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Diaphragm Motor Unit Recruitment in Rats

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Abstract

We hypothesized that considerable force reserve exists for the diaphragm muscle (DIAM) to generate transdiaphragmatic pressures (Pdi) necessary to sustain ventilation. In rats, we measured Pdi and DIAM EMG activity during different ventilatory (eupnea and hypoxia (10% O₂) – hypercapnia (5% CO₂)) and non-ventilatory (airway occlusion and sneezing induced by intranasal capsaicin) behaviors. Compared to maximum Pdi (Pdi_{max} - generated by bilateral phrenic nerve stimulation), the Pdi generated during eupnea (21±2%) and hypoxia-hypercapnia (28±4%) were significantly less (P<0.0001) than that generated during airway occlusion (63±4%) and sneezing (94±5%). The Pdi generated during spontaneous sighs was 62±5% of Pdi_{max}. Relative DIAM EMG activity (root mean square [RMS] amplitude) paralleled the changes in Pdi during different ventilatory and non-ventilatory behaviors (r²=0.78; p<0.0001). These results support our hypothesis of a considerable force reserve for the DIAM to accomplish ventilatory behaviors. A model for DIAM motor unit recruitment predicted that ventilatory behaviors would require activation of only fatigue resistant units.

Keywords

respiration; recruitment order; phrenic nerve; motor units

1. Introduction

The diaphragm muscle (DIAM) is the main inspiratory muscle in mammals and comprises different muscle fiber types organized by their innervation into motor units that vary in their mechanical and fatigue properties (Fournier & Sieck, 1988; Sieck, 1988). In adults, all muscle fibers within a DIAM motor unit are the same fiber type (Enad et al., 1989; Johnson & Sieck, 1993; Sieck et al., 1989a; Sieck et al., 1996). Motor units with slower contraction times and resistance to fatigue (type S) comprise fibers that express the slow isoform of myosin heavy chain (MyHC_{Slow}) and have higher mitochondrial volume density and oxidative capacity (Enad et al., 1989; Sieck et al., 1996). Three types of motor units display faster contraction times, but vary in their fatigue resistance. Fast motor units that are fatigue-resistant (type FR) comprise muscle fibers expressing the MyHC_{2A} isoform with higher mitochondrial volume density and oxidative capacity; those that are highly fatigable (type FF) comprise muscle fibers expressing both MyHC_{2B} and MyHC_{2X} isoforms with lower

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mitochondrial volume density and oxidative capacity; and those that are fatigue-intermediate (type FInt) comprise muscle fibers that express only the MyHC_{2X} isoform with intermediate mitochondrial volume density and oxidative capacity (Sieck et al., 1996). Fiber types comprising these motor units also display differences in mechanical properties including maximum specific force, force-calcium relationships that underlie submaximal activation (Geiger et al., 2000; Geiger et al., 1999) and cross bridge cycling kinetics (Sieck & Prakash, 1997).

In previous studies in cats (Fournier & Sieck, 1988; Sieck & Fournier, 1989) and hamsters (Sieck, 1991a; Sieck, 1994), we measured transdiaphragmatic pressure (Pdi) to estimate DIAM forces generated during different ventilatory and non-ventilatory behaviors. Maximum Pdi ($P_{di_{max}}$) was determined by bilateral phrenic nerve stimulation. In cats, Pdi generated during quiet breathing (eupnea) was ~12% $P_{di_{max}}$, whereas in hamsters, eupneic Pdi was ~27% $P_{di_{max}}$. When breathing was stimulated by exposing animals to a hypoxic (10% O₂) and hypercapnic (5% CO₂) gas mixture, DIAM forces increased to ~28% $P_{di_{max}}$ in cats. During sustained tracheal occlusion, DIAM forces increased to ~49% $P_{di_{max}}$ in cats and ~43% $P_{di_{max}}$ in hamsters. Only during short duration expulsive behaviors (e.g., coughing, sneezing) were maximal DIAM forces generated in both species. Based on these results, we hypothesized that considerable force reserve exists for the DIAM to generate Pdi necessary to sustain ventilation.

In cat DIAM, motor unit recruitment during these different ventilatory and non-ventilatory behaviors was estimated based on direct measurements of maximum forces generated by single type-identified motor units (Fournier & Sieck, 1988) and estimates of the proportion of different motor unit types within the DIAM (Sieck & Fournier, 1989; Sieck et al., 1986). In a model of DIAM neuromotor control, we assumed that motor units were recruited in an orderly fashion (type S first, followed by type FR, FInt and FF), that maximum force was generated by each recruited motor unit and that full recruitment of all motor units of a single type was achieved before recruitment of the next type (Sieck, 1991a; Sieck, 1994; Sieck & Fournier, 1989). In this model, eupneic Pdi required recruitment of only type S motor units, whereas the increased Pdi during exposure to hypoxia-hypercapnia required additional recruitment of type FR units. During sustained tracheal occlusion, additional recruitment of some type FInt motor units was required. Only during short duration expulsive behaviors was full recruitment of all motor unit types required. Our analyses in the cat and hamster clearly indicated that species differences exist in the relative Pdi generated during different motor behaviors (as a fraction of $P_{di_{max}}$). This most likely relates to differences in the mechanical properties of the respiratory system, e.g., varying lung and/or chest wall compliance.

The rat is a common animal model used extensively in physiological studies of respiratory neuromotor control. The purpose of the present study was to measure relative Pdi generated during different motor behaviors (e.g., eupnea, hypoxia-hypercapnia, sustained airway occlusion and sneezing). Based on this information, we estimated motor unit recruitment in the rat DIAM during these motor behaviors.

2.1. Methods

2.1. Animals and Experimental Groups

Eight adult male, Sprague-Dawley rats (~300g) were used. All experimental procedures were approved by the Institutional Animal Care and Use Committee, and were in accordance with the American Physiological Society Animal Care Guidelines. Rats were anesthetized via intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) for all procedures.

2.2. Transdiaphragmatic Pressure (Pdi)

Forces generated by the DIAM during different motor behaviors were estimated by measuring Pdi, using a modification of a previously reported technique (Sieck, 1991a; Sieck, 1994; Sieck & Fournier, 1989). In anesthetized animals, the abdomen was constrained using a custom-made binder that restricted outward movements of the abdomen during inspiration. The abdomen must be constrained in order to better approximate isometric conditions and thereby better achieve maximum Pdi. In addition, without abdominal constraint we found that Pdi measurements were far more variable. A dual barrel catheter, constructed with latex balloons attached to each opening, was inserted into the esophagus and stomach. The catheters were connected to a differential pressure transducer (DP45, Validyne, Northridge, CA). Positioning of the balloons in the mid-esophagus and stomach was confirmed by negative and positive pressures, respectively, during inspiration. The output of the differential pressure transducer was Pdi and this together with independent measures of thoracic and abdominal pressures were digitized at 400 Hz and recorded using custom-developed software in LabView (National Instruments, Austin, TX).

2.3. DIAM EMG Recording

To independently assess DIAM activation, EMG was recorded using previously described methods (Dow et al., 2006; Sieck & Fournier, 1990; Trelease et al., 1982). Briefly, pairs of multistrand fine wire, insulated (stripped to expose a ~2 mm segment at the tip), stainless steel electrodes (0.28 mm diameter - model AS631, Cooner Wire Inc., Chatsworth, CA) were implanted (~3 mm apart) into the midcostal regions of both right and left sides of the DIAM. Electrodes were implanted intramuscularly via an abdominal approach in anesthetized animals (see above). The loose ends were tunneled subcutaneously to the dorsum of the animal and externalized. Animals were allowed to recover for at least 3 days to avoid laparotomy-induced suppression of respiratory drive. At the time of recording, externalized electrodes were connected to a differential amplifier (Model 2124, DATA Inc.), and EMG signals were amplified, band pass filtered (20–1000 Hz), and digitally sampled at 2 kHz using a data acquisition board (National Instruments, Austin, TX). To assess DIAM EMG activity, the root-mean-squared (RMS) signals were computed using a 50-ms window. The duration of inspiration, respiratory rate, duty cycle (inspiratory time to total cycle time; $T_I:T_{tot}$) and peak amplitude were also determined. For each animal, DIAM EMG activity was normalized to the respective maximal value (RMS EMG).

2.4. DIAM Activity during Different Ventilatory and Non-ventilatory Behaviors

In anesthetized, spontaneously breathing rats, both Pdi and DIAM EMG activity were recorded during each of the following conditions: 1) eupnea (breathing room air), 2) hypoxia (10% O₂) – hypercapnia (5% CO₂), 3) sustained (45 s) airway occlusion induced by forced closure of the airway, and 4) sneezing induced by intranasal infusion of 10 µl of 30 µM capsaicin (Kamei et al., 1990). During each of these conditions, peak amplitudes of the Pdi and EMG RMS signals were averaged during eupnea and hypercapnia-hypoxia (usually ~2 min for each). During airway occlusion, measurements were averaged for the last 10 s of the 45 s occlusion period. During sneezing, peak values were calculated and found to approximate Pdi_{max} as determined using bilateral phrenic nerve stimulation at 75 Hz (0.5 ms duration pulses at 5 V in 300 ms trains repeated each s) elicited via a pair of silver electrodes applied to the phrenic nerve exposed in the neck. In all studies, we performed experiments in the following sequence: eupnea, hypoxia-hypercapnia, sneezing, airway occlusion and phrenic nerve stimulation. Phrenic nerve stimulation was performed last because additional surgery to expose the phrenic nerve in the neck is required. Animals were thus provided with a fairly long (15–20 min) recovery period following airway occlusion before phrenic nerve stimulation. In all cases, breathing rate and pulse oximetry measures had returned to

baseline levels. For each animal, both Pdi and DIAM EMG activity were normalized to the respective maximal values.

2.5. Estimate of Motor Unit Recruitment

The extent of motor unit recruitment during different ventilatory and non-ventilatory behaviors was estimated based on a model previously developed in the cat (Sieck & Fournier, 1989) and hamster (Sieck, 1991a; Sieck, 1994). Individual motor unit properties were not assessed but were approximated based on previous measurements of: 1) specific force (force per cross-sectional area) in single DIAM fibers (Geiger et al., 2002; Geiger et al., 2001b; Geiger et al., 2000; Geiger et al., 1999; Sieck, 1988), 2) cross-sectional areas of type-identified fibers (Lewis & Sieck, 1990; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997), 3) proportion of different fiber types, and 4) assumptions of the number of fibers innervated by each motoneuron and an innervation ratio ~15% greater at type FF and FInt motor units than at type S or FR units (Enad et al., 1989; Fournier & Sieck, 1988; Sieck, 1988; Sieck et al., 1989a; Sieck et al., 1996). In the model, it was assumed that motor units were recruited in an orderly manner, with type S recruited first, followed by type FR, type FInt and finally type FF units. We also assumed that all motor units of one type were fully activated before activation of the next motor unit type.

2.6. Statistical Analysis

Differences in Pdi and DIAM EMG activity across experimental groups and motor behaviors were evaluated using repeated-measures MANOVA. When appropriate, *post hoc* analyses were conducted using the Tukey-Kramer honestly significant difference (HSD) test. 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

3.1. Pdi during Different Motor Behaviors

Representative tracings of Pdi generated during different ventilatory and non-ventilatory motor behaviors are shown in Fig. 1. With bilateral phrenic nerve stimulation, Pdi_{max} was 37.3 ± 2.0 cm H₂O. As expected, Pdi increased progressively from eupnea (7.7 ± 0.5 cm H₂O) to hypoxia-hypercapnia (10.1 ± 1.4 cm H₂O), airway occlusion (23.4 ± 2.3 cm H₂O) and sneezing (34.8 ± 2.0 cm H₂O; $p < 0.0001$). Sneezing behavior was characterized by concomitant increases in esophageal and gastric pressures (Fig. 2).

The normalized Pdi (as a percentage of Pdi_{max}; Fig. 3) generated during eupnea was $21 \pm 2\%$, during hypoxia-hypercapnia $28 \pm 4\%$, during sustained airway occlusion $63 \pm 4\%$, and during sneezing $94 \pm 5\%$ of Pdi_{max} ($p < 0.0001$). During sneezing, Pdi was not significantly different from bilateral phrenic nerve stimulation, indicating that Pdi_{max} is generated in rats during sneezing induced by intranasal infusion of capsaicin. The Pdi generated during hypoxia-hypercapnia was not significantly different from that generated during eupnea, however the Pdi generated during all other motor behaviors was significantly greater than these two ventilatory conditions ($p < 0.05$).

It is worth noting that during spontaneous breathing conditions (i.e., eupnea and hypoxia-hypercapnia), sporadic large breaths (i.e., sighs) were evident in 6 (out of 8) animals. These sighs were relatively infrequent ($\sim 0.2/\text{min}$). However, the normalized Pdi generated during these sighs was relatively large ($62 \pm 5\%$ of Pdi_{max}; $p < 0.05$ compared to eupnea and hypoxia-hypercapnia), comparable to Pdi generated during sustained airway occlusion.

3.2. DIAM EMG Activity during Different Motor Behaviors

Representative DIAM EMG traces are shown in Fig. 1 during eupnea, hypoxia/hypercapnia, tracheal occlusion and sneezing. Sneezing induced by airway irritation with capsaicin resulted in a prolonged period of apnea before a large amplitude response that typically comprised an initial, lower level of EMG activity followed by high-burst activity of relative short duration (Fig. 1). In general agreement with Pdi findings (Fig. 3), RMS EMG amplitude increased progressively from eupnea ($26 \pm 4\%$ of maximal) to hypoxia/hypercapnia ($39 \pm 5\%$) to sustained airway occlusion ($69 \pm 4\%$) to sneezing ($p < 0.001$). Unlike Pdi measurements, the RMS EMG amplitude during hypoxia-hypercapnia was significantly greater than that during eupnea ($p < 0.05$). Other than this slight difference there was remarkable concordance between normalized Pdi and RMS EMG measures ($r^2 = 0.78$; $p < 0.0001$). Also in agreement with Pdi measurements, RMS EMG amplitude during sighs was $63 \pm 5\%$ of maximum ($p < 0.05$ compared to eupnea and hypoxia-hypercapnia), also comparable to that during sustained airway occlusion.

3.3. Ventilatory Parameters

Ventilatory parameters were determined from the DIAM EMG during eupnea and hypoxia/hypercapnia. Respiratory rate was $62 \pm 5 \text{ min}^{-1}$ during eupnea, increasing to $87 \pm 6 \text{ min}^{-1}$ during exposure to hypoxia-hypercapnia. The inspiratory burst duration was $0.38 \pm 0.01 \text{ s}$ and $0.46 \pm 0.02 \text{ s}$ during eupnea and hypoxia-hypercapnia, respectively ($p < 0.05$). Accordingly, the duty cycle during eupnea was $39\% \pm 2\%$ compared to $66\% \pm 4\%$ during hypoxia-hypercapnia ($p < 0.05$).

During non-ventilatory behaviors, burst duration was 0.70 ± 0.03 and $0.87 \pm 0.09 \text{ s}$ for sustained airway occlusion and sneezing, respectively ($p < 0.05$ compared to both eupnea and hypoxia-hypercapnia).

3.4. Motor Unit Recruitment

Figure 4 shows the maximal force produced by full activation of all muscle fibers in a DIAM motor unit of each type. Estimates of motor unit force were based on previously published information (Table 1) on specific force (force per cross-sectional area) in single DIAM fibers, cross-sectional areas of type-identified fibers and the proportion of different fiber types (Geiger et al., 2002; Geiger et al., 2001b; Geiger et al., 2000; Geiger et al., 1999; Lewis & Sieck, 1990; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997). This information can thus be used to generate a model estimating DIAM motor unit recruitment during different motor behaviors in rats, assuming an orderly and full recruitment of each motor unit type (Fig. 5). Based on this model, the Pdi generated during eupnea in rats would require the recruitment of only fatigue resistant type S and FR motor units. The Pdi generated during exposure to hypoxia-hypercapnia would require recruitment of all type S and FR motor units, and that generated during airway occlusion would require additional recruitment of nearly all type FInt units. In this model (Fig. 5), recruitment of type FF units would occur only during sneezing (i.e., during maximal or near maximal conditions).

4. Discussion

In rats, ventilation during eupnea and during exposure to hypoxia and hypercapnia can be sustained by generation of Pdi that is less than 30% of total force generating capacity of the DIAM. Thus, there is considerable force reserve in the rat DIAM for generation of Pdi required during ventilatory behaviors. This force reserve is necessary to accomplish non-ventilatory behaviors involved in deep sighs and clearing the airways (expulsive behaviors). A simple model was proposed for the recruitment of different motor unit types in the rat

DIAM to accomplish these different motor behaviors. In this model, generation of sustained Pdi during ventilatory behaviors requires recruitment of only fatigue resistant type S and FR DIAM motor units. Recruitment of more fatigable FIInt and FF motor units in the rat DIAM is required only during non-ventilatory behaviors that are typically infrequent and shorter in duration.

4.1. Diaphragm muscle force across behaviors

We found that as a percentage of Pdi_{max} (elicited by bilateral phrenic nerve stimulation), the Pdi generated by the rat DIAM during eupnea was 21% of Pdi_{max} compared to 12% of Pdi_{max} in the cat and 27% of Pdi_{max} in the hamster. In humans, Pdi generated by the DIAM during eupnea was estimated at ~10% of Pdi_{max} (Sieck, 1994). Thus, eupneic Pdi appears generally to scale with animal size. Exposure to hypoxia-hypercapnia represents the maximum requirement of ventilatory behaviors; yet, Pdi only increased to ~28% of Pdi_{max} in both rats and cats. Even during sustained airway occlusion, the forces generated by the DIAM amounted to only 63%, 49% and 43% of Pdi_{max} in the rat, cat and hamster, respectively. Differences in normalized Pdi and the reserve capacity for force generation by the DIAM across species may reflect the different ventilatory demands placed on this muscle, with more diverse behaviors being necessary in larger species as well as different mechanical properties of the respiratory system, e.g., varying lung and/or chest wall compliance.

The forces generated by the DIAM during ventilatory behaviors (i.e., eupnea and hypoxia-hypercapnia) could be generated by recruitment of only type S and FR motor units in rats (present study), cats (Sieck, 1991a; Sieck & Fournier, 1989) and hamsters (Sieck, 1994). Consistent with the disparate ventilatory demands, the proportion of fatigue-resistant motor units (type S and FR units) is ~65% in the rat and ~54% in the hamster, compared to ~34% in the cat (Sieck, 1991a; Sieck & Fournier, 1989). Interestingly, there were relatively large “sigh” breaths during eupnea and hypoxia-hypercapnia in the rat where the DIAM generated forces similar to those evoked during airway occlusion (~60% of Pdi_{max}), suggesting that recruitment of type FIInt motor units occurs during spontaneous ventilatory behaviors. In general agreement with previous studies in the cat and hamster (Sieck, 1991a; Sieck, 1994; Sieck & Fournier, 1989), Pdi generated in the rat during sneezing was near maximal, measured during bilateral phrenic nerve stimulation. Thus, only during short duration expulsive behaviors (e.g., sneezing or coughing) are maximal DIAM forces generated in all species. In a similar model of motor unit recruitment in the cat medial gastrocnemius (Walmsley et al., 1978), a large portion of the motor unit pool would also only be recruited during short-duration behaviors requiring high levels of force generation (e.g., jumping).

In other species, the latter stages of prolonged tracheal occlusion can elicit an “asphyxic” response, which is characterized by an intense expiratory effort immediately followed by a large inspiratory effort (Bucher et al., 1972). This type of autoresuscitative behavior is thought to be more relevant to the Heimlich maneuver than to expulsive behaviors such as coughing or sneezing (Bolser, 1991). Although the expiratory component of this behavior is blunted in anesthetized animals, the mechanics of the inspiratory efforts that were recorded in rats were not qualitatively different from other large inspirations and were not preceded by strong abdominal contractions (Fig. 2), indicating that an asphyxic response was not elicited in our rat preparations. In fact, the asphyxic response usually requires much longer periods of airway occlusion (~90 sec). Regardless, the sneezing behavior we observed in rats likely reflects physiological patterns of motor activation and, importantly, is near maximal (Pdi_{max}).

The mechanical forces generated by the DIAM correlated very well with electrical activation measured by peak RMS EMG amplitude (Fig. 3). Although RMS EMG amplitude in the

DIAM increased significantly during hypoxia-hypercapnia, Pdi increased only slightly compared to eupnea. However, with the increased ventilatory demand associated with hypoxia-hypercapnia, rats increased their respiratory rate, burst duration and therefore duty cycle (~2-fold). The correlation between Pdi and peak RMS EMG amplitude for the rat DIAM ($r^2 = 0.78$) suggests that DIAM EMG activity may be used as surrogate for Pdi measurements when these are impractical (e.g., in awake animals) and highlights the importance of measuring behaviors with near maximal activation (e.g., sneezing). Techniques permitting chronic EMG recordings (Trelease et al., 1982), if adapted to the rat, could be useful for assessment of DIAM function over time, e.g., during assessment of functional recovery following spinal cord injury (Sieck & Mantilla, 2009). Although EMG recordings were performed in the costal DIAM, activity and fiber type composition in costal and crural regions of the DIAM is similar (Oyer et al., 1989; Reid et al., 1992; Sieck, 1988), and thus, EMG activity of the costal region is representative of motor unit activation in the entire DIAM.

4.2. Modeling DIAM motor unit recruitment and force generation

The model of motor unit recruitment during the different ventilatory and non-ventilatory behaviors was estimated based on previous measurements of (Table 1): 1) specific force (force per cross-sectional area) in single DIAM fibers (Geiger et al., 2002; Geiger et al., 2001b; Geiger et al., 2000; Geiger et al., 1999; Sieck, 1988), 2) cross-sectional areas of type-identified fibers (Lewis & Sieck, 1990; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997), 3) proportion of different fiber types, and 4) assumptions of the number of fibers innervated by each motoneuron and an innervation ratio ~15% greater at type FF and Flnt motor units than at type S or FR units (Enad et al., 1989; Fournier & Sieck, 1988; Sieck, 1988; Sieck et al., 1989a; Sieck et al., 1996). Importantly, the muscle fibers included in a motor unit have homogeneous fiber type composition (Enad et al., 1989; Fournier & Sieck, 1988; Hamm et al., 1988; Nemeth et al., 1986; Sieck et al., 1996). The increasing slope of force development as additional motor units are recruited reflects proportionally larger force generation by these units (Fig. 4). The assumption that fatigue-resistant motor units are recruited first is supported by multiple studies in which phrenic motoneurons with slower axonal conduction velocities were shown to be recruited first during inspiratory efforts (Dick et al., 1987; Jodkowski et al., 1987; Jodkowski et al., 1988). Accordingly, we assumed that there is a sequential recruitment of motor units, with type S units being recruited first, followed by type FR, type Flnt and lastly type FF units. We did not confirm that this recruitment pattern matches predictions of the “size principle” nor has any other study exploring recruitment order in the phrenic motor unit pool.

Muscle force generated during a contraction reflects the number and type of motor units recruited (Burke et al., 1982; Enad et al., 1989; Fournier et al., 1991; Fournier & Sieck, 1988; Johnson & Sieck, 1993; Sieck, 1995; Sieck & Fournier, 1989). Both the cross-sectional area of type identified fibers and the maximum force of isolated fibers follow the rank order of $\text{MyHC}_{\text{Slow}} > \text{MyHC}_{2\text{A}} > \text{MyHC}_{2\text{X}} > \text{MyHC}_{2\text{B}}$ (Geiger et al., 2002; Geiger et al., 2001a; Geiger et al., 2000; Miyata et al., 1995; Sieck et al., 1996; Sieck & Prakash, 1997; Zhan et al., 1997). Thus, the relative contribution to overall muscle force attributable to all of the muscle fibers associated with a type-identified motor unit also follows the rank order: type S < type FR < type Flnt < type FF (Fournier & Sieck, 1988; Sieck, 1988; Sieck, 1991b; Sieck, 1994).

During an inspiratory burst, discharge frequencies may change at individual motor units (Butler et al., 1999; Kong & Berger, 1986). We are unable based on our techniques to determine onset and offset of individual motor units or their discharge frequency. It is also possible that motor unit discharge frequencies change across behaviors. Studies examining motor unit discharge rates during behaviors associated with high intensity contractions are

exceedingly difficult technically and thus, at present, there is a dearth of information regarding this important aspect of motor unit recruitment. We believe that the model presented herein represents a useful first approximation to the evaluation of motor unit plasticity.

4.3. Conclusions

The rat is a common animal model used extensively in physiological studies of respiratory neuromotor control. The present study measured relative Pdi generated during different motor behaviors (e.g., eupnea, hypoxia-hypercapnia, sustained airway occlusion and sneezing) providing estimates of motor unit recruitment in the rat DIAM during these motor behaviors. This information is necessary for the evaluation of the extent of motor recovery in conditions of injury or disease. Importantly, we also found that across motor behaviors, there was a high degree of correlation between relative RMS EMG and Pdi indicating that relative EMG measurements (normalized to maximal activity elicited during sneezing) may serve as a useful surrogate of DIAM force generation in future studies.

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Fig. 1.

Representative raw electromyographic (EMG), root mean square (RMS) EMG tracings and transdiaphragmatic pressure (Pdi) measurements obtained in a rat across different ventilatory and non-ventilatory behaviors: eupnea, hypoxia (10% O₂)/hypercapnia (5% CO₂), sustained airway occlusion and sneezing induced by airway irritation with capsaicin.

Fig. 2.

Representative raw esophageal (P_{eso}) and abdominal pressure (P_{gas}) measurements obtained during sneezing induced by airway irritation with capsaicin (see Methods).

Fig. 3.

Relationship between diaphragm muscle (DIAM) EMG activity (peak RMS EMG amplitude) and Pdi measurements across different ventilatory and non-ventilatory behaviors in adult rats. Bilateral phrenic nerve stimulation at 75 Hz was used to elicit Pdi_{max} . During sneezing, Pdi was not significantly different from Pdi_{max} and the RMS EMG was used as maximal. Both RMS EMG and Pdi increased progressively from eupnea to hypoxia-hypercapnia to airway occlusion to sneezing. The Pdi and RMS EMG generated during all motor behaviors was significantly different from that generated during eupnea or hypoxia-hypercapnia ($p < 0.05$).

Fig. 4.

Estimated force elicited by maximal activation of an individual motor unit of each type in the adult rat DIAM. Force was estimated based on previously published data on specific force (force per cross-sectional area) in single DIAM fibers, cross-sectional areas of type-identified fibers, and the number of fibers in the different motor unit types (Lewis & Sieck, 1990; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997) (Geiger et al., 2002; Geiger et al., 2001b; Geiger et al., 2000; Geiger et al., 1999).

Fig. 5. Model of motor unit recruitment for the rat DIAM. The extent of motor unit recruitment during different ventilatory and non-ventilatory behaviors was estimated based on a model previously developed in cats and hamsters (Sieck, 1991a; Sieck, 1994; Sieck & Fournier, 1989).

Table 1

Properties of Single Muscle Fibers and Fiber Type Composition in the Rat Diaphragm Muscle.

	Myosin Heavy Chain			
	<i>Slow</i>	<i>2A</i>	<i>2X</i>	<i>2B</i>
Cross-Sectional Area (μm^2) [*]	578 $\pm$ 16	672 $\pm$ 16	1673 $\pm$ 80	2452 $\pm$ 135
Fiber Proportions (%) [†]	36.4 $\pm$ 1.9	31.0 $\pm$ 1.4	24.4 $\pm$ 2.0	8.2 $\pm$ 1.7
Force (N/cm²) [‡]	9.8 $\pm$ 0.4	11.5 $\pm$ 0.6	14.4 $\pm$ 0.5	17.6 $\pm$ 0.6

^{*} Based on Lewis & Sieck, 1990; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997.

[†] Based on Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989b; Zhan et al., 1997.

[‡] Based on Geiger et al., 2002; Geiger et al., 2001b; Geiger et al., 2000; Geiger et al., 1999.