Title	Effect of hypercapnia on self-sustained muscle activity
Author(s)	Hatano, Kei; Shirakawa, Kazuki; Usuda, Noboru; Matsuura, Ryouta; Ohtsuka, Yoshinori; Yunoki, Takahiro
Citation	Respiratory physiology & neurobiology, 250, 24-30 https://doi.org/10.1016/j.resp.2018.02.002
Issue Date	2018-04
Doc URL	http://hdl.handle.net/2115/74457
Rights	© 2018. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	http://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	after_acceptance.pdf

[Instructions for use](#)

ABSTRACT

The aim of the present study was to determine the effect of hypercapnia on motor neuromuscular activity of the human triceps surae muscle. Nine subjects participated in trials in a normal breathing condition and a CO₂ rebreathing condition. In both conditions, in order to provoke self-sustained muscle activity, percutaneous electrical train stimulation was applied to the tibial nerve while each subject lay on a bed. Self-sustained muscle activity, which is an indirect observation of plateau potentials in spinal motoneurons, was measured for 30 sec after the train stimulation by using surface electromyography. The sustained muscle activity was increased by CO₂ rebreathing ($P < 0.05$). This finding suggests that motor neuromuscular activity may be linked to the respiratory system that is activated during hypercapnia.

Keywords

Hypercapnia, Central chemoreceptor, Self-sustained muscle activity, Plateau potentials

1   **Title: Effect of hypercapnia on self-sustained muscle activity**

2   List of authors: Kei Hatano^a; Kazuki Shirakawa^a; Noboru Usuda^a; Ryouta Matsuura^b;
3   Yoshinori Ohtsuka^c; Takahiro Yunoki^c

4
5   ^a Graduate School of Education, Hokkaido University, Sapporo, Japan

6   ^b Department of Health and Physical Education, Joetsu University of Education, Joetsu,
7   Japan

8   ^c Department of Human Developmental Sciences, Faculty of Education, Hokkaido
9   University, Sapporo, Japan

10
11   Corresponding author: Kei Hatano

12   Address: Graduate School of Education, Hokkaido University, Kita-11, Nishi-7, Kita-ku,
13   Sapporo 060-0811,

14   Japan

15   E-mail address: k-hatano@eis.hokudai.ac.jp

16

17

1. Introduction

Carbon dioxide (CO₂) is a strong stimulus to central chemoreceptors located in the brainstem for ventilatory response, which is a homeostatic function for maintaining the internal environment of the body (Whipp and Ward 1998). Hypercapnia results in the generation of depolarizing currents known as central respiratory drive potentials in spinal motoneurons of respiratory muscles to increase ventilation through the output of the respiratory center in the medulla (Butler 2007). Additionally, it has been reported that hypercapnia facilitated plateau potentials in hindlimb motoneurons of a decerebrate anesthetized cat (Kirkwood et al. 2002, 2005). This indicates the possibility that a change in arterial blood CO₂ pressure (PaCO₂) is involved not only in respiratory control but also in limb motoneuronal activity in animals. Plateau potentials are sustained depolarizations of spinal motoneurons that are facilitated via activation of L-type calcium channels and persistent sodium channels of motoneurons (Bennett et al. 1998; Lee and Heckman 1999; Li et al. 2004). However, there has been no study in which the relationship between hypercapnia and plateau potentials of limb muscle motoneurons in humans was investigated.

In previous studies, it has been shown that plateau potentials can be measured

by electromyography (EMG) signals in animals (Crone et al. 1988; Gorassini et al. 1999; Hounsgaard et al. 1988) and humans (Collins et al. 2001, 2002; Gorassini et al. 1998, 2002; Nozaki et al. 2003; Trajano et al. 2014; Walton et al. 2002). Nozaki et al. (2003) demonstrated that autonomous neural activity that is related to the plateau potentials of limb muscle motoneurons could be recorded as self-sustained muscle contractions, which are muscle activities that involuntarily continue after the end of percutaneous electrical train stimulation to a peripheral nerve, by using surface EMG. This indicates that sustained muscle contractions are an indirect observation of plateau potentials in spinal motoneurons. Therefore, self-sustained muscle activity, which is an enhanced sustained EMG activity, was induced in the present study by using the methods of Nozaki et al. (2003). If plateau potentials are associated with hypercapnia as indicated by Kirkwood et al. (2002, 2005), EMG activity of the sustained muscle activity in humans would be affected by hypercapnia. The purpose of the present study was to investigate whether hypercapnia induced by CO₂ rebreathing alters the self-sustained muscle activity of the human triceps surae muscle.

2. Methods

2.1. Subjects

Nine healthy males, with a mean ($\pm$ standard deviation) age of 29 ± 8.4 years, who had never had a nervous or motor disorder participated in the present study. All of the subjects provided informed consent for participation in the study. This study was approved by the Human Research Ethics Committee of the Graduate School of Education, Hokkaido University.

2.2. Experimental set-up

In the experiment, each subject lay on a bed on his left side, and the subject's right leg was placed on a cushion. The subject's right hip and knee joint were flexed approximately 60° . The subjects were instructed to remain relaxed throughout the experiment.

2.3. Recordings

Surface EMG signals were recorded from the soleus muscle (Sol) and from the

medial head (MG) and lateral head (LG) of the gastrocnemius muscle of the right leg via a bipolar electrode. The ground electrode was placed over the right caput fibulae. EMG signals were amplified (Amplifier: AB-611-J, Nihon-Kohden, Japan) with band-pass filtering between 1.5 Hz and 1 kHz and converted into digital signals at a sampling rate of 2 kHz using an analog-digital converter (LabChart 8, ADInstruments, Australia). Throughout the experiment, the subjects breathed through a face mask connected to a hot-wire flow meter and a respiratory gas analyzer (AE-280S, Minato Medical Science, Japan) to measure minute ventilation ($\dot{V}_E$) and end-tidal carbon dioxide pressure (PETCO₂). Respiratory variables were measured with the breath-by-breath mode. A zirconium sensor and infrared absorption analyzer were used to analyze inspired and expired fractions of O₂ and CO₂, respectively. Inspired and expired flows were measured using the hot-wire flow meter. The gas analyzer was calibrated with precision reference gas (O₂, 15.17%; CO₂, 4.92%). The flow meter was calibrated with a standard 2-liter syringe.

2.4. Experimental protocol

Each subject participated in trials in two respiratory conditions, a CO₂

1 rebreathing condition and a normal breathing condition, which were conducted on the
2 same day (Fig. 1). The subjects remained relaxed for 152 sec (- 90 to 62 sec) in each trial.
3 In the CO₂ rebreathing condition, respiratory dead space (1500 ml) was added to the
4 respiratory mask from 30 sec after the initiation of the trial to the end of the trial (- 60 to
5 62 sec) in order to make the subjects rebreathe expired CO₂. In the normal condition, each
6 subject remained rested and spontaneously breathed room air through the respiratory
7 mask. At 90 sec after the start of a trial in the both conditions, 2-sec train stimulation was
8 delivered to the right tibial nerve. Then each subject remained relaxed for 60 sec after the
9 2-sec train stimulation in both conditions. This trial was repeated 6 times with intervals
10 of 5-10 min. During each interval, maximal M-wave (M-max) and Hoffmann's reflex (H-
11 reflex) of the soleus muscle were measured three times and five times respectively. Three
12 of the 6 trials were performed in the CO₂ rebreathing condition. Three trials in each
13 condition were sequentially performed and the order of the two conditions was random
14 among subjects. It has been shown that PaCO₂ is increased by rebreathing expired air
15 through the increased respiratory dead space (Koppers et al. 2006; Smolka et al. 2014).

16
17 Electrical stimulation. While each subject remained reclined on the bed, the tibial nerve
18 was percutaneously stimulated by applying a rectangular electrical pulse of 1 msec in

duration to the popliteal fossa using a constant current stimulator (DS7AH, Digitimer Ltd, UK). Sustained muscle activity was elicited by 50-Hz electrical train stimulation for 2 sec (Nozaki et al. 2003). In this study, activity of the soleus muscle was mainly assessed sample. Thus, the stimulation intensity was 120% of the H-reflex threshold of the soleus muscle. The cathode of the stimulation electrodes was secured to the right leg popliteal fossa, and the anode was attached to the patella. The subjects were asked to ignore the electrical train stimulation as much as possible. In order to measure M-max during intervals between trials, the electrical stimulus delivered by the stimulator was increased gradually until the M-wave of the soleus muscle reached a plateau while the subject was at rest. The level of stimulation was then set 20% above this point to ensure maximal activation of these muscles. Additionally, the stimulus intensity of H-reflex was 120% of the H-reflex threshold of the soleus muscle during an interval.

Safety of subjects. In both respiratory conditions, the subjects were asked about their sensation of dyspnea after recording self-sustained muscle activity using the modified Borg scale (Borg 1982) in order to determine whether the experiment could be continued. Using a pulse oximeter (PULSOX-300i, Konica Minolta, Japan), arterial oxygen saturation of pulse oximetry (SpO₂) was measured noninvasively to check whether SpO₂

was within safety level (over 90%).

2.5. Data analysis

The root mean squares (RMSs) of EMG of the Sol, MG, and LG muscles were calculated from 10-sec and 30-sec windows immediately before and after the train stimulation. The RMSs in each respiratory condition were averaged by the 3 trials. The magnitudes of M-max and H-reflex were measured as peak-to-peak amplitude and averaged over 3 times and 5 times, respectively, for use in the following analyses. The H-reflex amplitude was evaluated as percentage of M-max amplitude.

In order to obtain continuous data for arterial carbon dioxide pressure (PaCO_2), PaCO_2 was calculated from PETCO_2 using the formula of Jones et al. (1979).

$$\text{Predicted PaCO}_2 (\text{PaCO}_{2\text{pred}}) = 5.5 + 0.90 \text{ PETCO}_2 - 0.0021 \text{ tidal volume (VT)}.$$

To obtain continuous averaged data for changes in $\dot{V}\text{E}$ and $\text{PaCO}_{2\text{pre}}$ in all subjects, the variables were interpolated second-by-second using the 3-dimensional spline technique. The interpolated data were for the period from 30 sec before the start of CO_2 rebreathing to 60 sec after the end of the train stimulation (i.e., a 152-sec period) in each trial.

2.6. Statistical analysis

Measured data are presented as means $\pm$ standard error (SE). A paired *t*-test was used to compare $\dot{V}E$, $PaCO_{2pred}$, sensation of dyspnea and SpO_2 between the two respiratory conditions and RMSs between pre- and post-train stimulation. Two-way repeated measures analysis of variance (ANOVA) was performed to examine the effects of the two respiratory conditions and three muscles on RMSs of the muscles during and before the self-sustained muscle activity period. To confirm that the activities of peripheral nerves were not different among all of the trials before the CO_2 rebreathing period, the effects of trials and respiratory conditions on the amplitudes of M-max and H-reflex were examined by using two-way repeated measures ANOVA. After ANOVA, the Bonferroni post hoc test was performed for multiple comparisons. If a significant interactive effect was indicated, a simple main effect test was performed. SPSS (Version 20, IBM, USA) was used for statistical analysis. Statistical significance was set at $P < 0.05$.

3. Results

Fig. 2A shows changes in $\dot{V}E$ and $PaCO_{2pred}$. Before the start of CO_2 rebreathing (30-sec period), $\dot{V}E$ and $PaCO_{2pred}$ were not different between the two conditions (Fig. 2B, a¹, b¹). During self-sustained muscle activity (Fig. 2B, a², b²), the variables in the CO_2 rebreathing condition ($\dot{V}E$: 17.3 ± 1.2 l/min, $PaCO_{2pred}$: 53.6 ± 0.7 mmHg) were significantly ($P < 0.05$) larger than those in the normal breathing condition ($\dot{V}E$: 8.9 ± 0.3 l/min, $PaCO_{2pred}$: 39.0 ± 0.7 mmHg). In the CO_2 rebreathing condition, $\dot{V}E$ and $PaCO_{2pred}$ were 1.9 ± 0.1 -times and 1.4 ± 0.02 -times larger, respectively, than those in the normal breathing condition. $PaCO_{2pred}$ continuously increased immediately after the start of CO_2 rebreathing, while $\dot{V}E$ increased gradually at a later timepoint than $PaCO_{2pred}$. $\dot{V}E$ and $PaCO_{2pred}$ did not reach a plateau during the CO_2 rebreathing period as shown in Fig. 2A. The sensation of dyspnea was 4.3 ± 0.4 (moderate – very severe) in the CO_2 rebreathing condition. SpO_2 decreased during CO_2 rebreathing ($93.6 \pm 0.7\%$) compared to that during normal breathing ($97.8 \pm 0.3\%$), but it did not reach a dangerous level.

M-max amplitudes, which were evoked before the start of each trial in both conditions, were 9.6 ± 1.3 mV, 9.9 ± 1.3 mV and 10.1 ± 1.2 mV in the normal breathing condition and 10.1 ± 1.2 mV, 9.9 ± 1.3 mV and 9.5 ± 1.3 mV in the CO_2 breathing condition. H-reflex amplitudes (%M-max) were $58.1 \pm 7.1\%$, $57.8 \pm 8.2\%$ and $54.9 \pm 7.5\%$ in the normal breathing condition and $53.5 \pm 6.7\%$, $53.9 \pm 6.3\%$ and $54.0 \pm 6.9\%$

1 mV in the CO₂ breathing condition. Two-way repeated measures ANOVA did not indicate
2 a significant interaction between conditions and trials for M-max and H-reflex (Fig. 3).
3 There were no differences in M-max and H-reflex in the soleus muscle measured before
4 electrical train stimulation among all trials and between conditions.

5 During the electrical train stimulation (1 msec pulse, 50 Hz, 2 sec) period, EMG
6 reflex responses were elicited in the Sol, MG and LG (Fig. 4). The first stimulus induced
7 a large reflex response, and then several subsequent responses were depressed (lower left
8 panel). However, the reflex response recovered with repetitive stimuli (lower right panel).

9 Fig. 5 shows typical data for self-sustained muscle activities in both respiratory
10 conditions that were induced following electrical train stimulation in the same subject.
11 We attempted to measure self-sustained muscle activities for 1 min. However, in three
12 subjects, the activities in several trials were suppressed within 1 min. Thus, RMSs of
13 EMG signals during the sustained muscle activity were evaluated for 30 sec after the end
14 of the train stimulation. RMSs increased from pre- to post-train stimulation in both the
15 normal breathing condition (Sol, from 1.6 ± 0.3 μ V to 11.1 ± 3.1 μ V; MG, from 1.6 ± 0.3
16 μ V to 5.4 ± 1.4 μ V; LG, from 2.2 ± 0.4 μ V to 8.7 ± 2.8 μ V; $P < 0.05$) and the CO₂
17 rebreathing condition (Sol, from 1.7 ± 0.3 μ V to 18.4 ± 4.9 μ V; MG, from 1.4 ± 0.2 μ V
18 to 9.2 ± 1.8 μ V; LG, from 1.7 ± 0.1 μ V to 10.7 ± 2.7 μ V; $P < 0.05$). In pre-train stimulation,

there were no main effects or interactions between conditions and muscles for RMSs. In post-train stimulation (i.e., during the sustained muscle activity), there was no significant interaction between conditions and muscles for RMSs. RMSs of the CO₂ rebreathing condition were significantly larger than those of the normal breathing condition for all muscles ($P < 0.05$), though there were no differences among muscles.

4. Discussion

The purpose of this study was to investigate the effect of hypercapnia on self-sustained muscle activity in humans. PaCO_{2pred} showed an exponential increase immediately after the start of CO₂ rebreathing, and $\dot{V}E$ showed a constant increase about 30 sec after the rise in PaCO_{2pred} (Fig. 2A). There were no differences between the two conditions in $\dot{V}E$ and PaCO_{2pred} before the start of the CO₂ rebreathing period (Fig. 2B, a¹, b¹), indicating that the internal environment of the body was likely to be the same in the two conditions until the start of CO₂ rebreathing. An increase in PaCO₂ is a stimulus to activate central chemoreceptors located in the medulla (Whipp and Ward 1998). Therefore, an increase of $\dot{V}E$ during CO₂ rebreathing is thought to be due to activation of the chemoreceptors (Fig. 2B, a², b²).

1   In order to induce self-sustained muscle activity, 2-sec train stimulation was
2 delivered to the right tibial nerve 3 times with intervals of 5-10 min in each condition. To
3 confirm that the electrical train stimulation electrode was in the same position in both
4 conditions, we measured M-max of the soleus muscle three times in each interval between
5 trials. In this study, the magnitude of M-max was not different among the trials (Fig. 3).
6 Thus, the stimulation electrode position was not likely to be different among the trials.
7 Also, the magnitude of H-reflex of the soleus muscle, which was measured five times at
8 the same time as measurement of M-max, was not different among the trials. This
9 suggests that activities of peripheral nerves were not different among the trials before the
10 CO₂ rebreathing period.

11   During the electrical train stimulation period, reflex responses were elicited in
12 the triceps surae muscles (Fig. 4). Nozaki et al. (2003) found that although the first
13 electrical stimulus induced the largest reflex response in the soleus muscle and several
14 subsequent responses were depressed as shown in the lower left panel of Fig. 4, responses
15 recovered with repetitive stimuli as shown in the lower right panel of Fig. 4. They
16 explained this phenomenon as depolarization of membrane voltage of motoneurons due
17 to the emergence of plateau potentials because this phenomenon resembled firing
18 frequency acceleration of motoneurons during elicitation of plateau potentials

1 (Hounsgaard et al. 1984, 1988). In the present study, we induced self-sustained muscle
2 activity by using the methods of Nozaki et al. (2003). Reflex responses, which are similar
3 to those in the previous study, were elicited during train stimulation. Therefore, we
4 probably induced neuromuscular activity as self-sustained muscle activity that is likely to
5 be associated with plateau potentials.

6 We attempted to measure self-sustained muscle activity of the triceps surae
7 muscle for 1 min in all subjects, but the sustained muscle activity was depressed within 1
8 min in three subjects. Thus, RMS of EMG of the Sol, MG, and LG muscles was calculated
9 from a 30-sec window immediately after the train stimulation. Although we could not
10 determine the cause of these results, we thought that sustained muscle activities of the
11 three subjects in several trials were suppressed by slight limb movement. Indeed, the
12 sustained muscle activity was suppressed after the measuring period by voluntary
13 dorsiflexion in our study. In a previous study, sustained muscle contraction that was
14 induced by the same methods as those used in this study was shown to be depressed by
15 electrical stimulation to an antagonist muscle nerve (Nozaki et al. 2003). Therefore, it is
16 possibly because of slight contraction of an antagonist muscle such as the tibialis anterior
17 muscle that the sustained muscle activity was depressed within 1 min in the three subjects.

18 Before the electrical train stimulation, RMSs of all muscles were not different

1 between the two conditions, while after stimulation, self-sustained muscle activities were
2 significantly larger for the CO₂ rebreathing condition (Fig. 6). This suggests that an
3 increase in sustained muscle activity was not due to differences in EMG activity before
4 the train stimulation between conditions.

5 Self-sustained muscle activity is an indirect measure of plateau potentials
6 (Nozaki et al. 2003). It is known that plateau potentials are facilitated via activation of L-
7 type calcium channels and persistent sodium channels of spinal motoneurons (Bennett et
8 al. 1998; Lee and Heckman 1999; Li et al. 2004). The channels are strongly activated by
9 serotonin (5-HT) released from serotonergic neurons in the medullary raphe (Harvey et
10 al. 2006a, 2006b; Perrier & Hounsgaard, 2003). Walton et al. (2003) demonstrated that
11 self-sustained firing of the limb muscle motor unit, which is associated with plateau
12 potentials, was facilitated by an increase in 5-HT via a dose of caffeine. Previous studies
13 have shown that the 5-HT system of the brain is involved in spinal motor output
14 (Heckman et al. 2005; Jacobs and Fornal 1993; Jacobs et al. 2002). In addition, plateau
15 potentials are likely to be a fundamental component in the maintenance of posture
16 (Hounsgaard et al. 1988; Lee and Heckman 1998). Furthermore, serotonergic neurons,
17 which are a part of central chemoreceptors, are activated by an increase in PaCO₂
18 (Corcoran et al. 2013; Mitchell et al. 2008; Veasey et al. 1995, 1997; Wang et al. 2002).

1 Speculatively, if serotonergic projection from the medulla to spinal motoneurons is
2 increased during a high PaCO₂ level of the body and if 5-HT can facilitate plateau
3 potentials of spinal motoneurons, an increase in self-sustained muscle activity might be
4 associated with activation of central chemoreceptors. However, there are several other
5 possibilities for the increase in sustained muscle activity.

6 First, there is a matter of an increase in ventilatory activity per se during CO₂
7 rebreathing that might have influenced the sustained muscle activity. Balzamo et al.
8 (1997) found that respiratory resistive breathing enhanced tonic vibratory response in an
9 arm and a leg. They suggested that activation of respiratory muscle afferents facilitated
10 limb motoneurons reflex. Second, the effect of a decrease in SpO₂ induced by CO₂
11 rebreathing on sustained muscle activity is not clear. It was shown that H-reflex amplitude
12 in an arm muscle was increased during hypoxia induced by inhalation of hypoxic gas
13 (Delliaux and Jammes 2006). This indicates that there is a direct or indirect effect of
14 hypoxia on excitability of motoneurons. Therefore, to establish that CO₂ is indeed
15 necessary to facilitate self-sustained muscle activity, the effect of hyperventilation at a
16 rate under normoxic/isocapnic conditions should be investigated. Third, there was an
17 increase in dyspnea sensation of the subjects during CO₂ rebreathing in the present study.
18 In previous studies, a slight increase of H-reflex amplitude in the soleus muscle occurred

1 with respiratory discomfort caused by doses of lobeline and/or inspiratory threshold
2 loading (Gandevia et al. 1998; Morélot-Panzini et al. 2007). It is possible that emotion
3 alters the function of afferent fibers. With regard to serotonergic neurons, a link between
4 the 5HT neuron system and the “emotional” system has been shown (Bowker et al., 1982;
5 Holstege and Kuypers 1987) Therefore, further research in sleeping subjects to remove
6 the emotional effect on sustained muscle activity may be required (Collins et al. 2001).

7   We measured self-sustained muscle activity for 30 sec in the two respiratory
8 conditions and evaluated the effect of hypercapnia on self-sustained muscle activity. Self-
9 sustained muscle activities were increased during CO₂ rebreathing. Kirkwood et al.
10 (2005) reported that plateau potentials triggered by central respiratory drive potentials
11 were observed in hindlimb motoneurons of a decerebrate anesthetized cat during
12 hypercapnia. They indicated that activation of the plateau potentials of spinal
13 motoneurons was dependent on the physiological state of the cat. Consequently, our study
14 is the first study showing that hypercapnia is likely to influence self-sustained muscle
15 activity originating from plateau potentials of triceps surae muscle motoneurons in
16 humans. We conclude that self-sustained muscle activity in humans may be linked to the
17 respiratory system, which is activated during hypercapnia.

1   **Acknowledgments**

2

3   This study was supported by a Grant-in-Aid for Scientific Research from the
4   Japanese Ministry of Education, Science, and Culture (JSPS KAKENHI Grant Number
5   17K01612).

6

References

- Balzamo, E., Vuillon-Cacciuttolo, G, Burnet, H., Jammes, Y. 1997. Influence of respiratory afferents upon the proprioceptive reflex of skeletal muscles in healthy humans. *Neurosci Lett* 236, 127–130.
- Bennett, D.J., Hultborn, H., Fedirchuk, B., Gorassini, M. 1998. Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *J Neurophysiol* 80, 2023–2037.
- Borg, G. 1982. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14, 377–381.
- Bowker, R.M., Westlund, K.N., Sullivan, M.C., Coulter, J.D. 1982. Organization of descending serotonergic projections to the spinal cord. *Prog Brain Res* 57. 239–65.
- Butler, J.E. 2007. Drive to the human respiratory muscles. *Respir Physiol Neurobiol* 159, 115–126.
- Collins, D.F., Burke, D., Gandevia, S.C. 2001. Large involuntary forces consistent with plateau-like behavior of human motoneurons. *J Neurosci* 21, 4059–4065.
- Collins, D.F., Burke, D., Gandevia, S.C. 2002. Sustained contractions produced by plateau-like behavior in human motoneurons. *J Physiol* 538, 289–301.

- 1 Corcoran, A.E., Richerson, B.G., Harris, M.B. 2013. Serotonergic mechanisms are
2 necessary for central respiratory chemoresponsiveness in situ. *Respir Physiol*
3 *Neurobiol* 186, 214–220.
- 4 Crone, C., Hultborn, H., Kiehn, O., Mazieres, L., Wigström, H. 1988. Maintained changes
5 in motoneuronal excitability by short-lasting synaptic inputs in the decerebrate cat.
6 *J Physiol* 405. 321–343.
- 7 Delliaux, S., Jammes, Y. 2006. Effects of hypoxia on muscle response to tendon vibration
8 in humans. *Muscle Nerve* 34. 754–761.
- 9 Gandevia, S.C., Butler, J.E., Taylor, J.L., Crawford, M.R. 1998. Absence of
10 viscerosomatic inhibition with injections of lobeline designed to activate human
11 pulmonary C fibres. *J Physiol* 511. 289–300.
- 12 Gorassini, M., Bennett, D.J., Kiehn, O., Eken, T., Hultborn, H. 1999. Activation patterns
13 of hindlimb motor units in the awake rat and their relation to motoneuron intrinsic
14 properties. *J Neurophysiol* 82. 709–717.
- 15 Gorassini, M.A., Bennett, D.J., Yang, J.F. 1998. Self-sustained firing of human motor
16 units. *Neurosci Lett* 247. 13–16.

- 1 Gorassini, M., Yang, J.F., Siu, M., Bennett, D.J. 2002. Intrinsic activation of human
2 motoneurons: possible contribution to motor unit excitation. *J Neurophysiol* 87.
3 1850–1858.
- 4 Harvey, P.J., Li, X., Li, Y., Bennett, D.J. 2006a. 5-HT₂ receptor activation facilitates a
5 persistent sodium current and repetitive firing in spinal motoneurons of rats with and
6 without chronic spinal cord injury. *J Neurophysiol* 96. 1158–1170.
- 7 Harvey, P.J., Li, X., Li, Y., Bennett, D.J. 2006b. Endogenous monoamine receptor
8 activation is essential for enabling persistent sodium currents and repetitive firing in
9 rat spinal motoneurons. *J Neurophysiol* 96. 1171–1186.
- 10 Heckman, C.J., Gorassini, M.A., Bennett, D.J. 2005. Persistent inward currents in
11 motoneuron dendrites: implications for motor output. *Muscle Nerve* 31. 135–156.
- 12 Holstege, J.C., Kuypers, H.G. 1987. Brainstem projections to spinal motoneurons: an
13 update. *Neuroscience* 23. 809–821.
- 14 Hounsgaard, J., Hultborn, H., Jespersen, B., Kiehn, O. 1984. Intrinsic membrane
15 properties causing a bistable behaviour of alpha-motoneurons. *Exp Brain Res* 55.
16 391–394.

- 1 Hounsgaard, J., Hultborn, H., Jespersen, B., Kiehn, O. 1988. Bistability of alpha-
2 motoneurons in the decerebrate cat and in the acute spinal cat after intravenous 5-
3 hydroxytryptophan. *J Physiol* 405. 345–367.
- 4 Jacobs, B.L., Fornal, C.A. 1993. 5-HT and motor control: a hypothesis. *Trends Neurosci*
5 16. 346–352.
- 6 Jacobs, B.L., Martín-Cora, F.J., Fornal, C.A. 2002. Activity of medullary serotonergic
7 neurons in freely moving animals. *Brain Res Brain Res Rev* 40. 45–52.
- 8 Jones, N.L., Robertson, D.G., Kane, J.W., 1979. Difference between end-tidal and arterial
9 PCO₂ in exercise. *J Appl Physiol* 47. 954–960.
- 10 Kirkwood, P.A., Lawton, M., Ford, T.W. 2002. Plateau potentials in hindlimb
11 motoneurons of female cats under anaesthesia. *Exp Brain Res* 146. 399–403.
- 12 Kirkwood, P., Denton, M.E., Wienecke, J., Nielsen, J., Hultborn, H. 2005. Physiological
13 roles for persistent inward currents in motoneurons: insights from the Central
14 Respiratory Drive. *Biocybern Biomed Eng* 25. 31–38.
- 15 Koppers, R.J., Vos, P.J., Folgering, H.T. 2006. Tube breathing as a new potential method
16 to perform respiratory muscle training: safety in healthy volunteers. *Respir Med* 100.
17 714–720.

- 1 Lee, R.H., Heckman, C.J. 1998. Bistability in spinal motoneurons in vivo: systematic
2 variations in rhythmic firing patterns. *J Neurophysiol* 80. 572–582.
- 3 Lee, R.H., Heckman, C.J. 1999. Paradoxical effect of QX-314 on persistent inward
4 currents and bistable behavior in spinal motoneurons in vivo. *J Neurophysiol* 82.
5 2518–2527.
- 6 Li, Y., Gorassini, M.A., Bennett, D.J. 2004. Role of persistent sodium and calcium
7 currents in motoneuron firing and spasticity in chronic spinal rats. *J Neurophysiol*
8 91. 767–783.
- 9 Mitchell, G.S., Turner, D.L., Henderson, D.R., Foley, K.T. 2008. Spinal serotonin
10 receptor activation modulates the exercise ventilatory response with increased dead
11 space in goats. *Respir Physiol Neurobiol* 161. 230–238.
- 12 Morélot-Panzini, C., Demoule, A., Straus, C., Zelter, M., Derenne, J.P., Willer, J.C.,
13 Similowski, T. 2007. Dyspnea as a noxious sensation: inspiratory threshold loading
14 may trigger diffuse noxious inhibitory controls in humans. *J Neurophysiol* 97. 1396–
15 1404.
- 16 Nozaki, D., Kawashima, N., Aramaki, Y., Akai, M., Nakazawa, K., Nakajima, Y., Yano,
17 H. 2003. Sustained muscle contractions maintained by autonomous neuronal activity
18 within the human spinal cord. *J Neurophysiol* 90. 2090–2097.

1 Perrier, J.F., Hounsgaard, J. 2003. 5-HT₂ receptors promote plateau potentials in turtle
2 spinal motoneurons by facilitating an L-type calcium current. *J Neurophysiol* 89.
3 954–959.

4 Smolka, L., Borkowski, J., Zaton, M. 2014. The effect of additional dead space on
5 respiratory exchange ratio and carbon dioxide production due to training. *J Sports*
6 *Sci Med* 13. 36–43.

7 Trajano, G.S., Seitz, L.B., Nosaka, K., Blazeovich, 2014. A.J. Can passive stretch inhibit
8 motoneuron facilitation in the human plantar flexors? *J Appl Physiol* (1985) 117.
9 1486–1492.

10 Veasey, S.C., Fornal, C.A., Metzler, C.W., Jacobs, B.L. 1995. Response of serotonergic
11 caudal raphe neurons in relation to specific motor activities in freely moving cats. *J*
12 *Neurosci* 15. 5346–5359.

13 Veasey, S.C., Fornal, C.A., Metzler, C.W., Jacobs, B.L. 1997. Single-unit responses of
14 serotonergic dorsal raphe neurons to specific motor challenges in freely moving cats.
15 *Neuroscience* 79. 161–169.

16 Wang, W., Bradley, S.R., Richerson, G.B. 2002. Quantification of the response of rat
17 medullary raphe neurones to independent changes in pH (o) and P (CO₂). *J Physiol*
18 540. 951–970.

- 1 Walton, C., Kalmar, J.M., Cafarelli, E. 2002. Effect of caffeine on self-sustained firing in
- 2 human motor units. *J Physiol* 545. 671–679.
- 3 Whipp, B.J., Ward, S.A. 1998. Determinants and control of breathing during muscular
- 4 exercise. *Br J Sports Med* 32: 199–211.
- 5

Figure 1. Schematic representation of the experimental protocol of the normal breathing condition and/or CO₂ rebreathing condition. The sustained muscle activity trial was repeated 6 times with intervals of 5-10 min. M-max and H-reflex of the soleus muscle were measured three times and five times, respectively, before the trial (*vertical arrows*). CO₂ rebreathing was performed from 30 sec after the initiation of the trial to the end of the trial (*horizontal arrow*). The vertical gray bar indicates the electrical train stimulation period.

Figure 2. A: Changes in minute ventilation ($\dot{V}_E$) and predicted arterial carbon dioxide pressure ($\text{PaCO}_{2\text{pred}}$) in the CO_2 rebreathing condition (*closed circle*) and normal breathing condition (*open circle*). A horizontal arrow indicates the CO_2 rebreathing period. Four squares drawn by dotted lines indicate the periods before CO_2 rebreathing (a^1 , b^1 ,) and during the self-sustained muscle activity period (a^2 , b^2). Vertical gray bars indicate the electrical train stimulation period. B: Comparisons of $\dot{V}_E$ and $\text{PaCO}_{2\text{pred}}$ between the CO_2 rebreathing condition (*black bar*) and normal breathing condition (*white bar*). a^1 and b^1 were before the CO_2 rebreathing period (data for 30 sec), and a^2 and b^2 were during the self-sustained muscle activity period (data for 1 min). Data are means $\pm$ SE for $n = 9$. * = significant difference between the two respiratory conditions ($P < 0.05$).

1   **Figure 3.** Comparison among all trials of the amplitudes of M-max and H-reflex in the
2   soleus muscle measured before electrical train stimulation. H-reflex is expressed as a
3   percentage of M-max. Data are means $\pm$ SE for $n = 9$.
4

1 **Figure 4.** EMG signals of the soleus muscle (Sol) and the medial head (MG) and lateral
2 head (LG) of the gastrocnemius muscle during the electrical train stimulation period
3 (*horizontal arrow*) in one subject. The first electrical stimulus induced the largest reflex
4 response in the soleus muscle, but several subsequent responses were depressed (*lower*
5 *left panel*) Then the reflex responses recovered with repetitive stimuli (*lower right panel*).
6

1   **Figure 5.** EMG signals in the soleus muscle (Sol) and the medial head (MG) and lateral
2   head (LG) of the gastrocnemius muscle in one subject. In the CO₂ rebreathing condition
3   (*left panel*) and the normal breathing condition (*right panel*), self-sustained muscle
4   activities were induced for 1 min after electrical train stimulation (*vertical gray bars*) to
5   the tibial nerve.

6

Figure 6. Comparisons of root mean squares (RMSs) of the soleus muscle (Sol) and the medial head (MG) and lateral head (LG) of the gastrocnemius muscle for 10 sec before the train stimulation (upper panel) and for the 30 sec after the train stimulation (lower panel) between the CO₂ rebreathing condition (*black bar*) and the normal breathing condition (*white bar*). Data are means $\pm$ SE for $n = 9$. * = significant difference between the two respiratory conditions ($P < 0.05$).

Figure 1

1
2
3

Figure 2

■ CO₂ rebreathing condition □ Normal breathing condition

Figure 3

Figure 4

Figure 5

■ CO₂ rebreathing condition □ Normal breathing condition

Figure 6