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RESEARCH ARTICLE

F-waves induced by motor point stimulation are facilitated during handgrip and motor imagery tasks

Naotsugu Kaneko[1,4,5] · Atsushi Sasaki[2,3] · Kai Lon Fok[4,5] · Hikaru Yokoyama[6] · Kimitaka Nakazawa[1] · Kei Masani[4,5]

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Abstract
The F-wave is a motor response elicited via the antidromic firings of motor nerves by the electrical stimulation of peripheral nerves, which reflects the motoneuron pool excitability. However, the F-wave generally has low robustness i.e., low persistence and small amplitude. We recently found that motor point stimulation (MPS), which provides the muscle belly with electrical stimulation, shows different neural responses compared to nerve stimulation, e.g., MPS elicits F-waves more robustly than nerve stimulation. Here, we investigated whether F-waves induced by MPS can identify changes in motoneuron pool excitability during handgrip and motor imagery. Twelve participants participated in the present study. We applied MPS on their soleus muscle and recorded F-waves during eyes-open (EO), eyes-closed (EC), handgrip (HG), and motor imagery (MI) conditions. In the EO and EC conditions, participants relaxed with their eyes open and closed, respectively. In the HG, participants matched the handgrip force level to 30% of the maximum voluntary force with visual feedback. In the MI, they performed kinesthetic MI of plantarflexion at the maximal strength with closed eyes. In the HG and MI, the amplitudes of the F-waves induced by MPS were increased compared with those in the EO and EC, respectively. These results indicate that the motoneuron pool excitability was facilitated during the HG and MI conditions, consistent with findings in previous studies. Our findings suggest that F-waves elicited by MPS can be a good tool in human neurophysiology to assess the motoneuron pool excitability during cognitive and motor tasks.

Keywords Motor point stimulation · F-wave · Motoneuron pool excitability · Handgrip · Motor imagery

Introduction

As the final common pathway, spinal motoneurons play an essential role in the control of human physical movement. Late latency motor responses such as F-waves and H-reflexes elicited by transcutaneous electrical stimulation applied to peripheral nerves can be used to investigate the excitability of the spinal motoneuron pool (i.e., motoneuron pool excitability). Specifically, the sum of the membrane properties of the spinal motoneurons in the motoneuron pool likely reflects the net synaptic inputs to the motoneurons. F-waves are evoked by the antidromic firing of motor neurons, i.e., activation of α-motoneurons via impulses that propagate along the motor axons in an antidromic manner (Fisher 1992; Mercuri et al. 1996; Panayiotopoulos and Chroni 1996; Mesrati and Vecchierini 2004). H-reflexes, on the other hand, are elicited by afferent conduction in Ia afferent fibers and subsequent efferent conduction in α-motoneurons (Misiaszek 2003). Both these motor responses are generally

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✉ Kei Masani
k.masani@utoronto.ca

1 Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro-ku, Tokyo 153-8902, Japan

2 Japan Society for the Promotion of Science, Tokyo 102-0083, Japan

3 Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Suita, Osaka 560-8531, Japan

4 Institute of Biomedical Engineering, University of Toronto, Toronto, ON M5S 3G9, Canada

5 Motion & Adaptation Science Laboratory, Lyndhurst Centre, KITE Research Institute – University Health Network, 520 Sutherland Drive, Toronto, ON M4G 3V9, Canada

6 Institute of Engineering, Tokyo University of Agriculture and Technology, Koganei-shi, Tokyo 184-8588, Japan

used to investigate motoneuron pool excitability, although different neural mechanisms generate them.

Methodologically, H-reflexes are generally robust, evoked by electrical stimulation with submaximal intensity, and rarely painful (McNeil et al. 2013). F-waves, on the other hand, are used infrequently compared to H-reflexes because they are generally lower in amplitude and persistence, i.e., less robust compared to H-reflex, and require the use of a supramaximal electrical stimulation, which is significantly painful (Mesrati and Vecchierini 2004; McNeil et al. 2013). However, F-waves are more sensitive to the motoneuron pool excitability than the H-reflexes (Milanov 1992) since the H-reflex involves synaptic activities in the loop, while F-waves are independent from them (McNeil et al. 2013). This is because the changes in H-reflexes involve modulation of the motoneuron pool excitability as well as synaptic activities such as post-activation depression and presynaptic inhibition, which are independent to the motoneuron pool excitability (Misiaszek 2003; Pierrot-Deseilligny and Burke 2012). Hence, although the H-reflex is a powerful tool frequently used for investigating neural mechanisms in the spinal cord, such as post-activation depression and presynaptic inhibition, a more reliable method of robustly eliciting F-waves is desirable to investigate the modulation of the motoneuron pool excitability.

The transcutaneous placement of the stimulus electrodes on mixed peripheral nerve branches to elicit F-waves and H-reflexes is referred to as peripheral nerve stimulation (PNS) in the present study. In many human neurophysiological studies, PNS is the most well-known technique to evoke compound action potentials like H-reflexes and F-waves. Another method of activating peripheral nerves is transcutaneous electrical stimulation applied on the muscle belly at the most responsive locations for inducing muscle activation, called the motor point, i.e., motor point stimulation (MPS). MPS is commonly used in clinical studies and neurorehabilitation but less often in human neurophysiological experiments. MPS induces orthodromic activation of motor nerves and evokes a muscular response termed the M-wave (Farina et al. 2004; Bickel et al. 2011; Maffiuletti et al. 2011). Simultaneously, MPS, as well as PNS, evokes an antidromic activation parallel to the α-motoneurons, which in turn activates the spinal motoneuron and then elicits F-waves. In our recent studies, it has been demonstrated that MPS elicit antidromic firing that activates the motoneuron cell bodies (Fok et al. 2020), and that MPS induces F-waves (Nakagawa et al. 2020; Kaneko et al. 2022).

The changes in the motoneuron pool excitability can be assessed by measuring the F-wave amplitude and persistence (Dengler et al. 1992; Rivner 2008). Previous studies in literature have used F-waves induced by PNS to investigate the changes in the motoneuron pool excitability during muscle contraction and motor imagery (MI) tasks (Taniguchi et al. 2008; Fujisawa et al. 2011; Takemi et al. 2015; Yamazaki et al. 2019). However, F-waves induced by PNS are not commonly used due to the use of supramaximal intensities leading to discomfort or pain. Alternatively, because the standard electrode sizes of MPS are larger than PNS (Heller et al. 1996; Nakazawa et al. 2004; Barsi et al. 2008; Stein et al. 2010; Popovic et al. 2011; Roche et al. 2011), MPS can be less painful and more comfortable (Kaneko et al. 2022). Therefore, if F-waves elicited by MPS truly reflect the motoneuron pool excitability, it can be a useful method instead of PNS in human neurophysiology.

Here, we tested whether F-waves elicited by MPS can be used to assess changes in the motoneuron pool excitability similar to F-waves elicited by PNS. Using PNS, it has been shown that the Jendrássik maneuver and MI facilitate the motoneuron pool excitability (Rossini 1999; Muellbacher et al. 2000; Hara et al. 2010). Therefore, we adopted both conditions assuming that the motoneuron pool is excited under these conditions compared to a resting condition to test whether F-waves induced by MPS reflected the expected changes.

Material and methods

Participants

Twelve non-disabled individuals (4 females, age: 27.5 ± 6.1 (23–46) years old and height: 166.8 ± 12.0 (150–182) cm, as mean ± standard deviation (SD) the range in parentheses) without any history of neurological disorders took part in the present study after giving their written informed consent. The local ethics committee of the University of Tokyo approved all experimental procedures of the study (No. 533-3). This study was conducted in conformance with the Declaration of Helsinki (1964).

Electromyographic (EMG) recording

We recorded EMG activity from the right SOL and tibialis anterior (TA) muscles. The skin was cleaned with alcohol and then bipolar Ag/AgCl surface electrodes were positioned on each recorded muscle with a distance of 20 mm between electrodes (Vitrode F-150S; Nihon Kohden, Tokyo, Japan). In addition, the ground electrode was attached around the right knee (GE Health Care Japan, Tokyo, Japan). The SOL recording electrodes were attached over the muscle belly of the SOL one-third of the distance from the medial malleolus to the fibular head (Fig. 1A). The EMG signals were amplified (multiplied by 1000) and filtered with a band-pass filter from 150 Hz to 1 kHz by means of a bio-amplifier system (MEG-6108; Nihon Kohden, Tokyo, Japan). As MPS causes large stimulus artifacts on the EMG signal, we decided to

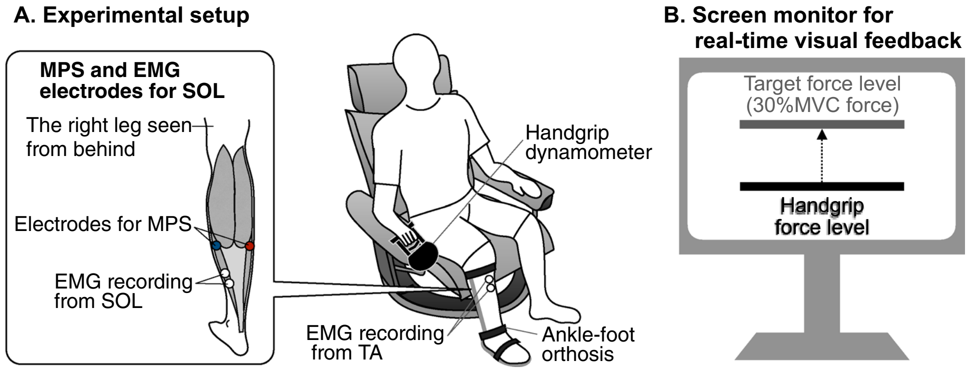

Fig. 1 Experimental setups (**A**). Blue and red stimulus electrodes, respectively, present the cathode and anode electrodes for motor point stimulation (MPS) for the soleus (SOL) muscle. White electrodes are placed for EMG recording from the SOL and tibialis anterior (TA) muscles. Participants sat in a chair, and the ankle joint of their tested right leg was fixed at 10 degrees plantarflexion using an ankle–foot orthosis. In experiment 1, the participants were asked to hold the handgrip dynamometer in their right hands. In front of the participants, a screen monitor displayed visual feedback of the handgrip force levels in real-time (**B**). In the Handgrip condition of experiment 1, the participants were instructed to match the handgrip force level to 30% of the maximum voluntary contraction (MVC) force

use this band-pass filter to sufficiently reduce the stimulus artifacts. This was identified during pilot testing as well as in reference to previous studies using similar high-pass filter with a cut-off frequency of 100 Hz or 200 Hz (Espiritu et al. 2003; Khan et al. 2012). The analog signals were digitized with an A/D converter (Power Lab, AD Instruments, NSW, Australia) at a sampling rate of 4 kHz.

Motor point stimulation (MPS)

Figure 1A indicates the locations of electrodes for EMG recording and MPS. We delivered MPS with constant current stimulation using a constant-current electrical stimulator (model DS7A, Digitimer, Welwyn Garden City, UK) with 500 μs pulse duration.

We used a hand-held pen type electrode with a ball end of approximately 1 cm diameter to identify the motor point for SOL as the position where SOL was activated by the lowest stimulus intensity (Gobbo et al. 2014). The initial stimulus intensity was set to 10 mA when probing to find the motor point. We used an oscilloscope to visually check the M-wave amplitude at the initial intensity and determined the position with the largest M-wave amplitude (i.e., the motor point). If the initial intensity did not induce M-waves, the stimulus intensity was increased by 2 mA and the position was searched in the same manner. The cathode electrode was then attached on the identified medial motor point and the anode over the lateral side of SOL. Both cathode and anode electrodes were 3.2 cm diameter circular electrodes. We identified a stimulation intensity for each participant to evoke an M-wave with the maximum amplitude (i.e., Mmax). The initial stimulus intensity was selected as 30 mA, and the stimulus intensity was gradually increased in 2-mA increments until no increases were observed in peak-to-peak amplitude of M-wave in SOL. We visually confirmed Mmax with an oscilloscope and identified a stimulus intensity to induce it (63.3 ± 19.4 (41–100) mA). For ten of the twelve participants, the stimulation intensity set to 20% above the intensity to elicit Mmax was used to induce an F-wave in this study ($n = 10$, 71.4 ± 15.1 (49–97) mA). A stimulation intensity of 100 mA was used for the other two participants because their stimulus intensity was set to 20% above the intensity to elicit Mmax exceeding 100 mA.

Experimental design and procedure

Participants were instructed to keep their bodies relaxed and not to contract the right leg muscles through the experiment. Using an ankle–foot orthosis, the right ankle joint was fixed at 10 degrees plantarflexion to prevent the tested right leg from moving. This study consisted of two experiments investigating the effects of motor and cognitive tasks on F-waves induced by MPS. We measured the F-waves in SOL during a handgrip task in experiment 1 and during kinesthetic MI of plantarflexion in experiment 2. In experiment 1, the handgrip task was performed with eyes-open to provide visual feedback; in experiment 2, the MI task was performed with eyes-closed to focus on kinesthetic MI. Therefore, the corresponding control condition in each experiment, and the F-waves in SOL were measured at rest with eyes-open (experiment 1) and eyes-closed (experiment 2). Experiment 2 was conducted following Experiment 1, with at least a 5-min break in between.

Experiment 1

At the beginning of Experiment 1, the participants were asked to perform the maximum voluntary contraction

(MVC) of their right-hand grip for 3 s. The MVC force of the handgrip was recorded with a grip force transducer on the handgrip dynamometer (FG-1002FA; Uchida Electronics, Japan). In Experiment 1, we investigated the following two conditions: eyes-open condition (EO) and handgrip condition (HG). In both conditions, the participants were instructed to hold the handgrip dynamometer in their right hand. A 32-inch screen monitor (697.7 × 392.3 mm, Multisync V321, NEC, Tokyo, Japan) was placed one meter in front of them. The monitor displayed visual feedback of the handgrip force levels in real-time (Fig. 1B), controlled via a custom-built LabVIEW program (National Instruments, USA). In the EO, the participants were asked to watch the monitor and match the handgrip force level to 0% of MVC force (i.e., not to perform handgrip). In the HG, they were instructed to watch the monitor and to match the handgrip force level to 30% of their MVC force as closely as possible for 1 min with real-time visual feedback of the handgrip force levels displayed on the monitor.

Measurement of motor responses induced by MPS consisted of four sessions. The EO and HG conditions were conducted in two sessions each, randomized across the participants. For each session, MPS was given a total of 10 times with 5-s intervals. In total, twenty responses were recorded in each condition. At a minimum, a 1-min break was included between each session to prevent fatigue. The timing of MPS was controlled by a custom-built LabVIEW program (National Instruments, USA).

Experiment 2

In Experiment 2, we investigated the following two conditions: eyes-closed condition (EC) and MI condition. In the EC, the participants were instructed to close their eyes and imagine nothing. In the MI condition, they were instructed to perform kinesthetic MI of plantarflexion at the maximal strength with their eyes closed. We set the strength of plantarflexion in the MI to the maximum because it is difficult to standardize the strength of the MI among participants. Before measuring motor responses, the participants were provided a training session for 1 min for the MI. We carefully asked them not to contract their lower-limb muscles and checked that the muscles were not active based on background EMG during the condition. We verbally confirmed that they could perform the kinesthetic MI as we asked during the training session.

Measurement of motor responses consisted of four sessions similar to Experiment 1. The EC and MI conditions were conducted in two sessions each, in a randomized order across the participants. A minimum of a 1-min break was included between each session to prevent a loss of concentration. For each session, MPS was given a total of 10 stimuli at 5-s intervals. In total, twenty responses were recorded in each condition.

After completing both experiments, participants were asked to contract SOL and TA to their maximum force against the experimenter's manual resistance for 3 s at least in the same position, to obtain the maximum voluntarily exerted EMG.

Data and statistical analyses

For offline data analysis, custom-written scripts were used (MATLAB 2019b, MathWorks, USA). Then, for all four conditions (i.e., EO, HG, EC, and MI), the peak-to-peak amplitudes of the early latency and late latency responses, i.e., M-waves and F-waves, were calculated. The time windows for analyzing the M-wave and F-wave amplitudes were determined individually by visual verification because the M-wave and F-wave latencies differ between participants. For the F-wave persistence, a detection threshold to define the detectable response (responses larger than the threshold) and the absent response (responses smaller than the threshold) was set as 30 μV (Zhou and Zhu 2000; Kurobe et al. 2021). The F-wave persistence was computed based on the numbers of detectable responses. We previously used a 25 Hz high-pass filter and a 50 μV detection threshold for offline data analysis to reduce stimulus artefacts and to correctly detect F-wave (Kaneko et al. 2022). The present study used a 150 Hz online high-pass filter, thus attenuating artifacts and using a lower threshold (i.e., 30 μV).

For the four conditions, the background EMG activities in SOL and TA were defined by computing the root mean square (RMS) value 50 ms before each MPS was applied. For Experiment 1, the MVC force of the handgrip was computed as the maximum value of the handgrip force. The handgrip force levels were computed as the average values of the handgrip force 50 ms before each MPS, and they were normalized according to the MVC force. The MVC values in SOL and TA were computed as the RMS values of the EMG signals.

All statistical analyses were carried out using statistical software (SPSS Statistics ver. 25, IBM Corp., USA). First, parametric tests were conducted since the Shapiro–Wilk tests showed that normal distributions were observed in the M-wave and F-wave amplitudes, F-wave persistence, and handgrip force levels. For experiment 1, paired *t*-tests were conducted to compare the amplitudes, persistence, and handgrip force levels in the EO and HG. For experiment 2, paired *t*-tests were performed comparing the amplitudes and persistence in the EC and MI. Second, non-parametric tests were performed because the Shapiro–Wilk tests showed that normal distributions were not observed in the background EMG activities in SOL and TA. Subsequently, Wilcoxon signed-rank tests were performed comparing the background

EMG activities in each muscle in the EO and HG for experiment 1 and the EC and MI for experiment 2. For all statistical tests, the significance level was set at $p < 0.05$. Data are described as the mean ± SD.

Results

Figure 2A shows the raw waveforms of M-waves and F-waves in the EO and HG for experiment 1. The average M-wave amplitude with SDs were 9.05 ± 4.74 mV and 9.08 ± 4.76 mV in the EO and HG, respectively. The average F-wave amplitudes with SDs were 87.05 ± 22.41 μV and 102.17 ± 35.39 μV in the EO and HG, respectively. In the EO and HG, the average F-wave persistence with SDs were 95.42 ± 6.56% and 97.50 ± 5.00%, respectively. Figure 2B, C shows the average M-wave and F-wave amplitudes and the F-wave persistence with SDs. Paired *t*-tests showed no difference in the M-wave amplitudes and F-wave persistence between the EO and HG ($p > 0.05$), and significant greater F-wave amplitudes in the HG than those in the EO ($p < 0.05$, Table 1).

Figure 2D shows the absolute voltages of the background EMG activities in SOL and TA. The average absolute voltages of the background EMG activity for SOL with SDs were respectively 2.780 ± 1.388 μV and 2.724 ± 1.296

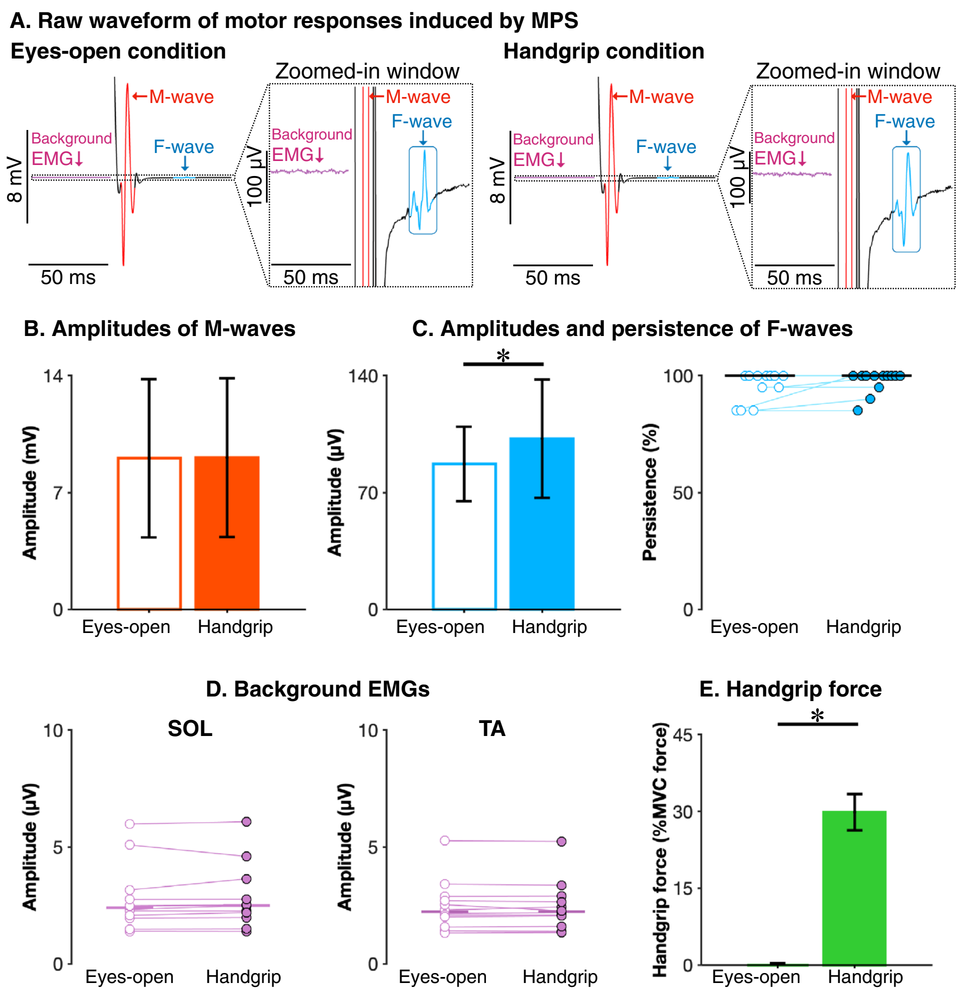

Fig. 2 Typical examples of mean raw waveforms of motor responses elicited by MPS obtained from SOL in the EO and HG conditions (**A**). MPS evokes an M-wave and F-wave. The peak-to-peak amplitudes of M-wave and F-wave were computed from the red and blue parts of the waveform, respectively. Background EMGs 50 ms before MPS were calculated from the purple parts of the waveform. The window on the right is a zoomed in *y*-axis view of the signal framed by the dashed lines on the left. We compared the average maximum M-wave amplitudes (i.e., Mmax) (**B**), the average F-wave amplitudes and persistence (**C**), SOL and TA background EMGs (**D**), and handgrip force levels between the EO (unfilled) and HG conditions (filled). For the amplitudes of M-waves (**B**) and F-waves (**C**, left) and handgrip force levels (**E**), bar graphs and error bars, respectively, indicate the average values and standard deviation (SD). For the persistence of F-waves (**C**, right) and background EMGs (**D**), each plot and line show individual data and the median value, respectively. Asterisks (*) present significant differences between the EO and HG conditions (p value < 0.05)

Table 1 Test statistic values, *p*-values, and effect sizes for paired *t*-tests and Wilcoxon signed-rank tests

	Paired *t*-tests			Wilcoxon signed-rank tests		
	M-wave amplitude (mV)	F-wave amplitude (μV)	Handgrip force (%)	F-wave persistence (%)	Background EMG (μV)	
					SOL	TA
EO versus HG	$t\,(11) = 1.342$	$t\,(11) = 2.962$	$t\,(11) = 28.76$	$Z = 1.633$	$Z = 1.883$	$Z = 0.157$
	$p = 0.206$	$p = 0.013$*	$p > 0.001$*	$p = 0.102$	$p = 0.060$	$p = 0.875$
	$r = 0.375$	$r = 0.666$	$r = 0.993$	$r = 0.471$	$r = 0.544$	$r = 0.045$
Eyes-EC versus MI	$t\,(11) = 0.978$	$t\,(11) = 2.844$		$Z = 1.219$	$Z = 1.412$	$Z = 0.784$
	$p = 0.349$	$p = 0.016$*		$p = 0.223$	$p = 0.158$	$p = 0.433$
	$r = 0.283$	$r = 0.651$		$r = 0.352$	$r = 0.408$	$r = 0.226$

EO eyes-open, *HG* handgrip, *EC* eyes-closed, *MI* motor imagery

Asterisks denote $p < 0.05$

in the EO and HG, while for TA they were respectively 2.425 ± 1.074 μV and 2.453 ± 1.058 μV. Wilcoxon signed-rank tests showed no difference in the background EMG activities in either muscle between the EO and HG ($p > 0.05$, Table 1). The average MVC with SDs in SOL and TA were 0.986 ± 0.688 mV and 0.697 ± 0.222 mV, respectively. The average background EMG values normalized to the MVC for SOL with SDs were, respectively, 0.388 ± 0.299% and 0.395 ± 0.300% in the EO and HG, whereas for TA, they were, respectively, 0.379 ± 0.167% and 0.376 ± 0.162%.

The average MVC force of the handgrip with SD was 294.4 ± 97.0 N (range: 167.3–435.5 N). Figure 2E shows the average handgrip force levels normalized to the MVC force. The average handgrip force levels with SDs were 0.0012 ± 0.0009% and 29.84 ± 3.60% in the EO and HG, respectively. A paired *t*-test showed that the handgrip force levels in the HG were significantly greater than those in the EO ($p < 0.05$, Table 1).

Figure 3A shows the raw waveforms of M-waves and F-waves in the EC and MI for experiment 2. The average M-wave amplitude with SDs were 9.04 ± 4.64 mV and 9.075 ± 4.710 mV in the EC and MI, respectively. The average F-wave amplitudes with SDs were 76.73 ± 26.44 μV and 101.46 ± 38.02 μV in the EC and MI, respectively. The average F-wave persistence with SDs were 93.75 ± 13.16% and 98.75 ± 3.11% in the EC and MI, respectively. Figure 3B, C shows the average M-wave and F-wave amplitudes and the F-wave persistence with SDs. Paired *t*-tests showed no difference in the M-wave amplitudes and F-wave persistence between the EC and MI ($p > 0.05$) and significant greater F-wave amplitudes in the MI than those in the EC ($p < 0.05$, Table 1).

Figure 3D shows the absolute voltages of the background EMG activities in SOL and TA. The average absolute voltages of the background EMG activity for SOL with SDs were, respectively, 2.724 ± 1.296 μV and 2.902 ± 1.309 in the EC and MI, whereas, for TA, they were, respectively, 2.364 ± 0.987 μV and 2.420 ± 0.895 μV. Wilcoxon signed-rank tests show no difference in the background EMG activities in either muscle between the EC and MI ($p > 0.05$, Table 1). The average background EMG values normalized to the MVC for SOL with SDs were, respectively, 0.388 ± 0.308% and 0.408 ± 0.312% in the EC and MI, whereas for TA, they were, respectively, 0.363 ± 0.159% and 0.375 ± 0.154%.

Discussions

The present study investigated whether F-waves elicited by MPS reflects the motoneuron pool excitability during handgrip and motor imagery tasks. We found that both HG and MI significantly increased the F-wave amplitudes in SOL ($p < 0.05$, Table 1). The background EMG activities in both SOL and TA muscles were lower than 1% MVC, showing that the muscles were at rest. Also, there was no difference in the background EMG activities in either muscle between the EC and MI and between the EO and HG ($p > 0.05$, Table 1). Thus, the background EMG activities did not influence the increase in the F-waves amplitudes during the HG and MI conditions. Previous studies reported that the Jendrássik maneuver, such as HG, and MI facilitate the motoneuron pool excitability (Rossini 1999; Muellbacher et al. 2000; Hara et al. 2010). Therefore, these results demonstrated that the increase in the F-waves amplitudes induced by MPS reflects the facilitation of the motoneuron pool excitability during both HG and MI, which is in line with our hypothesis. These results suggest that MPS can be a tool to evaluate motoneuron pool excitability in human neurophysiology.

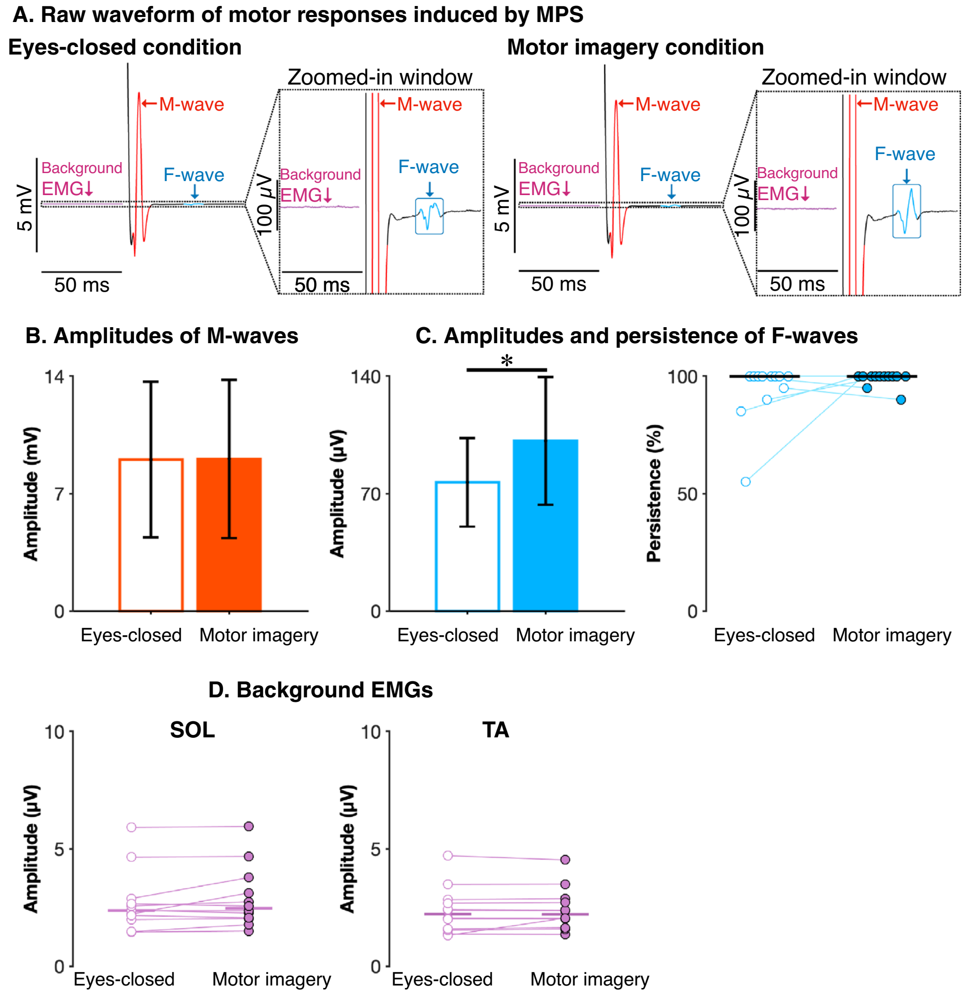

Fig. 3 Typical examples of mean raw waveforms of motor responses elicited by MPS obtained from SOL in the EC and MI conditions (**A**). The peak-to-peak amplitudes of M-wave and F-wave were computed from the red and blue parts of the waveform, respectively. Background EMGs 50 ms before MPS were calculated from the purple parts of the waveform. The window on the right is a zoomed in *y*-axis view of the signal framed by the dashed lines on the left. We compared the average maximum M-wave amplitudes (i.e., Mmax) (**B**), the average F-wave amplitudes and persistence (**C**), and SOL and TA background EMGs (**D**) between the EC (unfilled) and MI conditions (filled). For the amplitudes of M-waves (**B**) and F-waves (**C**, left), bar graphs and error bars, respectively, indicate the average values and standard deviation (SD). For the persistence of F-waves (**C**, right) and background EMGs (**D**), each plot and line show individual data and the median value, respectively. Asterisks (*) represent significant differences between the EC and MI conditions (*p* value < 0.05)

Advantages of using MPS as the measurement tool to evaluate motoneuron pool excitability

There are two advantages of using MPS as the measurement tool to induce F-waves which reflect motoneuron pool excitability in human neurophysiology. First, MPS is more comfortable and less painful than PNS when using a supramaximal intensity to induce F-waves (Kaneko et al. 2022). Second, F-waves elicited by MPS have higher amplitude and persistence compared to those induced by PNS, indicating more robust F-waves when using MPS (Kaneko et al. 2022). For PNS, F-wave measurement is used less frequently than an H-reflex technique because F-waves are generally more painful and less robust compared to H-reflex. The comfort of measurement and robustness of F-waves elicited by MPS would reduce the number of stimuli, resulting in a reduction of the experiment time and participant burden. F-waves are less dependent on synaptic activities such as post-activation depression and presynaptic inhibition (McNeil et al. 2013) and therefore, are more susceptible to the motoneuron pool excitability than the H-reflexes (Milanov 1992). Furthermore, F-waves may primarily reflect the excitability of the high-threshold spinal motoneuron, while H-reflexes may reflect that of the low-threshold spinal motoneuron (Pierrot-Deseilligny and Burke 2012). Therefore, an F-wave measurement may investigate a part of the motoneuron pools that is not examined by an H-reflex measurement. Taken together, MPS is a better tool for evaluating motoneuron pool excitability than PNS for physiological and clinical purposes.

The present study focused on F-waves in the SOL because the SOL is the most popular muscle for H-reflex and F-wave measurements. However, MPS can be applied to lower-limb muscles other than SOL (e.g., TA and the thigh muscles) (Botter et al. 2011), where PNS is not easily applicable to induce F-waves. While further studies need to determine whether F-waves can be evoked by MPS in other lower-limb muscles besides the SOL, F-waves elicited by MPS could be utilized to investigate the changes in motoneuron pool excitability in a greater number of muscles compared to ones elicited by PNS.

Upper-limb muscle contractions facilitate motoneuron pool excitability in SOL

Previous studies have shown that motor execution of upper-limb muscle contractions like handgrip increases the excitability of spinal reflex circuits in the lower-limb muscles (Dowman and Wolpaw 1988; Tazoe et al. 2005; Masugi et al. 2019; Kato et al. 2019; Sasaki et al. 2020). The increase during upper-limb muscle contractions partly reflects facilitation of motoneuron pool excitability in the lower-limb muscles. A previous study reported that voluntary muscle contractions of an upper limb increase the F-wave amplitudes in the contralateral upper limb muscles (Muellbacher et al. 2000) and the motor responses induced by transcutaneous electrical stimulation to the spinal cord (Stedman et al. 1998). These results suggested upper-limb motor execution facilitates the motoneuron pool excitability in the contralateral upper limb muscles.

Our present study investigated whether upper-limb muscle contractions modulate F-waves induced by MPS. Our results showed an increase in the F-wave amplitudes during the HG ($p<0.05$, Table 1, Fig. 2C), suggesting facilitation of the motoneuron pool excitability. Furthermore, we confirmed that participants performed the HG as we instructed (Fig. 2E). Therefore, our results showed the HG facilitates the motoneuron pool excitability, in line with previous findings, indicating that F-waves elicited by MPS can be used for investigating changes in the motoneuron pool excitability during motor tasks such as muscle contractions.

Motor imagery facilitates motoneuron pool excitability

Previous studies demonstrated that MI increases the F-wave amplitudes during MI, indicating facilitation of motoneuron pool excitability (Rossini 1999; Hara et al. 2010). In this study, we demonstrated that the amplitudes of F-wave elicited by MPS increased in the MI, compared to the EO. The increase of the F-wave amplitudes during MI was consistent with the results of previous studies (Rossini 1999; Hara et al. 2010). Our results showed that F-waves elicited by MPS can be used for assessing changes in the motoneuron pool excitability during cognitive tasks.

Previous studies demonstrated that MI of plantarflexion increases the H-reflex amplitudes (Kiers et al. 1997; Hale et al. 2003; Cowley et al. 2008). However, another study reported that MI of plantarflexion does not change H-reflexes (Aoyama and Kaneko 2011). Thus, the changes in H-reflexes during MI of plantarflexion are inconsistent, and more detailed research has been needed. In addition to H-reflexes, MI increases tendon reflex (Bonnet et al. 1997), short latency stretch reflex (Li 2004; Aoyama and Kaneko 2011), and posterior-root spinal reflex (Nakagawa et al. 2020). Thus, it was suggested that MI facilitates excitabilities of spinal motoneurons and/or interneurons. In this study, we demonstrated that F-waves elicited by MPS can identify the facilitation of motoneuron pool excitability. Facilitation of motoneuron pool excitability would show one aspect of the modulation of spinal neural circuits shown by the previous studies mentioned above. However, a previous study showed that MI of plantarflexion did not change F-waves in the SOL (Liepert and Neveling 2009). The F-wave persistence can explain the contradiction between the previous study and our current study. The previous study reported that the F-wave amplitudes during rest and MI were 80 ± 40 µV with the persistence of $70 \pm 10\%$ and 90 ± 50 µV with $69 \pm 15\%$, respectively (Liepert and Neveling 2009), while the F-wave amplitudes and persistence during the EC and MI in our study were 76.73 ± 26.44 µV with $93.75 \pm 13.16\%$ and 101.46 ± 38.02 µV with 98.75 ± 3.11. Thus, the F-wave amplitude was comparable, but the persistence was higher in our study compared to the previous study. Our previous study showed that MPS evokes F-waves with higher persistence than PNS (Kaneko et al. 2022). Therefore, F-waves elicited by MPS with higher persistence would detect the increase of F-wave amplitudes during MI of plantarflexion.

Previous studies reported that MI increases both F-wave amplitude and persistence (Rossini 1999; Hara et al. 2010). However, in our study, the F-wave persistence did not change. This may be because of the high F-wave persistence induced by MPS which were above 90% during control conditions (EO: $95.42 \pm 6.56\%$, EC: $93.75 \pm 13.16\%$). A previous study reported that the F-wave persistence in the upper-limb muscles was from 75 to 76.2% at rest and from 83.3 to 89.5% during MI (Rossini 1999). Another study showed the persistence increased significantly from $32.5 \pm 11.9\%$ at rest to $58.3 \pm 15.2\%$ during MI even though they set the threshold at 20 µV, which is lower than 30 µV used in our present study (Hara et al. 2010). Therefore, higher F-wave persistence at rest may make it harder to detect the modulation of persistence during tasks, although there was room for an increase of a few percent. Further studies are needed to investigate the detailed characteristics of F-waves elicited by MPS (i.e., the recruitment curves of amplitudes and persistence and sensitivity to modulation during tasks at each phase of recruitment curves).

Possible mechanisms behind handgrip and motor imagery increasing F-wave amplitudes

The increase in F-wave amplitudes during HG and MI reflects the facilitation of the motoneuron pool excitability. This is probably due to an increase of the net synaptic inputs to the motoneuron pool when muscle force exertion at remote muscles or subthreshold motor descending command to the target muscle occurs This then increases the subliminal fringe and makes the motoneuron pool more sensitive. Given that F-waves are induced by the activation of spinal motoneurons via impulses that antidromically propagate along the motor axons (Fisher 1992; Mercuri et al. 1996; Panayiotopoulos and Chroni 1996; Mesrati and Vecchierini 2004), an increased sensitivity of net synaptic input to the motoneurons would be a plausible reason for the change in F-waves during HG and MI. Thus, the changes in the net synaptic inputs are likely caused by motor-related cortical outputs induced by HG and MI.

Alternatively, there is a possibility that HG and MI work as diverting activities. In the EO and EC conditions, participants kept their bodies relaxed, however they likely anticipated supramaximal MPS which is uncomfortable. This anticipation and anxiety about MPS may have an inhibitory effect on the net synaptic inputs to the spinal motoneurons. HG and MI may reduce the participants' attention to MPS, which may result in removing the inhibitory effect. This may reduce the activation threshold of the net synaptic inputs, leading to greater F-waves. To determine whether facilitation of the motoneuron pool excitability was due to HG and MI acting as a motor task or a diverting task, it is necessary to investigate the effects of non-motor related tasks such as mental arithmetic on F-waves in future.

Another possible mechanism from the abovementioned synaptic mechanism may be that HG or MI alters persistent inward currents (PICs) through dendritic persist sodium and L-type calcium in the spinal motoneurons (Pierrot-Deseilligny and Burke 2012). Motor-related cortical outputs generated during HG and MI can influence the subcortical areas such as the spinal cord and possibly the monoaminergic neuromodulators. This may affect PICs to make motoneuron responses to given inputs more sensitive. As a result, HG and MI reduces the activation thresholds of motoneurons to facilitate the motoneuron pool excitability at a subthreshold level.

Conclusions

We demonstrated facilitation of the motoneuron pool excitability during handgrip and motor imagery tasks by using F-waves induced by MPS. Our results suggest that MPS can be a tool for assessing motoneuron pool excitability in human neurophysiology.

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Data availability Data presented in this manuscript were newly collected for this study. Due to data privacy issues, they are unavailable to the community in public open repositories. The datasets produced in the current study are available from the corresponding author upon reasonable request. In such cases, considerations will be given based on the review of reasons for the data request and the procedures to ensure data privacy.

Declarations

Conflict of interest We declare that there is no financial disclosure and conflict of interest.

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