Effects of lower-limb vibration on intracortical and spinal excitability in healthy subjects

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Abstract

We examined the effects of lower-limb segmental muscle vibration (SMV) on intracortical and spinal excitability in 13 healthy participants (mean age: 34.9 ± 7.8 years, 12 males, 1 female). SMV at 30 Hz was applied to the hamstrings, gastrocnemius, and soleus muscles for 5 min. Paired-pulse protocols were used to investigate motor-evoked potentials (i.e., test MEP), short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF) from the abductor hallucis muscle (AbdH). These assessments were compared to the results of a control experiment (i.e., non-vibration) in the same participants. F-waves were evaluated from the AbdH on the right (vibration side) and left (non-vibration side) sides, and we calculated the ratio of the F-wave amplitude to the M-response amplitude (F/M ratio). These assessments were obtained before, immediately after, and 10, 20, and 30 min after SMV. No change was observed immediately after SMV, but there was a significant decrease in SICI over time (before vs. 30 min after, p = 0.021; immediately after vs. 30 min after, p = 0.015). There were no significant changes in test MEP, SICF, or the F/M ratio. SMV might cause a decrease in SICI over time in the AbdH of healthy subjects.

Introduction

Vibration is a non-invasive technique that enables modulation of the central nervous system (CNS) via peripheral stimulation (Murillo et al. 2014). Vibratory stimulation can be applied as focal muscle vibration (fMV) or whole-body vibration (WBV). The equipment used for fMV is small, portable, and suitable for local stimulation. In contrast, WBV is conducted with the subject standing on a platform. The vibration is mediated through the activation of muscle spindles and transmission by Ia fibers, modulating cortical or spinal excitability. In healthy subjects, at the cortical level, changes in excitability in the primary motor cortex and intracortical regions, and sensorimotor integration have been reported during or after fMV and WBV, respectively (Krause et al. 2016; Lapole et al. 2012, 2015a, b; Mileva et al. 2009; Rosenkranz et al. 2003). At the spinal level, it has been reported that H-reflex (HR) amplitudes are decreased during or after fMV and WBV, respectively (Krause et al. 2016; Lapole et al. 2012; Souron et al. 2019).

Recently, regarding the pathophysiology of spasticity, Li et al. (2021) proposed that "when damages occur to the motor cortex and its descending cortico-reticulo-spinal tract (cortico-RST) after stroke on one hemisphere, dorsolateral or medial cortico-RST excitatory/inhibitory imbalance, eventually, spinal motor neurons and the stretch reflex circuitry are hyperexcitable or may be spontaneously firing". In addition, Afzal et al. (2019) suggested that the increased excitability of the stretch reflex after stroke in comparison with normal subjects may be partially due

to activation of the cortico-RST. However, there are few reports on the relationship between the cortical and spinal excitability in humans after stroke, further verification is needed. A recent review of clinical research found scattered reports on the use of fMV (Mortaza et al. 2019) or WBV (Alashram et al. 2019; Huang et al. 2017) for the treatment of spasticity in CNS disorders. The American Heart Association/American Stroke Association guidelines recommend botulinum toxin, oral antispasticity agents, neuromuscular electrical stimulation, and fMV (Winstein et al. 2016). With regard to upper-limb spasticity in stroke patients, fMV for upper-limb spasticity has been reported to improve the clinical assessment (i.e., muscle tone and motor function), and decrease F-wave parameters [i.e., F-wave amplitude and ratio of F-wave amplitude to the M-response amplitude (F/M ratio)] (Noma et al. 2009, 2012). These changes remained until at least 30 min after the end of fMV, indicating an acute effect of fMV. fMV for 30 min × 3 days for upper-limb spasticity has been reported to improve the clinical assessment (i.e., muscle tone and motor function), and increase short-interval intracortical inhibition (SICI) (Marconi et al. 2011). These changes remained until at least two weeks after the end of fMV, indicating a long-term effect.

On the other hand, clinical studies on the effects of fMV on lower limb spasticity in stroke patients have not been reported (Celletti et al. 2020). While several clinical effects of WBV on lower-limb spasticity in stroke patients have been reported (Brogardh et al. 2012; Chan et al. 2012; Huang et al. 2017; Pang et al. 2013; Park et al. 2018; Tankisheva et al. 2014), the detailed mechanism of action, including effects at the cortical and spinal levels, is

unclear. Although WBV has been performed with the subject standing on a platform, some stroke patients could not stand on a platform without support due to standing instability (Chan et al. 2012). Therefore, we developed a new vibration method (which uses a WBV platform) to deliver vibrations directly to the spastic muscles of a hemiplegic lower limb for 5 min in stroke patients (i.e., vibration stimulation of the "segmental" muscle of the lower limbs in the sitting position, not the whole body in the standing position) (Miyara et al. 2014). We reported an improvement in muscle tone and range of motion with segmental muscle vibration (SMV), as well as a decrease in F-wave parameters (Miyara et al. 2018) and an increase in bilateral sensory motor cortical activation during voluntary ankle dorsiflexion of the affected limb using functional near-infrared spectroscopy (fNIRS) (Miyara et al. 2020). However, fNIRS only captures changes in cerebral blood flow, and the specific role of the cortical excitability with SMV in reducing lower-limb spasticity was unclear.

In the present study, we aimed to examine the temporal effects of SMV, which is useful in reducing spasticity in stroke patients, on intracortical and spinal excitability in the lower limbs of healthy subjects as a preliminary step in investigating the mechanism of reduced spasticity in stroke patients. In this study, paired-pulse transcranial magnetic stimulation (TMS) and F-wave were used to investigate cortical and spinal excitability with SMV. Paired-pulse TMS allows for the evaluation of SICI (Kujirai et al. 1993) and short-interval intracortical facilitation (SICF) (Tokimura et al. 1996). With regard to the timing of the assessment, few previous studies have examined temporal

effects immediately after the vibration intervention (Celletti et al. 2020). In this study, based on the results of previous studies on F-wave (Miyara et al. 2018; Noma et al. 2009, 2012), we evaluated the temporal effects up to 30 min after SMV.

Materials and methods

Participants

Thirteen healthy participants (mean age: 34.9 ± 7.8 years, 12 males, 1 female) participated in this study. The exclusion criteria were metal in the body, a history of neurological disorders, including epilepsy, pregnancy or the possibility of pregnancy, and medical management problems during vibration stimulation. Informed consent for study participation was obtained from each participant prior to study commencement. Experimental procedures were approved by the ethics committee of Kagoshima University (No. 190042) and were consistent with the Declaration of Helsinki guidelines.

Experimental paradigm

We used a comparative before-and-after intervention trial. The experimental protocol is shown in Fig. 1. All participants received the vibration on the right lower limb for 5 min (Fig. 2b). The paired-pulse TMS (vibrated side)

104 and F-wave (both sides) were assessed before, immediately after (Post0), and 10 (Post10), 20 (Post20), and 30 min
105 (Post30) after the vibration intervention. Similarly, the paired-pulse TMS assessments were performed in a control
106 experiment (i.e., non-vibration) in the same participants. The paired-pulse TMS (vibration, non-vibration) and F-wave
107 (both sides recording) evaluations were performed on different days (Fig. 1). During the evaluation sessions,
108 participants remained seated on a chair with a backrest in a relaxed position with 80° hip flexion, 80° knee flexion,
109 10° ankle plantar flexion and their feet on the floor (Fig. 2c).
110
111 **SMV**
112 SMV was delivered via a vibrating platform (Powerplate®, Performance Health Systems UK Ltd., UK) (Fig. 2a).
113 During the intervention, each participant sat with the hip joints flexed at approximately 90° and with the knee joints
114 extended at 0° (Fig. 2b) (Miyara et al. 2014, 2018, 2020). In the previous study (Miyara et al. 2014, 2018, 2020), SMV
115 was applied to both lower limbs. In contrast, in the present study, SMV was performed on the unilateral lower limb to
116 exclude the effect of vibration on the contralateral lower limb. The lower limb on the non-vibrating side avoided
117 contact with the vibrating platform. SMV was applied at 30 Hz (4-8 mm amplitude) to the right hamstring,
118 gastrocnemius, and soleus muscles for 5 min. On the other hand, for the control experiment (i.e., non-vibration), the
119 same posture was maintained for 5 min without SMV. The frequency and intervention time were based on our previous

SMV studies that confirmed their effectiveness in stroke patients (Miyara et al. 2014, 2018, 2020). The frequency was set to 30 Hz, which has been shown to be effective in previous studies of WBV on lower-limb spasticity in stroke patients (Pang et al. 2013). The intervention time was set at 5 min, which has been shown to be effective in previous studies of fMV on upper-limb spasticity in stroke patients (Noma et al. 2009, 2012).

Assessment

Electromyogram (EMG) recording

Electromyographic activity was recorded using silver–silver chloride electrodes positioned in a belly-tendon montage on the skin overlying the abductor hallucis muscle (AbdH). The skin area was rubbed with alcohol before the electrode was applied, and skin resistance was kept below 5kΩ. The signal was amplified and filtered (20–5000 Hz) for on-line analysis (Neuropack MEB-2200; Nihon Kohden, Tokyo).

Transcranial magnetic stimulation (TMS)

We recorded motor evoked potentials (MEP) from the right AbdH. As the target muscle, we first tried the soleus muscle, which is a common spastic muscle in the lower limb. However, we could not record stable motor evoked potentials (MEP) in the soleus muscle in many subjects, because of a high threshold. Therefore, we chose the AbdH,

from which MEPs can be recorded for a relatively low threshold. The AbdH has the same tibial innervation as the gastrocnemius muscle, and could be considered to be a vibrated muscle because it was visibly shaking during vibratory stimulation. For these reasons, the AbdH seems to be an appropriate target muscle. A Magstim 200 stimulator (Magstim Co., Dyfed, UK) with a double cone coil was used for transcranial magnetic stimulation. The coil was placed tangentially over the motor cortex at the optimal site for the right AbdH. This was defined as the location where stimulation at a slightly suprathreshold intensity elicited the largest MEP in the AbdH. The coil was positioned over the leg area of the motor cortex along the nasal-inion axis to induce a postero-anterior current. This position was marked on the scalp and used throughout the experiment. The resting motor threshold (rMT) was defined as the lowest stimulus output capable of producing MEPs with peak-to-peak amplitudes greater than 50μV in more than 50% of the 10 trials (Rossini et al. 2015). The intensity of the test stimulus was set to evoke MEP of approximately 1.0 mV peak-to-peak and MEP in the relaxed muscle was measured as an index of corticospinal excitability (i.e., test MEP).

Intracortical excitability

Paired-pulse TMS was used to assess intracortical excitability, with inter-stimulus intervals (ISI) of 1.5 and 3 ms (Tokimura et al. 1996; Kujirai et al. 1993). The ISI of SICI was set to 3ms. A subthreshold conditioning stimulus precedes a suprathreshold test stimulus. The ISI of SICF was set to 1.5ms. A suprathreshold conditioning stimulus

precedes a subthreshold test stimulus. SICF is mediated by the recruitment of interneuronal circuits generating later I waves that are generated via interneurons in the motor cortex (Higashihara et al. 2020). There seems to be no relationship between SICF and ICF (Wagle-Shukla A et al. 2009; Van den Bos MAJ et al. 2018). We chose SICI and SICF as our assessment items, because SICI and SICF appear to be mediated by the recruitment of I waves that are generated via interneurons in the motor cortex (Higashihara et al. 2020). The intensity of the test stimulus for SICI and the conditioning stimulus for SICF was set to evoke MEP of approximately 1.0 mV peak-to-peak. The conditioning stimulus for SICI and the test stimulus for SICF were sub-threshold and set at 80 % of the active motor threshold (aMT). For aMT, a minimal response of 200μV was necessary in 50% of all trials (Ridding et al. 1995) while the participant performed isometric toe flexion. In the relaxed muscle, under the above three conditions (i.e., test MEP, SICF and SICI), the inter-stimulus interval was randomly determined at a frequency of about once every 4-6 seconds, and 12 stimuli were delivered under each condition. The control experiment (i.e., non-vibration) was conducted with the same setup. We calculated the amplitudes of SICI and SICF as the average conditioned MEP amplitude, expressed as a percentage of the average unconditioned MEP amplitude.

F-wave

A one-channel recording from the AbdH allowed comparison of evoked compound muscle action potentials (CMAPs)

and F-waves in the right (vibration side) and left (non-vibration side) lower limbs to evaluate the effect of SMV. A Nihon-Kohden Neuropack system (Nihon Kohden Co. Ltd., Tokyo) was used with a band-pass filter of 20 Hz to 5 kHz, with the sensitivity set at 5 mV and 500μV/division, respectively. Paired Ag–AgCl surface electrodes were taped to the belly and tendon of the AbdH. The tibial nerve was stimulated at 1 Hz from the medial malleolus. Stimuli were 0.2 ms in duration and ranged from 16 to 25 mA, at an intensity 20% higher than that at which the largest CMAPs could be elicited (Eisen and Odusote 1979; Miranov 1992). In total, 112 F-waves were recorded following supramaximal percutaneous electrostimulation for each session. Peak-to-peak measurements were made of the M-response amplitude and the 112 averaged F-wave amplitudes for each lower limb. We calculated the ratio of F-wave amplitude to the M-response amplitude (F/M ratio).

Statistical analyses

To confirm the differences of each condition (rMT, aMT, intensity of the test stimulus and conditioning stimulus) of between conditions (SMV vs. non-vibration), a paired t-test was performed.

To analyze test MEP, SICI and SICF, a two-factor repeated measures analysis of variance (ANOVA) was used to analyze the effects of intervention (SMV vs. non-vibration) and time (before, immediately after, 10, 20, and 30 min after). Post hoc comparisons were conducted with a paired t-test using a criterion of $p < 0.05$ with Bonferroni's

correction for multiple comparisons. Between-time differences in the test MEP, SICI and SICF (SMV vs. non-vibration) were analyzed using the paired t-test.

To analyze the F/M ratio, two-factor repeated measures ANOVA was used to analyze the effects of condition (vibration side vs. non-vibration side) and time (before, immediately after, 10, 20, and 30 min after). Between-time differences in the F/M ratio (vibration side vs. non-vibration side) were analyzed using the paired t-test. The results are presented as the mean ± standard deviation of the mean (SD). Data were analyzed using IBM SPSS Statistics ver. 27.0. $P < .05$ indicated statistical significance.

Results

None of the participants experienced discomfort before, during, or after SMV intervention. The paired-pulse TMS outcomes were analyzed in all 13 participants. Due to pain and discomfort caused by the electrical stimulation in two participants, F-wave outcomes were analyzed in 11 participants. Prior to undertaking paired-pulse TMS studies, rMT and aMT were assessed. In SMV, the rMT was 46.5 ± 5.3 % (mean ± standard deviation) and aMT was 38.4 ± 4.0 % as a percentage of the maximum stimulator output. The intensity of the test stimulus was 55.2 ± 8.3 %, and the intensity of the conditioning stimulus was 32.9 ± 4.9 %. In non-vibration, the rMT was 44.8 ± 6.0 % (mean ± standard deviation) and aMT was 36.0 ± 5.9 % as a percentage of the maximum stimulator output. The intensity of the test stimulus was

52.2 ± 6.3 %, and the intensity of the conditioning stimulus was 31.0 ± 5.2 %. Between differences in each condition (SMV vs. non-vibration) showed significant (rMT, $p = 0.098$; aMT, $p = 0.014$; the intensity of the test stimulus, $p = 0.016$; the intensity of the conditioning stimulus, $p = 0.023$).

Temporal changes in the test MEP amplitudes

Two-factor repeated measures ANOVA showed no significant interaction (intervention × time) ($F_{4,48} = 0.631$, $p = 0.643$) or main effect of intervention ($F_{1,12} = 0.873$, $p = 0.368$) in the test MEP amplitudes. There were significant main effects of time ($F_{4,48} = 3.861$, $p = 0.008$). Between-time differences in the test MEP (SMV vs. non-vibration) were not significant (see Fig. 3).

Intracortical excitability

Temporal changes in SICI

Fig. 4a shows the change in SICI after SMV intervention. Two-factor repeated measures ANOVA showed significant interaction (intervention × time) ($F_{4,48} = 2.717$, $p = 0.040$), main effect of intervention ($F_{1,12} = 16.358$, $p = 0.002$) and time ($F_{4,48} = 4.351$, $p = 0.004$). Post hoc testing showed a significant decrease in SICI in SMV (Before vs. Post30, $p = 0.021$; immediately after vs. Post30, $p = 0.015$). Between-time differences in SICI (SMV vs. non-vibration) were

significant (Post10, $p = 0.015$; Post20, $p < 0.001$; Post30, $p = 0.012$).

Temporal changes in SICF

Fig. 4b shows the change in SICF after SMV intervention. Two-factor repeated measures ANOVA showed no significant interaction (intervention × time) ($F_{4,48} = 0.311$, $p = 0.869$), main effect of intervention ($F_{1,12} = 2.007$, $p = 0.182$) or time ($F_{4,48} = 2.421$, $p = 0.061$). Between-time differences in SICF (SMV vs. non-vibration) were not significant.

Temporal changes in the F/M ratio

Fig. 5 shows the change in the F/M ratio after SMV intervention. Two-factor repeated measures ANOVA showed no significant interaction (condition × time) ($F_{4,40} = 0.129$, $p = 0.971$), main effect of condition ($F_{1,10} = 2.677$, $p = 0.133$) or time ($F_{4,40} = 1.437$, $p = 0.240$). Between-time differences in the F/M ratio (vibration side vs. non-vibration side) were not significant ($n = 11$).

Discussion

In the present study, there was no significant change immediately after 5 min of SMV. However, over time, there was

a trend toward a decrease in SICI at the cortical level and a significant decrease was seen 30 min after SMV. In contrast, no significant changes in test MEP amplitudes or SICF were observed. This result regarding the test MEP amplitudes indicated that the SMV intervention had no confirmed effect on corticospinal excitability. At the spinal level, there was no significant difference in the F/M ratio. This is the first study to examine the temporal changes in intracortical and spinal excitability induced by SMV for the unilateral lower limbs in healthy subjects.

Effects of SMV on test MEP, SICI and SICF

For test MEP, there were significant main effects of time. This may have been due to body movements when subjects placed their foot on the platform of the vibration device. This may not affect the main results of the present study.

The present results regarding SICI show that SMV has a direct effect on intracortical circuits (Before vs. Post30, $p = 0.021$; immediately after vs. Post30, $p = 0.015$). In addition, there was no significant change in non-vibration, and a clear difference was observed in the group comparison with SMV. In contrast, there was no significant change in SICF. Previous studies on fMV that applied 80 Hz vibration to the upper limbs in healthy subjects showed a significant increase in MEP amplitude and a decrease in SICI in vibrated muscles (Rosenkranz et al. 2003) and a significant decrease in MEP amplitude and an increase in SICI in non-vibrated muscles (Rosenkranz and Rothwell, 2003), which suggested a specific effect during vibration. Christova et al. (2011) reported that 25 Hz whole-hand vibration for 20

248 min in healthy subjects produced a significant increase in MEP recruitment curves, and a significant decrease in SICI
249 until 1 hour after vibration. In a report on fMV of the lower limb in healthy subjects, 50 Hz vibration of the Achilles
250 tendon produced a significant increase in maximal MEP amplitude, but had no effect on SICI during vibration. The
251 authors suggested that the vibration-induced increase in corticospinal excitability in the soleus muscle is not mediated
252 by changes in SICI, in contrast to the reports in the upper limbs (Lapole et al. 2015b). On the other hand, in a report
253 during WBV, Mileva et al. (2009) reported that the tibialis anterior muscle (TA) MEP amplitude and SICI were
254 significantly increased, while the soleus muscle MEP amplitude was unchanged. The authors stated that the differences
255 between the results for the two muscles could be explained by functional differences (dorsiflexion versus plantar
256 flexion) and/or by differences in the strength of corticospinal projections to motor neurons (Perez et al. 2004).

257 The present study differs from previous studies in several methodological respects [e.g., method of vibration
258 application (focal or segmental or whole-limb, standing), frequency, time of intervention, target muscle (in this study,
259 remote muscles were evaluated instead of the vibrated muscles), and time of evaluation (during or after vibration)].
260 Our results were similar to those reported for the upper limb (decrease in SICI) (Christova et al. 2011), since we
261 observed a significant decrease in SICI at 30 min after SMV.

262 The temporal relationship between the process of vibration and changes in SICI remains unclear. For a trend similar
263 to that seen in our results, Christova et al. (2011) suggested that long-term potentiation (LTP) (i.e., changes in synaptic

efficacy) may play a role in outlasting effects. In addition, although the types of peripheral stimulation are different, Golaszewski et al. (2010) reported a significant decrease in SICI using whole-hand electrical stimulation 1 hour after stimulation, but not immediately after. While the reason for the delayed excitability potentiation is not clear, Golaszewski et al. (2010) stated that it may be functional evidence of intracortical synaptic reorganization. They suggested that the neural basis for these long-lasting effects might involve a LTP mechanism. In animal experiments using cats, Kaneko et al. (1994) confirmed that somatosensory inputs from area 2 to layer II/III pyramidal cells in the motor cortex are transmitted to layer V pyramidal cells, including Betz cells, and to adjacent layer II/III pyramidal cells. They suggested that certain areas of the somatosensory cortex produce long-term changes in the activity of certain output cell groups in the motor cortex. In this study, similar to previous studies (Christova et al. 2011; Golaszewski et al. 2010; Kaneko et al. 1994), the involvement of LTP was suggested as a hypothesis to explain why SICI decreased significantly over time.

Effects of SMV on F-wave parameters

No significant changes in the F/M ratio were observed for 30 min after WBV. F-wave parameters are more sensitive to changes in lower motor neuron excitability associated with spasticity than T and H-reflexes (Miranov 1992). The F-wave amplitude and the F/M ratio are correlated with motor neuron excitability, and are increased in spastic patients

(Eisen and Odusote 1979; Fisher 1988). For these reasons, we have used F-waves to assess spinal-level excitability in lower-limb spasticity in stroke patients (Miyara et al. 2018), and we used the same assessment in the present study. In addition, Lin et al. (2004) concluded that a sample size of 50-75 F-waves is needed to approximate the amplitude and area results of 100 F-waves with 25% accuracy. In the current study, F-waves were measured 112 times, suggesting that this was a reasonable sample size.

The present results are consistent with those reported by Espiritu and Lapole et al. (Espiritu et al. 2003; Lapole et al. 2012). Espiritu et al. (2003) reported that 50 Hz vibration of TA and abductor pollicis brevis muscle (APB) for 10 min induced long-lasting depression of the HR, but no significant change in F-wave parameters (area and latency). The authors suggested that F-waves provide a flawed measure of the excitability of the motoneuron pool. Lapole et al. (2012) reported that 50 Hz vibration of the Achilles tendon for 1 h induced acute depression of the HR amplitudes, but no significant change in F-wave parameters (persistence and amplitude). Based on these results, they suggested that a presynaptic inhibitory mechanism is primarily involved and is not affected by motor neuron excitability.

Several other authors have examined the effects of fMV at the spinal level using HR. Rocchi et al. (2018) applied 100 Hz vibrations to the flexor carpi radialis (FCR), biceps brachialis, APB, and extensor carpi radialis for 30 min. As a result, fMV applied over the FCR induced a long-term decrease in HR without modifying the three phases of reciprocal inhibition (RI). On the other hand, Souron et al. (2019) used thoracic motor evoked potentials (TMEP) as

296 the most direct way to test motor neuron excitability in response to synaptic inputs as a more reliable approach than
297 F-waves (McNeil et al. 2011). They concluded that presynaptic mechanisms are not involved in the depression of
298 spinal excitability after local vibration (100 Hz, 30 min for the Achilles tendon). They suggested that depressed spinal
299 excitability relies on postsynaptic changes with potentially decreased motoneuron excitability. We did not record the
300 HR as an indicator of spinal cord excitability because it was difficult to record the HR from the target AbdH. It may
301 be necessary to record the HR from the soleus to investigate the effect of vibratory stimulation on the spinal motor
302 neuron pool in a future study.
303
304 **Implications for clinical studies**
305 SICI is a GABAA receptor-mediated inhibition (Ziemann et al. 1996). The inhibitory neurotransmitter GABA may
306 play an important role in cortical plasticity. In animal studies in adult rats, changes in GABA activity may initiate the
307 functional recovery seen after stroke-induced brain injury (Jacobs and Donoghue, 1991). In upper-limb spasticity in
308 chronic stroke patients, a decrease in SICI is associated with greater motor impairment and worse dexterity (Ding et
309 al. 2019). In a clinical study using fMV, an association between an improvement of spasticity and an increase in SICI
310 has been reported. In randomized controlled trials using vibration, the addition of vibration to physiotherapy (Marconi
311 et al. 2011) and to robotic rehabilitation (Calabrò et al. 2017) has been shown to be superior for improving spasticity

and motor function, and increasing SICI. Calabrò et al. (2017) suggested that the improvement of spasticity might depend on modulation of the motor cortex and spinal cord excitability (i.e., increased inhibitory output from the motor cortex to the spinal cord level, as suggested by increased SICI and a decreased H/M ratio).

On the other hand, we have not found any reports on the relationship between clinical assessment and the cortical or spinal level in stroke lower-limb spasticity. Our findings in the present study confirmed a delayed decrease of SICI under the application of SMV to a lower limb for 5 min in healthy subjects. This means that the application of SMV to the lower limbs in healthy subjects can alter intracortical inhibition. SMV may also alter SICI in stroke patients, because SMV has been proven to reduce spasticity of the paralyzed lower limb in stroke patients (Miyara et al. 2014, 2018, 2020). Since there are reports of reduced spasticity and increased SICI (Calabro et al. 2017; Marconi et al. 2011), SMV may increase SICI as an acute and cumulative effect. Future studies of the effect of SMV on SICI in the lower limbs of hemiplegic patients will help to elucidate the mechanisms of functional recovery and reduction of spasticity, and lead to the development of new treatments.

Limitations

This study has some limitations. First, there are limitations in the design of the study, which had a small sample size. Increasing the sample size will be needed to clarify our findings. Second, we chose AbdH as the target muscle based

on the trend in preliminary experiments (i.e., more stable MEPs were observed in AbdH than in soleus muscle). However, in the case of AbdH, there is no direct exposure to the vibratory stimulus, and we cannot deny the possibility that this influenced the results. Third, we could not calculate MEP/M ratio and therefore could not assess the MEP accurately because we did not measure the amplitude of the M-wave amplitudes with our MEP assessment. Fourth, this study included only healthy subjects. The relationship between the decrease in SICI in stroke-related hemiplegic legs and improved spasticity and motor function requires further investigation. To verify the effectiveness of this SMV method, clinical studies on stroke patients are required.

Conclusions

In this study, we investigated the effects of SMV on intracortical and spinal excitability in the unilateral lower limbs of healthy subjects. SMV did not cause effects at the spinal level, but SICI tended to decrease at the cortical level and significantly decreased after 30 min of SMV. The results of this study indicate that the application of SMV to the lower limb can have an important effect on changes in intracortical excitability, and may provide basic knowledge for future clinical studies. Future studies in larger study populations or stroke patients will be needed to apply these findings to clinical practice.

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Author contributions

KM and SE conceived and designed the experiments. KM, SE, KK, AM and TK collected the data, and KM, SE and AM analyzed the data. KM wrote the manuscript, and SE, KK, AO and MS reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Availability of data and material

Data are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The experimental procedures were approved by the local ethics committee and were consistent with the Declaration of Helsinki guidelines.

Informed consent

Informed consent for study participation was obtained from each participant prior to study commencement.

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493 **Figure captions**

494 **Fig. 1** Experimental protocol

495 Paired-pulse transcranial magnetic stimulation (TMS) was used to assess the motor evoked potential (MEP) from the

496 right abductor hallucis muscle (AbdH). We assessed the short-interval intracortical inhibition (SICI) at an inter-

497 stimulus intervals (ISI) of 3 ms and short-interval intracortical facilitation (SICF) at an ISI of 1.5 ms as an index of

498 intracortical excitability. F-wave was used to assess the compound muscle action potentials (CMAPs) and F-waves

499 from the AbdH in the right (vibration side) and left (non-vibration side) lower limbs. The time course was set to be

500 before, immediately after (Post0), and 10 min (Post10), 20 min (Post20), and 30 min (Post30) after segmental muscle

501 vibration (SMV). The control experiment (i.e., non-vibration) was conducted with the same participants: (a) (b) (c)

502 were performed on separate days

503

504 **Fig. 2** Segmental muscle vibration (SMV) intervention and paired-pulse transcranial magnetic stimulation (TMS)

505 evaluation

506 (**a**) Vibration device. (**b**) Posture while using 5 min SMV to the right hamstring, gastrocnemius, and soleus muscles.

507 (**c**) Paired-pulse transcranial magnetic stimulation (TMS) evaluation

508

Fig. 3 Temporal changes in the test motor-evoked potential (MEP) amplitudes

The MEP amplitudes were unchanged after segmental muscle vibration (SMV). The black (dotted) line and markers (circle, square) represent the mean data of all participants (n = 13). Values indicate mean ± standard deviation of the mean (SD).

Fig. 4 Temporal changes in short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF)

(**a**) Changes in SICI. The SICI showed a decreasing trend with time after segmental muscle vibration (SMV). (**b**) Changes in SICF. SICF were unchanged after SMV. The black (dotted) line and markers (circle, square) represent the mean data of all participants (n = 13). Values indicate mean ± SD. *Significant at $p < 0.05$. ††Significant differences between SMV and non-vibration are indicated at $p < 0.01$. †Significant at $p < 0.05$

Fig. 5 Temporal changes in the ratio of the F-wave amplitude to the M-response amplitude (F/M ratio)

The F/M ratios on the vibration side and non-vibration side were unchanged after segmental muscle vibration (SMV). The black (dotted) line and markers (circle, square) represent the mean ± SD of all participants (n = 11)