Whole-hand water flow stimulation increases motor cortical excitability: a study of transcranial magnetic stimulation and movement-related cortical potentials

Daisuke Sato,1,2 Koya Yamashiro,1,2 Hideaki Onishi,1,3 Baba Yasuhiro,2 Yoshimitsu Shimoyama,2 and Atsuo Maruyama1,2

1Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, Niigata City, Japan; 2Department of Health and Sports, Niigata University of Health and Welfare, Niigata City, Japan; and 3Department of Physical Therapy, Niigata University of Health and Welfare, Niigata City, Japan

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Sato D, Yamashiro K, Onishi H, Yasuhiro B, Shimoyama Y, Maruyama A. Whole-hand water flow stimulation increases motor cortical excitability: a study of transcranial magnetic stimulation and movement-related cortical potentials. J Neurophysiol 113: 822–833, 2015. First published November 5, 2014; doi:10.1152/jn.00161.2014.—Previous studies examining the influence of afferent stimulation on corticospinal excitability have demonstrated that the intensity of afferent stimulation and the nature of the afferents targeted (cutaneous/proprceptive) determine the effects. In this study, we assessed the effects of whole-hand water immersion (WI) and water flow stimulation (WF) on corticospinal excitability and intracortical circuits by measuring motor evoked potential (MEP) recruitment curves and conditioned MEP amplitudes. We further investigated whether whole-hand WF modulated movement-related cortical activity. Ten healthy subjects participated in three experiments, comprising the immersion of participants’ right hands with (whole-hand WF) or without (whole-hand WI) water flow, and no immersion (control). We evaluated MEP recruitment curves produced by a single transcranial magnetic stimulation (TMS) pulse at increasing stimulus intensities, short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF) using the paired TMS technique before and after 15 min of intervention. Movement-related cortical potentials (MRCPs) were evaluated to examine primary motor cortex, supplementary motor area, and somatosensory cortex excitability upon movement before and after whole-hand WF. After whole-hand WF, the slope of the MEP recruitment curve significantly increased, whereas SICI decreased and ICF increased in the contralateral motor cortex. The amplitude of the Bereitschaftspotential, negative slope, and motor potential of MRCPs significantly increased after whole-hand WF. We demonstrated that whole-hand WF increased corticospinal excitability, decreased SICI, and increased ICF, although whole-hand WI did not change corticospinal excitability and intracortical circuits. Whole-hand WF modulated movement-related cortical activity, increasing motor cortex activation for the planning and execution of voluntary movements.

whole-hand water flow stimulation; primary motor cortex; corticospinal excitability; intracortical circuit; movement-related cortical potentials

TRANSCRANIAL MAGNETIC STIMULATION (TMS) is used to examine the effect of afferent sensory input from the hand on the excitability of the human motor cortex. Motor evoked potentials (MEPs) are affected by preceding electrical stimuli to mixed (Bertolasi et al. 1998; Deuschl et al. 1991; Tokimura et al. 2000) or cutaneous (Maertens de Noordhout et al. 1992; Maertens et al. 1992; Ridding and Taylor 2001; Rossini et al. 1996) nerve. In addition, continuous afferent input can alter cortical maps and modulate corticomotor excitability. Studies on humans (Kaelin-Lang et al. 2002; Ridding and Taylor 2001) have shown that a period of sensory stimulation increases corticomotor excitability for a period outlasting the stimulus. In vivo experiments identified the anatomic substrate for this cross-systemic plasticity as topographically and functionally specific reciprocal connections between the primary motor cortex (MI) and the primary somatosensory cortex (SI) (Rocco and Brumberg 2007). Changes in synaptic efficiency through timing-dependent associative neuronal activities have been proposed as the physiological basis of this sensory stimulation-driven prolonged increase in excitability (Feldman 2000). Mechanical, electrical, and magnetic afferent stimuli are employed to influence motor-controlling structures. Changes in motor map organization, corticospinal excitability, sensorimotor organization, and intracortical circuits are achieved after periods of continuous afferent input using TMS (Christova et al. 2011; Rosenkranz et al. 2003; Rosenkranz and Rothwell 2003). The predominant effects exhibit a somatotopic organization, and the largest changes in MEPs are evident in muscles nearest to the site of stimulation. Because afferent stimulation increases in corticospinal excitability are not associated with changes in F-waves (Chen et al. 1999; Christova et al. 2011; Golaszewski et al. 2012; Rosenkranz and Rothwell 2003), it is likely that they originate at the cortical level.

For therapeutic applications, it is advantageous to deliver sensory stimuli to a large area rather than a single muscle or nerve. Water immersion (WI) can alter numerous physiological parameters depending on the physical characteristics such as hydrostatic pressure and temperature. Moreover, partial WI can ameliorate edema, improve blood flow (Fothergill et al. 1998), and relieve pain (Kakigi 1994). We investigated whether whole-body WI changes sensory and motor cortical excitability caused by increased cutaneous afferent input (Sato et al. 2012a; Sato et al. 2012b; Sato et al. 2013) and showed that this afferent input modulates sensorimotor integration (Sato et al. 2013). However, corticospinal excitability and intracortical circuits were unchanged because MEPs were measured in hands that were not in water. According to results on the effects of cutaneous afferent input on MI excitability, stimulus
intensity may be an important parameter in the modulation of corticomotor excitability. For example, electrical stimuli at intensities greater than motor threshold increase the motor excitability of corticomotor pathways to the stimulated muscles. In contrast, the results are equivocal regarding the effects of electrical stimuli at intensities less than the motor threshold, but sufficient to induce sensory perception, on the excitability of corticomotor pathways (Chipchase et al. 2011). Moreover, the results of experiments using neurography show that proprioceptive input has effects on sensorimotor cortex (Heath et al. 1976; Jones and Porter 1980) and muscle vibration that produces 1a afferent activation can induce lasting effects on cortical excitability. (Rosenkranz et al. 2003; Rosenkranz and Rothwell 2003). Therefore, in the present study, we considered whole-hand WI and water flow stimulation (WF) utilizing a water flow device originally made for partial WI. Whole-hand WI results in projection to a wide area of hand corticomotor representation, and touching water with the whole hand generates afferent input from cutaneous afferents (type II fiber group) that project to the SI, mainly to the Brodmann area 3B. Additionally, we expected that whole-hand WF elicits not only the greater cutaneous input than whole-hand WI, but also the proprioceptive input induced by skin and muscle movement that increases MI excitability.

We investigated whether whole-hand WF has vibrational effects on the skin and the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles in experiment 1 (EX 1). We determined the effects of whole-hand WI and WF on corticospinal excitability and intracortical circuits by measuring MEP recruitment curves in experiments 2 and 3 (EX 2 and 3) as well as the conditioned MEP amplitudes in experiments 4 and 5 (EX 4 and 5). Therefore, we anticipated increased excitability within the MI contralateral to the stimulation-exposed hand following whole-hand WF in the vibrated skin and muscle. Moreover, in humans, changes in MEP amplitude are associated with movement-related cortical activity (Holler et al. 2006; Rossi et al. 2000). High-frequency transcutaneous electrical nerve stimulation (TENS), which inhibits MI excitability (Mima et al. 2004), causes a deterioration of movement-related cortical activity visualized using magnetoencephalography such as motor field (MF) and motor evoked field one (MEF1) (Murakami et al. 2010). The inhibition of MI excitability using low-rate repetitive TMS (rTMS) decreases the amplitude of the negative slope (NS) by ~300–400 ms before voluntary movement (Rossi et al. 2000). We investigated whether whole-hand WF modulates movement-related cortical activity in experiment 6 (EX 6).

MATERIALS AND METHODS

We performed six experiments. In EX 1, we used a piezosensor to determine whether WF stimulation induces skin and muscle movement. In EX 2 and EX 3, we measured MEP recruitment curves to evaluate corticospinal excitability in two hand muscles (FDI and ADM muscles) before and after interventions. In EX 4 and EX 5, we measured short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) to evaluate intracortical excitability in those muscles before and after intervention. In EX 6, we measured movement-related cortical potentials (MRCPs) to evaluate motor cortical activation associated with voluntary movement in the vibrated muscle before and after intervention.

Participants

We enrolled three healthy right-handed male volunteers aged 20–22 yr in EX 1 and 10 healthy right-handed male volunteers aged 19–25 yr in EX 2, EX 4, and EX 6. Eight and nine of these latter subjects participated in EX 3 and EX 5, respectively. Informed consent was obtained from all participants, and the study was conducted in accordance with the Declaration of Helsinki and with approval granted by the local ethics committee.

Intervention

The interventions (15 min each) were as follows: nonimmersion (control), whole-hand WI, and whole-hand WF. In all interventions, subjects were instructed to place the right hand in the sluicing device, keeping it relaxed. The left hand was placed on a soft support beside the body and kept relaxed. The hands were fixed in the same position for all interventions using a belt to avoid muscle contractions. For whole-hand WF, WF was applied to the palm of the right hand with a sluicing device (Japan Aqua Tec, Sasebo, Nagasaki, Japan; Fig. 1) at ~40 l/min. Participants were instructed to focus their gaze at the wall facing them throughout the experiments to divert their attention from their right hand. For each intervention, the ambient temperature

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Fig. 1. A: the sluicing device used in this study. B: whole-hand water flow stimulation intervention. The water jet is within the black circle in A.
was 29°C ± 1°C, and water temperature was 33°C ± 1°C. Ambient and water temperatures were modulated to avoid changing skin temperature. During whole-hand WI and WF, we monitored the electromyograph (EMG) for any muscle contraction in FDI and ADM muscle, besides possible voluntary activation, the occurrence of the tonic vibration reflex (Hagbarth and Eklund 1968; Marsden et al. 1969). During whole-hand WF there was no evidence of a tonic vibration reflex in the EMG.

**Experimental protocol in EX 1.** Using two piezosensors (DTI–028K/L; Tokyo Sensor), we measured whether the WF stimulation induces skin and muscle vibration on the right FDI muscle and right ADM muscle. The two piezosensors were attached with double-faced tape and covered with a waterproof transparent film (Tegaderm Hydrocolloid Dressing; 3M Japan, Tokyo, Japan) (Fig. 2). The signals were recorded at a sampling rate of 2,000 Hz during WF stimulation. A fast Fourier transform was used to determine the frequency power spectrum of the piezosensor signal.

**Experimental protocol in EX 2–6.** The experimental sequence included a preassessment intervention for 15 min and postassessment immediately after the intervention in EX 2 through EX 6. During pre- and postassessment, the right hand was placed relaxed on a desk to the right side. The experimental protocols are presented in Fig. 3. The three interventions were randomly performed in counterbalanced order across participants. All participants underwent this protocol with an interval of at least 5 days between experiments.

**TMS Assessments and EMG Recordings**

TMS was performed with two Magstim 200 magnetic stimulators (Magstim, Dyfed, UK) connected by a Y-cable to a figure-eight coil with an external wing diameter of 9 cm. The coil was held with the handle pointing backward laterally at ~45° to the sagittal plane and was optimally positioned to obtain MEPs in the right FDI muscle for EX 2 and EX 4 and in the right ADM muscle for EX 3 and EX 5. The site was marked on the skull to allow the experimenter to reposition the coil in the same spot before each measurement. With this coil orientation, the induced current in the brain flowed in a posterior-anterior direction.

Surface muscle responses were acquired via surface electrodes placed over the right FDI (EX 2 and EX 4) and right ADM muscles (EX 3 and EX 5) using disposable adhesive silver/silver chloride surface electrodes 9 mm in diameter. The active electrode was placed over the muscle belly and the reference over the interphalangeal joint of the index finger and digitus minimus. Signals were amplified and filtered (gain × 1,000, 5–1 kHz; AB-601G; Nihon Kohden, Tokyo, Japan) and transferred through a Micro 1401 Laboratory Interface Computer for further analysis. The all electrodes were covered with a waterproof transparent film (Tegaderm Hydrocolloid Dressing; 3M Japan, Tokyo, Japan) (Fig. 2). The signals were recorded at a sampling rate of 2,000 Hz during WF stimulation. A fast Fourier transform was used to determine the frequency power spectrum of the piezosensor signal.

**Pre and Post assessment**

**TMS data analysis in EX 2 and 3.** We evaluated the amplitude of MEPs produced by a single TMS pulse at increasing stimulus intensities (MEP recruitment curve). The TMS intensities were 50, 80, 90, 100, 110, 120, 130, and 150% of the rMT, determined for each participant. These intensities remained the same in pre- and post-measurements. The MEP recruitment curve was determined at rest. Eight pulses were delivered for each stimulus intensity every 4 s, and stimulus intensities were randomly administered. To avoid collecting startle and reflex responses, we excluded the first MEP for each trial from the analysis.

**TMS data analysis in EX 4 and 5.** SICI and ICF were studied by the techniques of Kujirai et al. (1993) and Ziemann et al. (1996c). Paired TMS pulses were administered through the same stimulating coil over the left motor cortex and the effect of the first (conditioning) stimulation on the second (test) stimulation was measured. As previously reported (Christova et al. 2011; Golaszewski et al. 2012; Golaszewski et al. 2010), the amplitude of the test MEP increased after prolonged afferent stimulation. Because the SICI and ICF are affected by the size of the test MEP, we adjusted the stimulus intensity in this experiment so that the MEP amplitude remained the same throughout for water-immersed conditions with water-flowing. Conditioning stimulus (CS) was set at 90% of the aMT. Test stimulus (TS) intensity was adjusted to elicit an unconditioned test MEP in the relaxed right FDI and ADM with ~1 mV peak-to-peak amplitude. TS was applied every 4 s. The following ISIs were selected: 3 and 10 ms. Each block of trials consisted of three different stimuli: TS alone, TS plus CS at 3 ms, and TS plus CS at 10 ms. The order of these stimuli was randomized by a computer, and 12 trials of each type were recorded per block.

**Electroencephalograph and EMG recording.** Brain electrical potentials were recorded in a continuous mode using a SynAmps amplifier system and SCAN 4.3 software (Neuroscan, El Paso, TX, USA). Electroencephalographs (EEG) were recorded using five scalp electrodes placed at C3/C4, Fz, Cz, and Pz according to the 10–20 system. The left earlobe was used as a reference, and the ground
electrode was at Fpz. Electrode impedance was maintained <5 KΩ. Band-pass filters were set to 0.1–100 Hz, with a sampling rate of 1,000 Hz. EMG recordings were performed using pairs of 9 mm diameter Ag-AgCl surface electrodes placed over the belly of the right FDI muscle and the reference electrodes over the ipsilateral proximal interphalangeal joint (belly-tendon technique). The EMG signals were also amplified, and a high-pass filter was set to 10 Hz, with a sampling rate of 1,000 Hz.

Analysis of movement-related cortical potentials in EX 6. Participants performed brisk abduction movements with their right-hand index finger. Each movement was repeated voluntarily at irregular self-paced intervals exceeding 6 s. Participants were instructed not to count or engage in any other rhythmic activity during the recording. During measurements the right hand and arm were fixed in the same position by a belt to prevent other muscle contractions. One session comprised 100 epochs of movement. A practice session before the recording consisted of 10 movements.

For the analysis of MRCP, epochs from 2,200 ms before the voluntary EMG onset (−2,200 ms) to 1,000 ms after EMG onset (1,000 ms) were segmented. The initial 200 ms (−2,200 to −2,000 ms) were assigned as baseline. Voluntary EMG onset (0 ms) was marked visually in each sweep. Trials during which EMG activity exceeded the mean of the resting EMG plus three times standard deviation were excluded. A total of 75–90 artifact-free trials were used for each experimental condition. The amplitudes of MRCPs were analyzed in four periods, from 1,500 to 500 ms before EMG onset as Bereitschaftspotentials (BP) (Kornhuber and Deecke 1965), from 500 to 0 ms before the EMG onset as NS (Shibasaki et al. 1980), from −10 to 50 ms at EMG onset as motor potentials (MP; Kornhuber and Deecke 1965), and −300 ms after EMG onset as widespread positivity, with the maximum at the contralateral central area (P + 300; Ikeda and Shibasaki 1992).

Skin Temperature Measurements

In our preliminary study, the amplitude of the test MEP increased during 15-min WI due to decreased skin temperature. To correlate the effects of changes in skin temperature with global neuromodulatory changes, skin temperatures from the dorsal surfaces of both hands were continuously measured through the experiments with a temperature logger (LT-8; Gram, Japan), and the values were averaged during each trial (pre and post).

Data Analysis and Statistics

For the rMT (expressed as percentage of maximum stimulator output), we conducted two-factorial repeated-measures analysis of variance (ANOVA) with the within-subject factors “time” (two levels: pre and post) and “intervention” (three levels: control, whole-hand WI, and whole-hand WF). Follow-up ANOVAs for each intervention separately with within-subject factor time were conducted when the follow-up ANOVA revealed a significant main effect of time.

We analyzed skin temperature by repeated-measures ANOVA with the within-subject factors “hand” (two levels: right and left hand) and “time” (two levels: pre and post) and the between-subject factor “intervention” (three levels: control, whole-hand WI, and whole-hand WF).

All MEPs were expressed as peak-to-peak amplitudes. The peak-to-peak amplitude of the test MEP by a single (unpaired) TMS stimulus was termed MEP<sub>TEST</sub>. The two conditioned MEP values were MEP<sub>PRE</sub> and MEP<sub>POST</sub>. For the analysis of MEP recruitment curve, MEP amplitudes were analyzed by repeated-measures ANOVA with the within-subject factors “stimulus intensity” (eight levels: from 50% to 150% rMT) and “time” (two levels: pre and post) and the between-subject factor “intervention” (three levels: control, whole-hand WI, and whole-hand WF). If a significant interaction with a group in the three-factorial ANOVA was found, we conducted follow-up two-factorial ANOVAs separately for each group with the within-subject factors “stimulus intensity” and “time”.

For the analysis of SICI and ICF, we used repeated-measures ANOVA to assess the effect of whole-hand WF on SICI and ICF with the within-subject factors “ISI” (two levels: SICI and ICF), “time” (two levels: pre and post), and “intervention” (three levels: control, whole-hand WI, and whole-hand WF). If a significant interaction with a group in the three-factorial ANOVA was found, we conducted follow-up two-factorial ANOVAs separately for each ISI with the within-subject factors “intervention” and “time”.

For the analysis of MRCPs, we used repeated-measures ANOVA to assess the effect of whole-hand WF on BP, NS’, MP, and P + 300 components with the within-subject factors “electrode” (five levels: Fz, Cz, C3, C4, and Pz) and “time” (two levels: pre and post).

If the assumption of sphericity was violated in Mauchly’s sphericity test, the degree of freedom was corrected with Greenhouse-Geisser’s correction coefficient epsilon, and F and P values were recalculated. Post hoc tests (Bonferroni-Dunn) were performed, and the significance level was set at 5%.

RESULTS

Vibrational Effect of Whole-hand WF on the Skin and Muscles

Figure 4 presents the power spectrum during whole-hand WF in the FDI and ADM muscles. In all three subjects, whole-hand WF stimulation induced skin and muscle vibrations ranging from 15 to 150 Hz on the FDI muscle and no detectable effect on the ADM muscle. Additionally, the highest spectrum power of ~70 Hz was detected only in the FDI muscle in contrast to the ADM muscle.

Motor Threshold and Skin Temperature

Table 1 presents the pre- and post-assessment rMT and aMT values in both muscles. Repeated-measures ANOVA revealed a lack of interactions and effects as follows: For rMT in the FDI muscle, there was no interaction between “intervention” and “time” [F(2,18) = 3.14, P > 0.05] and no main effects of “intervention” [F(2,18) = 0.19, P > 0.05] and “time” [F(2,18) = 2.52, P > 0.05]. For rMT in the ADM muscle, there was no interaction between “intervention” and “time” [F(2,18) = 1.81, P > 0.05] and no main effects of “intervention” [F(2,18) = 1.29, P > 0.05] and “time” [F(2,18) = 0.13, P > 0.05]. For skin temperature, there was no interaction between “intervention” and “time” [F(2,18) = 0.94, P > 0.05], “intervention” and “hand” [F(2,18) = 2.11, P > 0.05], and “time” and “hand” [F(1,9) = 0.03, P > 0.05]. There were no main effects of “intervention” [F(2,18) = 0.09, P > 0.05], “time” [F(1,9) = 0.05, P > 0.05], and “hand” [F(1,9) = 3.61, P > 0.05].

M EP Recruitment Curve in EX 2 and 3

Fig. 5 presents pre- and post-MEP recruitment curves for all interventions. For the analysis of the FDI muscle in EX 2, repeated-measures ANOVA revealed a reliable interaction between “intervention” and “time” [F(2,18) = 4.10, P < 0.05] and “intervention” and “stimulus intensity” [F(7,63) = 2.34, P < 0.05]. Furthermore, there were significant main effects...
of “stimulus intensity” \[F(7,63) = 89.99, P < 0.001\]. No other effects were reliable. Follow-up separate ANOVAs for each intervention revealed main effects on all interventions of “stimulus intensity” \[F(7,63) = 83.04, P < 0.05; F(7,63) = 65.69, P < 0.05; \] and \[F(7,63) = 69.27, P < 0.05\] for control, whole-hand WI, and whole-hand WF, respectively. Although the control and whole-hand WI did not show any effects on amplitudes after “time” \[F(1,9) = 2.93, P = 0.121; F(1,9) = 1, P = 0.342\] and no interaction effects of “stimulus intensity” and “time” \[F(7,63) = 0.86, P = 0.541; F(7,63) = 0.681, P = 0.688\], there was an interaction effect of “stimulus intensity” and “time” \[F(7,63) = 2.34, P < 0.05\] for whole-hand WF. Post hoc comparisons of each intensity revealed that at midrange and higher intensities, post-MEP increases were significant compared with pre-MEP increases \(P < 0.05\), Bonferroni-corrected. For the ADM muscle in EX 3, repeated-measures ANOVA revealed a reliable interaction between “stimulus intensity” and “time” \[F(7,49) = 3.93, P < 0.05\] and a significant main effect of “stimulus intensity” \[F(7,49) = 103.64, P < 0.001\]. No other effects were reliable.

Paired-pulse Stimulation in EX 4 and 5

Figure 6 shows the changes in SICI and ICF in both muscles. Table 1 presents pre- and postassessment of TMS intensities of TS and CS in both muscles. For the FDI muscle in EX 4, repeated-measures ANOVA revealed a reliable interaction between “intervention” and “time” \[F(2,18) = 15.87, P < 0.05\] and revealed significant main effects of “ISI” \[F(1,9) = 132.15, P < 0.05\], “intervention” \[F(2,18) = 4.41, P < 0.05\], and “time” \[F(1,9) = 20.93, P < 0.05\]. No other effects were reliable. Follow-up separate ANOVAs of SICI and ICF revealed the interaction of “intervention” and “time” \[F(2,18) = 19.90, P < 0.05; F(2,18) = 7.48, P < 0.05\]. Furthermore, main effects of “time” \[F(1,9) = 22.33, P < 0.05; F(1,9) = 6.69, P < 0.05\] and “intervention” \[F(2,18) = 3.73, P < 0.05; F(2,18) = 3.84, P < 0.05\] for SICI and ICF were noted. Post hoc comparisons revealed that significant differences were observed between post-SICI and post-ICF compared with pre-SICI and pre-ICF, respectively, in whole-hand WF \((P < 0.05\), Bonferroni-corrected). For the ADM muscle analyzed in EX 5, repeated-measures ANOVA revealed no significant interaction or significant main effects of “ISI” \[F(1,8) = 59.31, P < 0.05\].
ANOVAs for SICI and ICF revealed no significant interaction between each stimulus intensity, the mean (SE) of MEP amplitude has been plotted. *Significant difference (P < 0.05) between before and after the intervention.

Table 1. TMS intensities in EX 3 and 4

<table>
<thead>
<tr>
<th></th>
<th>FDI Muscle in EX 3</th>
<th>ADM Muscle in EX 4</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>rMT, %</td>
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<tr>
<td>control</td>
<td>46.7 ± 1.9</td>
<td>46.7 ± 1.9</td>
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<tr>
<td>whole-hand WI</td>
<td>46.2 ± 1.8</td>
<td>46.2 ± 1.8</td>
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<tr>
<td>whole-hand WF</td>
<td>46.6 ± 2.1</td>
<td>45.6 ± 2.1</td>
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<tr>
<td>aMT, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>36.1 ± 2.0</td>
<td>36.1 ± 2.0</td>
</tr>
<tr>
<td>whole-hand WI</td>
<td>35.3 ± 2.0</td>
<td>35.3 ± 2.0</td>
</tr>
<tr>
<td>whole-hand WF</td>
<td>36.3 ± 2.1</td>
<td>36.3 ± 2.1</td>
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<tr>
<td>TS, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>64.4 ± 2.5</td>
<td>64.4 ± 2.5</td>
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<tr>
<td>whole-hand WI</td>
<td>64.4 ± 2.6</td>
<td>64.4 ± 2.6</td>
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<tr>
<td>whole-hand WF</td>
<td>63.8 ± 2.7</td>
<td>61.9 ± 2.6</td>
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<tr>
<td>CS, %</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>32.5 ± 1.6</td>
<td>32.5 ± 1.6</td>
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<tr>
<td>whole-hand WI</td>
<td>31.8 ± 1.4</td>
<td>31.8 ± 1.5</td>
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<tr>
<td>whole-hand WF</td>
<td>32.7 ± 1.7</td>
<td>32.7 ± 1.6</td>
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Means ± SE. EX, experiment; FDI, first dorsal interosseus; ADM, abductor digiti minimi; MP, resting motor threshold; aMT, active motor threshold; TS, test stimulus; CS, conditioning stimulus; gain, rMT; percent of motor threshold; TS, test stimulus; CS, conditioning stimulus; Wi, water immersion; WF, water flow stimulation.

and “time” [F(1,8) = 10.81, P < 0.05]. Follow-up separate ANOVAs for SICI and ICF revealed no significant interaction of “intervention” and “time” [F(2,16) = 2.28, P > 0.05; F(2,16) = 0.36, P > 0.05].

Figure 7 presents the grand average of MRCP waveforms before and after whole-hand WF. The earliest activity, beginning 1.5 s before EMG onset, was negative and sustained when recorded at the frontal, central, and parietal electrode sites. Table 2 presents the latency and amplitude of each MRCP component. The BP component, typically observed in MRCP studies, was recorded on all electrodes 1,000–1,500 ms before EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4, 36) = 5.30, P < 0.05] and “time” [F(1,9) = 15.84, P < 0.05] with no significant interactions [F(4, 36) = 1.87, P > 0.05]. Post hoc comparisons revealed that BP amplitude in each electrode was significantly different between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The NS component peaked on the vertex immediately before EMG onset. Repeated-measures ANOVA revealed significant main effects of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions between “electrode” and “time” [F(4,36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The MP component was recorded on all electrodes immediately after EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05]. Post hoc comparisons revealed that MP amplitude in each electrode was significantly different between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions [F(4, 36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The MP component was recorded on all electrodes immediately after EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions [F(4, 36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The MP component was recorded on all electrodes immediately after EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions [F(4, 36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The MP component was recorded on all electrodes immediately after EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions [F(4, 36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected). The MP component was recorded on all electrodes immediately after EMG onset. Repeated-measures ANOVA revealed a main effect of “electrode” [F(4,36) = 12.59, P < 0.05] and “time” [F(1,9) = 17.98, P < 0.05] with no significant interactions [F(4, 36) = 1.09, P > 0.05]. Post hoc comparisons revealed that the NS amplitude of each electrode significantly differed between before-and-after whole-hand WF (P < 0.05, Bonferroni-corrected).
12.80, $P < 0.05$] and “time” [$F(1,9) = 39.50, P < 0.05$] with no significant interactions [$F(4,36) = 0.17, P > 0.05$]. Post hoc comparisons revealed that the MP amplitude measured by each electrode significantly differed between before-and-after whole-hand WF ($P < 0.05$, Bonferroni-corrected). The P 300 component, typically observed in MRCP studies, was recorded on all electrodes 250–470 ms after EMG onset. Repeated-measures ANOVA revealed a significant interaction between “electrode” and “time” [$F(4,36) = 3.66, P < 0.05$] with no main effect of “electrode” [$F(4,36) = 2.28, P > 0.05$] and “time” [$F(1,9) = 1.40, P > 0.05$]. Post hoc comparisons revealed that the P 300 amplitude at Cz significantly differed between before-and-after whole-hand WF ($P < 0.05$, Bonferroni-corrected).

**DISCUSSION**

In the present study, we investigated the effects of whole-hand WI, with and without WF, on corticospinal excitability and intracortical inhibitory and excitatory circuits. Whole-hand WI alone did not change MEP recruitment curves, SICI, or ICF in both FDI and ADM muscles. In contrast, whole-hand WI with WF increased MEP recruitment curves and ICF and decreased SICI only in FDI muscle. These results suggest that...
whole-hand WF modulates corticospinal excitability as well as intracortical inhibitory and excitatory circuits in vibrated muscle. Furthermore, we controlled the water and ambient temperatures and found no change in skin temperatures during the experiment, strongly suggesting that the changes in MEP recruitment curves, SICI, and ICF were possibly not influenced by changes in body temperature. Moreover, whole-hand WF increased the BP, NS, and MP amplitudes and decreased the $P + 300$ amplitude at Cz without changing latencies, suggesting that whole-hand WF modulates movement-related preparatory and executive cortical activity.

WI such as whole-hand WI and WF can alter numerous physiological parameters depending on physical characteristics such as hydrostatic pressure and temperature. In recent years, WI has been used as a rehabilitation for improving ADL ability in frail elderly (Sato et al. 2009; 2007). Additionally, several studies have shown that whole-body WI could change neural activity, such as activation in the SI and multimodal sensory processing (Sato et al. 2012b) and sensorimotor integration (Sato et al. 2013). Partial WI can also provide relief from edema, improve blood flow (Fothergill et al. 1998), and relieve pain (Kakigi 1994). However, the effects of partial WI on corticospinal excitability and intracortical excitability are not well known. Moreover, the results of this study revealed no effect on rMT; however, stable MEP amplitude increases were found after whole-hand WF. rMT is believed to reflect neuronal membrane excitability because it is increased by drugs that block voltage-gated sodium channels (Ziemann et al. 1996b) but not by drugs

**Table 2. The amplitude and latency of MRCP components before and after whole-hand WF**

<table>
<thead>
<tr>
<th></th>
<th>Amplitude, $\mu$V</th>
<th>Latency, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bereitschaftspotential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fz pre</td>
<td>$-5.16 \pm 0.96$</td>
<td>$-138.70 \pm 181.72$</td>
</tr>
<tr>
<td>post</td>
<td>$-7.11 \pm 1.09^*$</td>
<td>$-1375.30 \pm 172.75$</td>
</tr>
<tr>
<td>Cz pre</td>
<td>$-4.17 \pm 0.61$</td>
<td>$-1287.60 \pm 194.31$</td>
</tr>
<tr>
<td>post</td>
<td>$-6.66 \pm 0.52^*$</td>
<td>$-1291.60 \pm 151.78$</td>
</tr>
<tr>
<td>Pz pre</td>
<td>$-3.11 \pm 0.42$</td>
<td>$-1313.10 \pm 179.35$</td>
</tr>
<tr>
<td>post</td>
<td>$-4.42 \pm 0.35^*$</td>
<td>$-1296.40 \pm 171.84$</td>
</tr>
<tr>
<td>C3 pre</td>
<td>$-3.48 \pm 0.68$</td>
<td>$-1279.10 \pm 187.81$</td>
</tr>
<tr>
<td>post</td>
<td>$-6.14 \pm 0.47^*$</td>
<td>$-1242.30 \pm 160.84$</td>
</tr>
<tr>
<td>C4 pre</td>
<td>$-3.23 \pm 0.48$</td>
<td>$-1341.60 \pm 123.93$</td>
</tr>
<tr>
<td>post</td>
<td>$-5.40 \pm 0.58^*$</td>
<td>$-1390.50 \pm 123.37$</td>
</tr>
<tr>
<td><strong>Negative slope</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fz pre</td>
<td>$-9.82 \pm 1.48$</td>
<td>$-83.30 \pm 16.21$</td>
</tr>
<tr>
<td>post</td>
<td>$-13.32 \pm 1.45^*$</td>
<td>$-69.50 \pm 18.84$</td>
</tr>
<tr>
<td>Cz pre</td>
<td>$-7.16 \pm 0.88$</td>
<td>$-129.50 \pm 24.36$</td>
</tr>
<tr>
<td>post</td>
<td>$-11.66 \pm 0.91^*$</td>
<td>$-102.00 \pm 15.91$</td>
</tr>
<tr>
<td>Pz pre</td>
<td>$-5.10 \pm 0.59$</td>
<td>$-156.50 \pm 36.59$</td>
</tr>
<tr>
<td>post</td>
<td>$-7.80 \pm 0.82^*$</td>
<td>$-118.30 \pm 17.55$</td>
</tr>
<tr>
<td>C3 pre</td>
<td>$-6.41 \pm 0.97$</td>
<td>$-134.90 \pm 28.95$</td>
</tr>
<tr>
<td>post</td>
<td>$-9.23 \pm 0.81^*$</td>
<td>$-107.50 \pm 15.59$</td>
</tr>
<tr>
<td>C4 pre</td>
<td>$-5.30 \pm 0.69$</td>
<td>$-128.20 \pm 24.82$</td>
</tr>
<tr>
<td>post</td>
<td>$-9.08 \pm 1.27^*$</td>
<td>$-102.10 \pm 23.13$</td>
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<tr>
<td><strong>Motor potentials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fz pre</td>
<td>$-8.98 \pm 1.41$</td>
<td>$71.90 \pm 14.82$</td>
</tr>
<tr>
<td>post</td>
<td>$-14.73 \pm 1.70^*$</td>
<td>$72.00 \pm 14.00$</td>
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<tr>
<td>Cz pre</td>
<td>$-6.37 \pm 0.89$</td>
<td>$6.50 \pm 8.56$</td>
</tr>
<tr>
<td>post</td>
<td>$-11.76 \pm 0.82^*$</td>
<td>$2.90 \pm 15.19$</td>
</tr>
<tr>
<td>Pz pre</td>
<td>$-4.81 \pm 0.51$</td>
<td>$-7.80 \pm 11.67$</td>
</tr>
<tr>
<td>post</td>
<td>$-8.07 \pm 0.79^*$</td>
<td>$-17.90 \pm 15.96$</td>
</tr>
<tr>
<td>C3 pre</td>
<td>$-6.34 \pm 1.30$</td>
<td>$16.10 \pm 13.72$</td>
</tr>
<tr>
<td>post</td>
<td>$-9.94 \pm 0.93^*$</td>
<td>$26.50 \pm 13.82$</td>
</tr>
<tr>
<td>C4 pre</td>
<td>$-4.53 \pm 0.59$</td>
<td>$20.30 \pm 20.99$</td>
</tr>
<tr>
<td>post</td>
<td>$-8.84 \pm 1.31^*$</td>
<td>$21.60 \pm 19.66$</td>
</tr>
<tr>
<td>$P + 300$ Fz pre</td>
<td>$7.63 \pm 3.32$</td>
<td>$329.80 \pm 27.93$</td>
</tr>
<tr>
<td>post</td>
<td>$7.88 \pm 3.57$</td>
<td>$377.80 \pm 22.05$</td>
</tr>
<tr>
<td>Cz pre</td>
<td>$8.06 \pm 1.65$</td>
<td>$334.80 \pm 22.95$</td>
</tr>
<tr>
<td>post</td>
<td>$5.25 \pm 2.20^*$</td>
<td>$360.30 \pm 21.04$</td>
</tr>
<tr>
<td>Pz pre</td>
<td>$4.98 \pm 1.26$</td>
<td>$335.80 \pm 27.94$</td>
</tr>
<tr>
<td>post</td>
<td>$4.72 \pm 1.57$</td>
<td>$332.00 \pm 29.46$</td>
</tr>
<tr>
<td>C3 pre</td>
<td>$6.72 \pm 2.26$</td>
<td>$338.60 \pm 23.49$</td>
</tr>
<tr>
<td>post</td>
<td>$7.40 \pm 2.23$</td>
<td>$358.30 \pm 25.42$</td>
</tr>
<tr>
<td>C4 pre</td>
<td>$5.64 \pm 1.40$</td>
<td>$306.50 \pm 19.13$</td>
</tr>
<tr>
<td>post</td>
<td>$2.70 \pm 1.71^*$</td>
<td>$355.40 \pm 25.56$</td>
</tr>
</tbody>
</table>

Means $\pm$ SE. MRCP, movement-related cortical potential. *Significant difference compared with pre.
that influence neuronal synaptic transmission. Compared with rMT, MEP recruitment curves assess neurons that are intrinsically less excitable or spatially further from the center of activation by TMS (Hallett 1999). Many previous reports have shown that afferent input such as electrical nerve stimulation (Schabrun et al. 2012; Thompson et al. 2011), electrical skin stimulation (Golaszewski et al. 2012; Golaszewski et al. 2010), and muscle vibration (Rosenkranz et al. 2003; Rosenkranz and Rothwell 2003) increases MEP recruitment curves. Some of these increases are considered due to increased excitability of spinal mechanisms (Claus et al. 1988a; b). In addition, recent studies comparing the effect of afferent input on responses to TMS have suggested that peripheral electrical and vibration stimulation influence the excitability of cortical mechanisms (Kossev et al. 1999; Rosenkranz et al. 2003; Rosenkranz and Rothwell 2003). Although we cannot exclude spinal contributions to these results, our data on SICI, ICF, and MRCP are compatible with additional action on the MI.

As the paired-pulse technique gives access to the motor cortex independently of spinal or peripheral mechanisms, it allows evaluation of the intracortical circuits. There is good evidence that the interaction between a subthreshold conditioning stimulus and a suprathreshold test stimulus at short ISIs (1–5 ms) relies on the activation of γ-aminobutyric acid particularly GABAA circuits in the motor cortex (Hanajima et al. 1998; Ziemann et al. 1996a; b). The circuit underlying intracortical facilitation is less well understood, and considered mediated by glutamate (Liepert et al. 1997). Moreover, the downregulation of inhibitory neural circuits appears to play a critical role in strengthening excitatory synapses (Hess and Donoghue 1994). Therefore, our findings suggest that whole-hand WF has a direct effect on the excitability of the intracortical circuit responsible for SICI and ICF at a cortical level.

Our results show that whole-hand WF increases corticospinal excitability and ICF and decreases SICI, although whole-hand WI alone did not affect corticospinal excitability and intracortical circuits. One explanation for these differential effects is that stimulus intensity in whole-hand WF is sufficient to change corticospinal excitability and intracortical circuits. Golaszewski et al. (2012) explored the modulatory effects of whole-hand electrical stimulation at different stimulus intensities and frequencies and found that whole-hand electrical stimulation at the sensory threshold intensity at 50 Hz increased corticospinal excitability and ICF and decreased SICI, whereas stimulation at the sensory threshold intensity at 2 Hz did not elicit significant excitability changes. In whole-hand WI, the cutaneous input from water was due to hydrostatic pressure alone, which is considered insufficient to elicit changes in excitability. Another explanation for these differential effects is that changes in corticospinal excitability and intracortical circuits might require greater cutaneous input from skin and proprioceptive input from muscle spindles. Rosenkranz and Rothwell (2003) compared the effects of muscle vibration and cutaneous digital nerve stimulation on corticospinal excitability, SICI, and ICF and found that low-amplitude muscle vibration decreased SICI in motor input to that muscle, whereas specific digital nerve stimulation had no effect on SICI in the digital muscle. Based on these results, the authors concluded that the effects on SICI depend on the modality of the afferent sensory stimulus. In particular, the muscle spindle input activated by vibration has a greater effect on the cortical circuits controlling SICI than cutaneous input from digital nerve stimulation. The latter explanation is consistent with the different distribution of cutaneous and proprioceptive afferents to areas of the SI. The input from low-threshold mechanoreceptors and cutaneous receptors primarily reach areas 3b and l (Kaas 1993), whereas muscle vibration produces afferent input that reaches both area 3a (Heath et al. 1976; Hore et al. 1976) and area 4 of the motor cortex directly (Jones and Porter 1980). Thus, muscle spindle input may have a stronger and more selective influence on the motor cortex than cutaneous input. In the present study, the results of the EX 1 shows whole-hand WF might involve not only greater cutaneous input induced by skin movement but also proprioceptive input induced by vibration of FDI muscle. The increased corticospinal and intracortical excitability would be attributed to proprioceptive input from muscle spindle in the FDI muscle. Unfortunately, we were unable to show an optimal stimulation protocol for whole-hand WF; however, our results show that whole-hand WF is beneficial for changing corticospinal excitability and intracortical circuits.

There is some limitations to these results. First, in the EX 1, as the piezosensor was attached to the muscle belly it is possible that these data include both skin and muscle vibration. Second, in EX 2–5, the MEP recordings in FDI and ADM were conducted in separate experiments, and the order of these experiments was not randomized. Therefore, it is possible that there is an order effect for these two conditions with subjects having a modified response to subsequent exposures to the water flow condition. Third, in EX 2 and 3, the interstimulus interval might be too short and lead to hysteresis effects on MEPs recruitment curve (Moller et al. 2009).

Effects on Movement-related Cortical Activity

We investigated whether increased MI excitability induced by whole-hand WF involves the natural motor cortical activation associated with voluntary movement and found that even movement-related preparatory brain activity was modulated by continuous afferent input such as whole-hand WF. However, because accelerometric parameters were not recorded during the finger movement, the possibility that tiny kinematic changes occurred during the production of each movement after whole-hand WF cannot be eliminated.

Using event-related EEG recordings, Rossi et al. (2000) showed that 1 Hz rTMS of the motor cortex can modify premovement cortical activity. In five healthy volunteers, suprathreshold “inhibitory” 1 Hz rTMS of the MI produced a significant decrement of NS’ amplitude. Despite differences in the motor paradigms used, subthreshold “facilitatory” 5 Hz rTMS of the MI increased the amplitude of the late segment of the contingent negative variation (Holler et al. 2006). Murakami et al. (2010) investigated the movement-related cortical magnetic field before and after high-frequency TENS, which decreases the MI excitability (MEP by TMS), showing that high-frequency TENS causes a significant amplitude reduction of MF. Therefore, the observed changes (increases in NS’ and MP) are specific to whole-hand WF because the MI excitability at rest increased only after whole-hand WF (EX 2–5). We considered the negative potentials detected before movement to indicate the overall excitability and inhibitory synaptic activity.
activity required for the sequential planning and execution of voluntary movements.

In particular, the NS' primarily relates to MI activity (Kristeva-Feige et al. 1994; Neshige et al. 1988; Urbano et al. 1996). The peak MP usually occurs immediately before the EMG peak but shortly after EMG onset (Shibasaki and Barrett 1980), and this component localizes to the precentral region contralateral to the side of movement (Neshige and Luders 1988). Therefore, increased NS' and MP after whole-hand WF may reflect increased activity of the cortical areas participating in preparing for voluntary movement and execution. Our results show that whole-hand WF has specific effects on corticospinal excitability and intracortical circuits. In the MI of cats trained to make voluntary forelimb movements, Neafsey et al. (1978) identified the neurons that change their firing rate within 500 ms before movement onset as pyramidal tract neurons located in the medial cortex. Arezzo and Vaughan (1980) also reported this component in humans and monkeys, beginning 100 ms before movement with maximal amplitude overlaying the contralateral precentral gyrus. Therefore, increased NS' and MP amplitudes after whole-hand WF are possibly induced by the motor cortex, although other sites, such as subcortical and spinal, may exist.

The initial component of negative potentials before movement (BP) reflects the preparatory activity of the SMA (Ball et al. 1999; Cui et al. 1999; Shibasaki and Ikeda 1996), occurring 1–1.5 s before EMG onset, reaches its maximum value at the midline precentral-parietal region, and is widely and symmetrically distributed (Shibasaki et al. 1980). Increased BP after whole-hand WF may reflect the increased activity of the SMA in preparation for movement. Naito et al. (1999) investigated whether muscle and skin vibration at 10, 70–80, and 220–240 Hz changes cortical activities in sensorimotor neurons and the SMA and found that vibration at 70 Hz increased regional cerebral blood flow in the SMA. Another PET study has showed that passive finger movement, which induces proprioceptive input, increases cortical activities in the sensorimotor cortex and SMA (Radovanovic et al. 2002). Other studies show that the MI and SMA mediate the processing of afferent input during cyclical passive wrist and elbow movement (Radovanovic et al. 2002; Weiller et al. 1996). On the other hand, Christova et al. (2013) examined whether whole-hand flutter stimulation with a frequency of 25 Hz evoked lasting neuro-modulatory changes within the sensorimotor network during finger tapping and showed no change in SMA activity. This differences with the present study would be attributed to stimulation frequency and motor task as well as stimulation category (flutter stimulation vs. water flow stimulation). Whole-hand WF in the present study induces muscle and skin vibration at ~70 Hz, which is a higher frequency than that in the previous study of Christova et al.(2013). Besides differences in the stimulation frequency, differences in the motor task may account for these effects. In the study of Christova et al., self-paced finger tapping was used as a motor task though whole-hand flutter stimulation was applied to the right-hand palm. Therefore, the increased BP after whole-hand WF may be attributed to skin and muscle vibration at a higher stimulation frequency applied to agonist muscle (EX 1).

Moreover, we showed that whole-hand WF decreased the amplitude of P + 300 elicited from index finger abduction movement. The P + 300 response is a postmovement compo-

**Conclusion**

In this study, we demonstrated that whole-hand WF increased corticospinal excitability, decreased SICI, and increased ICF, although whole-hand WI did not change corticospinal excitability and intracortical circuits. Moreover, whole-hand WF modulated movement-related cortical activity and increased, in particular, the activation of the motor cortex required for the sequential planning and execution of voluntary movements.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: D.S. and A.M. conception and design of research; D.S. and K.Y. performed experiments; D.S. and K.Y. analyzed data; D.S., H.O., and A.M. interpreted results of experiments; D.S. prepared figures; D.S. drafted manuscript; D.S., K.Y., H.O., B.Y., Y.S., and A.M. edited and revised manuscript; D.S., K.Y., H.O., B.Y., Y.S., and A.M. approved final version of manuscript.
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