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Voluntary Breathing Influences Corticospinal Excitability of Nonrespiratory Finger Muscles

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Li S, Rymer WZ. Voluntary breathing influences corticospinal excitability of nonrespiratory finger muscles. *J Neurophysiol* 105: 512–521, 2011. First published December 15, 2010; doi:10.1152/jn.00946.2010. The present study aimed to investigate neurophysiologic mechanisms mediating the newly discovered phenomenon of respiratory–motor interactions and to explore its potential clinical application for motor recovery. First, young and healthy subjects were instructed to breathe normally (NORM); to exhale (OUT) or inhale (IN) as fast as possible in a self-paced manner; or to voluntarily hold breath (HOLD). In *experiment 1* ($n = 14$), transcranial magnetic stimulation (TMS) was applied during 10% maximal voluntary contraction (MVC) finger flexion force production or at rest. The motor-evoked potentials (MEPs) were recorded from flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), and abductor digiti minimi (ADM) muscles. Similarly, in *experiment 2* ($n = 11$), electrical stimulation (ES) was applied to FDS or EDC during the described four breathing conditions while subjects maintained 10%MVC of finger flexion or extension and at rest. In the exploratory clinical experiments (*experiment 3*), four patients with chronic neurological disorders (three strokes, one traumatic brain injury) received a 30-min session of breathing-controlled ES to the impaired EDC. In *experiment 1*, the EDC MEP magnitudes increased significantly during IN and OUT at both 10%MVC and rest; the FDS MEPs were enhanced only at 10%MVC, whereas the ADM MEP increased only during OUT, compared with NORM for both at rest and 10%MVC. No difference was found between NORM and HOLD for all three muscles. In *experiment 2*, when FDS was stimulated, force response was enhanced during both IN and OUT, but only at 10%MVC. When EDC was stimulated, force response increased at both 10%MVC and rest, only during IN, but not OUT. The averaged response latency was 83 ms for the finger extensors and 79 ms for the finger flexors. After a 30-min intervention of ES to EDC triggered by forced inspiration in *experiment 3*, we observed a significant reduction in finger flexor spasticity. The spasticity reduction lasted for ≥ 4 wk in all four patients. TMS and ES data, collectively, support the phenomenon that there is an overall respiration-related enhancement on the motor system, with a strong inspiration–finger extension coupling during voluntary breathing. As such, breathing-controlled electrical stimulation (i.e., stimulation to finger extensors delivered during the voluntary inspiratory phase) could be applied for enhancing finger extension strength and finger flexor spasticity reduction in poststroke patients.

INTRODUCTION

Human breathing is a unique motor act. Humans can breathe automatically (automatic breathing), e.g., during sleep, whereas

breathing can be controlled voluntarily when needed (voluntary breathing), such as singing and speech. Automatic breathing is believed to originate in the brain stem via the pontomedullary respiratory oscillator. A descending bulbospinal projection from the oscillator synapses with the spinal cord anterior horn cells with rhythmic projections to the respiratory muscles to cause automatic breathing. The oscillator could function automatically without any peripheral feedback and responds to changes only in pH and P_{CO_2} (cf. Guz 1997). In contrast, cortical inputs are required during voluntary breathing. Spinal motoneurons receive corticospinal inputs originating from a discrete region of the motor cortex, where the respiratory muscles are represented. These cortical areas are clearly identified in humans (Colebatch et al. 1991; Gandevia and Rothwell 1987; Maskill et al. 1991; Sharshar et al. 2004). Clinical evidence strongly suggests that bulbospinal fibers project separately from the relevant corticospinal fibers. For instance, patients with brain stem lesions (Plum and Leight 1981) or very high cervical cord lesions (Davis and Plum 1972; Lahuerta et al. 1992) can breathe voluntarily, but lack automatic breathing when drowsy or asleep. Corticospinal pathways bypass the brain stem respiratory centers and provide direct cortical control to the spinal respiratory motoneurons during voluntary breathing (Corfield et al. 1998). During normal functioning, spinal motoneurons are able to integrate these different sources, including descending cortico- and bulbospinal inputs and peripheral afferent inputs into a segmental interneuronal network (Aminoff and Sears 1971).

During voluntary breathing, humans need to voluntarily suppress autonomic control of breathing (Guz 1997; Haouzi et al. 2006) through voluntary cortical activation (the “cortical respiratory center”). Briefly, brain imaging studies (Colebatch et al. 1991; Evans et al. 1999; Fink et al. 1995; Macey et al. 2003, 2004; Maskill et al. 1991; Ramsay et al. 1993; Smejkal et al. 1999, 2000) have demonstrated extensive respiratory involvement of cortical areas bilaterally, including the primary motor cortex (M1), the premotor cortex, and the supplementary motor areas. It is important to point out that the breathing-associated cortical areas within the primary motor cortex are distinctly different from the motor cortical areas for nonrespiratory skeletal muscles. Fink et al. (1995) observed that, in addition to activation of the M1 “leg” areas, there was also activation in the different areas within M1 previously shown to be associated with voluntary breathing during and after modest leg exercise in normal adult subjects. As such, the breathing-associated M1 activation could act in concert directly and/or indirectly with the descending motor drive from M1 to the

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nonrespiratory skeletal muscles (Guz 1997), thus influencing the motor functions of these muscles.

A recent series of behavioral studies have demonstrated that voluntary breathing could influence motor functions of nonrespiratory muscles, in both small muscles (finger flexors; Li and Laskin 2006; Li and Yasuda 2007) and large muscles (knee extensors; Ikeda et al. 2009). For example, the maximal voluntary contraction (MVC) force of finger flexors was significantly greater (~10%) during forced expiration than that during normal or forced inspiration. MVC change was accompanied by changes in flexor/extensor cocontraction ratio (Li and Laskin 2006). A hypothesis of respiratory-motor interactions was proposed to account for the observed voluntary breathing effect on behavioral measures (Li and Laskin 2006). The hypothesis stated that corticospinal excitability was enhanced for finger extensors by forced inspiration and for finger flexors by both forced inspiration and expiration. Accordingly, the primary aim was to test the coupling hypothesis by quantifying modulation of corticospinal excitability during voluntary breathing using transcranial magnetic stimulation (TMS) and electrical stimulation (ES) in a variety of tasks in normal subjects.

Electrical stimulation, including electromyographic (EMG)-triggered ES, is commonly used to enhance finger extensor strength and to reduce finger flexor spasticity, thus promoting hand function recovery in stroke patients. Its effect on spasticity reduction, which may last up to a few hours after the intervention, remains controversial (Bakhtiyari and Fatemy 2008; Chae 2003). According to the finger extension-inspiration coupling, when delivered to the finger extensors on inspiratory phases of voluntary breathing cycles, ES was hypothesized to induce enhanced response, thus maximizing the electrical stimulation effect. As such, the respiratory-motor intrinsic coupling could potentially be used to enhance finger extension strength and spasticity reduction in stroke survivors. Therefore the secondary aim was to examine chronic stroke patients using the same ES protocol to explore its potential clinical applications.

METHODS

The present study consisted of three experiments. Modulations of corticospinal excitability of finger flexors and extensors were examined using TMS (*experiment 1*) and ES (*experiment 2*). We further examined the clinical application of the coupling in patients with neurological disorders using the same ES protocol (*experiment 3*). General descriptions of subject characteristics, breathing instructions, and recordings are provided first. Detailed procedures, measurements, and analysis are described in each experiment in the following sections.

Inclusion/exclusion criteria

We recruited 14 subjects for *experiment 1* (5 male, 9 females, mean: 25.5 yr of age; age range: 23–28 yr) and 11 subjects for *experiment 2* (3 males, 8 females, mean: 28.2 yr of age; age range, average: 24–39 yr). Some subjects ($n = 3$) participated in both experiments. The main inclusion criterion was healthy adults. The exclusion criteria included subjects 1) with a history of neurological disorders or skeletal-muscular injury in the upper extremities; 2) with a history of brain injury, or seizure, and recurring headaches; 3) with intracranial metallic or magnetic objects, a pacemaker, or any other implanted devices; or 4) with hypertension or respiratory disease, such

as asthma and chronic obstructive pulmonary disease. As a pilot study, six patients with varying degrees of chronic neurological impairment, but meeting other exclusion criteria, were recruited (see details in *Experiment 3*) to test the proposed hypothesis. All subjects gave written informed consent. The study was compliant with Health Insurance Portability and Accountability Act (1996) regulations and was approved by our institutional review board.

Instructions on voluntary breathing

During testing, subjects were seated on an adjustable chair and breathed through a facemask connected to a pneumotach system (Series 1110A; Hans Rudolph, Kansas City, MO) to monitor breathing. The four different breathing conditions and the instructions for each were 1) normal breathing (NORM): no specific instructions on breathing; 2) forced expiration (OUT): to exhale once as fast as possible in a self-paced manner; 3) forced inspiration (IN): to inhale once as fast as possible in a self-paced manner; and 4) breath holding (HOLD): voluntarily holding breath after a trial began. The breath-holding was not associated with any preceding active inspiration or expiration. These four conditions were tested in both *experiments 1* and *2*. During IN and OUT conditions, subjects were explicitly instructed to dissociate forced inspiration and forced expiration within a breathing cycle (i.e., forced inspiration only without preceding forced expiration during IN) and forced expiration only without preceding forced inspiration during OUT. Subjects were allowed to have 8 to 10 practice trials to familiarize themselves with these instructions. These instructions were the same as in previous studies (Ikeda et al. 2009; Li and Laskin 2006; Li and Yasuda 2007). Subjects did not report signs of hypoxemia, such as headache, after breathing through a facemask.

Measurement and recordings

Main measurements include muscle forces, EMG signals, and breathing signals. Disposable surface electrodes (DelSys, Boston, MA) were placed over the muscle bellies of flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), and abductor digiti minimi (ADM) muscles to obtain EMG signals in *experiment 1*. The EMG signals were amplified, digitized, and high-pass filtered at 10 Hz and low-pass filtered at 500 Hz. Four unidirectional piezoelectric force sensors (208C02; PCB Piezotronics, Depew, NY) were used to measure individual finger flexion forces in a pressing device in *experiment 1* and in a customized finger force measurement apparatus in *experiments 2* and *3*. Breathing data were used to monitor voluntary breathing and to trigger external devices (TMS: Magstim 200², Magstim, Whitland, South West Wales, UK; ES: Grass 10DSCMA, Grass Instrument, Quincy, MA). A desktop computer was used for data acquisition and processing. All signals were sampled at 1,000 Hz by a 16-bit A/D board (PCI 6229; National Instruments, Austin, TX) using customized LabVIEW software. Data were saved for off-line analysis using a customized MatLab software.

Experiment 1: to quantify modulation of corticospinal excitability of finger muscles using TMS

After being comfortably seated, the shoulders were kept at about 45° of abduction in the frontal plane and 45° of flexion in the sagittal plane and the elbow joints at nearly 135° of flexion on the table. The right forearm, wrist, and hand were in one of the following two positions: 1) The forearm was in the neutral position; the wrist was stabilized by two vertical bars on the table, with the hand in a functional position: slightly extended wrist (~20°), slightly curved finger joints (~20°) (Fig. 1A). In this neutral position of the forearm and wrist, subjects were instructed to completely relax hand muscles, i.e., at rest trials, to avoid the effect of gravitational force on either finger flexors or extensors. 2) As in previous studies (Li and Laskin

2006; Li and Yasuda 2007), subjects placed the fingertips on four force sensors on a customized finger force device with fingers slightly curved ($\sim 20^\circ$ flexion of interphalangeal joints). The position of the sensors can be adjusted within a steel frame (140×90 mm) to fit the individual. Subjects were asked to relax prior to a trial and the weights of fingers were offset to zero at the beginning of a trial. Surface electrodes were placed on the muscle bellies of FDS, EDC, and ADM of the right hand.

In the preceding described configuration, subjects breathed through a facemask. First, the maximal inspiration and expiration airflow rates were measured (i.e., the highest magnitude from three attempts). Subjects were then instructed to produce finger flexion MVC with all four fingers at fingertips during NORM. The highest value from three MVC attempts was selected as the MVC. A red target line was created

and displayed on the computer screen, corresponding to 10% of the MVC. The target level was well within the range (~ 6 to 30% MVC) in which force responses to TMS were reported to be linear (Danion et al. 2003; Li et al. 2004b), such that potential modulation of force responses were reasonably observed and compared with a constant background force.

The method and procedures of application of TMS were the same as those reported in previous studies (Danion et al. 2003; Li 2007; Li et al. 2004b, 2009). Briefly, focal TMS was performed with a figure-of-eight-shaped stimulation coil (mean diameter of each wing 45 mm) connected to a magnetic stimulator, with the maximal magnetic field strength of 2.2 Tesla. The intersection of the coils was placed tangentially about 2 cm to the left of the vertex (Cz), with the handle pointing posteriorly and laterally at a 45° angle away from the midline. In this way, the current induced in the neural tissue is directed approximately perpendicular to the line of the central sulcus in a direction parallel to the midline between the two coils. This configuration provides optimal activation of the corticospinal pathways transynaptically (Brasil-Neto et al. 1992). First, the stimulus intensity was set at 60% of the stimulator output. The optimal position for eliciting the largest EMG response in the finger flexors was found by moving the coil over the scalp in steps of 1 cm. The optimal position was then marked with a pen to ensure constant positioning of the coil throughout the experiment. By keeping the coil at the optimal location, the intensity of the stimulation was slowly decreased until the motor threshold (MT) was found. The MT was defined as the lowest stimulus capable of evoking ≥ 5 of 10 discernable MEPs with the amplitude of $\geq 50 \mu\text{V}$ during normal breathing. The coil position and orientation were ensured with double-sided adhesive tape; besides, at all times, the coil position was stabilized by an experimenter. The stimulation intensity was kept the same at 150% of the resting MT.

A 10-s trial began with two computer-generated tones. Subjects either rested in a neutral position or produced 10% MVC finger flexion force in the described position, respectively. During finger flexion force production trials, subjects waited about 1 s to offset the finger weights. Subjects then started to produce a force matching the displayed target line as accurately as possible and sustained that level of force until TMS was delivered. The following breathing conditions were tested: 1) NORM, 2) IN, 3) OUT, and 4) HOLD. During NORM and HOLD breathing conditions, TMS pulses were randomly delivered between 3.5 and 7.5 s of a 10-s trial with an interval of 1 ms. During IN and OUT breathing conditions, the TMS was externally triggered when forced inspiration or expiration airflow rate reached 40% of the corresponding maximal airflow. Note that the subject

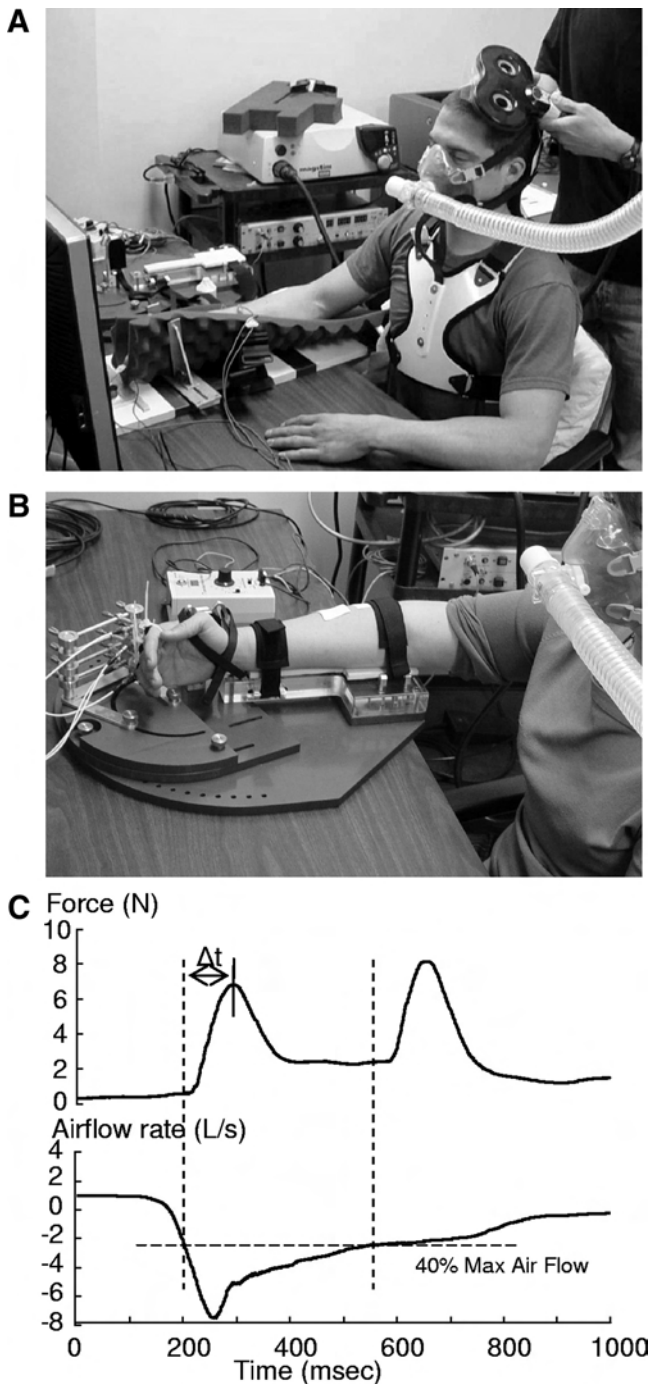


FIG. 1. A: transcranial magnetic stimulation (TMS) experimental setting (*experiment 1*). A subject breathed through a facemask. His test forearm (right) rested in the neutral position while the wrist was supported by a customized device in the neutral position with fingers naturally curved. A cervicothoracic brace was used to help support his head. The head movement was limited during voluntary breathing. The experimenter held a coil throughout the experiment. B: breathing-controlled electrical stimulation (ES) experimental setting in *experiment 2*. A subject breathed through a facemask. Her right forearm and wrist were stabilized in the neutral position in a customized finger force device. Her proximal phalanges were stabilized in loops that were connected to unidirectional force sensors. Surface electrodes were placed on finger extensors. C: triggering electrical stimulation by airflow rate during forced expiration. ES was triggered and delivered at 40% of maximal expiration airflow, as represented by the intersections of dashed vertical and horizontal lines. The background force was defined as the mean finger force averaged over a 100-ms window prior to the ES delivery. The ES-induced force response was the difference between the peak response and background force. The latency (Δt) of the increment was defined as the difference between the time of ES delivery and the time of peak response. Note that there were 2 spikes triggered by forced expiration. As shown, the second force response was likely triggered again by the inspiration airflow rate during the decreasing phase. We measured only the peak response and latency of the first peak, since there may be other mechanisms for the second peak.

initiated a forced inspiration (IN) or expiration (OUT) in a self-paced manner.

Specific and explicit instructions on breathing and TMS delivery were given to each subject. No movements were allowed at two upper limbs, shoulder joints, and the head. To ensure no body and head movement during voluntary breathing, a cervicothoracic brace (Lerman Minerva cervical orthosis; Trulife, Dublin, Ireland; see Fig. 2A) was used to stabilize the trunk, neck, and head. In our pilot study (two young healthy males), we monitored EMG activities from muscles on the test side, including the biceps, triceps, anterior deltoid, and pectoralis major. We observed EMG silence in these muscles during IN and OUT except for a small EMG burst in the anterior deltoid muscle coinciding with initiation of forced expiration during OUT. Sufficient practice (8–10) trials were allowed for subjects to understand and familiarize themselves with procedures and instructions. Each condition has 10 trials. Blocks of rest and 10%MVC trials were randomized. Within each block (rest, 10%MVC), four breathing conditions (IN, OUT, NORM, HOLD) were further randomized.

Data analysis and statistics. EMG signals from FDS, EDC, and ADM muscles, force, and airflow rate data were recorded. The methods for measuring of magnitude and latency of MEPs due to focal TMS were the same as previously described (Danion et al. 2003; Li 2007; Li et al. 2004b). Briefly, from the rectified, unfiltered EMG signal, the background EMG (EMG_{BG}) was defined as the mean EMG calculated from -100 ms to the moment of TMS application (t_0). The size of the MEP was defined as the difference between the peak EMG (within 50 ms after TMS application) and EMG_{BG} in the rectified signal. The MEP size was expressed in absolute units (AUs) (Danion et al. 2003). Similarly, the background force (F_{BG}) was defined as the mean force from -100 ms to t_0 . The TMS-induced force response was defined as the difference between the induced peak force and F_{BG} . The force response was measured in Newtons (N). Variables were averaged across 10 trials for each condition. A repeated-measures ANOVA was performed to examine the background EMG activities and the effect of breathing on the MEP magnitude for three muscles. The factors were BREATH (4 levels: NORM, IN, OUT, HOLD) and FORCE (2 levels: rest and 10%MVC). Since force data were recorded only at 10%MVC, a one-way ANOVA with the factor of BREATH was used to compare the background force as well as the finger flexion

force response. Tukey post hoc comparisons were performed to determine the specific locus of the main effect.

Experiment 2: to quantify modulation of corticospinal excitability of finger muscles using ES

Similar to *experiment 1*, the breathing signal was used to trigger electrical stimulation to finger flexors or extensors. A similar protocol was used (see *Experiment 1* for details) with the following differences.

Finger force device. A customized finger force device (Fig. 1B) was used. The right forearm was stabilized in the neutral position by Velcro straps at the proximal and distal sites. The palm was also stabilized by Velcro straps with the wrist joint in about 20° of extension. The metacarpophalangeal (MCP) joints were stabilized at about 20° of flexion with the shaft of the proximal phalanges against force sensors with plastic bands. The distal parts of the fingers were instructed to be naturally curved during isometric finger extension/flexion against force sensors. Subjects were explicitly instructed to maintain a constant force production to match a predisplayed target (10%MVC flexion or extension) on the screen before the ES delivery, and to relax after the delivery. ES delivery to FDS or EDC was randomized as blocks.

Electrical stimulation (ES). Two carbonized-rubber electrodes were attached to the skin overlying the muscle belly of the target muscles (FDS or EDC). The cathode (3×5 cm) was placed over the muscle belly just distal to the origin. The anode (1.5×3 cm) was attached to a site distal to the cathode. The sites for the anode and cathode were determined by assessing which position elicited the largest finger force response with minimal wrist response. A single square-wave pulse with 0.1-ms duration was delivered each trial. The intensity of electrical stimulation was determined based on the following criteria: 1) isolated finger flexion/extension responses were detected with minimal involvement of wrist joint responses; and 2) maximal tolerance of the subject to the evoked pain. The absolute magnitude of stimulation intensity varied across subjects, but the intensity was kept the same across different conditions for the same subject since our purpose was to examine the effect of voluntary breathing on evoked responses within subjects.

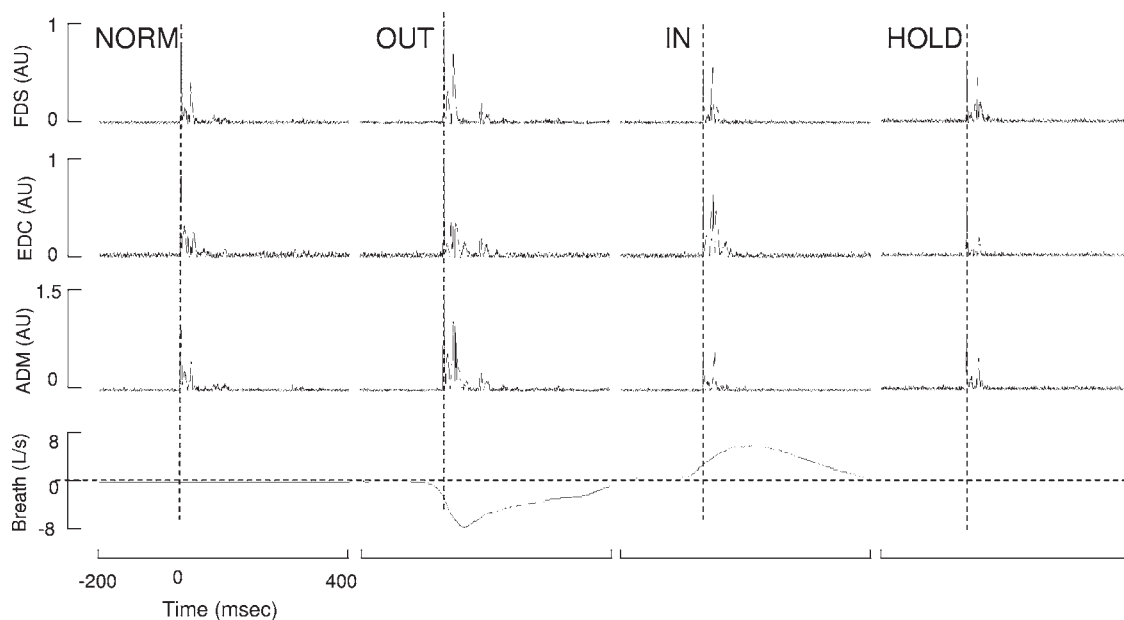


FIG. 2. Typical trials at rest in *experiment 1*. Electromyographic (EMG) responses of a single trial from resting flexor digitorum superficialis (FDS), extensor digitorum communis (EDC), and abductor digiti minimi (ADM) muscles during 4 breathing conditions (breathe normally [NORM], exhale [OUT], inhale [IN], voluntarily hold breath [HOLD]) are plotted. FDS, EDC, and ADM correspond to the 1st (top), 2nd, and 3rd traces, respectively. Vertical lines are aligned with the moment of TMS application. Note that baseline EMG activities were similar across different breathing condition for each muscle.

Data analysis and statistics. The same method as *experiment 1* was used to calculate the two dependent variables: the background force prior to ES (F_{BG}) and the ES-induced force response for FDS or EDC stimulation. The response was averaged across five trials for each condition. Latency of the evoked response was also measured. The latency (Fig. 1C) was defined as the interval between the moment of ES delivery and the moment of peak force response. Similarly, a repeated-measures ANOVA was performed with factors of BREATH (4 levels: Norm, HOLD, IN, OUT) and FORCE (2 levels: rest and 10%). Background forces were compared with factors BREATH and FORCE. The latency was compared with that reported in the literature.

Experiment 3: to explore clinical applications of the respiratory–motor coupling

To provide further evidence for the coupling hypothesis and to explore its potential clinical use, we performed a preliminary clinical study. First, we tested two chronic stroke patients using the same ES protocol as with the *experiment 2*. The results indicated a similar pattern of voluntary breathing effects on ES-induced responses as observed in healthy subjects. Second, we further investigated the potential intervention effect of voluntary breathing-triggered electrical stimulation on finger flexor spasticity reduction in four patients and followed up for 4 wk.

RESULTS

Experiment 1

In this experiment, motor-evoked potentials (MEPs) from finger muscles were recorded and compared across different breathing conditions. The effect of voluntary breathing on TMS motor threshold, if any, was not the interest of this study and was not measured.

Finger flexion force and EMG responses to TMS were significantly modulated during voluntary breathing. Typical trials were plotted in Fig. 2. No changes in background EMG activities were found. Differences in F_{BG} in 10%MVC trials were within the range of 1 to 3% of the averaged F_{BG} among conditions. The background forces were not significantly different across all tested conditions. Averaged across subjects, a 2×4 two-way repeated-measures ANOVA (FORCE, BREATH) on MEP indicated a main effect of breathing for all three muscles [FDS, $F(3,39) = 6.98$, $P < 0.001$; EDC, $F(3,39) = 14.00$, $P < 0.0001$; ADM, $F(3,39) = 4.71$, $P = 0.007$, respectively]. Separate post hoc tests (Tukey's honestly significance difference) revealed different patterns of MEP modulations (Fig. 3). The FDS MEP magnitude increased significantly during IN and OUT in 10%MVC, but not at rest, whereas the EDC MEPs increased significantly during IN and OUT at both rest and 10%MVC, compared with NORM and HOLD. The ADM MEP increased only during OUT, compared with NORM, for both rest and 10%MVC. No effects of FORCE or significant FORCE \times BREATH interactions were found for the ADM MEP. No difference was found between NORM and HOLD for all three muscles. In 10%MVC trials, the flexion force response was also measured. A one-way ANOVA indicated a significant effect of BREATH [$F(3,39) = 13.38$, $P < 0.0001$]. Post hoc tests showed that the force response increased in both IN and OUT conditions than that in NORM and HOLD ($P < 0.028$).

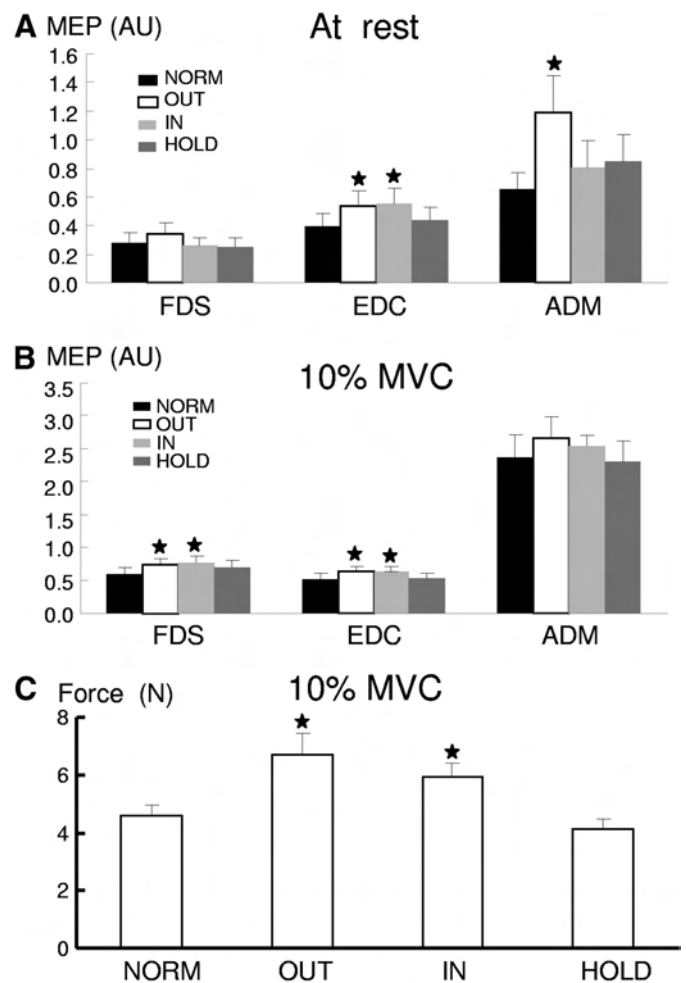


FIG. 3. Modulations of motor-evoked potentials (MEPs) and force responses during voluntary breathing under 4 breathing conditions: NORM, OUT, IN, and HOLD. MEPs from FDS, EDC, and ADM muscles were measured at rest and during 10% maximal voluntary contraction (MVC) trials. Force responses were measured only at 10%MVC trials. Average and SEs are presented. Asterisks indicate significant difference as compared with NORM ($P < 0.05$).

Experiment 2

ES-induced finger force responses were significantly modulated by voluntary breathing, but differently for the finger flexors and finger extensors. Typical trials of force response were presented in Fig. 4. Differences in background forces across conditions were about 2–3% of the target force at each force level. No significant differences were found across conditions. When the finger extensors were electrically stimulated (Fig. 5A), on average, ANOVAs (FORCE \times BREATH) indicated main effects of FORCE [$F(1,10) = 9.10$, $P = 0.013$] and BREATH [$F(3,30) = 3.46$, $P = 0.028$], but no significant interaction was found. Post hoc tests revealed that the extension force response was greater during IN than that during NORM at both rest and 10%MVC. No difference was found among other comparisons. During electrical stimulation of the finger flexors (Fig. 5B), there were main effects of FORCE [$F(1,10) = 40.68$, $P < 0.0001$] and BREATH [$F(3,30) = 7.78$, $P < 0.001$]. There was also a significant interaction of BREATH \times FORCE [$F(3,39) = 6.22$, $P = 0.002$]. Post hoc tests showed the flexion force responses were significantly increased during IN and OUT compared with NORM and

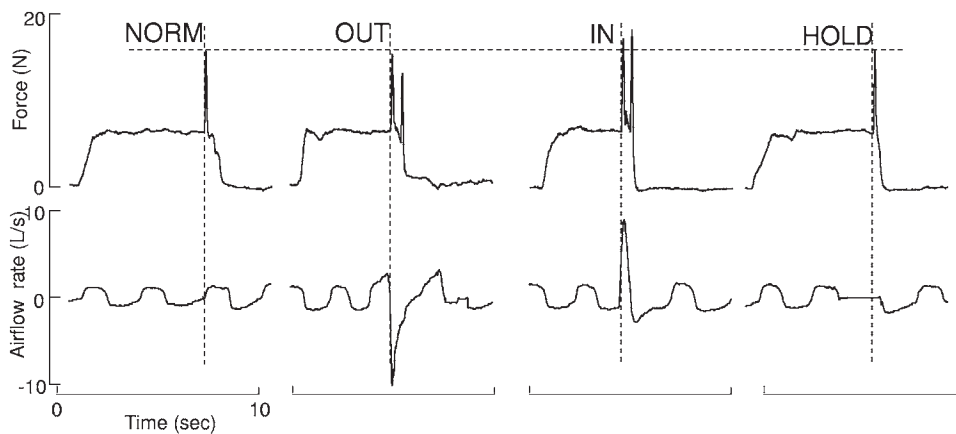


FIG. 4. Typical trials of ES-induced force responses during electrical stimulation of finger extensors during 10% MVC finger extension force production (*experiment 2*). Background force (about 100 ms prior to the ES delivery) was similar across 4 breathing conditions: NORM, OUT, IN, and HOLD. Vertical lines are aligned with the ES application. Horizontal line indicates the magnitude of response during NORM. The second spike during IN and OUT may be triggered by the decreasing phase of voluntary breathing (see Fig. 3). This spike is not analyzed.

HOLD only in 10% MVC trials; no difference at rest was found among all breathing conditions. In both flexion and extension trials, the force responses were not different between NORM and HOLD. In addition, we measured latency of the ES-induced response. The latency was 83 ms (range: 61–119 ms) for the finger extensors and 79 ms (range: 51–115 ms) for the finger flexors.

Experiment 3

Two chronic stroke patients were tested using the same ES protocol as that in *experiment 2*. Patient 1 (male, 75 yr of age, right cerebrovascular accident [CVA]/left hemiplegia for 22 yr) had intact sensation and weak voluntary finger extension. Patient 2 (male, 60 yr of age, left CVA/right hemiplegia for 2 yr and 2 mo) had intact sensation but without voluntary

finger/wrist extension. Patient 1 produced 10% and 30% MVC background force following the visual target, whereas patient 2 was at rest when ES was delivered to the finger extensors. As summarized in Table 1, when normalized to NORM, the ES-induced response was 107% during OUT and 117% during IN in patient 1, but 123% during OUT and 428% during IN in patient 2. The results revealed a pattern of modulations in chronic stroke patients similar to those of healthy subjects.

Furthermore, a pilot intervention study was performed in a third patient (patient 1 in Table 2). Similarly, ES was triggered when the inspiration airflow reached its 40% maximal value. Breaks were allowed as needed during a 30-min intervention. The patient initiated each trial in a self-paced manner with verbal cueing from the experimenter. Each patient received a total of approximately 100 trials of breathing-controlled ES. A series of 10 electrical pulses were delivered to the finger extensors when the patient was at rest to measure the baseline response before and immediately after the intervention (Fig. 6). The duration and intensity of the pulses were the same as those used during the intervention. As shown in Fig. 6, the ES-induced response was doubled immediately after the intervention.

To follow-up on the long-term effect on spasticity reduction, we recruited three additional patients (patients 2–4 in Table 2) with hemiplegia. All four patients had chronic neurological disorders (three stroke, one traumatic brain injury) that occurred ≥ 18 mo prior to the intervention. Patients had various levels of finger flexors spasticity (Modified Ashworth Scale ranges from 1⁺ to 4, examined by the first author). Two patients had intact sensation, one patient had diminished sensation, and the other patient had no sensation. Each patient received a nearly 30-min ES to the finger extensors triggered by forced inspiration using the same protocol as described in *Experiment 2*. The impaired forearm and hand of the third patient was secured in the device when ES was delivered, whereas the forearm and hand of other patients rested on the

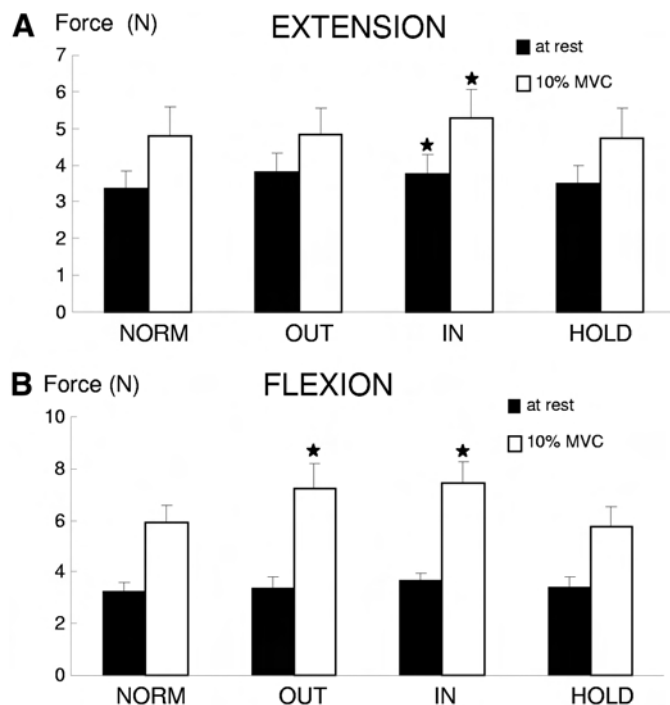


FIG. 5. Modulations of ES-induced force responses in finger extensors (A) and flexors (B). The force responses were measured at rest and 10% MVC trials under 4 breathing conditions: NORM, OUT, IN, and HOLD. Average and SEs are presented. Asterisks indicate significant difference as compared with NORM ($P < 0.05$).

TABLE 1. ES-induced finger extension force increment (in Newtons) and normalized to that during normal breathing (NORM)

Patient	ES-Induced Force Increment		
	NORM	OUT	IN
1			
10% MVC	6.5	6.9 (108.3%)	7.2 (113.9%)
30% MVC	9.3	9.4 (104.8%)	10.5 (119.4%)
Average	7.9	8.3 (106.6%)	8.9 (116.6%)
2 Rest	4.4	5.4 (122.9%)	18.7 (427.6%)

TABLE 2. Patient characteristics

Patient	Age, yr	Gender	Impaired	Etiology	History	Preintervention			Modified Ashworth Scale (Finger Flexors)		
						Sensation	Active ROM	Extensors' Strength	Pre	Post	At 4 wk
1	69	M	Rt	Stroke	22 mo	Intact	90–70°	4/5	1 ⁺	0	0
2	75	F	Rt	Stroke	19 mo	No	N/A	0	4	2	2
3	27	M	Lt	TBI	13 yr	Intact	N/A	0	3	2	2
4	53	F	Lt	Stroke	48 mo	Diminished	N/A	0	3	1 ⁺	1 ⁺

Sensation was tested in the impaired forearm and hand. Active range of motion (ROM) of metacarpophalangeal joints was measured. The Modified Ashworth Scale for the impaired finger flexors was measured before (Pre) and immediately after (Post) the intervention and 4 wk after the intervention. M, male; F, female; Rt, right hemiplegia; Lt, left hemiplegia; TBI, traumatic brain injury; ROM, range of motion. Extensors' strength was measured using manual muscle tests (scale: 0–5).

experimental table because of high tone of their finger flexors. All patients were able to tolerate the intervention with a facemask. They did not report any side effects, such as dizziness. On average, the Ashworth score was 2.9 before and 1.4 immediately after the intervention. Patients were followed-up regularly with their routine activities and therapies for 4 wk. Reduction in finger flexors spasticity lasted for ≥ 4 wk. The Ashworth score remained the same (1.4) at the end of 4-wk follow-up. Significant reduction in spasticity has dramatically improved hand function in the third patient (Fig. 7). This patient regained his hand function close to normal immediately after the 30-min intervention; he reported that he could cut meat with a knife and button shirts using his impaired hand. More strikingly, the recovery retained throughout the follow-up.

DISCUSSION

Overall respiration-related motor enhancement with a strong finger extension–inspiration coupling

A unique pattern of respiratory–motor interactions was observed. Both TMS and ES induced-responses, examined with

the same background activity, showed overall respiration-related motor enhancement, e.g., TMS-induced flexion force increment and MEP enhancement in FDS and EDC and ES-induced force increment in finger flexors during both IN and OUT. TMS and ES data supported a strong inspiration–finger extension coupling, however. When subjects were at rest, forced expiration (OUT) led to enhanced MEPs in FDS (increased, but not significant statistically), EDC, and ADM, but no significant change in ES-induced force increment in finger extension/flexion. In contrast, forced inspiration (IN) resulted in significantly enhanced MEPs only in EDC and ES-induced force increment in the finger extensors, but not the finger flexors. Furthermore, ES-induced force increment was observed in the finger extensors only during IN but not OUT during 10%MVC finger extension trials. The finding of no significant change in ES-induced force increment during OUT, but such increment only in the finger extensors during IN, suggest a strong intrinsic inspiration–finger extension coupling. Taken together, data from *experiments 1 and 2* support the idea that there is an overall respiration-related enhancement on the motor system, with a strong intrinsic

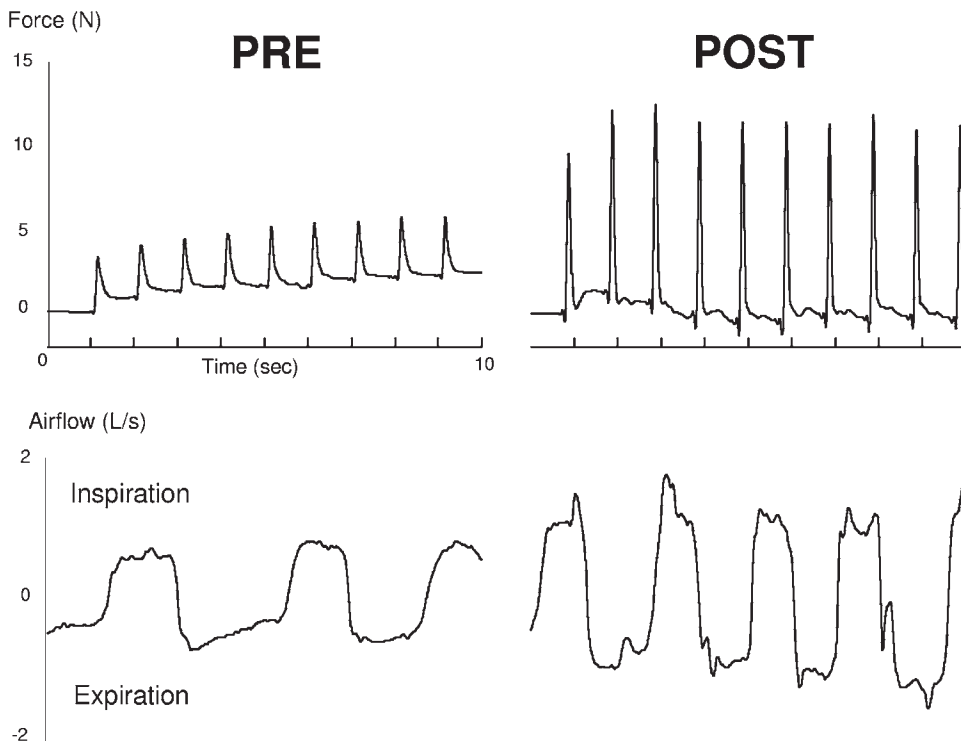


FIG. 6. ES-induced force response in a stroke patient during normal breathing before (PRE) and after (POST) a 30-min ES intervention to the finger extensors. The same ES intensity was used pre- and postintervention. Notice that ES-induced response was doubled after the intervention.

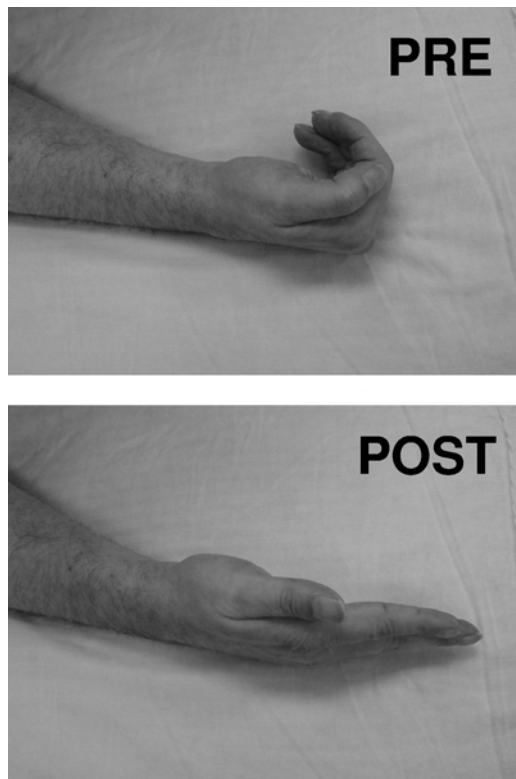


FIG. 7. The impaired hand before (PRE) and after (POST) a 30-min breathing controlled ES to the finger extensors. The patient (male, 69 yr of age, left CVA/right hemiplegia for 2 yr) has weak voluntary finger extension and intact sensation. His finger flexor spasticity was 1⁺. He was able to actively extend his metacarpophalangeal joints from 90 to 70° of flexion. His flexor spasticity decreased to minimum and voluntary finger extension returned close to normal immediately after the 30-min intervention. His hand function recovers close to normal. He is now able to cut meat with a knife and button shirts using his impaired hand after the intervention.

inspiration–finger extension coupling during voluntary breathing.

Findings from this study could provide direct neurophysiological evidence to explain results in a previous study of the effect on voluntary breathing on finger forces (Li and Laskin 2006). It has been reported that finger flexion MVCs were significantly increased in OUT, but not in IN. According to current findings, the overall respiration-related motor enhancement mechanism facilitates finger flexion and results in an enhanced MVC during forced expiration. This overall respiration-related enhancement is balanced by the finger extension–inspiration coupling effect during forced inspiration. As a result, no change in the finger flexion MVC is observed. Activation of finger extensors during finger flexion MVC tasks is relatively low (Li et al. 2001). There is a limitation that the finger extension–inspiration coupling observed at low levels of force production is used to explain finger flexion MVC data.

During automatic breathing, inspiration is an active process, whereas expiration is passive, primarily assisted by recoil force of the lung tissues. Respiratory afferents from the diaphragm and intercostal muscles project onto the somatosensory cortex (Gandevia and Macefield 1989; Zifko et al. 1995). These cortical projections, when activated during voluntary breathing, could influence skeletal motor drive. In addition to similar cortical areas activated during active inspiration, active expiration also involves additional activation of large M1 areas

(Guz 1997). It is likely that active expiration imposes a general excitatory effect on the motor system. In a previous study (Filippi et al. 2000), TMS was delivered during the expiratory or inspiratory phase of normal breathing, or during maximal inspiration, whereas MEPs were recorded only from ADM and were compared across these conditions. No significant increase in the ADM MEP magnitude was observed during maximal inspiration (Filippi et al. 2000). The current observation of enhanced MEPs in EDC, but not in FDS or ADM at rest, during forced inspiration was consistent with and expanded the reported finding (Filippi et al. 2000). It further suggested that corticospinal excitability for EDC be more easily influenced by voluntary breathing and there exists a finger extension–inspiration coupling. This coupling is in accordance with previous reports of neurophysiologic mechanisms mediating respiratory–motor interactions. For instance, resistive loaded inspiration significantly enhanced tonic vibratory response in the extensor digitorum (Balzamo et al. 1997), but did not affect contraction of biceps brachialis (Fontanari et al. 1996). The latency of median nerve components of somatosensory evoked potentials (SEPs) was lengthened by inspiratory, but not by expiratory, resistive loaded breathing, suggesting possible inhibitory effects of forceful inspiration on the wrist/finger flexors (Balzamo et al. 1999).

The ES latency, the interval between the ES delivery and the force response peak, could suggest possible origins of the induced force responses. The latency was 83 ms (range: 61–119 ms) for the finger extensors and 79 ms (range: 51–115 ms) for the finger flexors. The range was consistent with a previous report (Yue et al. 2000). The monosynaptic pathway (e.g., tendon reflex) for the finger flexors is about 24 ms (Li et al. 2004a). It takes about 17 ms for the induced muscle force to peak as a consequence of electrical stimulation (Yue et al. 2000). As such, the ES-induced response may have sufficient time (about an additional 40 ms) for supraspinal mechanisms of induced response (cf. Dick et al. 1987; Maluf et al. 2007). The ES data, in combination with the TMS data, provide direct neurophysiological evidence of the respiration-related motor enhancement with a strong finger extension–inspiration coupling, although they do not distinguish origins of modulation of corticospinal excitability of finger muscles during voluntary breathing. Future studies, such as H-reflex and H/M ratio, may be helpful to differentiate the cortical and spinal mechanisms. The ES data, however, provided a rationale for potential clinical applications of the respiratory–motor interactions, particularly the finger extension–inspiration coupling.

Potential clinical applications

According to the finger extension–inspiration coupling, when delivered to the finger extensors during forced inspiration, ES increases corticospinal excitability specifically for the finger extensors. As such, possibly via reciprocal inhibition mechanisms, the breathing-controlled ES (BreESim) could avoid excessive finger flexor–extensor coactivation problems in stroke patients (Kamper and Rymer 2001) and, in turn, lead to reduction of flexor hypertonia. Similar to this idea, ES, when combined with motor point block for antagonist muscles (hybrid FES therapy), has shown greater functional improvement and spasticity reduction than used alone for stroke rehabilitation (Hara et al. 2000). In the preliminary study, a similar

pattern of finger force responses was observed in chronic stroke patients. More intriguingly, a single-session treatment of BreESTim has led to an immediate reduction and a long-lasting effect on finger flexor spasticity, subsequently resulting in improvement in hand function. As opposed to the commonly observed short-term reduction by EMG-triggered ES (Bakhtiyari and Fatemy 2008; Chae 2003), the long-lasting effect on spasticity reduction after a single session of BreESTim provides further neurophysiologic evidence for the finger extension–inspiration coupling. Since fingers are not mechanically linked with respiration, such significant effects are not expected in chronic stroke patients if there is no intrinsic physiological coupling. Furthermore, the primitive clinical study also demonstrates potential clinical application of BreESTim. During BreESTim, a second ES stimulation occurred in a majority of trials, likely triggered by the airflow signal during the descending phase (Fig. 1C). This unintentional stimulation should be taken into account when planning the dosage of BreESTim in the future study. On the other hand, this primitive clinical study raises more questions regarding pathophysiology and treatment of spasticity—e.g., Why is there an immediate spasticity reduction? and Why does it last so long? To better understand the underlying mechanisms and to generalize its clinical use, further studies are needed, e.g., to compare BreESTim and EMG-triggered ES, to objectively quantify the spasticity of flexors using standard laboratory measures and to correlate laboratory measures with functional outcomes in a larger patient population.

In summary, the present findings support a neurophysiologic basis of respiratory–motor interactions. Specifically, there exists an overall respiration-related enhancement on the motor system, with a strong finger extension–inspiration coupling. In an exploratory preliminary study, electrical stimulation to the finger extensors timed to the inspiratory phase of voluntary breathing, breathing-controlled electrical stimulation (BreESTim), has demonstrated a long-lasting effect on spasticity reduction with subsequent improvement in hand function recovery in patients with chronic neurologic disorders (*experiment 3*). On the other hand, the observed long-lasting effect imposes scientifically challenging questions regarding its specific underlying mechanisms of BreESTim, as well as the pathophysiology of spasticity.

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DISCLOSURES

Method and apparatus of breathing-controlled electrical stimulation for skeletal muscles [inventor: S. Li, pending patent, application number 12146176].

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