HIGHLIGHTED TOPIC | Hypoxia

Recruitment and plasticity in diaphragm, intercostal, and abdominal muscles in unanesthetized rats

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Navarrete-Opazo A, Mitchell GS. Recruitment and plasticity in diaphragm, intercostal, and abdominal muscles in unanesthetized rats. J Appl Physiol 117: 180–188, 2014. First published May 15, 2014; doi:10.1152/japplphysiol.00130.2014.—Although rats are a frequent model for studies of plasticity in respiratory motor control, the relative capacity of rat accessory respiratory muscles to express plasticity is not well known, particularly in unanesthetized animals. Here, we characterized external intercostal (T2, T4, T5, T6, T7, T8, T9 EIC) and abdominal muscle (external oblique and rectus abdominis) electromyogram (EMG) activity in unanesthetized rats via radiotelemetry during normoxia (Nx: 21% O2) and following acute intermittent hypoxia (AIH: 10 × 5-min, 10.5% O2; 5-min intervals). Diaphragm and T2–T5 EIC EMG activity, and ventilation were also assessed during maximal chemoreceptor stimulation (MCS: 7% CO2, 10.5% O2) and sustained hypoxia (SH: 10% O2). In Nx, T2 EIC exhibits prominent inspiratory activity, whereas T4, T5, T6, and T7 EIC inspiratory activity decreases in a caudal direction. T8 and T9 EIC and abdominal muscles show only tonic or sporadic activity, without consistent respiratory activity. MCS increases diaphragm and T2 EIC EMG amplitude and tidal volume more than SH (0.94 ± 0.10 vs. 0.68 ± 0.05 ml/100 g; < 0.001). Following AIH, T2 EIC EMG amplitude remained above baseline for more than 60 min post-AIH (i.e., EIC long-term facilitation, LTF), and was greater than diaphragm LTF (41.5 ± 13% vs. 19.1 ± 2.0% baseline; < 0.001). We conclude that 1) diaphragm and rostral T2–T5 EIC muscles exhibit inspiratory activity during Nx; 2) MCS elicits greater ventilatory, diaphragm, and rostral T2–T5 EIC muscle activity vs. SH; and 3) AIH induces greater rostral EIC LTF than diaphragm LTF.

intercostal; abdominal; long-term facilitation; intermittent hypoxia; breathing; EMG; telemetry

THE SPATIAL AND TEMPORAL DISTRIBUTION of inspiratory and expiratory activity among intercostal muscles has been extensively investigated in cats, dogs, and humans (12, 14, 48). The pattern of respiratory muscle activation in these species can be described by two general principles: 1) the activity within each muscle layer follows the distribution of mechanical advantage in that layer; and 2) inspiratory activation is restricted to the external layer and the parasternal muscle, whereas expiratory activation is restricted to the internal layer (10). However, rats appear to diverge from these principles in important aspects.

In unanesthetized rats, cephalic rib cage muscles (scalenes medius, parasternal, and external and internal intercostal muscles) always exhibit inspiratory activity, regardless of the state of consciousness (33). On the other hand, muscles of the midthoracic region (less so, the caudal region) may be inspiratory or expiratory; and some areas of a single intercostal layer can even be activated during both inspiration and expiration (33) during quiet breathing. Moreover, only two studies have investigated the effect of hypoxia and hypercapnia on diaphragm and external intercostal muscles in unanesthetized rats. During REM sleep, mean diaphragm electromyogram (EMG) activity was reported to be greater during sustained hypoxia (10% O2) vs. hypercapnia (5% CO2) or combined hypercapnia and hypoxia (10% O2 and 4% CO2) (11.3 vs. 9.3 and 8.3 arbitrary units, respectively) (34). Respiratory frequency was also greater during sustained hypoxia vs. hypercapnia during all sleep states in this study (34). On the other hand, gas concentrations had minimal effect on external intercostal EMG activity in unanesthetized rats, with the exception of the 2nd and 5th external intercostal muscles, which exhibited robust increases in EMG activity when breathing 5% CO2 (33). Important measurements that were not made in these pioneering studies of unanesthetized rats include: 1) simultaneous measurements of ventilation with diaphragm and intercostal EMG activity during sustained hypoxia vs. maximum chemoreceptor stimulation; and 2) responses to stimuli known to elicit respiratory plasticity, such as long-term facilitation (LTF) induced by acute intermittent hypoxia (AIH). To date, our efforts have largely focused on AIH-induced plasticity in phrenic motor neurons and the capacity to increase tidal volume. However, to fully understand AIH-induced respiratory plasticity and its therapeutic potential in conditions such as spinal injury, we must know the contributions of muscles that assist the diaphragm in generating tidal volume, such as the inspiratory intercostal muscles.

AIH-induced LTF in the phrenic motor system has been extensively studied in anesthetized and paralyzed rats (18, 31, 36, 37). Respiratory motor plasticity is less well studied in unanesthetized, spontaneously breathing rats, although AIH elicits persistent increase in ventilation (ventilatory long-term facilitation, vLTF) (39, 40) and diaphragm activity (diaphragm long-term facilitation, diaLTF) (45). Based on studies in anesthetized cats, there appears to be greater capacity for LTF in inspiratory intercostal vs. phrenic activity (21); however, there are no reports concerning the impact of AIH on LTF in inspiratory muscles in unanesthetized rats.

Physiological measurements collected from unrestrained conscious, unstressed animals better represent the normal state of an animal since they are unaffected by chemical (e.g., anesthesia), surgical (e.g., decerebration), or psychological factors (e.g., stress). Indeed, the United States Food and Drug
Administration (FDA) suggests that such measurements are more physiologically relevant and more accurately reflect responses in humans (17). In this regard, radiotelemetry systems offer considerable advantage as a means of monitoring respiratory muscle EMG activity since radiotelemetry can be used in chronically instrumented and unrestrained animals. The main purpose of the present investigation is to study the recruitment and plasticity in respiratory muscles (diaphragm, external intercostal, and abdominal muscles) using radiotelemetry to assess EMG activity in unanesthetized, spontaneously breathing rats. We tested three specific hypotheses: 1) EIC muscles (T2, T4, T5 EIC) show inspiratory activity whereas caudal T6, T7, T8, T9 EIC and abdominal muscles show only expiratory activity during normoxia; 2) maximum chemoreceptor stimulation (combined 7% CO2 and 10.5% O2) elicits greater EMG activity and tidal volume vs. sustained hypoxia alone; and 3) AIH elicits LTF in rostral external intercostal muscles (T2, T4, T5) but not in caudal segments (T6–T9 EIC) or abdominal muscles.

METHODS

Animals

All experiments were performed on 3- to 4-mo-old, male Sprague-Dawley rats (320–360 g, colony 211, Harlan, Indianapolis, IN). Animals were individually housed in a controlled environment (12:12 h light/dark cycle). The Animal Care and Use Committee at the School of Veterinary Medicine, University of Wisconsin, approved all experimental procedures used in this study.

Experimental Preparation

Surgical preparation. Sterile surgery was performed under anesthesia induced with isoflurane in 100% O2. The rats were treated with buprenorphine (0.03 mg/kg), carprofen (Rimadyl, 5 mg/kg), and enrofloxacin (Baytril, 4 mg/kg) subcutaneously to minimize potential surgery. Body temperature was maintained at 36.5–37.5°C using a rectal probe and external heating pad. A cannula was inserted into the trachea, and the animals were artificially ventilated by 10.220.33.6 on October 30, 2017 http://jap.physiology.org/ Downloaded from by 10.220.33.6 on October 30, 2017

concentration in the chamber less than 0.5% at all times; 95% of a

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were exposed to AIH (AIH n = 8). For the second round of experiments targeting R-Dia, T6, T7 EIC, and external oblique muscles, 4 rats were used as time controls (TC n = 4) and 6 rats were exposed to AIH (AIH n = 6). In the third group targeting R-Dia, T8, T9 EIC, and rectus abdominus muscles, 4 rats were used as time control (TC n = 4) and 6 rats were used exposed to AIH (AIH n = 6). Rats had ad libitum access to food and water throughout experiments. Chamber temperature was 22.5–24.5°C. Chamber O2 and CO2 concentrations (inflow and outflow) gases were continuously monitored during experiments.

**Sustained hypoxia and maximum chemoreceptor stimulation.** Normoxic (21% O2), hypoxic (10.5% O2), and hypercapnic conditions (7% CO2) were established in plethysmography chambers (see above) by mixing O2, N2, and CO2 via a custom-made, computer-controlled system of mass flow controllers to obtain the desired inspired gas concentrations. After 30 min acclimation, baseline during normoxia was recorded for 20 min, followed by maximum chemoreceptor stimulation (MCS: 10.5% O2 with 7% CO2) for 20 min (see Fig. 2A). Combined hypcapnia and hypoxia is a powerful stimulus to chemoreflexes, giving a standardized high drive to breathing for comparison among animals; however, this stimulus should not be regarded as the maximal respiratory muscle activity since higher levels can be achieved during respiratory defense reflexes such as cough (31a).

One week after MCS, the same rats were exposed to 20-min normoxia, followed by sustained hypoxia (SH: 10.5% O2) for 20 min (see Fig. 2B). Time control (TC) rats were exposed to 20 min of normoxia to match the MCS/SH protocol. Unlike MCS, rats were poikilocapnic in the SH protocol; thus arterial PCO2 levels presumably decreased during SH. Gas flow through the chamber was 4 l/min to enable control of inspired gas composition and prevent CO2 accumulation within the chamber (CO2 < 0.5%). For both MCS and SH, targeted muscles were the R-Dia, as well as the T2, T4 and T5 EIC muscles; 4 rats were exposed to TC, and 6 rats underwent SH and MCS. EMG assessment and plethysmography were performed simultaneously in each experiment. All volumes were corrected for chamber and body temperature (via telemetry), an important correction since body temperature changes appreciably during hypoxia in rats.

**Data Analyses**

EMG signals were filtered (100–624 Hz), rectified, integrated (100 ms), and quantified in arbitrary units using Neuroscore software. Mean integrated amplitude, respiratory frequency, and minute activity (amplitude × frequency) were averaged in all rat groups. For AIH experiments, peak amplitude and burst frequency were averaged every 20 min before (60 min) and after AIH (60 min). For the MCS/SH experiments, values were averaged before (20 min) and during MCS/SH (20 min). Values during active rat movement or during sniffing behavior were excluded from analysis. Data obtained during the pretreatment period represent baseline values. All values during MCS/SH and after AIH are expressed as a percent change from this baseline. For MCS/SH experiments, tidal volume and respiratory frequency were expressed as absolutes values.

Respiratory variables (diaphragm/external/abdominal amplitude, respiratory frequency, tidal volume, minute activity) were compared for time (pre- and post-AIH, or pre- and during MCS or SH) and treatment (TC vs. AIH and TC vs. MCS/SH) using a two-way, repeated-measures ANOVA with Fisher’s LSD post hoc tests (SigmaStat version 2.03, Systat Software, San Jose, CA). Differences were considered significant if P < 0.05. All values are expressed as means ± 1 SE.

**RESULTS**

**External Intercostal and Abdominal Muscle Activity During Baseline**

Right diaphragm EMG activity was used as an indicator of inspiratory activity to characterize activity of the intercostal (T2, T4, T5, T6, T7, T8, and T9) and abdominal muscles (external oblique and rectus abdominus). EMG activity during baseline (normoxia) conditions differed among thoracic external intercostal muscles (Fig. 1). The second thoracic external intercostal muscle (T2 EIC) showed prominent inspiratory activity in all rats studied, with minimal tonic (nonrespiratory) activity. In contrast, T4 and T5 EIC exhibited both inspiratory and tonic activity, whereas T6 and T7 EIC showed minimal inspiratory activity superimposed on tonic activity; and T8 and T9 EIC muscles showed only tonic activity, with no appreciable inspiratory or expiratory modulation. Similarly, the external oblique and rectus abdominus muscles exhibited tonic activity exclusively, with no clear evidence for respiratory phasic activity.

**Motor Activity During Sustained Hypoxia and Maximum Chemoreceptor Stimulation**

To investigate the ability to recruit EIC and abdominal muscle respiratory activity during SH and MCS, diaphragm and T2, T4, and T5 EIC EMG activity were recorded. After baseline normoxia (20 min), rats were exposed to MCS, which significantly increased inspiratory EMG activity in Dia, T2, T4, and T5 EIC muscles (102 ± 2%, 98 ± 3%, 72 ± 3%, and 53 ± 2% above baseline, respectively; P < 0.001; Figs. 2A and 3). A similar, decreasing pattern was observed when rats were exposed to sustained hypoxia (10.5% O2), although this was not as powerful as MCS to increase breathing in unanesthetized rats. SH increased EMG amplitude in the Dia, T2, and T4 EIC muscles (56 ± 2%, 55 ± 2%, 20 ± 3% baseline, respectively; P < 0.001; Figs. 2B and 3) but not in T5 EIC which was

![Fig. 1. Representative traces of diaphragm (Dia) and second, fourth, fifth, sixth, seventh, eighth, and ninth external intercostal (T2, T4, T5, T6, T7, T8, T9 EIC) muscles as well as abdominal muscles (RA: rectus abdominus, EO: external oblique) raw EMG activity during normoxia in unanesthetized rats. The T2 EIC muscle shows inspiratory activity that is phase-locked with the diaphragm. There is a rostrocaudal decrease of inspiratory activity from T2 through T7 EIC muscles and, inversely, a rostrocaudal increase of tonic activity from T2 through T9. Abdominal muscles show tonic activity exclusively.](http://jap.physiology.org/ by 10.2203/33.6 on October 30, 2017)
unaffected by SH relative to baseline or TC rats (5 ± 1% vs. 5 ± 2%, respectively; \(P = 0.563\); Figs. 2B and 3).

**Ventilation During Sustained Hypoxia and Maximum Chemoreceptor Stimulation**

MCS elicits the greatest increase in tidal volume vs. SH and TC (0.94 ± 0.1 vs. 0.68 ± 0.05 and 0.48 ± 0.03 ml/100 g, respectively; \(P < 0.001\); Fig. 4A). Respiratory frequency during MCS was also greater than SH or TC (133 ± 1 vs. 118 ± 2 and 75 ± 3 breaths/min, respectively, \(P < 0.001\); Fig. 4B). Accordingly, MCS elicits greater minute ventilation (tidal volume \(\times\) respiratory frequency) than SH (126 ± 5 vs. 80 ± 2 ml/100 g \(\cdot\) min\(^{-1}\), respectively, \(P < 0.001\)).

**Effect of Acute Intermittent Hypoxia**

A sustained increase in integrated diaphragm (Dia) and second, fourth, and fifth external intercostal (T2, T4, T5 EIC) muscle activity was observed 0–60 min post-AIH (19 ± 2% baseline, \(P < 0.001\); Figs. 5A and 6A), confirming reports of diaphragm long-term facilitation (Dia LTF) (45). External T2 intercostal EMG amplitude also exhibited LTF, although its magnitude was over double that of Dia LTF (T2 EIC LTF: 41 ± 1% baseline; \(P < 0.001\); Figs. 5A and 6A). Respiratory frequency (14 ± 1% above baseline; \(P = 0.004\); Fig. 5B) and minute activity (diaphragm: 40 ± 2% baseline; T2 EIC: 63 ± 2% baseline; both \(P < 0.001\); Fig. 5C) were also increased 60 min post-AIH (i.e., frequency and minute activity LTF). On the other hand, continuous normoxia (i.e., time controls) did not significantly affect post-treatment EMG amplitude, frequency, or minute activity in either diaphragm or T2 EIC muscles (all \(P > 0.05\); Figs. 5 and 6C). T4 and T5 EIC muscles exhibited considerable variability in their response following AIH. In T4 EIC, three rats increased EMG amplitude 60 min post-AIH (Fig. 6A), but three did not. T5 EIC muscles showed increased (\(n = 1\); Fig. 6A), no change in (\(n = 3\)), or decreased EMG amplitude (\(n = 2\); Fig. 6B) 60 min post-AIH. The presence or absence of T4/T5 LTF, or overt inhibition (long-term inhibition, LTI) was not completely predictable but appeared related to the rat posture when AIH was administered. A curled up position favored LTF (Fig. 6A), whereas an extended position favored LTI (Fig. 6B). Semi-curler postures exhibited no change in T4/T5 EIC activity vs. baseline 60 min post-AIH. This postural difference suggests that ribcage rotation influences respiratory behavior in midthoracic EIC muscles. Neither T6, T7, T8, or T9 EIC muscles (or the rectus abdominus) exhibited any tendency to increase or decrease respiratory-related activity 60 min post-AIH (Fig. 7, C and D). Interestingly, the external oblique muscle was inhibited during AIH protocol, but not following AIH (Fig. 8). Due to the highly variable tonic activity, the lack of apparent respiratory activity and the profound influence of posture, we did not quantify the magnitude of this inhibition.

**DISCUSSION**

Here, we report five major findings: 1) diaphragm and second external intercostal muscles show exclusively inspiratory activity in all conditions studied; 2) from T4 caudal to T9 EIC, there is progressively less inspiratory activity, and progressively more tonic (nonrespiratory) activity; 3) combined hypoxia and hypercapnia is a more powerful stimulus than hypoxia alone in all variables studied; 4) moderate AIH elicits consistent diaphragm LTF, but even greater LTF in the second external intercostal muscle; and 5) AIH elicits LTF, LTI, or no change in midthoracic external intercostal muscle activity, a differential response that appears related to rat posture. Collectively, these data advance our understanding concerning the regulation of respiratory muscles in unanesthetized and undisturbed rats.

**Respiratory (Phasic) Activity in Normoxia**

Phasic respiratory activity in intercostal muscles depends on the particular muscle under study, the animal’s posture, the extent of chemoreflex activation, and the presence of ongoing respiratory plasticity. It is particularly difficult to characterize the respiratory function of individual external intercostal muscles. Nevertheless, consistent with previous studies in rats and dogs (15, 33), the second EIC muscle always exhibited prominent inspiratory activity during normoxia, with a progressive, decreasing rostrocaudal gradient of inspiratory activity from T4 to T7. Similar results were found in dogs, where inspiratory...
EIC activity decreases rapidly from the second to sixth interspaces, and actually reverses to expiratory activity by the 8th to 10th interspaces (7). We did not find expiratory activity in midcostal (T6, T7 EIC) or caudal thoracic segments (T8, T9 EIC) in rats. This finding is consistent with intercostal nerve activity in anesthetized rats, which exhibits greater inspiratory discharges in the more rostral external intercostal nerves (5). Intercostal nerve recordings in anesthetized rats confirm both inspiratory and expiratory activity in caudal external and internal intercostal nerves (5), and individual thoracic motoneurons can be excited in both phases of respiration (6).

Variability of inspiratory/expiratory activity in midthoracic and caudal EIC muscles may relate to the convergence of bulbo-spinal synaptic pathways onto these motor neurons, cross-talk between the external and internal intercostal muscles, and spinal sensory inputs. The external intercostals muscles in the caudal interspaces are particularly thin, so the expiratory activity recorded in some studies could also result from electrical cross talk in the EMG recordings. Legrand and De Troyer (29) examined the pattern of activity of the caudal canine external intercostal muscles using selective denervation procedures. In agreement with previous observations (3, 28), phasic expiratory discharges were recorded from the external intercostal muscles in the ventrolateral portion of the caudal interspaces. However, expiratory activity was unchanged after section of the external intercostal nerves, and disappeared only after section of the internal intercostal nerve in the same interspace; thus expiratory discharges in the external intercostal muscles of caudal segments were due to impulses traveling via internal vs. external intercostal nerves. Moreover, the dorsoventral gradient of expiratory activity in caudal external intercostals may explain the lack of expiratory activity observed in this study. In dogs (11) and cats (24), the external intercostals in the caudal interspaces have larger masses dorsally (vs. ventrally); therefore, an already reduced expiratory activity may be masked by tonic activity in our ventrally implanted rats. Further, we cannot rule out that the signal recorded form rostral T2 external intercostal and external oblique muscles may be partially contaminated with signal from T2 internal intercostal and internal oblique muscles, which is a limitation of EMG recordings in freely moving, unanesthetized animals.

Details concerning central respiratory drive in spinal thoracic motoneurons are still unresolved. Intercostal activity appears to be driven by a network of spinal interneurons, with only rare monosynaptic inputs from brain stem respiratory neurons (35, 42). Thus high-frequency stimulation of the T2 to T5 spinal cord in cervical hemisected, anesthetized dogs activates inspiratory external intercostal motor neurons in a remarkably physiological manner (14). Interestingly, the vast majority of individual thoracic interneurons produce relatively weak effects on EIC activity (25, 26), suggesting that a few, key interneurons drive thoracic motor neurons (32, 42). The impact of these spinal interneurons and their tendency to elicit inspiratory, expiratory, or combined activity at different levels of the thoracic spinal cord has not been adequately studied.

**Maximum Chemoreceptor Stimulation vs. Sustained Hypoxia**

Maximum chemoreceptor stimulation (combined hypoxia/hypercapnia) is a stronger stimulus to respiratory muscle activity than sustained hypoxia alone, in both diaphragm and rostral external intercostal muscles. These results contradict previous studies in rats showing that, during REM sleep, hypoxia is a more potent stimulus than combined hypoxia/hypercapnia (34). We did not analyze EMG activity in different sleep states, which may account for some observed differences. In contrast, studies done in unanesthetized dogs (4), cats (19, 22), and humans (43) show that hypercapnia alone is a more powerful stimulus to minute ventilation vs. hypoxia, and that hypercapnia and hypoxia have synergistic effects on minute ventilation (43), consistent with our studies.

**Fig. 4.** Absolute values of tidal volume (VT, A) per 100 g rat and respiratory frequency (breaths/min, B) during MCS, SH, and TC groups. MCS is a more powerful stimulus than SH for VT and respiratory frequency. Values are means ± SE.

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<tr>
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<th>VT (ml/100 g)</th>
<th>Respiratory Frequency (breaths/min)</th>
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<tr>
<td>TC</td>
<td>0.8 ± 0.2</td>
<td>140 ± 20</td>
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<tr>
<td>SH</td>
<td>0.6 ± 0.1</td>
<td>120 ± 15</td>
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<tr>
<td>MCS</td>
<td>1.0 ± 0.3</td>
<td>160 ± 25</td>
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*Significantly different from TC; †significantly different from baseline; ‡significantly different from SH; P < 0.001.

**Fig. 5.** Changes in diaphragm (Dia) and second external intercostal (T2 EIC) peak amplitude (A), respiratory frequency (B), and calculated values of minute activity (amplitude × frequency) (C) 0–60 min post-AIH, expressed as percent change from baseline after acute intermittent hypoxia (AIH) and continuous normoxia (time controls, TC) in unanesthetized rats. There is a significant increase in amplitude above baseline in diaphragm and T2 EIC EMG amplitude (A) indicative of robust diaphragm and T2 EIC long-term facilitation (DiaLTF and T2 EIC LTF, respectively). Moreover, a significant increase in respiratory frequency (B) and minute activity (C) in diaphragm and T2 EIC is observed after AIH, representing LTF for frequency and minute activity. Time control rats receiving normoxia do not show a change in amplitude compared with baseline. Values are means ± SE. *Significantly different from TC; †significantly different from baseline; ‡significantly different from diaphragm; P < 0.001.
Effect of Acute Intermittent Hypoxia in External Intercostal and Abdominal Muscles

We confirm that AIH elicits a long-lasting increase in diaphragm peak amplitude above baseline, i.e., long-term facilitation (DiaLTF) (45). The cellular mechanisms of phrenic LTF have been explored extensively (1, 2, 30). Here, we report for the first time that AIH elicits a similar long-lasting increase in peak EMG activity in the second external intercostal (T2 EIC) muscle (i.e., T2 EIC LTF) in unanesthetized, spontaneously breathing and poikilocapnic rats. Interestingly, the in-
crease in peak EMG amplitude in T2 EIC is more than double Dia LTF, suggesting a relatively greater contribution of inspiratory intercostal muscles to ventilation after intermittent hypoxia.

Although the specific cause is unknown, variability in AIH effects on midthoracic (fourth and fifth) EIC may relate to several factors. First, more caudal thoracic segments may be more involved in postural adjustments as rats navigate their world, compromising respiratory function. Studies in cats (13, 16) and humans (23, 41, 47) demonstrate that head and trunk rotation affect the degree of respiratory activity in middle and lower thoracic intercostal muscles. Second, there may be differences among sleep states that were not accounted for in this study. LTF is bigger during NREM vs. quiet wakefulness (45), and this effect may be greater in some respiratory muscles vs. others; failure to account for sleep state is one limitation of this study (a logistics issue due to limited biopotential leads per transmitter). LTF expression may depend on the degree of inspiratory activity at the time of exposure, which is affected by both posture and sleep stage. When cats adopt a curled position, inspiratory intercostal muscle activity is greater on the concave-upward vs. the convex-downward side (13). In rats, inspiratory activity of second and fifth external intercostal muscles is greater when the rat is in curled-up vs. in an extended position (33). Indeed, our rats were free to adopt different postures during experimental conditions, potentially influencing the results on one or both sides. In general, we observed that a curled position during AIH increased EMG inspiratory activity and was accompanied by AIH-induced LTF in the T4 and T5 EIC muscles. In contrast, an extended position favored long-term inhibition in the T5 EIC muscle, whereas rats in a semi-curled posture exhibited no LTF following AIH. However, the association between posture and the presence of LTF or LTI remains to be investigated.

AIH-induced LTF is elicited routinely in inspiratory muscles, such as the diaphragm and T2 EIC muscle. LTF may be an exclusive and general property of inspiratory motor nerve activity, as suggested by Fregosi and Mitchell (21). In cats, repeated carotid sinus nerve stimulation evokes serotonin-independent LTF in both phrenic and inspiratory internal intercostal (parasternal) nerve activity in anesthetized cats. Further, carotid sinus nerve stimulation-evoked LTF of inspiratory intercostal nerve activity exceeds that observed in phrenic nerve activity (21), similar to our results for T2 EIC LTF vs. Dia LTF.

The four abdominal muscles with significant respiratory function in quadrupeds and humans are rectus abdominis, external oblique, internal oblique, and transversus abdominis. Under normoxic conditions the muscles explored in this study show almost exclusively tonic activity, although weak expiratory activity may have been obscured by postural changes. Our results are consistent with unanesthetized dogs, where expiratory EMG activity is rarely seen in the external oblique, and is absent in rectus abdominis muscles (8, 49). Posture also affects the extent of expiratory activity in abdominal muscles. Expiratory activity in canine external oblique muscles is reduced considerably when lying in a prone position (vs. standing) (8). Similar postural issues may help explain the absence of phasic abdominal muscle activity in the rats of this study, since they were always in a prone position.

We observed that EMG activity in external oblique was actually inhibited during hypoxia, consistent with previous studies. For example, peak EMG amplitude in external oblique increases during hypercapnia, but is inhibited during hypoxia in dogs (49), and isocapnic hypoxia inhibits iliohypogastric nerve activity in cats (20).

One purpose of this study was to characterize external intercostal and abdominal muscle activity in unanesthetized and unstressed animals using a minimally invasive radiotelemetry system; although most findings were consistent with earlier studies, differences with prior accounts may have arisen from the use of telemetry vs. traditional wire-implantation techniques used in those earlier studies. Radiotelemetry is expected to cause less animal stress vs. externalized wires, which restrain movement and leave an open (possibly infected) exit site. Telemetry represents the most physiologically relevant and humane method for monitoring of physiological parameters in conscious, freely moving laboratory animals (27, 38). Some have claimed that radiotelemetry minimizes the number of animals required for a given study by up to 60–70% (46), and reduces research costs by increasing the number of variables monitored from a single animal. One powerful experimental advantage is that EMG radiotelemetry permits virtually continuous data collection for weeks to months. This latter feature is critical for studies in chronic disease models, such as spinal cord injury or motor neuron disease.
disease. Further, radiotomometry permits investigation of drug effects in freely moving animals, enabling collection of physiological data in more relevant conditions and, possibly, more reliable clinical translation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.A.N.-O. and G.S.M. conception and design of research; A.A.N.-O. performed experiments; A.A.N.-O. analyzed data; A.A.N.-O. and G.S.M. interpreted results of experiments; A.A.N.-O. prepared figures; A.A.N.-O. drafted manuscript; A.A.N.-O. and G.S.M. edited and revised manuscript; A.A.N.-O. and G.S.M. approved final version of manuscript.

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