.001$) after DNV.

4. DISCUSSION

In the present study, we found that in rats, DIAM hemiparalysis induced by unilateral DNV did not affect the Pdi generated during ventilatory behaviors and did not affect normal ventilation (as determined by blood gas levels). However, neural drive to the contralateral DIAM (RMS_{75}) increased significantly to compensate for the loss of force contributed by the right paralyzed DIAM. These results indicate that the force reserve of the contralateral DIAM is sufficient to sustain ventilation in the rat even after unilateral DIAM paralysis. In contrast, we found that higher force, non-ventilatory DIAM motor behaviors requiring greater than ~50% Pdi_{max} were compromised after unilateral DIAM DNV. These results suggest that essential airway clearance behaviors (e.g., coughing, sneezing) may be affected when the force reserve of the whole DIAM is compromised.

There are a number of conditions that may induce unilateral hemidiaphragm paralysis including: pulmonary malignancy (Welvaart et al., 2011), postoperative thoracic surgery complications (Smith et al., 2013), indwelling central venous catheters (Takasaki and Arai, 2001), and high cervical spinal cord injury (Goshgarian, 2003; Gransee et al., 2013; Mantilla et al., 2013a; Mantilla et al., 2013b; Mantilla and Sieck, 2009; Miyata et al., 1995; Vinit et al., 2006; Zhan et al., 1997). In all these conditions, the substantial reserve capacity of the DIAM for generating force may be significantly compromised, even if ventilation appears unimpaired. Thus, Pdi assessments conducted across a range of ventilatory and non-ventilatory behaviors are necessary to fully evaluate the functional impact of respiratory and neuromuscular diseases that affect DIAM strength.

4.1. Changes in Neural Drive to the Contralateral DIAM and Ventilatory Patterns after DNV

After DNV, respiratory rate increased by 35–47% together with a 21–24% increase in inspiratory duration and a 63% increase in duty cycle. During eupnea, there was also a 12% decrease in PIF and 17% decrease in V_t . At the same time neural drive to the contralateral DIAM (RMS_{75}) increased by 60%. This change in neural drive to the contralateral DIAM during eupnea is consistent with our previous reports (Mantilla et al., 2013b; Miyata et al., 1995; Prakash et al., 1999). In two separate rat models of DIAM hemiparalysis, unilateral DNV, and tetrodotoxin-induced phrenic nerve conduction blockade, we found that EMG RMS_{peak} of the intact, contralateral DIAM increased by ~50% during eupnea relative to measures before hemiparalysis (Miyata et al., 1995; Prakash et al., 1999). In the present

study, EMG RMS_{peak} of the contralateral DIAM increased by 60% during eupnea after DNV. Such an increase in EMG RMS_{peak} might be expected since normally, the two sides of the DIAM act synergistically to generate Pdi. Accordingly, the increase in contralateral DIAM motor output after unilateral DNV is most likely the result of a compensatory increase in central drive to the phrenic motoneuron pool following synergist removal. The increase in RMS_{75} in the left DIAM found post-DNV supports such an increase in central drive (i.e., a compensatory loading effect). Unilateral DIAM DNV would amount to a substantial increase in load on the intact side of the DIAM. Consistent with such an increase in load, PIF decreased by 12%, while inspiratory duration increased after unilateral DNV. Thus, during eupnea it is likely that the velocity of shortening of the intact DIAM decreased in response to an increased load.

4.2. Motor Unit Recruitment after DNV

The changes in neural drive and breathing pattern after DIAM hemiparalysis are likely a compensatory strategy to maintain adequate ventilation using only the contralateral pool of DIAM motor units. As in skeletal muscles, motor units in the DIAM comprise motor neurons and the muscle fibers they innervate (Fournier and Sieck, 1988a; Sieck, 1994; Sieck and Fournier, 1989). The properties of both phrenic motor neurons and muscle fibers are matched (Enad et al., 1989; Sieck, 1994) and critically determine the efficacy of the central nervous system in accomplishing specific motor tasks. During neural activation, motor units are recruited in an orderly fashion, based on the intrinsic electrophysiological properties of motoneurons such that, for a given synaptic input, smaller more excitable motor neurons innervating more fatigue resistant muscle fibers (type I and IIa fibers comprising type S and type FR motor units) are recruited before larger motor neurons innervating more fatigable muscle fibers (type IIx and IIb fibers comprising type FInt and type FF motor units) (Butler et al., 1999; Henneman et al., 1965; Mantilla and Sieck, 2011; Sieck and Fournier, 1989). The recruitment order of motor units is not likely to change after acute DIAM hemiparalysis. Thus, after DNV, a larger fraction of the contralateral DIAM motor unit pool is required to accomplish ventilatory behaviors reflected by the marked increase in RMS_{75} . However, the decreased total number of fatigue resistant motor units available after unilateral DNV may affect ventilatory behaviors as well. With a reduced total number of available fatigue resistant motor units and an insufficient number on the contralateral side, it may become necessary to recruit more fatigable motor units to perform ventilatory behaviors unless breathing strategy changes. After unilateral DNV, with a 50% decrease in the total number of DIAM motor units that can contribute to Pdi generation, it is not surprising that the ability of the DIAM to generate Pdi during higher force, non-ventilatory behaviors is compromised. What is surprising is that the Pdi_{max} induced by phrenic nerve stimulation was reduced by only 37% after DNV.

In previous studies from our lab, a simple model was proposed for the recruitment of different motor unit types in the DIAM when accomplishing a range of ventilatory and non-ventilatory behaviors. In this model, eupnea is accomplished by recruitment of type S and type FR motor units across species (Fournier and Sieck, 1988a; Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck and Fournier, 1989). Typically, recruitment of more fatigable (FInt and FF) motor units in the rat DIAM is required only during higher force,

non-ventilatory behaviors that are infrequent and shorter in duration. Following unilateral DNV, it is likely that an increase in central drive to the contralateral phrenic motor neuron pool is necessary to recruit additional motor units to maintain adequate ventilation. This may impinge on recruitment of more fatigable (FInt and FF) motor units, but this is difficult to assess.

4.3. Compensatory Respiratory Plasticity after DNV

Neural plasticity is an essential aspect of respiratory control, providing adaptive mechanisms at all levels (central, spinal, peripheral) in response to inadequate ventilation (Feldman et al., 2013; Mantilla and Sieck, 2003). Indeed, compensatory respiratory plasticity following unilateral DIAM hemiparalysis has been studied extensively for more than a century (Fuller et al., 2008; Golder et al., 2003; Golder et al., 2001; Goshgarian, 2003; Mantilla et al., 2013a; Mantilla et al., 2014a; Mantilla et al., 2013b; Miyata et al., 1995; Porter, 1895; Prakash et al., 1999; Sieck, 1994; Zhan et al., 1997). However, the precise mechanisms responsible for the regulation and integration of central respiratory drive have not yet been fully elucidated.

Unlike other neural systems involving precise control of motor behaviors, the neural circuitry controlling DIAM activation is unique because the phrenic motor neurons must be activated rhythmically and repeatedly to maintain ventilation. The coordination, and timing, of neural drive to the phrenic motoneuron pool is complex and integration may occur at various levels within the central nervous system. A compensatory increase in central respiratory drive or adaptations at the motor unit level all serve to preserve breathing capacity after perturbation (Butler, 2007; Johnson and Mitchell, 2013; Mantilla and Sieck, 2003, 2009; Miyata et al., 1995; Zhan et al., 1997).

A myriad of sensory afferent inputs have the potential to directly affect respiratory motor output, including detection of acute changes in arterial blood gases (O_2 , CO_2 pH) via central (Richerson et al., 2005) and peripheral chemoreceptors (Kumar and Prabhakar, 2007), and changes in lung volume via pulmonary stretch receptors (Schelegle and Green, 2001). Collectively, these afferent signals may modulate the central pattern generator for respiration (Feldman et al., 2013; Mantilla et al., 2014b). In this respect, these compensatory mechanisms remained intact after unilateral DNV since ventilation was maintained with an increase in central drive to contralateral phrenic motor neuron pool.

The reduced PIF and rate of lung inflation was associated with a 19% decrease in V_t . Changes in lung volume may impact DIAM force generating capacity by altering the force-length relationship. Lung inflation beyond FRC causes a shortening of the DIAM and reduction in force development (De Troyer et al., 2005; Mier et al., 1990). However, it is unlikely that this occurred in the present study since the abdomen was bound to minimize changes in FRC. A comprehensive assessment of Pdi in response to stimulation of the phrenic nerves across a range of lung volumes above and below FRC before DNV was beyond the scope of the present study, but any change in FRC induced by DIAM hemiparalysis could certainly complicate Pdi generation. To avoid such complications, measurements were made with the abdomen bound to limit changes in FRC and to maintain isometric conditions across motor behaviors (Greising et al., 2013; Mantilla et al., 2010;

Sieck and Fournier, 1989). In a previous study (Zhan et al., 1995), we directly examined changes in DIAM length (using sonomicrometry) induced by unilateral DNV. We found that there was no change in static resting length of the DIAM (e.g., as would be imposed by an increase in end-expiratory lung volume) and only very minimal changes in rhythmic passive length of the paralyzed DIAM imposed by continued activation of the intact contralateral side. These passive length changes depended on DIAM region with passive stretch in the costal region and passive lengthening in the sternal region due to differences in fiber orientation. After performing these *in vivo* measurements of DIAM length, the DIAM was excised and *in vitro* experiments were then conducted, in which it was demonstrated that the passive length changes of the paralyzed DIAM would have had minimal impact on the force generation.

After DNV, a compensatory loading effect on the contralateral DIAM may contribute to the observed increase in central drive to contralateral phrenic motor neurons. Typically, in skeletal muscle, muscle spindles (group Ia) and Golgi tendon organs (group Ib) respond to changes in muscle length and force, respectively. Thus, an increase in central respiratory drive after acute DNV may have been mediated by input from muscle spindles and/or Golgi tendon organs emanating from the DIAM or chest wall. Muscle spindles are rarely found in the DIAM with most if present, being found in the crural portion of the DIAM (Duron et al., 1978; Langford and Schmidt, 1983). The majority of proprioceptive afferents in the DIAM arise from Golgi tendon organs (Road, 1990) situated near the tendinous portion of the crural and ventral costal DIAM regions. It is possible that paralysis contralateral DIAM after DNV resulted in withdrawal of inhibitory input emanating from Golgi tendon organs on that side. Such an effect of Golgi tendon organ input has been previously reported as the phrenic-to-phrenic inhibitory reflex (De Troyer et al., 1999; De Troyer, 1998; Gill and Kuno, 1963).

5. Conclusions

Unilateral DIAM DNV and paralysis does not affect Pdi generation during ventilatory behaviors, while Pdi generation during higher force, non-ventilatory behaviors is significantly compromised. The maintenance of Pdi during ventilatory behaviors is the result of an increase in neural drive to the contralateral DIAM. These results are consistent with the presence of a large reserve capacity for ventilatory functions of the DIAM. There are a number of conditions that result in DIAM weakness, and the large reserve capacity of the DIAM for ventilatory functions may obscure the impact of weakness on the performance of important higher force, non-ventilatory functions during disease progression. Thus, it is important to fully evaluate DIAM motor performance in ventilatory and non-ventilatory motor behaviors.

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PRE **POST** **Figure 1.**

Representative measurements of transdiaphragmatic pressure (Pdi), raw diaphragm (DIAM) EMG (both left, L and right, R), and root mean squared (RMS) EMG before (PRE) and after (POST) unilateral (right, R) DNV in an adult male rat across 1) ventilatory behaviors: eupnea, hypoxia (10% O₂)/hypercapnia (5% CO₂) and spontaneous deep breaths; and 2) higher force, non-ventilatory behaviors: sustained airway occlusion and sneeze.

Figure 2.

Normalized Pdi (A and B) and RMS EMG (C and D) during ventilatory and higher force, non-ventilatory behaviors before (Pre, white bars) and after (Post, black bars) sham (A and C) or DNV (B and D) surgery. Note that compared to values before surgery, Pdi generated during higher force, non-ventilatory behaviors (airway occlusion, and sneezing) were significantly reduced after DNV. In addition, RMS_{peak} EMG increased significantly during eupnea and hypoxia-hypercapnia after DNV. * $p < 0.05$, values are means $\pm$ SE.

Figure 3. Relationship between DIAM EMG (normalized to RMS_{peak}) and Pdi (normalized to Pdi_{max}) measurements in rats across different ventilatory and higher force, non-ventilatory behaviors before and after unilateral DNV

Figure 4.

Neural drive to the DIAM during different ventilatory and higher force, non-ventilatory behaviors was estimated by measuring the EMG RMS value at 75 ms after the onset of activity (RMS_{75}). In addition, the peak EMG RMS value (RMS_{peak}) was measured. Both values were normalized to the estimated maximal RMS values (RMS_{max}) based on measures during airway occlusion. A) Averaged EMG RMS traces obtained during eupnea before surgery (intact) and after sham surgery or unilateral DNV. Dashed line at 75 ms indicates the point at which RMS_{75} was calculated. B) Normalized EMG RMS_{75} (white bars) and

RMS_{peak} (black bars) values during eupnea increased significantly after DNV. C) Averaged EMG RMS traces during hypoxia-hypercapnia before (intact), and after sham surgery or unilateral DNV. D) Normalized EMG RMS₇₅ (white bars) and RMS_{peak} (black bars) values during hypoxia-hypercapnia increased significantly after DNV. E) Averaged EMG RMS traces during airway occlusion before (intact) and after sham surgery or unilateral DNV. F) Normalized EMG RMS₇₅ (white bars) and RMS_{peak} (black bars) values during airway occlusion did not change after DNV. * $p < 0.05$, values are means $\pm$ SE.

Table 1

Ventilatory parameters before (pre) and after (post) unilateral DNV.

Ventilatory parameter	Pre-DNV	Post-DNV
Respiratory rate (min^{-1})	61 ± 5	90 ± 5 *
Tidal volume (ml)	1.6 ± 0.1	1.3 ± 0.1 *
Ventilation (ml min^{-1})	90.1 ± 5.9	112.1 ± 10.0
Peak inspiratory flow (ml s^{-1})	9.0 ± 0.6	8.0 ± 0.6 *
Peak expiratory flow (ml s^{-1})	7.5 ± 0.4	7.4 ± 0.5
Inspiratory time (s)	0.28 ± 0.0	0.34 ± 0.0
Expiratory time (s)	0.7 ± 0.1	0.5 ± 0.0 *

Values are means $\pm$ SE.

*
p<0.05

Table 2

Arterial blood gas values before (pre) and after (post) unilateral DNV indicate that ventilation was maintained after DNV.

Blood gas value	Pre-DNV	Post-DNV
pH	7.41 $\pm$ 0.01	7.41 $\pm$ 0.01
PCO ₂ (mmHg)	39.7 $\pm$ 0.9	37.9 $\pm$ 0.8
PO ₂ (mmHg)	94.0 $\pm$ 1.5	92.4 $\pm$ 1.0
HCO ₃ ⁻ (mM)	24.2 $\pm$ 0.5	23.4 $\pm$ 0.6
SaO ₂ (%)	98 $\pm$ 0	95 $\pm$ 0

Values are means $\pm$ SE.