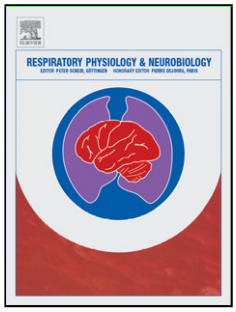

Title: Voluntary breathing increases corticospinal excitability of lower limb muscle during isometric contraction

Author: Kazuki Shirakawa Takahiro Yunoki Roghayyeh Afroundeh Chang-Shun Lian Ryouta Matsuura Yoshinori Ohtsuka Tokuo Yano

PII: S1569-9048(15)30016-1
DOI: http://dx.doi.org/doi:10.1016/j.resp.2015.07.003
Reference: RESPNB 2517

To appear in: *Respiratory Physiology & Neurobiology*

Received date: 16-3-2015
Revised date: 4-7-2015
Accepted date: 4-7-2015

Please cite this article as: Shirakawa, Kazuki, Yunoki, Takahiro, Afroundeh, Roghayyeh, Lian, Chang-Shun, Matsuura, Ryouta, Ohtsuka, Yoshinori, Yano, Tokuo, Voluntary breathing increases corticospinal excitability of lower limb muscle during isometric contraction.Respiratory Physiology and Neurobiology http://dx.doi.org/10.1016/j.resp.2015.07.003

Voluntary breathing increases corticospinal excitability of lower limb muscle during isometric contraction

Kazuki Shirakawa[a], Takahiro Yunoki[b], Roghayyeh Afroundeh[c], Chang-Shun Lian[a], Ryouta Matsuura[d], Yoshinori Ohtsuka[b], Tokuo Yano[b]

[a] Graduate School of Education, Hokkaido University, Sapporo, Japan

[b] Department of Human Developmental Sciences, Faculty of Education, Hokkaido University, Sapporo, Japan

[c] Department of Sport Science and Physical Education, Payame Noor University, Tehran, Iran

[d] Department of Health and Physical Education, Joetsu University of Education, Joetsu, Japan

Corresponding author: Kazuki Shirakawa

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

E-mail: kazuki.shirakawa@edu.hokudai.ac.jp

1. Introduction

Respiratory control during exercise is a vital function for maintaining the internal environment of the body. However, this homeostatic function is not the only role of the respiratory system during exercise. For instance, activation of respiratory afferents by resistive loaded breathing or a respiratory stimulant has been reported to alter the tonic vibratory reflex in the extensor digitorum and vastus lateralis (Balzamo et al., 1997) and the H reflex in the soleus (Gandevia et al., 1998). At the same time, Gandevia et al. (1998) demonstrated that only when, in addition to the stimulation of respiratory afferents, respiratory discomfort occurred, corticospinal excitability of the biceps brachii muscle increased. This indicates that change in the excitability of the limb muscle motoneuron pool induced by activation of respiratory afferents may be modulated by increased descending excitation occurring with respiratory sensations or arousal (Gandevia et al., 1998). Furthermore, it has been proposed that in a voluntary exercise with respiratory muscle fatigue, central motor output to exercising limbs is inhibited through the activation of respiratory muscle metaboreceptors (Dempsey et al., 2006). Thus, it appears that the respiratory control system is involved in the formation process of voluntary and reflex limb movements through an interaction between activation of respiratory afferents and excitability

of the limb muscle motor cortex and/or spinal motoneurons.

In order to maintain pulmonary ventilation against respiratory resistive load or respiratory muscle fatigue, it would be necessary to increase the motor command to respiratory muscles. The respiratory motor command is output from not only the brainstem respiratory center but also the breathing-associated cortical motor area (Butler, 2007; Gandevia and Plassman, 1988; Gandevia and Rothwell, 1987). In fact, activation of the breathing-associated cortical motor area has been identified during resistive loaded breathing (Ramsay et al., 1993) as well as during voluntary breathing (Colebatch et al., 1991; Macefield et al., 1991; Petersen et al., 2011). Using a transcranial magnetic stimulation (TMS) technique, Li and Rymer (2011) found that voluntary breathing increased the corticospinal excitability (motor-evoked potentials, MEPs) of finger muscles during 10% maximal voluntary contraction. Since the electrical stimulation-induced force response of finger muscles during the same breathing maneuver provided a sufficient time for supraspinal mechanisms to become involved to affect the response, they suggested the possibility that activation of the breathing-associated cortical motor areas could enhance the motor functions of nonrespiratory muscles (Li and Rymer, 2011). In practice, they demonstrated that electrical stimulation to finger extensors delivered during voluntary inspiration was

efficacious in enhancing finger extension strength and reducing finger flexor spasticity in post-stroke patients. Thus, it is conceivable that the respiratory system has a role in the control of limb movements through not only the activation of respiratory afferents but also a supraspinal mechanism such as activation of the breathing-associated cortical motor areas. However, it is not known whether the increase in MEPs by voluntary breathing occurs in contracting muscles other than finger muscles (Li and Rymer, 2011). It has been reported that cortical stimulation results in larger excitatory postsynaptic potentials (EPSPs) in spinal motoneurons for finger muscles than for proximal arm or lower limb muscles (Palmer and Ashby, 1992; Phillips and Porter, 1964). Since this suggests that the strength of corticospinal projections to finger muscles is greater than that to other limb muscles (Chen et al., 1998), there is a possibility that an increase in MEP induced by voluntary breathing (Li and Rymer, 2011) is a reaction specific to finger muscles. Therefore, in the present study, we examined the effects of voluntary breathing on the corticospinal excitability of a leg muscle by using the TMS technique. If an increase in MEP by voluntary breathing occurs in other limb muscles, clinical applications reported by Li and Rymer (2011) might be able to be extended to muscles other than finger muscles.

2. Methods

2.1. Subjects

Seven healthy males participated in this study (means ± SD: age, 22 ± 1 yr; height, 172.1 ± 4.9 cm; body mass, 66.8 ± 4.6 kg). Each subject gave written informed consent following an explanation regarding the experimental procedures and potential risks involved. This study was compliant with the Declaration of Helsinki and was approved by our institutional review board. Intense exercise and alcohol intake were prohibited for a period of 24 h prior to a test.

2.2. Experimental design

Throughout the entire experiment, subjects maintained a seated position on an adjustable chair with a backrest board. It was possible to prevent movement of the upper body during knee extension tasks by using this chair, which was equipped with a stopper to fix the position of the shoulders and head. After the knee angle had been set at 90 degrees, maximal voluntary contraction (MVC) during 5-s isometric knee extension of the right leg was measured. Three trials were performed for each measurement, and the highest value was

used as MVC.

After that, each subject performed 5-s isometric knee extension of the right leg at the intensity of 10% MVC. This knee extension trial (= “10% MVC”) was carried out under three different breathing conditions: 1) normal breathing (NORM), with no specific instructions on breathing; 2) forced inspiration (IN), which involved inhaling once as fast as possible from the resting expiratory level at about 2 s after the start of 10% MVC; and 3) forced expiration (OUT), which involved exhaling once as fast as possible from the resting inspiratory level at about 2 s after the start of 10% MVC. Each breathing condition was repeated 7 times in random order. Thus, each subject performed 10% MVC 21 times in total with 1-min rest periods between trials. Transcranial magnetic stimulation (TMS) was applied to the cortical representation of the right vastus lateralis (VL) during 10% MVC performed under the designated breathing condition. The subjects were instructed not to move their head during the knee extension and breathing.

2.3. Measurement and recordings

During the 10% MVC test, subjects breathed through a mouthpiece connected to a hot-wire flow meter (AE-280s, Minato Medical Science) to measure respiratory flow. Knee

extension force was measured using a load cell (LC1205-K500, A&D) that was connected to a wire and belt fixed over the ankle joint. Both signals of the knee extension force and respiratory flow were converted into digital signals at a sampling rate of 1 kHz using an analog-digital converter (MacLab/8s, ADInstruments). The signal of the knee extension force was processed with a low-pass filter of 40 Hz and displayed on a PC monitor so that subjects could produce the required knee extension force (10% of MVC). A red target line corresponding to 10% of MVC was created and displayed on the PC monitor.

By using the modified Borg scale for the assessment of rating of perceived exertion (RPE) (Borg, 1982), we asked each subject to assess effort senses of breathing (Breath-RPE) and legs (Leg-RPE) during the breathing task immediately after each 10% MVC.

A surface electromyogram (EMG) was recorded from the right VL with a bipolar EMG sensor that had an interelectrode distance of 20 mm (SX230, Biometrics Ltd.). Before attachment of the EMG sensor, the skin was shaved, abraded, and cleaned with alcohol in order to reduce skin impedance. The sensor was placed longitudinally over the muscle belly. The ground electrode was placed over the styloid process of the right wrist. The raw EMG signals were amplified using an amplifier imbedded in the EMG sensor (bandwidth = 20–450 Hz; common mode rejection ratio, CMRR > 96 dB; input impedance > $10^{13}\Omega$; gain = 1,000)

and converted into digital signals at a sampling rate of 2 kHz.

2.4. Transcranial magnetic stimulation (TMS)

A transcranial magnetic stimulator (Magstim 200^2, Magstim) with a double-cone coil (110 mm in diameter) was used to elicit MEPs in the right VL. The optimal coil position and stimulation intensity were determined prior to the experiment as follows. The intersection of the TMS coil was aligned tangentially with the sagittal plane, with the center of the coil being < 2 cm to the left on the vertex (Cz). The coil was oriented so that the induced current flow within the cortex was in a posterior-to-anterior direction. During this procedure, the magnetic stimulator was set at about 45% of maximal output and the coil was moved over the vertex until the position evoking the largest MEP in the right VL was found while the subjects were performing isometric knee extension at 10% of MVC. The optimal location for the stimulation was determined as the position where the largest MEP was observed. The position was marked on a tight-fitting swimming cap that was placed on the subject's head to ensure constant positioning of the coil throughout the experiment. Subsequently, active motor threshold (AMT) intensity (mean intensity: 38.7 ± 4.6% of maximum stimulator output) was determined. AMT was defined as the lowest intensity of the stimulator that elicited an MEP

clearly distinguishable from the background EMG in five out of ten pulses during isometric knee extension at 10% of MVC. Then, throughout the experiment (10% MVC trial), the intensity of the stimulation was set at 120% of the AMT (MEP amplitude in NORM: 547.7 ± 152.1 μV).

2.5. Data processing

Raw EMG signals were full-wave-rectified and then background integrated EMG (iEMG) was calculated over a 100-ms window prior to the TMS delivery during the 10% MVC trial by using offline analysis software (Chart v5.5.6, ADInstruments). The background force was defined as the mean knee extension force averaged over the same period. The background iEMG and force were normalized as a proportion of iEMG and mean force calculated over a 100-ms interval of the peak during the MVC trial. The twitch evoked by TMS was defined as the difference between the evoked peak force and background force. The respiratory flow at the time of TMS (RF_{TMS}) was normalized as a proportion of maximal respiratory flow in each trial. As a result of manual operation, TMS in voluntary breathing (IN and OUT) was delivered in the latter (declining flow) part of the breath (see Fig. 1), and RF_{TMS} in IN, OUT, and NORM were 48.3 ± 10.0%, 37.6 ± 8.3%, and 8.2 ± 2.8% of the

maximal respiratory flow, respectively. The average value of peak inspiratory flow during normal breathing was calculated using one respiratory flow prior to TMS in each NORM, and the time when inspiratory flow exceeded this averaged value was defined as the onset of voluntary breathing in IN. Similar processing was also performed for determining the onset of voluntary breathing in OUT. The size of the MEP was defined as EMG peak-to-peak amplitude after TMS application. Although 7 MEPs were calculated in each breathing condition, the MEP with background iEMG that was farthest from the median value was excluded from further data analysis. According to a procedure used in previous studies (Danion et al., 2003; Li and Rymer, 2011), MEPs were normalized so that the average MEP size for each subject (3 conditions * 6 trials = 18 MEPs) was equal to 100, and the MEP size was expressed in arbitrary units (AUs) (Danion et al., 2003; Li and Rymer, 2011).

2.6. Statistical analyses

Results are presented as means ± standard deviation (SD). One-way repeated measures ANOVA was used to evaluate the effects of breathing conditions. When a main effect was found, the group means were compared by using Fisher's LSD. A paired t-test was used to compare the time from onset of voluntary breathing to TMS for two voluntary

breathing conditions. Pearson's correlation coefficient (r) was determined in order to examine the bivariate correlation. For examining correlation between RPEs and MEP, Spearman's rank correlation coefficient (rs) was used. Statistical significance was set at $P < 0.05$.

3. Results

Representative EMG, knee extension force, and respiratory flow during a 10% MVC trial are shown in Figure 1. There were no significant differences between the conditions for background iEMG and force before TMS. A significant condition effect was found in MEP ($F_{2, 12} = 7.91$, $p < 0.01$). MEPs in IN and OUT were significantly higher than that in NORM ($p < 0.01$, Fig. 2), while no difference in MEP was found between IN and OUT. Although there was no significant difference in twitch evoked by TMS between the conditions (NORM = 21.1 ± 8.8 N, IN = 23.5 ± 11.0 N, OUT = 23.2 ± 11.5 N), there was a significant correlation between twitch evoked by TMS and MEP ($r = 0.248$, $p < 0.01$).

There was a significant main effect of conditions on Breath-RPE ($F_{2, 12} = 11.1$, $p < 0.01$). Breath-RPEs in IN (3.39 ± 2.20) and OUT (3.23 ± 1.98) were significantly higher than that (0.29 ± 0.57) in NORM ($p < 0.01$). No difference in Breath-RPE was found between IN and OUT. There was no significant difference in Leg-RPE between the conditions (NORM:

0.76 ± 0.51, IN: 0.92 ± 0.65, OUT: 0.93 ± 0.71).

There were significant positive correlations between RF_{TMS} and MEP ($r = 0.337$, $p < 0.001$) and between MEP and Breath-RPE ($rs = 0.299$, $p < 0.001$). Figure 3 shows the correlations between RF_{TMS} and MEP ($r = 0.746$, $p < 0.001$) and between MEP and Breath-RPE ($rs = 0.619$, $p < 0.01$), which are presented by plotting each subject's mean value calculated in each condition. There was no significant correlation between Leg-RPE and MEP.

There was a significant difference in time from onset of voluntary breathing to TMS between IN (546 ± 94 ms) and OUT (432 ± 126 ms) ($p < 0.05$). There was a significant positive correlation between MEP and time from onset of voluntary breathing to TMS only in OUT ($r = 0.312$, $p < 0.05$).

4. Discussion

The main findings of the present study were as follows: 1) MEP increased during 10% MVC with voluntary breathing (IN and OUT) and 2) there were significant correlations between MEP and respiratory flow (RF_{TMS}) and between MEP and Breath-RPE. MEP could be influenced by both muscle fatigue and background muscle activity. However, there was no

evidence for muscle fatigue given that there were no significant differences in background muscle force, background iEMG, and Leg-RPE between the breathing conditions. Therefore, the present results suggest that voluntary breathing causes an increase in corticospinal excitability in lower limb muscles as well as in finger muscles (Li and Rymer, 2011).

There was no significant difference in the MEPs between IN and OUT, while voluntary breathing increased VL MEP. This result indicates that an increase in motor drive to the VL by voluntary breathing is not dependent on respiratory phase. In contrast, a previous study demonstrated that VL tonic vibratory reflex at rest was increased by inspiratory resistive load and decreased by expiratory resistive load (Balzamo et al., 1997). In addition, it has been shown that recruitment of high-frequency motor units in the VL during voluntary isometric contractions sustained at 80% of maximal force was not modified by inspiratory loading but was reduced by expiratory loading (Fontanari et al., 1996). Based on data that had been obtained in animal experiments (Deshpande and Devanandan, 1970; Schiemann and Schomburg, 1972), they (Balzamo et al., 1997; Fontanari et al., 1996) postulated a mechanism by which motor drive to leg muscles was inhibited by activation of pulmonary vagal afferents during the expiratory phase. Furthermore, a recent study (Hudson et al. 2012) has suggested that activations of pulmonary and non-pulmonary respiratory

afferents associated with increases in lung volume augmented excitability of the corticospinal pathway to scalene muscles during both voluntary inspiration and expiration. In the present study, since TMS was delivered in the latter part of voluntary breathing (see Fig. 1), lung volume at which TMS was delivered during OUT must have been lower than that during IN. If an increase in respiratory afferent inputs with increases in lung volume has a facilitative effect on VL as well as on scalene muscles, MEP in the VL would be lower in OUT than in IN. However, there was no significant difference in MEP between IN and OUT. Moreover, there was a significant positive correlation between MEP and time from onset of voluntary breathing to TMS only in OUT. It has been shown that voluntary expiration induced increases in regional cerebral blood flow (reflecting neuronal activation) in motor areas (e.g., primary motor cortex, supplementary motor area, etc.) that overlapped with, but were more extensive than, those for voluntary inspiration (Ramsay et al. 1993). Therefore, in the present study, the effect of activation of the breathing-associated cortical motor area on VL corticospinal excitability might have been greater in OUT than in IN. Although cortical effects of respiratory afferents (Hudson et al., 2012) cannot be ruled out, it is thought that the effect of voluntary breathing on motor drive to the VL cannot be explained by respiratory afferents alone.

Due to the technical limitation of the present study, there was a difference of about 10% in RF_{TMS} between IN and OUT. Likewise, there was a significant difference in time from onset of voluntary breathing to TMS between OUT (432 ± 126 ms) and IN (546 ± 94 ms). Although the contribution of respiratory afferents might be greater as the time from onset of voluntary breathing to TMS becomes longer, we cannot verify whether the time difference (about 100 ms) had an effect on the results showing that MEPs were similar in IN and OUT.

One possible mechanism for the increased VL MEP during voluntary breathing is a non-specific facilitation that occurs during maneuvers such as voluntary teeth clenching (VTC) (Miyahara et al., 1996) or facial muscle contraction (Andersen et al., 1999), that is, during the classical Jendrassik manoeuver (JM). It has conventionally been thought that JM causes facilitation of responses in remote muscles through intraspinal mechanisms (e.g., reduction of pre-synaptic inhibition (Dowman and Wolpaw, 1988; Zehr and Stein, 1999)). In the present study, in addition to the identical effect on VL activity of different volitional respiratory muscle contractions (i.e., IN and OUT), there was a significant positive correlation between RF_{TMS} and MEP (Fig. 3). The increase in respiratory flow must have been associated with an increased activation of respiratory muscles including accessory

respiratory muscles. Thus, the augmented MEP in this study might be explained by a remote effect of voluntary respiratory muscle activation on spinal cord excitability.

Regarding the remote effect of JM-like voluntary muscle activation, recent studies have suggested that VTC can cause facilitation of responses in remote muscles through mechanisms at both a cortical level and a spinal level (Furubayashi et al., 2003; Sugawara et al., 2005; Sugawara and Kasai, 2002). Furubayashi et al. (2003) demonstrated that motor cortical excitability, rather than spinal cord excitability, is important for the facilitation of responses in remote muscles just after the onset of VTC. Therefore, we cannot exclude the possibility that a supraspinal mechanism was involved in the increase in VL MEP. In the present study, there was a significant correlation between respiratory effort and MEP (Fig. 3). Respiratory effort is a respiratory sensation differing from the sensation of dyspnea and the sensation of air hunger that arise from various afferent sources (Lansing et al., 2000; Manning and Schwartzstein, 1995; Stendardi et al., 2005). When ventilatory volume is matched in two different conditions, respiratory effort becomes lower in a ventilatory condition with afferent input (e.g., high CO_2-induced ventilation) than in a condition without it (e.g., voluntary ventilation) (Demediuk et al., 1992; Lansing et al., 2000; Schwartzstein et al., 1989). Thus, respiratory effort is determined not by the breathing work done but by the

execution of voluntary breathing. Since respiratory muscles as well as limb muscles can be controlled by the motor cortex, respiratory effort is believed to increase depending on central command that is sent from the motor cortex to respiratory muscles (Lansing et al., 2000; Stendardi et al., 2005). Indeed, voluntary breathing has been reported to activate cortical regions, including the primary motor area (M1), premotor area and supplementary motor area, associated with respiratory muscles (Colebatch et al., 1991; Macefield et al., 1991; Petersen et al., 2011). Therefore, there is a possibility that activity of M1 associated with trunk muscles (e.g., respiratory muscles and accessory respiratory muscles) activated its neighboring M1 associated with lower muscles (e.g., VL), resulting in the observed increase in VL MEP.

In the present study, there was a significant correlation between MEP and twitch evoked by TMS. This suggests the possibility that an increase in corticospinal excitability of the VL by voluntary breathing induces an increase in TMS-evoked force response of the VL. However, there was no significant difference in VL twitch (force response) evoked by TMS between the three breathing conditions. One possible explanation for this may be related to coactivation of antagonist muscles. TMS might have also increased the evoked force in antagonist muscles with voluntary breathing, which then canceled out the effect of larger

MEPs for the VL (Sidhu et al. 2009, Todd et al. 2003). In contrast, TMS-evoked force response of finger muscles has been reported to become greater during voluntary breathing than during normal breathing (Li and Rymer, 2011). Leg muscles may be more susceptible than hand muscles to this potential antagonistic effect. It has been shown that cortical stimulation results in larger excitatory postsynaptic potentials (EPSPs) in spinal motoneurons for finger muscles than for proximal arm or lower limb muscles (Palmer and Ashby, 1992; Phillips and Porter, 1964). Although this suggests that the strength of corticospinal projections to finger muscles is greater than that to other limb muscles (Chen et al. 1998), voluntary breathing (IN and OUT) increased MEP in the VL as well as in finger muscles (Li and Rymer, 2011). Therefore, the difference in strengths of corticospinal projections is unlikely to be responsible for the discrepancy regarding the TMS-evoked force response between their study (Li and Rymer, 2011) and the present study.

The results of the present study suggest that voluntary breathing causes an increase in corticospinal excitability in lower limb muscles as well as in finger muscles (Li and Rymer, 2011). Li and Rymer (2011) demonstrated that electrical stimulation to finger extensors delivered during voluntary inspiration was efficacious in enhancing finger extension strength and reducing finger flexor spasticity in post-stroke patients. Therefore, the

present results offer the clinical possibility of the above-mentioned application (rehabilitation intervention) being able to be extended to lower muscles.

In conclusion, it was confirmed that increase in MEP during isometric exercise with voluntary breathing occurs in a lower muscle (VL) as well as in finger muscles (Li and Rymer, 2011). There was a significant correlation between MEP and Breath-RPE. The results suggest that activation of the breathing-associated cortical areas with voluntary breathing is involved in the increase in corticospinal excitability of the VL.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 24700606.

References

Andersen, B., Rösler, K.M., Lauritzen, M., 1999. Nonspecific facilitation of responses to transcranial magnetic stimulation. Muscle Nerve 22, 857–863.

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.

Borg, G., 1982. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14, 377–381.

Butler, J.E., 2007. Drive to the human respiratory muscles. Respir Physiol Neurobiol 159, 115–126.

Chen, R., Tam, A., Bütefisch, C., Corwell, B., Ziemann, U., Rothwell, J.C., Cohen, L.G., 1998. Intracortical inhibition and facilitation in different representations of the human motor cortex. J Neurophysiol 80, 2870–2881.

Colebatch, J.G., Adams, L., Murphy, K., Martin, A.J., Lammertsma, A.A., Tochon-Danguy, H.J., Clark, J.C., Friston, K.J., Guz, A., 1991. Regional cerebral blood flow during volitional breathing in man. J Physiol 443, 91–103.

Danion, F., Latash, M.L., Li, S., 2003. Finger interactions studied with transcranial magnetic stimulation during multi-finger force production tasks. Clin Neurophysiol 114, 1445–1455.

Demediuk, B.H., Manning, H., Lilly, J., Fencl, V., Weinberger, S.E., Weiss, J.W., Schwartzstein, R.M., 1992. Dissociation between dyspnea and respiratory effort. Am Rev Respir Dis 146, 1222–1225.

Dempsey, J.A., Romer, L., Rodman, J., Miller, J., Smith, C., 2006. Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol 151, 242–250.

Deshpande, S.S., Devanandan, M.S., 1970. Reflex inhibition of monosynaptic reflexes by stimulation of type J pulmonary endings. J Physiol 206, 345–357.

Dowman, R., Wolpaw, J.R., 1988. Jendrassik maneuver facilitates soleus H-reflex without change in average soleus motoneuron pool membrane potential. Exp Neurol 101, 288–302.

Fontanari, P., Vuillon-Cacciuttolo, G., Balzamo, E., Zattara-Hartmann, M.C., Lagier-Tessonnier, F., Jammes, Y., 1996. Resistive loaded breathing changes the motor drive to arm and leg muscles in man. Neurosci Lett 210, 130–134.

Furubayashi, T., Sugawara, K., Kasai, T., Hayashi, A., Hanajima, R., Shiio, Y., Iwata, N., Ugawa, Y., 2003. Remote effects of self-paced teeth clenching on the excitability of hand motor area. Exp Brain Res 148, 261–265.

Gandevia, S.C., Butler, J.E., Taylor, J.L., Crawford, M.R., 1998. Absence of viscerosomatic inhibition with injections of lobeline designed to activate human pulmonary C fibres. J Physiol 511, 289–300.

Gandevia, S.C., Plassman, B.L., 1988. Responses in human intercostal and truncal muscles to motor cortical and spinal stimulation. Resp Physiol 73, 325–337.

Gandevia, S.C., Rothwell, J.C., 1987. Activation of the human diaphragm from the motor cortex. J Physiol 384, 109–118.

Hudson, A.L., Taylor, J.L., Anand, A., Gandevia, S.C., Butler, J.E., 2012. Evoked corticospinal output to the human scalene muscles is altered by lung volume. Respir Physiol Neurobiol 180, 263–268.

Lansing, R.W., IM, B.S.H., Thwing, J.I., Legedza, A.T., Banzett, R.B., 2000. The perception of respiratory work and effort can be independent of the perception of air hunger. Am J Respir Crit Care Med 162, 1690–1696.

Li, S., Rymer, W.Z., 2011. Voluntary breathing influences corticospinal excitability of nonrespiratory finger muscles. J Neurophysiol 105, 512–521.

Macefield, G., Gandevia, S.C., 1991. The cortical drive to human respiratory muscles in the awake state assessed by premotor cerebral potentials. J Physiol 439, 545–558.

Manning, H.L., Schwartzstein, R.M., 1995. Pathophysiology of dyspnea. N Engl J Med 333, 1547–1553.

Miyahara, T., Hagiya, N., Ohyama, T., Nakamura, Y., 1996. Modulation of human soleus H reflex in association with voluntary clenching of the teeth. J Neurophysiol 76, 2033–2041.

Palmer, E., Ashby, P., 1992. Corticospinal projections to upper limb motoneurones in

humans. J Physiol 448, 397–412.

Petersen, N.C., Taylor, J.L., Murray, N.P.S., Gandevia, S.C., Butler, J.E., 2011. Differential effects of low-intensity motor cortical stimulation on the inspiratory activity in scalene muscles during voluntary and involuntary breathing. Respir Physiol Neurobiol 175, 265–271.

Phillips, C.G., Porter, R., 1964. The pyramidal projection to motoneurones of some muscle groups of the baboon's forelimb. Prog Brain Res 12, 222–245.

Ramsay, S.C., Adams, L., Murphy, K., Corfield, D.R., Grootoonk, S., Bailey, D.L., Frackowiak, R.S., Guz, A., 1993. Regional cerebral blood flow during volitional expiration in man: a comparison with volitional inspiration. J Physiol 461, 85–101.

Schiemann, B., Schomburg, E.D., 1972. The inhibitory action of type J pulmonary receptor afferents upon the central motor and fusimotor activity and responsiveness in cats. Exp Brain Res 15, 234–244.

Schwartzstein, R.M., Simon, P.M., Weiss, J.W., Fencl, V., Weinberger, S.E., 1989. Breathlessness induced by dissociation between ventilation and chemical drive. Am Rev Respir Dis 139, 1231–1237.

Sidhu, S.K., Bentley, D.J., Carroll, T.J., 2009. Cortical voluntary activation of the human

knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle Nerve 39, 186–196.

Stendardi, L., Grazzini, M., Gigliotti, F., Lotti, P., Scano, G., 2005. Dyspnea and leg effort during exercise. Respir Med 99, 933–942.

Sugawara, K., Furubayashi, T., Takahashi, M., Ni, Z., Ugawa, Y., Kasai, T., 2005. Remote effects of voluntary teeth clenching on excitability changes of the human hand motor area. Neurosci Lett 377, 25–30.

Sugawara, K., Kasai, T., 2002. Facilitation of motor evoked potentials and H-reflexes of flexor carpi radialis muscle induced by voluntary teeth clenching. Hum Mov Sci 21, 203–212.

Todd, G., Taylor, J.L., Gandevia, S.C., 2003. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. J Physiol 551, 661–671.

Zehr, E.P., Stein, R.B., 1999. Interaction of the Jendrassik maneuver with segmental presynaptic inhibition. Exp Brain Res 124, 474–480.

Figure Legends

Figure 1. Representative electromyogram (EMG), knee extension force (Force) and respiratory flow (Flow) during isometric knee extension of the right vastus lateralis (VL) at the intensity of 10% MVC performed under the three breathing conditions (NORM, IN, OUT). Vertical dotted lines denote the moment of transcranial magnetic stimulation (TMS) application.

Figure 2. Motor-evoked potentials (MEPs) in the three breathing conditions (NORM, IN, OUT). MEP was recorded from the right vastus lateralis (VL) during isometric knee extension at the intensity of 10% MVC. Data from individual subjects (closed circles, n = 7) and the group (open circle, mean) are shown. Asterisks (*) indicate significant difference compared with NORM ($p < 0.01$)

Highlights

1. Voluntary breathing increased MEP of lower limb muscle during isometric contraction. ► 2. There was a correlation between MEP and effort sense of breathing. ► 3. Activation of breathing-associated cortical areas may be

involved in increased MEP.

Figure 3. Relationships between respiratory flow at which TMS was delivered (RF_{TMS}) and motor-evoked potential (MEP) (upper panel, r = 0.746, p < 0.001) and between effort sense of breathing (Breath-RPE) and MEP (lower panel, rs = 0.619, p < 0.01). Data presented are each subject's mean value calculated in each condition (NORM: circles, IN: squares, OUT: triangles).

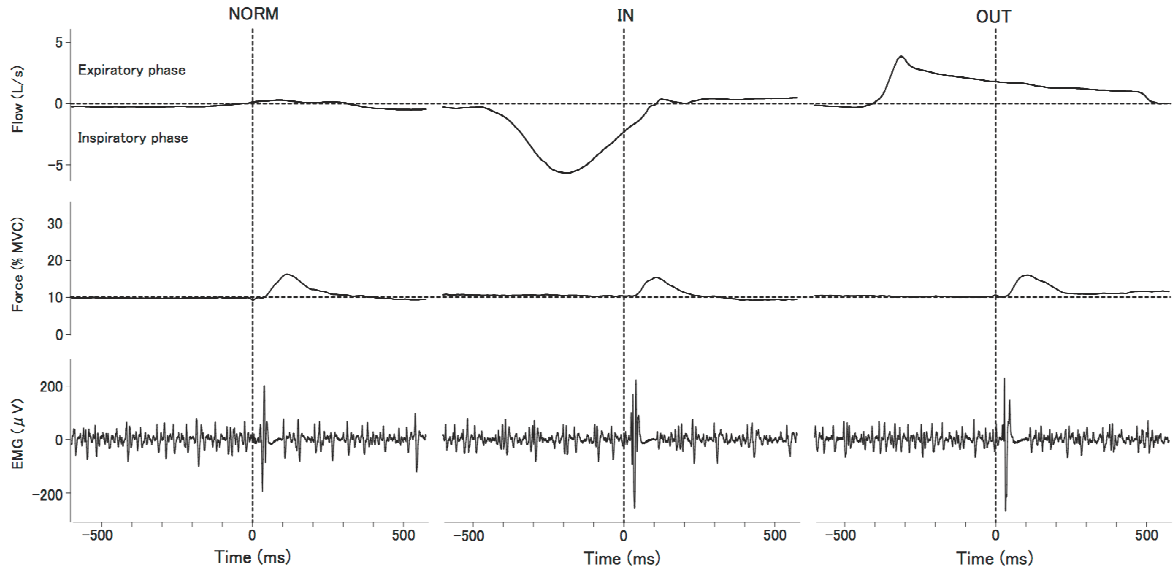

Figure 1

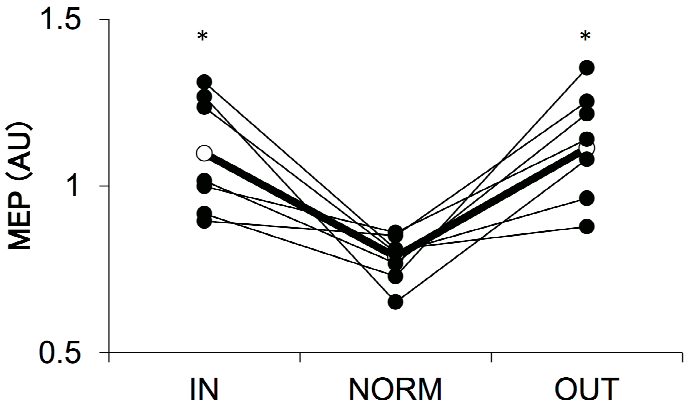

Figure 2

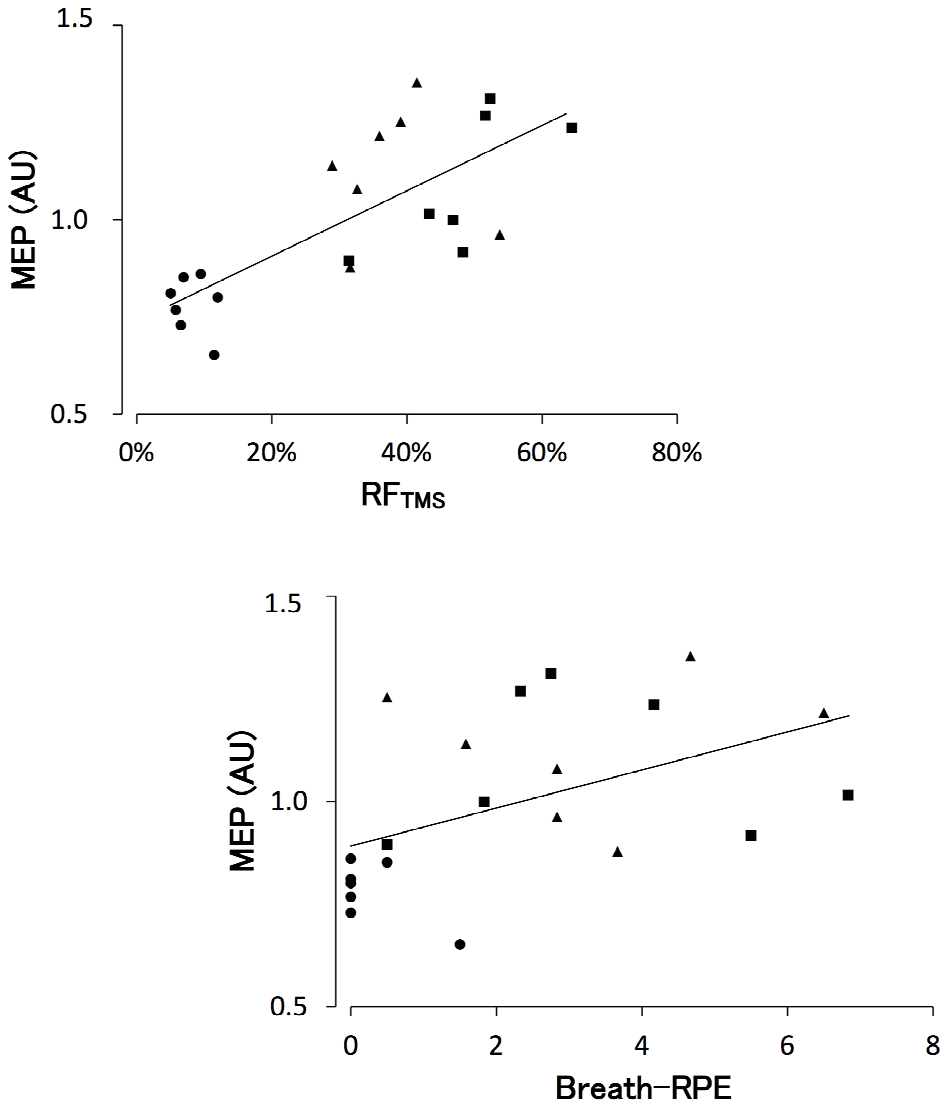

Figure 3