Published in final edited form as:

Respir Physiol Neurobiol. 2013 January 15; 185(2): 400–409. doi:10.1016/j.resp.2012.08.020.

Non-Stationarity and Power Spectral Shifts in EMG Activity Reflect Motor Unit Recruitment in Rat Diaphragm Muscle

Yasin B. Seven^a, Carlos B. Mantilla^{a,b}, Wen-Zhi Zhan^a, and Gary C. Sieck^{a,b}

^aDepartment of Physiology & Biomedical Engineering, Mayo Clinic, Rochester, MN, 55905, USA

^bDepartment of Anesthesiology, Mayo Clinic, Rochester, MN, 55905, USA

Abstract

We hypothesized that diaphragm muscle (DIAM) by a shift in the EMG power spectral density (PSD) to higher frequencies reflects recruitment of more fatigable fast-twitch motor units and motor unit recruitment is reflected by EMG non-stationarity. DIAM EMG was recorded in anesthetized rats during eupnea, hypoxia-hypercapnia (10% O₂-5% CO₂), airway occlusion, and sneezing (maximal DIAM force). Although power in all frequency bands increased progressively across motor behaviors, PSD centroid frequency increased only during sneezing ($p < 0.05$). The non-stationary period at the onset of EMG activity ranged from ~70 ms during airway occlusion to ~150 ms during eupnea. Within the initial non-stationary period of EMG activity 80–95% of motor units were recruited during different motor behaviors. Motor units augmented their discharge frequencies progressively beyond the non-stationary period; yet, EMG signal became stationary. In conclusion, non-stationarity of DIAM EMG reflects the period of motor unit recruitment, while a shift in the PSD towards higher frequencies reflects recruitment of more fatigable fast-twitch motor units.

Keywords

Motor Unit Recruitment; Power Spectrum; Diaphragm Muscle; Stationarity; Electromyography; Neuromotor Control

1. Introduction

Motor units are the final common pathway of neuromotor control, each comprising a single motor neuron and the group of muscle fibers it innervates. In the rat diaphragm muscle (DIAM) there are ~250–300 motor units on each side (Mantilla et al., 2009), and these motor units vary markedly in their contractile and fatigue properties (Sieck et al., 1989; Sieck et al., 1996). As in other skeletal muscles, motor control of DIAM force is achieved by recruitment of motor units and/or by modulation of discharge frequency (Sieck, 1991; Sieck et al., 1984).

© 2012 Elsevier B.V. All rights reserved.

Corresponding author: Gary C. Sieck, Ph.D., Department of Physiology & Biomedical Engineering, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN, 55905, USA, Phone (507) 775-0993, sieck.gary@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The compound EMG is the summation of individual motor unit action potentials (MUAP), and thus contains information about both recruitment and frequency coding of motor units during muscle activation (Keenan et al., 2005). The force generated by a muscle correlates well with integrated (e.g., root-mean-squared value - RMS) EMG activity, especially if longer window periods (>50 ms) for integration are used. For example, in rat DIAM, RMS EMG strongly correlates with transdiaphragmatic pressure generation across a range of motor behaviors (Mantilla et al., 2010). However, the RMS EMG does not provide specific information regarding the recruitment and/or frequency coding of individual motor units.

Fine wire recordings of MUAPs can be used to assess recruitment and discharge frequency of individual motor units. However, only the activities of 2–5 motor units can be recorded simultaneously, which in the case of the rat DIAM represents <2% of the entire motor unit pool; thus presenting a sampling problem when trying to characterize the entire pool. Moreover, as force increases, it becomes more difficult to distinguish the discharge of single motor units. Thus, single unit recording techniques, although previously used (Lee et al., 2009; Rice et al., 2011; Sieck et al., 1984), are inherently limited in characterizing neuromotor control.

Power spectral density (PSD) analyses of EMG activity may provide information on motor unit recruitment and perhaps frequency coding. According to a mathematical model of EMG, EMG PSD is influenced by the conduction velocity of recruited muscle fibers (Lindstrom and Magnusson, 1977). In single frog muscle fibers, action potential conduction velocities were shown to be directly proportional to fiber size (Hakansson, 1956, 1957). Action potentials of motor units with larger muscle fibers and faster conduction velocities likely have shorter MUAP duration, contributing to higher frequencies of the EMG PSD. DIAM motor units that are recruited during inspiration (at lower force levels) are likely to be slow- and fast-twitch fatigue resistant units composed of type I and IIa fibers that are significantly smaller than type IIx and/or IIb fibers comprising more fatigable fast-twitch motor units (Geiger et al., 2000; Miyata et al., 1995; Prakash et al., 2000; Sieck et al., 1989). Accordingly, we hypothesized that as the level of generated force increases, DIAM motor units will be recruited that have larger muscle fiber cross-sectional areas associated with shorter MUAP durations. Furthermore, we hypothesized that the DIAM EMG PSD during more forceful, non-ventilatory motor behaviors (e.g., sneezing) will have higher frequency content than eupnea, reflecting the additional recruitment of more fatigable fast-twitch motor units.

A requirement of PSD analysis is that the EMG signal is stationary at least in wide-sense, i.e., with respect to calculated mean and mean square values (Bilodeau et al., 1997; Mananas et al., 2001; Papoulis, 1984). While motor units are being recruited, the EMG signal is non-stationary; thus, in addition to being a criterion for PSD analysis, non-stationarity of the EMG signal should provide information regarding motor unit recruitment. We hypothesized that by analyzing the stationarity of EMG segments that are shorter (e.g., 10 ms) than the expected MUAP interpulse interval (~20–100 ms) the influence of frequency coding is minimized. Thus, motor unit recruitment is reflected by non-stationarity of the EMG signal. Combining analyses of PSD (what type of motor units are recruited) and non-stationarity (timing of motor unit recruitment) would thus provide a global assessment of DIAM motor unit recruitment across ventilatory and non-ventilatory behaviors.

2. Methods

2.1 Animals

A total of 27 adult, male, Sprague-Dawley rats with an approximate weight of 320 g were used for this study. All experimental procedures and techniques were approved by the

Institutional Animal Care and Use Committee, and were in accordance with the American Physiological Society Animal Care Guidelines. During all procedures, rats were anesthetized with an intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg).

2.2 Ventilatory and Non-Ventilatory Behaviors

Compound and single motor unit DIAM EMG were recorded during the following ventilatory and non-ventilatory motor behaviors: 1) eupnea (breathing in room air), 2) hypoxia-hypercapnia (breathing 10% O₂ and 5% CO₂ in N₂), 3) airway occlusion (forced closure of airways for ~40 s), and 4) sneezing (induced by intranasal administration of 10 μ l of 30 mM capsaicin) as described previously (Mantilla et al., 2011; Mantilla et al., 2010). Figure 1 illustrates representative DIAM EMG activity during the different motor behaviors. In analyses of DIAM EMG activity during eupnea and hypoxia-hypercapnia, inspiratory-related EMG bursts (~60 breaths per min) were recorded during a 2-min period and all spontaneous deep breaths ("sighs"; amplitude > 2 times eupneic average) were excluded. During airway occlusion, animals displayed repeated inspiratory efforts of increasing amplitude. Peak EMG activity during the last 10-s period of occlusion (reflecting ~2–5 bursts) was analyzed. During sneezing, EMG activity reflecting the largest bursts (the number of burst varied across animals ~2–5) was used for analyses. In a previous study, we found that this sneezing-related EMG activity approximated maximum DIAM activation as measured by Pdi generation (Mantilla et al., 2010). Animals were allowed to recover for at least 5 min after each behavior.

In addition to DIAM EMG recordings, respiratory rate and blood O₂ levels were also measured using a pulse oximeter (MouseOX, Starr Life Sciences Corp., Oakmont, PA). A return to baseline respiratory rate and normoxic levels was assured before analyzing the next motor behavior. During all recordings body temperature was monitored and maintained at 37°C using a heating pad.

2.3 Compound DIAM EMG Recordings

Intramuscular DIAM EMG was recorded in 15 rats to monitor DIAM activation during different ventilatory and non-ventilatory conditions as described previously (Mantilla et al., 2011; Mantilla et al., 2010; Miyata et al., 1995; Sieck and Fournier, 1990). Two pairs of insulated, stainless steel fine-wire electrodes with a diameter of 0.28 mm (AS631, Cooner Wire Inc., Chatsworth, CA) were implanted into the mid-costal regions of right and left sides of the DIAM. Approximately 2 mm of insulation was removed at the apposition point of the electrodes with the muscle. The distal ends of the electrodes were tunneled subcutaneously to the dorsum of the animal where they were externalized and connected via gold pin connectors to differential preamplifiers (Model 2124, DATA Inc.). After amplification (2000 $\times$), the EMG signals were bandpass filtered (20–1000 Hz) and digitally sampled (2 kHz) using a data acquisition board (National Instruments, Austin, TX) controlled by a custom-made program (LabView 8.2; National Instruments, Austin, TX). Root-mean-squared (RMS) EMG activity was calculated to evaluate the amplitude of DIAM activation (Mantilla et al., 2011; Mantilla et al., 2010). All RMS values were normalized to the maximal EMG activity, which was observed during sneezing (Mantilla et al., 2011; Mantilla et al., 2010). All EMG measurements were made with the animal in a supine position. No attempt was made to gate ECG artifacts; however, DIAM EMG recordings with substantial ECG contamination (i.e., contributing more than 3% to the total power of the eupneic DIAM EMG signal) were excluded.

2.4 Stationarity Analysis of DIAM EMG

In order to interpret power spectral analysis, it is necessary that the EMG signal is at least wide-sense stationary (Bilodeau et al., 1997; Papoulis, 1984); i.e. the mean and mean square values should be relatively unchanging (<2 standard deviation variance) across the specified window period (Duchene and Goubel, 1993). The reverse arrangement test, a non-parametric test for stochastic variables (Bendat and Piersol, 2010), was used to determine the window length where EMG is wide-sense stationary (Bilodeau et al., 1997). Mean square values were computed in non-overlapping 10-ms segments. This segment duration was selected since DIAM motor unit discharge rates have been reported to be typically less than 100 Hz (Sieck, 1989, 1990; Sieck et al., 1984); unpublished observations in the rat). Thus, a segment duration of 10 ms is less than the minimum interval between individual MUAPs. Accordingly, each EMG segment will not be influenced by motor unit discharge rate. Varying window lengths (30 to 300 ms) were used to assess the duration of stationarity. For example, for a window length of 60 ms, the mean square values of 6 consecutive segments were obtained.

Reverse arrangements, defined as the number of the inequalities where $x_i > x_j$ for $i < j$, were determined (Bendat and Piersol, 2010). For example, for a window length of 100 ms, $10 \times (10-1)/2 = 45$ comparisons were made. The z-score for EMG mean square values within a given window length was calculated using the following equation (Siegel and Castellan, 1988):

$$z = \frac{R - \frac{n(n-1)}{4}}{\sqrt{\frac{2n^3 + 3n^2 - 5n}{72}}}$$

where R is the total number of reverse arrangements and n is the number of segments within a window (e.g.; $n=10$ for a window size of 100 ms). The null hypothesis was that the mean square values from each segment are independent samples of a random variable and a z-score of $|z| > 1.96$ was used to reject the null hypothesis and states that the segments are not independent and part of a trend (two-tailed $\alpha > 0.05$). Accordingly, the EMG signal was classified as stationary or non-stationary according to the z-score for each window length. For each window size, the ratio of stationary EMG bursts to the total number of bursts (% stationarity) was computed for each motor behavior and animal. Within a DIAM EMG burst stationarity was determined: 1) during the initial 250 ms of EMG activity, with a moving average window length of 80 ms (8 consecutive 10 ms segments that was assessed progressively across the 250 ms period by adding one leading segment and dropping the trailing segment); and 2) around the peak of DIAM EMG activity, where motor unit recruitment is most likely to plateau (Kong and Berger, 1986) and before motor unit de-recruitment at the end of the burst occurs. Around the peak, window length was increased progressively increased (from 30 to 300 ms) in both directions. The proportion of DIAM EMG bursts satisfying stationarity criteria was determined.

2.5 Frequency Domain Analysis

During window periods within each DIAM EMG burst where stationarity criteria were satisfied, the PSD of the EMG was calculated using the periodogram method that utilizes the fast Fourier transform. From the PSD, centroid frequency (f_c), median frequency (f_{med}), power in different frequency bands and high frequency/low frequency ratio (H/L ratio) were computed. H/L ratio was calculated as the ratio of the power within the 4th (highest) quartile band of frequencies in the eupneic PSD to the 1st (lowest) quartile of eupneic PSD.

Centroid frequency (f_c) was calculated as:

$$f_c = \frac{\sum_0^{1000} f \cdot PSD(f)}{\sum_0^{1000} PSD(f)}$$

High/low ratio (H/L ratio) was calculated as:

$$H/L \text{ Ratio} = \frac{\sum_{f_2}^{1000} PSD(f)}{\sum_{20}^{f_1} PSD(f)}$$

where f_1 is the 1st quartile frequency and f_2 is the 3rd quartile frequency obtained from eupneic EMG signal obtained from the respective animal.

2.6 Single Motor Unit EMG Recordings and Analysis

Single motor unit DIAM EMG was recorded in 12 rats to distinguish the activity of DIAM motor units during different ventilatory and non-ventilatory conditions. Three pairs of insulated, stainless steel fine-wire electrodes with a diameter of 40 μm (Moleculoy, Bridgeport Insulated Wire Co., Bridgeport, CT) were inserted into the DIAM to simultaneously record discharges of 2–6 motor units. The distal ends of the fine-wire electrodes were connected via gold pin connectors to differential amplifiers (Model EMG100C, Biopac Systems Inc., Goleta, CA.). The EMG signal was amplified (2000 $\times$), bandpass filtered (100–5000 Hz) and digitally sampled (20 kHz) using a data acquisition board (National Instruments, Austin, TX) controlled by a custom-made program (LabView 8.2; National Instruments, Austin, TX). Compound EMG activity was also recorded as described above. All measurements were made with the animal in a supine position. The waveforms of single MUAPs were discriminated via thresholding and template matching using a customized application in MATLAB (Mathworks Inc., Natick, MA). The motor unit recruitment onset delay was calculated as the delay of the first MUAP of each motor unit from the onset of compound DIAM EMG activity. MUAP duration was calculated using averaged MUAP waveform.

The timing of discharge of MUAPs may coincide thereby increasing the mean square EMG value within a 10 ms segment. However, if this coincident discharge is random, the influence on mean square EMG values will not be consistent across EMG bursts. In contrast, if there is synchrony or temporal coupling of the discharge of MUAPs due to common drive, the mean square EMG values will be affected. To assess the temporal coupling of MUAP discharge, cross-correlation analyses were performed. A point-process cross-correlation histogram was calculated for each motor unit pair as described previously (Sieck et al., 1984) using 6 ms bin width for a window of 150 ms. If peaks in the cross-correlation histograms were at least 3 standard deviations greater than the mean value, a temporal association between the discharge of MUAP pairs was considered positive.

2.7 Statistical Analysis

Repeated measures were obtained for DIAM EMG activity during a given motor behavior for each animal. Thus, within each animal, mean $\pm$ standard deviation (SD) for each DIAM EMG parameter was calculated across motor behaviors. As mentioned above, stationarity was assessed for varying window lengths, and the % stationarity of DIAM bursts meeting stationarity criteria was determined. To perform PSD calculations, an arbitrary threshold of 80% stationarity was set to validate stationarity for each window length. Differences in

power densities in specific frequency bands, f_c and H/L ratio during different ventilatory and non-ventilatory behaviors were evaluated using a repeated measures univariate F-test with Greenhouse-Geisser adjustment (Quinn and Keough, 2002; Twisk, 2003). Tukey-Kramer Honestly Significant Difference test was used to analyze differences *post hoc* when appropriate. All statistical calculations were performed using statistical software (JMP 8.0, SAS Institute Inc., Cary, NC). Differences were considered significant if the *p* value is less than 0.05.

3. Results

Figure 1 shows a typical raw DIAM EMG recordings and RMS values for a single animal during different ventilatory and non-ventilatory behaviors. As can be seen, EMG activity and RMS values increased progressively as DIAM efforts became more forceful. These results were consistent with previous reports from our lab (Mantilla et al., 2011; Mantilla et al., 2010). The duration of DIAM EMG activity varied across different ventilatory and non-ventilatory behaviors. The average duration of inspiratory DIAM EMG activity during eupnea was 270 ± 23 ms, compared to 277 ± 17 ms during hypoxia-hypercapnia and 490 ± 35 ms during airway occlusion. The average duration of DIAM EMG activity during induced sneezing was 754 ± 51 ms (Figure 1).

The RMS of DIAM EMG increased significantly and progressively across motor behaviors - in rank order from eupnea, hypoxia-hypercapnia, airway occlusion and sneezing ($p < 0.01$). Peak RMS activity observed during each motor behavior normalized to the maximum RMS value observed during sneezing was $34 \pm 4\%$ during eupnea, $42 \pm 6\%$ during hypoxia-hypercapnia and $75 \pm 8\%$ during airway occlusion. However, for the initial 75 ms from the onset of DIAM EMG activity, the RMS EMG value was comparable for eupnea ($25 \pm 2\%$), hypoxia-hypercapnia ($28 \pm 4\%$) and sneezing ($30 \pm 7\%$) but significantly higher during airway occlusion ($47 \pm 5\%$; $P < 0.05$ compared to eupnea). For each motor behavior, EMG activity progressively increased as reflected by the ratio of the peak RMS value during the motor behavior to the RMS at 75 ms: 1.50 ± 0.15 during eupnea; 1.60 ± 0.14 during hypoxia-hypercapnia (NS compared to eupnea); 1.86 ± 0.19 during airway occlusion ($P < 0.01$ compared to eupnea); and 4.16 ± 0.65 during sneezing; $P < 0.01$ compared to eupnea).

3.1 Stationarity of DIAM EMG Activity

Stationarity was assessed at the onset (Figure 5) and around the peak RMS EMG activity (Figure 2) across motor behaviors. At the onset of DIAM EMG activity, stationarity of the EMG decreased immediately across all motor behaviors (Figure 5). The duration of non-stationarity at the onset of EMG activity was ~ 150 ms during eupnea, ~ 100 ms during hypoxia-hypercapnia and sneezing, and ~ 80 ms during airway occlusion.

Around the peak of RMS EMG activity, the maximum window duration encompassing stationary EMG bursts was evaluated across motor behaviors (Figure 2). At a window length of 150 ms around the peak RMS, EMG activity was stationary for all motor behaviors. For both eupnea and hypoxia-hypercapnia, DIAM EMG bursts were stationary for window durations of only 150–160 ms, and then EMG activity became rapidly non-stationary as window size increased. During airway occlusion and sneezing, DIAM EMG bursts remained stationary for slightly longer window periods (~ 170 – 180 ms).

3.2 Power Spectral Density Parameters during Different Motor Behaviors

PSD was calculated for each motor behavior around the peak EMG activity using a window period of 150 ms. Figure 3 demonstrates representative PSD for a single animal during different ventilatory and non-ventilatory behaviors. During each motor behavior, the coefficient of variation (burst-to-burst variability) within animals for f_c was $\sim 4\%$. The mean

f_c was 311 ± 11 Hz, 306 ± 12 Hz, 309 ± 11 Hz and 333 ± 14 Hz around the peak during eupnea, hypoxia-hypercapnia, airway occlusion and sneezing, respectively (Figure 4A). Peak f_c increased significantly during sneezing compared to eupnea, hypoxia-hypercapnia and occlusion ($p < 0.01$).

Different frequency bands were defined by quartiles in PSD of the DIAM EMG during eupnea for each animal. Power across all frequency bands increased progressively from eupnea to hypoxia-hypercapnia to airway occlusion ($p < 0.01$). However, during sneezing power increased primarily in the highest frequency band compared to during airway occlusion ($p < 0.01$). The H/L ratio was calculated as the ratio of power in the 4th quartile band to that in the 1st quartile band for each behavior. As shown in Figure 4B, the H/L ratio was comparable to eupnea during hypoxia-hypercapnia (0.94 ± 0.04) and airway occlusion (1.04 ± 0.08). In contrast, the H/L ratio increased significantly during sneezing compared to all other motor behaviors (1.40 ± 0.12 ; $p < 0.05$).

3.3 Single Motor Unit Recordings

Representative single motor unit recordings with compound EMG activity are shown in Figure 6. Activities of 55 single motor units were analyzed in 12 rats. In all cases, the discharge of at least two motor units could be discriminated. Across all motor behaviors, MUAPs were easier to discriminate at the onset of DIAM EMG activity, since the number of recruited motor units at the onset was lower. The number of recruited motor units increased as the level of DIAM force (RMS EMG activity) increased. Cross-correlation analyses of the discharge of pairs of motor units revealed no significant peaks. Thus, synchrony of motor unit activity appeared to be minimal.

During eupnea, the activities of 27 motor units were recorded. The onset delay of motor unit recruitment was calculated as the delay between the onset of the compound DIAM EMG activity and the first MUAP of each motor unit. Of the 27 eupneic motor units recorded, 26 were recruited within the initial 150 ms corresponding the period of non-stationarity of the EMG signal during eupnea (Figure 7A).

During hypoxia-hypercapnia, the motor units active during eupnea were still discriminated, but an additional 14 motor units were recruited (Figure 7B). The onset delay of those motor units active during eupnea was shortened such that all were recruited during the initial 100 ms corresponding to the period of non-stationarity of the EMG signal. In contrast to motor units recruited during eupnea, those units recruited during hypoxia-hypercapnia always had longer onset delays – i.e., higher recruitment thresholds. Yet, the onset delays of 12 of the 14 units recruited during hypoxia-hypercapnia was less than 100 ms.

During airway occlusion, the DIAM motor units recruited during eupnea and hypoxia-hypercapnia could still be discriminated, but an additional 7 motor units were recruited (Figure 7C). Generally, the onset delay of those motor units active during eupnea and hypoxia-hypercapnia was further shortened during airway occlusion such that the onset delay of 38 of the 41 units was less than 80 ms. Again, this pattern of motor unit recruitment corresponded to the period of non-stationarity of the EMG signal. All of the 7 of those motor units recruited during airway occlusion had onset delays > 100 ms. The index of stationarity of the DIAM EMG signal decreased in those animals in which the activity of these motor units were recorded (7 of 12 animals).

During the initial period of sneezing, those motor units active during eupnea and hypoxia-hypercapnia could still be discriminated. However, as the sneezing effort increased, it became much more difficult to discriminate MUAPs due to summation. It was possible to

discriminate the activities of an additional 7 higher threshold motor units. These motor units were recruited near the peak of EMG activity associated with sneezing.

Considering the motor units consistently recruited across all DIAM motor behaviors (27 motor units), the average onset delay of these units was 75 ms during eupnea, 29 ms during hypoxia-hypercapnia and 27 ms during airway occlusion. Earlier recruitment of motor units was consistent with a shorter period of non-stationarity in the DIAM EMG signal during hypoxia-hypercapnia and airway occlusion compared to eupnea.

MUAP durations of motor units ranged from 1.0 to 5.6 ms. For the motor units recruited only during hypoxia-hypercapnia and airway occlusion, MUAP durations were similar to those recruited during all three behaviors including eupnea. This is consistent with the similar F_c of the PSD of DIAM EMG activity across these motor behaviors. In contrast, for those high threshold motor units recruited at the peak of sneezing EMG activity, the MUAP durations were shorter (2.7 ± 0.2 ms) compared to those of units recruited during the other motor behaviors (3.5 ± 0.3 ms; $p < 0.05$).

Once recruited, motor units progressively increased their firing frequencies until the peak RMS EMG value for that motor behavior. Thus, an increase in motor unit discharge frequency did not affect the stationarity of EMG signal.

4. Discussion

The results of this study support our hypotheses that: 1) a shift in the DIAM EMG PSD during sneezing – a more forceful, non-ventilatory motor behavior - reflects the recruitment of more fatigable fast-twitch motor units; and 2) during the period of motor unit recruitment across ventilatory and non-ventilatory behaviors the DIAM EMG is non-stationary.

The shift in the DIAM EMG PSD to higher frequencies during sneezing was indicated by an increase in f_c and H/L ratio, both measures of central tendency of the PSD. In contrast, power across all frequency bands of DIAM EMG increased progressively from eupnea to hypoxia-hypercapnia to airway occlusion. While power continued to increase across all frequency bands during sneezing, the increase in power was disproportionately greater in the highest frequency band. These results support the observation that more forceful DIAM motor units (e.g., type FInt and FF) have a higher recruitment threshold and are recruited only during more forceful, non-ventilatory behaviors (Fournier and Sieck, 1988). Consistent with the shift in the PSD during sneezing, we recorded from seven single motor units that had a higher recruitment threshold and were recruited only during sneezing.

The MUAP duration of these high threshold motor units was significantly shorter than the MUAP duration of lower threshold units, which might underlie the rightward shift in the DIAM PSD during sneezing. The period of non-stationarity at the onset of DIAM EMG activity ranged from ~70 ms during airway occlusion to ~150 ms during eupnea. Single motor unit studies confirmed that a significant portion of motor units was recruited within the initial non-stationary period of DIAM EMG activity (~96% for eupnea, ~95% for hypoxia-hypercapnia and ~80% for airway occlusion). Once motor units were recruited, their firing frequencies continued to progressively increase beyond the initial 150 ms period; yet, the DIAM EMG signal became stationary. These results would suggest that the physiological range of motor unit discharge rates does not influence the stationarity of the EMG signal.

4.1 Power Spectral Density Shifts during Different Motor Behaviors

Ever since Piper's seminal work (Piper, 1912), frequency-domain analyses of EMG signals have been employed under a variety of conditions to assess neuromotor control. A rightward shift in the PSD of limb muscle EMG signals toward higher frequencies was previously reported during increasing levels of force generation (Arendt-Nielsen and Mills, 1985; Solomonow et al., 1990). Thus, the shift in the DIAM EMG PSD toward higher frequencies during sneezing is consistent with these previous studies.

One important assumption when using Fourier transforms to obtain PSD is that the EMG signal is stationary during the window period of analysis. Indeed, power spectral analyses can be considered valid only for stationary segments (Bilodeau et al., 1997; Hary et al., 1982; Merletti et al., 1992; Papoulis, 1984; Xiong and Shwedyk, 1987). Most previous studies reporting PSD analyses of DIAM EMG signals assumed stationarity, but did not explicitly test for stationarity (Cairns and Road, 1998; Hussain et al., 1991). In the present study, stationarity was verified for 150 ms around the peak of RMS EMG of each motor behavior. This window period provided frequency resolution of ~ 7 Hz.

Lindstrom and Magnusson (1977) modeled the contribution of activation of muscle fibers of varying size on the EMG power spectrum. In their model, EMG PSD is a function of the action potential conduction velocity of active muscle fibers, which, based on cable properties, varies in proportion to muscle fiber diameter (Hakansson, 1956, 1957). Thus, the size distribution of muscle fibers and their recruitment during different motor behaviors will affect the EMG PSD (Gerdle et al., 1991; Gerdle et al., 2000; Gerdle et al., 1988; Kupa et al., 1995; Wakeling et al., 2002). As larger muscle fibers with faster conduction velocities are recruited, the EMG PSD will shift to higher frequencies. In the rat DIAM, type I (slow-twitch motor units) and type IIa (fast-twitch, fatigue resistant motor units) fibers are approximately one third the cross sectional area of type IIx and/or IIb fibers comprising more fatigable fast-twitch motor units (Aravamudan et al., 2006; Geiger et al., 2000; Lewis et al., 1986; Mantilla et al., 2008; Prakash et al., 1993; Sieck and Fournier, 1989; Verheul et al., 2004). It is likely that slow-twitch and fast-twitch, fatigue resistant motor units are recruited first during ventilatory behaviors, and that more fatigable fast-twitch motor units are recruited only during more forceful non-ventilatory behaviors (Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck, 1991; Sieck and Fournier, 1989). In the present study, the shift in DIAM EMG PSD to higher frequencies during sneezing is consistent with the recruitment of motor units having shorter MUAP durations and likely reflects activation of larger type IIx and/or IIb muscle fibers comprising more fatigable fast-twitch motor units.

A shift in the DIAM EMG PSD in pigs to higher frequencies (increased f_c) was reported during increased force generation induced by inspiratory resistive loading (IRL – comparable to airway occlusion in the present study) (Hussain et al., 1991). In their study, the PSD was computed from the entire inspiratory EMG burst (inspiratory durations varying from 340 ms to >1 s), but stationarity of the EMG signal was not verified. In rabbits, a sliding window of 200ms period was used (overlapping by 100 ms), beginning at the onset of inspiratory effort to analyze DIAM EMG spectral shifts during IRL (Cairns and Road, 1998). They reported a small increase in f_c of DIAM EMG PSD as inspiration progressed from onset that was enhanced during IRL. Unfortunately, they did not evaluate stationarity of the EMG signal, and it is likely that 200 ms window period exceeded the period of stationarity. Thus, the shift they reported may have reflected a biasing of the EMG power spectra related to non-stationarity of the EMG signal. In the present study, we did not find a significant shift toward higher frequencies (f_c or H/L ratio) during sustained airway occlusion, which if anything would be an extreme case of IRL. It is possible that the non-stationarity of the EMG signals in these previous studies may have affected their results. Regardless, the results of these previous studies as well as the present study support a shift

in DIAM EMG PSD to higher frequencies with increased DIAM force generation requiring recruitment of high threshold fast-twitch fatigable motor units.

4.2 Stationarity of EMG Signal

Stationarity can be assumed if a stochastic signal has time-independent statistical properties such as mean, variance, or mean square values (Bendat and Piersol, 2010; Papoulis, 1984). Previous studies of EMG activity in other skeletal muscles, tested stationarity by calculating mean square values (Bilodeau et al., 1997; Duchene and Goubel, 1993; Mananas et al., 2001). During dynamic contractions where different motor units are recruited at different times, it has been reported that segmentation of respiratory-related EMG activity of the human sternocleidomastoid muscle into small window periods (<400 ms) was necessary to assume stationarity (Mananas et al., 2001; Mananas et al., 2002). Therefore, stationarity of the compound EMG signal is affected by motor unit recruitment.

To date, stationarity was used only as a criterion to apply PSD analysis rather than as a tool to assess muscle activation. Several factors contribute to time-varying conditions within a burst of DIAM activity. Both recruitment and frequency coding of motor units underlie the increase in DIAM EMG activity and force generation across different motor behaviors (Mantilla and Sieck, 2011; Nail et al., 1972; Sieck, 1989). With recruitment of motor units, there is an increased probability for summation of MUAPs that would increase mean square value (outcome measure affecting stationarity), and thus affect stationarity of the EMG. The present study showed that DIAM EMG was non-stationary at the onset of activity for a period ranging between 70–150 ms across motor behaviors. Single motor unit recordings confirmed that this was the period during which units were predominantly recruited. Beyond this initial period of non-stationarity, the EMG signal became stationary despite an increase in the frequency of motor unit discharge. Therefore, these findings support our assumption that by analyzing stationarity of EMG segments that are shorter (e.g., 10 ms) than the expected MUAP interpulse interval (~20–100 ms), the influence of frequency coding is minimized. Thus, we conclude that only motor unit recruitment is reflected by non-stationarity of the EMG signal.

4.3 Motor Unit Recruitment

Of those motor units recorded during eupnea (27 units), 96% were recruited within the initial 150 ms period. These motor units continued to be recruited across all motor behaviors, with a progressively shorter recruitment onset delay for hypoxia-hypercapnia and airway occlusion. Most of those 14 additional motor units recruited during hypoxia-hypercapnia also had shorter onset delays (within 100 ms), but longer than the onset delays of the lower threshold motor units recruited during eupnea. During airway occlusion, the recruitment of an additional 7 motor units was observed with onset delays >100 ms. In those animals where additional motor unit recruitment was observed during airway occlusion (7 of 12 animals) the DIAM EMG signal became non-stationary during the period corresponding to the recruitment of these motor units. Therefore, there was clear evidence of a consistent recruitment order of DIAM motor units across motor behaviors as force increased. The increasing number of motor units recruited was evident by an increase in EMG RMS across all frequencies. The progressive shortening of the recruitment onset delays as DIAM force increased was reflected by a shortening in the period of non-stationarity of the EMG signal indicating the timing of motor unit recruitment. During sneezing, additional high threshold motor units were recruited but the effects of the extent and timing of unit recruitment on the summated EMG precluded a clear analysis of effects on the timing of EMG stationarity. However, recruitment of high threshold motor units during sneezing caused a shift in the EMG spectral content toward higher frequencies due to the fact that these high threshold units had shorter MUAP durations and greater amplitudes.

During ventilatory behaviors phrenic motor neurons receive excitatory input from medullary premotor neurons. It is likely that this input causes activation of phrenic motor neurons in a size-dependent fashion according to Henneman's size principle by which motor neurons are recruited in an orderly fashion based on intrinsic, size-related electrophysiological properties of motor neurons (Henneman et al., 1965). The fact that EMG RMS values during the initial 75 ms period after onset of activity were comparable during eupnea, hypoxia-hypercapnia and sneezing was not surprising and suggests that inspiratory drive affecting motor unit recruitment and discharge rates are similar for early-onset low threshold units across these motor behaviors.

In contrast to all other motor behaviors, it was surprising that the RMS value at 75 ms during airway occlusion was greater. In addition, the period of non-stationarity during airway occlusion was the shortest (~70 ms). These findings are consistent with the shorter recruitment delay of motor units that was observed during airway occlusion. The greater initial recruitment of DIAm motor units during airway occlusion could reflect either an increased drive from non-inspiratory-related synaptic input or increased excitability of motor neurons. It was previously shown that the force threshold of motor unit recruitment during ballistic contractions of limb muscles is lower (Desmedt and Godaux, 1978) and it is possible that the motor effort during airway occlusion is comparable to ballistic contractions in limb muscles.

5. Conclusion

The results of the present study support the combined use of an assessment of non-stationarity of EMG signal to reflect the period of motor unit recruitment, and a shift in the PSD towards higher frequencies to reflect the recruitment of more fatigable fast-twitch motor units. Recordings of single motor unit discharge confirmed the utility of the analysis of non-stationarity for defining the period of motor unit recruitment. However, using single motor unit discharge as an alternative has significant limitations. Most importantly, it is only possible to record the activities of 2–5 units simultaneously; representing <2% of the phrenic motor unit pool. Moreover, using these recording techniques, it is difficult to distinguish the discharge of single motor units at high force levels due to the summation of MUAPs. Thus, such unitary recording techniques cannot provide information regarding how the entire population of DIAm motor units is recruited in relation to each other.

Acknowledgments

This work was supported by National Institutes of Health grant HL096750 and the Mayo Clinic.

Abbreviations List

DIAm	Diaphragm muscle
ECG	Electrocardiogram
EMG	Electromyogram/Electromyography
f_c	Centroid frequency
H/L ratio	Ratio of power in high frequency vs. low frequency bands
IRL	Inspiratory resistive loading
MUAP	Motor unit action potential
Pdi	Transdiaphragmatic pressure

PSD	Power spectral density
RAT	Reverse arrangements test
RMS	Root-mean-squared

References

- Aravamudan B, Mantilla CB, Zhan WZ, Sieck GC. Denervation effects on myonuclear domain size of rat diaphragm fibers. *Journal of Applied Physiology*. 2006; 100:1617–1622. [PubMed: 16410375]
- Arendt-Nielsen L, Mills KR. The relationship between mean power frequency of the EMG spectrum and muscle fibre conduction velocity. *Electroencephalogr Clin Neurophysiol*. 1985; 60:130–134. [PubMed: 2578364]
- Bendat, JS.; Piersol, AG. *Random Data: Analysis and Measurement Procedures*. 4. John Wiley & Sons; New York: 2010.
- Bilodeau M, Cincera M, Arsenault AB, Gravel D. Normality and stationarity of EMG signals of elbow flexor muscles during ramp and step isometric contractions. *J Electromyogr and Kinesiol*. 1997; 7:87–96. [PubMed: 20719694]
- Cairns AM, Road JD. High-frequency oscillation and centroid frequency of diaphragm EMG during inspiratory loading. *Respiratory Physiology*. 1998; 112:305–313.
- Desmedt JE, Godaux E. Ballistic contractions in fast or slow human muscles: discharge patterns of single motor units. *J Physiol*. 1978; 285:185–196. [PubMed: 745068]
- Duchene J, Goubel F. Surface electromyogram during voluntary contraction: processing tools and relation to physiological events. *Critical reviews in biomedical engineering*. 1993; 21:313–397. [PubMed: 8243094]
- Fournier M, Sieck GC. Mechanical properties of muscle units in the cat diaphragm. *Journal Of Neurophysiology*. 1988; 59:1055–1066. [PubMed: 3367195]
- Geiger PC, Cody MJ, Macken RL, Sieck GC. Maximum specific force depends on myosin heavy chain content in rat diaphragm muscle fibers. *J Appl Physiol*. 2000; 89:695–703. [PubMed: 10926656]
- Gerdle B, Henriksson-Larsen K, Lorentzon R, Wretling ML. Dependence of the mean power frequency of the electromyogram on muscle force and fibre type. *Acta Physiol Scand*. 1991; 142:457–465. [PubMed: 1835248]
- Gerdle B, Karlsson S, Crenshaw AG, Elert J, Friden J. The influences of muscle fibre proportions and areas upon EMG during maximal dynamic knee extensions. *Eur J Appl Physiol*. 2000; 81:2–10. [PubMed: 10552260]
- Gerdle B, Wretling ML, Henriksson-Larsen K. Do the fibre-type proportion and the angular velocity influence the mean power frequency of the electromyogram? *Acta Physiol Scand*. 1988; 134:341–346. [PubMed: 2976240]
- Hakansson CH. Conduction velocity and amplitude of the action potential as related to circumference in the isolated fibre of frog muscle. *Acta Physiol Scand*. 1956; 37:14–34. [PubMed: 13339449]
- Hakansson CH. Action potentials recorded intra- and extracellularly from the isolated frog muscle fibre in Ringer's solution and in air. *Acta Physiol Scand*. 1957; 39:291–312. [PubMed: 13444043]
- Hary D, Belman MJ, Propst J, Lewis S. A statistical analysis of the spectral moments used in EMG tests of endurance. *Journal of Applied Physiology*. 1982; 53(3):779–783. [PubMed: 7130002]
- Henneman E, Somjen G, Carpenter DO. Functional significance of cell size in spinal motoneurons. *Journal Of Neurophysiology*. 1965; 28:560–580. [PubMed: 14328454]
- Hussain SN, Clement MG, Vanelli G, Albertini M, Aguggini G. The effect of level of contraction on the electromyographic power spectrum of the diaphragm in pigs. *Experimental Physiology*. 1991; 76:765–775. [PubMed: 1742015]
- Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. *Journal of Applied Physiology*. 2005; 98:120–131. [PubMed: 15377649]

- Kong FJ, Berger AJ. Firing properties and hypercapnic responses of single phrenic motor axons in the rat. *J Appl Physiol*. 1986; 61:1999–2004. [PubMed: 3027021]
- Kupa EJ, Roy SH, Kandarian SC, De Luca CJ. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *Journal of Applied Physiology*. 1995; 79:23–32. [PubMed: 7559225]
- Lee KZ, Reier PJ, Fuller DD. Phrenic motoneuron discharge patterns during hypoxia-induced short-term potentiation in rats. *Journal Of Neurophysiology*. 2009; 102:2184–2193. [PubMed: 19657076]
- Lewis MI, Sieck GC, Fournier M, Belman MJ. Effect of nutritional deprivation on diaphragm contractility and muscle fiber size. *Journal of Applied Physiology*. 1986; 60:596–603. [PubMed: 3949661]
- Lindstrom L, Magnusson R. Interpretation of Myoelectric Power Spectra: A Model and Its Applications. *Proc IEEE*. 1977; 65:653–662.
- Mananas MA, Fiz JA, Morera J, Caminal P. Analyzing dynamic EMG and VMG signals of respiratory muscles. *IEEE Eng Med Biol Mag*. 2001; 20:125–132. [PubMed: 11838244]
- Mananas MA, Jane R, Fiz JA, Morera J, Caminal P. Influence of estimators of spectral density on the analysis of electromyographic and vibromyographic signals. *Med Biol Eng Comput*. 2002; 40:90–98. [PubMed: 11954714]
- Mantilla CB, Seven YB, Hurtado-Palomino JN, Zhan WZ, Sieck GC. Chronic assessment of diaphragm muscle EMG activity across motor behaviors. *Respir Physiol Neurobiol*. 2011; 177:176–182. [PubMed: 21414423]
- Mantilla CB, Seven YB, Zhan WZ, Sieck GC. Diaphragm motor unit recruitment in rats. *Respir Physiol Neurobiol*. 2010; 173:101–106. [PubMed: 20620243]
- Mantilla CB, Sieck GC. Phrenic motor unit recruitment during ventilatory and non-ventilatory behaviors. *Respir Physiol Neurobiol*. 2011; 179:57–63. [PubMed: 21763470]
- Mantilla CB, Sill RV, Aravamudan B, Zhan WZ, Sieck GC. Developmental effects on myonuclear domain size of rat diaphragm fibers. *Journal of Applied Physiology*. 2008; 104:787–794. [PubMed: 18187618]
- Mantilla CB, Zhan WZ, Sieck GC. Retrograde labeling of phrenic motoneurons by intrapleural injection. *Journal of Neuroscience Methods*. 2009; 182:244–249. [PubMed: 19559048]
- Merletti R, Knaflitz M, DeLuca CJ. Electrically evoked myoelectric signals. *Critical reviews in biomedical engineering*. 1992; 19:293–340. [PubMed: 1563271]
- Miyata H, Zhan WZ, Prakash YS, Sieck GC. Myoneural interactions affect diaphragm muscle adaptations to inactivity. *Journal of Applied Physiology*. 1995; 79:1640–1649. [PubMed: 8594024]
- Nail BS, Sterling GM, Widdicombe JG. Patterns of spontaneous and reflexly-induced activity in phrenic and intercostal motoneurons. *Exp Brain Res*. 1972; 15:318–332. [PubMed: 5070223]
- Papoulis, A. *Probability, Random Variables, and Stochastic Processes*. McGraw-Hill; New York: 1984.
- Piper, HE. *Elektrophysiologie Menschlicher Muskeln*. Springer; Berlin: 1912.
- Prakash YS, Fournier M, Sieck GC. Effects of prenatal undernutrition on developing rat diaphragm. *Journal of Applied Physiology*. 1993; 75:1044–1052. [PubMed: 8226510]
- Prakash YS, Mantilla CB, Zhan WZ, Smithson KG, Sieck GC. Phrenic motoneuron morphology during rapid diaphragm muscle growth. *Journal of Applied Physiology*. 2000; 89:563–572. [PubMed: 10926639]
- Quinn, GP.; Keough, MJ. *Experimental design and data analysis for biologists*. Cambridge University Press; 2002.
- Rice A, Fuglevand AJ, Laine CM, Fregosi RF. Synchronization of presynaptic input to motor units of tongue, inspiratory intercostal, and diaphragm muscles. *Journal Of Neurophysiology*. 2011; 105:2330–2336. [PubMed: 21307319]
- Sieck, GC. Recruitment and frequency coding of diaphragm motor units during ventilatory and non-ventilatory behaviors. In: Swanson, GD.; Grodins, FS.; Hughson, RL., editors. *Respiratory Control*. Plenum Press; New York: 1989. p. 441-450.

- Sieck, GC. Conceptual model of ventilatory muscle recruitment and diaphragmatic fatigue. In: Khoo, MCK., editor. *Modeling and Parameter Estimation in Respiratory Control*. Plenum Press; New York: 1990. p. 113-123.
- Sieck GC. Neural control of the inspiratory pump. *NIPS*. 1991; 6:260–264.
- Sieck GC, Fournier M. Diaphragm motor unit recruitment during ventilatory and nonventilatory behaviors. *Journal of Applied Physiology*. 1989; 66:2539–2545. [PubMed: 2745316]
- Sieck GC, Fournier M. Changes in diaphragm motor unit EMG during fatigue. *Journal of Applied Physiology*. 1990; 68:1917–1926. [PubMed: 2163376]
- Sieck GC, Fournier M, Enad JG. Fiber type composition of muscle units in the cat diaphragm. *Neuroscience Letters*. 1989; 97:29–34. [PubMed: 2521928]
- Sieck GC, Fournier M, Prakash YS, Blanco CE. Myosin phenotype and SDH enzyme variability among motor unit fibers. *Journal of Applied Physiology*. 1996; 80:2179–2189. [PubMed: 8806928]
- Sieck GC, Trelease RB, Harper RM. Sleep influences on diaphragmatic motor unit discharge. *Experimental Neurology*. 1984; 85:316–335. [PubMed: 6745377]
- Siegel, S.; Castellan, J. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill; 1988.
- Solomonow M, Baten C, Smit J, Baratta R, Hermens H, D'Ambrosia R, Shoji H. Electromyogram power spectra frequencies associated with motor unit recruitment strategies. *Journal of Applied Physiology*. 1990; 68:1177–1185. [PubMed: 2341343]
- Twisk, JWR. *Applied longitudinal data analysis for epidemiology: a practical guide*. Cambridge University Press; 2003.
- Verheul AJ, Mantilla CB, Zhan WZ, Bernal M, Dekhuijzen PN, Sieck GC. Influence of corticosteroids on myonuclear domain size in the rat diaphragm muscle. *Journal of Applied Physiology*. 2004; 97:1715–1722. [PubMed: 15234958]
- Wakeling JM, Kaya M, Temple GK, Johnston IA, Herzog W. Determining patterns of motor recruitment during locomotion. *J Exp Biol*. 2002; 205:359–369. [PubMed: 11854372]
- Xiong FQ, Shwedyk E. Some aspects of nonstationary myoelectric signal processing. *IEEE Trans Biomed Eng*. 1987; 34:166–172. [PubMed: 3557495]

Highlights

- The rat diaphragm muscle (DIAM) comprises different types of motor units, which are recruited to accomplish a wide range of motor behaviors.
- Stationarity and power spectral density (PSD) of DIAM EMG and single motor unit recordings were analyzed during different motor behaviors.
- Non-stationarity of DIAM EMG reflects the timing of motor units recruitment.
- PSD shifted towards higher frequencies during sneezing, reflecting recruitment of more fatigable fast-twitch motor units.

Figure 1.

Sample tracings of rat diaphragm muscle (DIAM) electromyographic (EMG) activity and root-mean-squared (RMS) values during different motor behaviors: eupnea, hypoxia-hypercapnia (10% O₂ - 5% CO₂), airway occlusion and sneezing. RMS EMG was computed using a window size of 50 ms.

Figure 2.

Stationarity of DIAM EMG around the peak RMS EMG as a function of window size. Stationarity was calculated for each motor behavior using reverse arrangements test (see text for details). Approximately 80% of EMG bursts satisfy stationarity for ~150 ms around the peak. The percentage of stationary bursts around the peak decreased at a fast rate as the window period was increased above 150ms.

Figure 3. Sample power spectral densities (PSD) around the peak RMS EMG activity during eupnea, hypoxia-hypercapnia, airway occlusion and sneezing. All traces were computed using a window size of 150 ms from the same animal across all behaviors.

Figure 4.

A) Centroid frequency (f_c in Hz) and calculated for 150 ms around the peak EMG activity
 B) H/L Ratio (the ratio of power in 4th eupneic quartile relative to the 1st eupneic quartile) during eupnea, hypoxia-hypercapnia, airway occlusion and sneezing. Both F_c and H/L ratio increased during sneezing compared to other motor behaviors ($p < 0.05$). Data are mean $\pm$ SD within animal. *, Significantly different compared to eupnea, hypoxia-hypercapnia and airway occlusion.

Figure 5.

Stationarity of diaphragm muscle (DIAM) EMG the initial 250 ms period of diaphragm EMG activity for A) a single animal, and B) across animals (mean $\pm$ standard error). Stationarity was calculated for each motor behavior using reverse arrangements test (see text for details). DIAM EMG burst is non-stationary for 150 ms, 100 ms, 80 ms and 100 ms for eupnea, hypoxia-hypercapnia, airway occlusion and sneezing.

Figure 6.

Sample single motor unit recordings with compound EMG activity during eupnea. The motor unit action potentials represented with their averaged waveforms, are recruited at the initial 150 ms of DIAM EMG activity.

Figure 7.

The histogram of motor unit recruitment onset delay during A) eupnea, B) hypoxia-hypercapnia and C) airway occlusion. The motor unit recruitment onset delay was calculated as the time period between the first MUAP of each motor unit from the onset of compound DIAM EMG activity. Twenty six of 27 motor units were recruited within the initial 150 ms of compound DIAM EMG activity where eupneic EMG burst was non-stationary. Thirty nine of 41 motor units were recruited within the initial 100 ms of compound DIAM EMG activity where hypoxic-hypercapnic EMG burst was non-stationary. Thirty eight of 48 motor units were recruited within the initial 80 ms of compound DIAM EMG activity where EMG burst was non-stationary during airway occlusion.

Figure 8.

Sample motor unit action potentials of two motor units which were recruited during (1) eupnea and (2) around the peak EMG activity of sneezing. The duration of sample motor unit action potentials were 3.8 ms and 3.2 ms during eupnea and sneezing, respectively. The motor unit action potentials are represented with their averaged waveforms.