Original article

Effects of long-duration paired-pulse electrical stimulation on excitability of the corticospinal tract in healthy subjects

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Abstract

Afferent input from peripheral nerves plays an important role in modulating corticospinal excitability. It is known that afferent input induced by paired-pulse electrical stimulation (ppES) with an interval between two doublets of 5 ms significantly increases corticospinal excitability, with short-duration ppES having a greater effect than single-pulse electrical stimulation (sES). In contrast, long-duration ppES was inefficient in modulating corticospinal excitability. It is possible that the stimulation frequency was effective in modulating the change in corticospinal excitability induced by long-duration ppES. The present study investigated the effect of long-duration (20 min) ppES on the corticospinal excitability using transcranial magnetic stimulation (TMS). In Experiment 1, motor-evoked potentials (MEPs) were measured before and after (immediately, 5, 10, 15, and 20 min after) sES at 30 Hz (120% sensory threshold or 120% motor threshold) or ppES with an interval between two doublets of 5 ms (120% sensory threshold, 30 Hz). In Experiment 2, MEPs were measured before and after sES at 15 or 60 Hz (120% sensory threshold) or ppES at 15 or 60 Hz (120% sensory threshold, an interval between two doublets of 5 ms). In Experiment 3, MEPs were measured before and after ppES with an interval between two doublets of 10 or 15 ms (120% sensory threshold, 15 Hz). Application of ppES at 30 Hz with an interval between two doublets of 5 ms significantly increased MEP, as did sES at 120% motor threshold. Moreover, ppES at 15 and 60 Hz with an interval between two doublets of 5 ms significantly increased MEP, and ppES at 15 Hz with an interval between two doublets of 15 ms significantly decreased MEP. These results suggest that corticospinal excitability increased after ppES is induced by paired-pulse paradigm and not by the total number of pulses.

Introduction

Peripheral nerve electrical stimulation (PES) can be used to successfully treat motor disorders

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following stroke. Langhorne et al. [1] reported that PES improved motor function of paralyzed limbs, whereas Everaert et al. [2] reported that PES was effective in increasing corticospinal excitability. Therefore, changes in motor function induced by PES could be associated with increased corticospinal excitability.

It has recently been shown that the delivery pattern of afferent inputs from the periphery plays an important role in modulating corticospinal excitability. For example, Saito et al. [3] reported that short-duration (5s) paired-pulse stimulation (ppES, 5 ms interval between two doublets) at an inter-train interval of 100 ms (10 Hz) increased corticospinal excitability. Conversely, long-duration (20 min) ppES (5 ms interval between two doublets) at an inter-train interval of 100 ms (10 Hz) had no effect on corticospinal excitability [3]; however, the reason for the lack of effect remains unknown. However, it is generally accepted that PES modulates the corticospinal excitability based on stimulation frequency. PES above the motor threshold at 10 Hz increases corticospinal excitability [4-9], whereas some reports suggest that stimulation at 10 Hz do not change corticospinal excitability regardless of above the motor threshold [10,11]. Taken together, these results indicate that there is variation in the effect of PES at 10 Hz. On the other hand, PES above the motor threshold at 30 Hz increases corticospinal excitability [12-15], whereas PES below the motor threshold at 30 Hz reduces corticospinal excitability [15]. Moreover, Chipchase et al. [13] showed that although stimulation at low and high motor intensities at the same frequency (10 Hz) did not induce a change in corticomotor excitability, while motor stimulation at a frequency of 30 Hz increased corticomotor excitability. Thus, compared with the effect of PES at 10 Hz, the effect of PES at 30 Hz on corticospinal excitability may be relatively stable depending on the stimulation intensity. Considering a systematic review showed that MEP changes following PES depend on stimulation frequency [16], long-duration ppES may increase corticospinal excitability when the stimulation frequency is set to 30 Hz. Moreover, the number of excitation pulses of ppES will be more than double that of a single stimulus if set at the same condition. A previous study reported that MEP immediately increased after short-duration ppES [3] is to the same extent as that after PES above the motor threshold as reported by Sasaki et al. [15]. Thus, long-duration ppES with an interval between two doublets of 5 ms is expected to increase corticospinal excitability to the same extent as sES above the motor threshold. However, the total number of pulses is different between ppES at 30 Hz and PES at 30 Hz. Considering that corticospinal excitability was modulated depending on the PES frequency [10, 17], corticospinal excitability was also modulated by the total number of pulses of ppES. In addition, short-duration ppES with an interval between two doublets of 10 ms had no effect on corticospinal excitability, and short-duration ppES with an interval between two doublets of 15 ms tended to decrease it [3]; therefore, long-duration ppES with an interval between two doublets of 10 ms may have no effect on corticospinal excitability, whereas long-duration ppES with an interval between two doublets of 15 ms may significantly reduce corticospinal excitability.

Therefore, in Experiment 1, we investigated the effect of long-duration (20 min) ppES at 30 Hz on corticospinal excitability. Considering the possibility that ppES parameters modulate corticospinal excitability, long-duration ppES is expected to change corticospinal excitability when the frequency is set to 30 Hz. We hypothesize that with the same frequency, the effect of sES and ppES will be different. Thus, in Experiment 2, we investigated the role of the total number of pulses in modulating corticospinal excitability after long-duration ppES. Additionally, we investigated the effect of the interval between two doublets on modulating corticospinal excitability induced by

long-duration ppES in Experiment 3.

Materials and Methods

A total of 42 healthy subjects (39 males and three females; mean ± standard deviation, 22.6 ± 5.8 years; age range, 20-43 years) participated in this study. Four subjects participated in all experiments. In addition, two subjects participated in Experiments 1 and 2, one subject participated in Experiments 1 and 3, and two subjects participated in Experiments 2 and 3. This study was conducted in accordance with the principles of the Declaration of Helsinki, and all protocols were approved by the Ethics Committee of Niigata University of Health and Welfare (Approval No: 17505-140703). Throughout the experiment (both TMS measurement and PES application), subjects took a comfortable sitting posture in a chair with an armrest, placing their hands at rest in a joint supination position.

1. Peripheral electrical stimulation (PES)

In this study, single electrical stimulation (sES) or ppES was applied to the right ulnar nerve at the wrist using a bipolar electrode (the anodal electrode was set to the distal side and the cathodal electrode was set to the proximal side) connected to an electrical generator (SEN-7203; Nihon Kohden Co., Tokyo, Japan) through an isolator (SS-104; Nihon Kohden Co.). The stimulation was delivered with a pulse duration of 0.2 ms. The duty cycle was set to an on and off time of 4 s and 6 s, respectively, based on previous work [13,15]. A bar type stimulation electrode (length: 55 mm, width: 15 mm, distance between electrodes: 20 mm) was used.

2. Experiment 1: Effect of sES and ppES on corticospinal excitability

Fourteen subjects participated in this experiment (13 males and 1 female; mean ± standard deviation, 22.6 ± 6.0 years; age range, 20-43 years). In the sES condition, interval between two single pulses was set to 33.3 ms (stimulus frequency: 30 Hz). The stimulation intensity was set to (i) 120% motor threshold (sES-motor_30 Hz) or (ii) 120% sensory threshold (sES-sensory_30 Hz). The motor threshold was set at the lowest intensity that evoked a twitch of the first dorsal interosseous (FDI) muscle, while the sensory threshold was set at the lowest intensity that the subject could perceive. The total number of pulses was 14400 in each stimulus condition.

In the ppES condition, ppES was delivered with an interval between two doublets of 5 ms and an inter-train interval of 33.3 ms (Figure 1). The stimulation intensity was 120% of the sensory threshold (ppES-sensory_30 Hz). The total number of pulses was 28800. We did not match the number of pulses in ppES-sensory_30 Hz with that in sES-motor_30 Hz and sES-sensory_30 Hz because we matched the stimulus frequency and duration of ppES with that of sES.

3. Experiment 2: Effect of stimulation frequency of sES and ppES on corticospinal excitability

Thirteen subjects participated in this experiment (13 males; mean ± standard deviation, 22.7 ± 6.1 years; age range, 20-43 years). In the sES condition, the stimulation intensity was set to 120% of the sensory threshold. Stimulation was delivered with an interval between two single pulses of (i) 66.6 ms (stimulation frequency: 15 Hz, sES-sensory_15 Hz) or (ii) 16.6 ms (stimulation frequency: 60 Hz, sES-sensory_60 Hz). The total number of pulses was 7200 in the sES-sensory_15 Hz condition and 28800 in the sES-sensory_60 Hz condition.

In the ppES condition, the stimulation intensity was set to 120% of the sensory threshold. Stimulation was applied with an interval between two doublets of 5 ms and an inter-train interval of (i) 66.6 ms (stimulation frequency: 15 Hz, ppES-sensory_15 Hz) or (ii) 16.6 ms (stimulation frequency: 60 Hz, ppES-sensory_60 Hz; Figure 1). The total number of pulses was 14400 in the

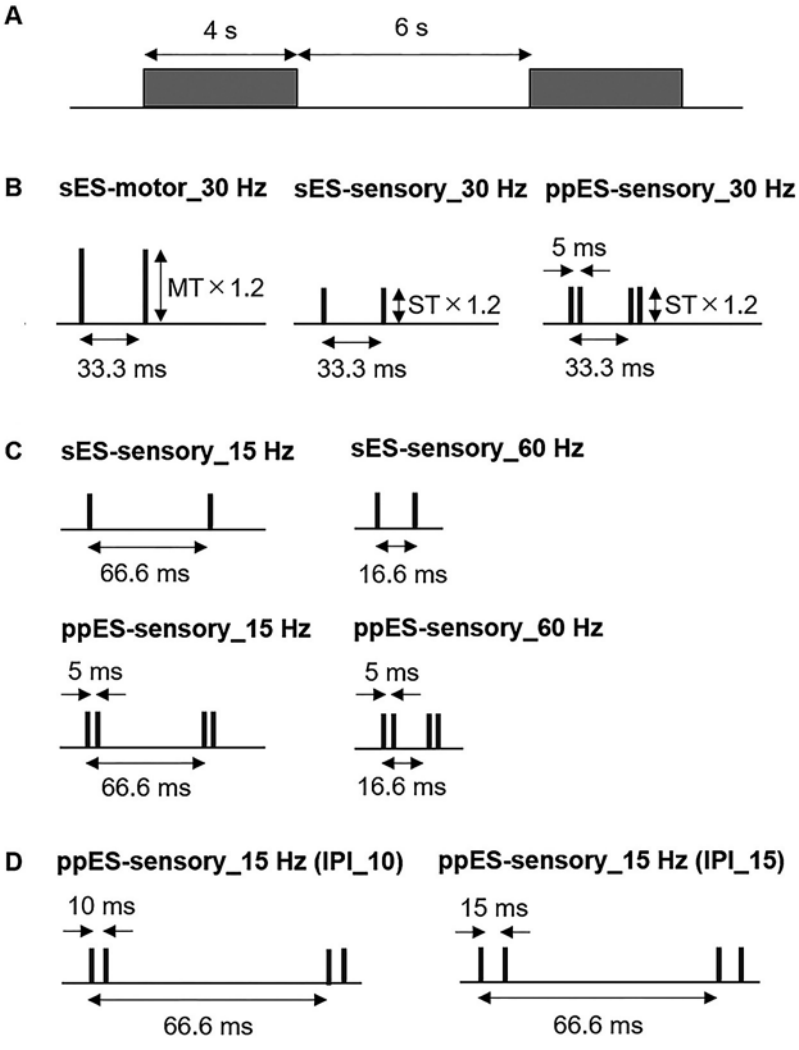

Figure 1. Stimulation parameters of peripheral nerve electrical stimulation (PES).

A. The stimulation cycle of peripheral nerve electrical stimulation was 4 s on and 6 s off.

B. Stimulation parameters of Experiment 1: The stimulation pulse interval was 33.3 ms (frequency: 30 Hz); the intensity was 120% of the motor threshold value (sES-motor_30 Hz) and 120% of the sensory threshold value (sES-sensory_30 Hz). For the stimulation parameters of paired-pulse electrical stimulation (ppES), the interval between two doublets was set to 5 ms, the train interval was set to 33.3 ms (frequency: 30 Hz), and the stimulation intensity was set to 120% of the sensory threshold value (ppES-sensory_30 Hz).

C. Stimulation parameters of Experiment 2: The stimulation intensity of a single stimulation was 120% of the sensory threshold and the two conditions were stimulation pulse intervals of 66.6 ms (frequency: 15 Hz, sES-sensory_15 Hz) and 16.6 ms (frequency: 60 Hz, sES-sensory_60 Hz). The stimulus intensity of ppES was 120% of the sensory threshold and the two conditions of the stimulus train were 66.6 ms (ppES-sensory_15 Hz) and 16.6 ms (ppES-sensory_60 Hz).

D. Stimulation parameters of Experiment 3: The two intervals between two doublets conditions of paired-pulse electrical stimulation (ppES) were 10 ms [ppES-sensory_15 Hz (10 ms)] and 15 ms [ppES-sensory_15 Hz (15 ms)].

ppES-sensory_15 Hz condition and 57600 in the ppES-sensory_60 Hz condition.

4. Experiment 3: Effect of stimulation pulse interval of ppES on corticospinal excitability

Fifteen subjects participated in this experiment (13 males and 2 females; mean ± standard deviation, 22.5 ± 5.8 years; age range, 20-43 years). In the ppES condition, the stimulation intensity of ppES was 120% of the sensory threshold. The inter-train interval was 66.6 ms (stimulation frequency: 15 Hz). The interval between two doublets was set to (i) 10 ms [ppES-sensory_15 Hz (10 ms)] or (ii) 15 ms [ppES-sensory_15 Hz (15 ms); Figure 1]. The total number of pulses was 14400 in each stimulus condition.

5. Electromyography recordings

Surface electromyography (EMG) was recorded from the right FDI muscle via disposable Ag / AgCl electrodes in a belly-tendon montage. Signals from the EMG electrodes were amplified (×100) by an amplifier (A-DL-720-140, 4 Assist, Tokyo, Japan), filtered (high pass, 20 Hz), digitized at 4 kHz using an A/D converter (Power Lab 8/30, AD Instruments, Colorado Springs, CO, USA), and then stored on a lab computer for later offline analysis (LabChart7, AD Instruments).

6. Measurement of MEP

Transcranial magnetic stimulation (TMS) was delivered through a figure-eight coil (diameter, 9.5 cm) connected to a Magstim 200 stimulator (Magstim, Dyfed, UK). The coil was held tangentially to the skull over the left M1 area with the handle pointing posterolaterally at 45° to the sagittal plane. The TMS coil was placed over the left M1 at the position producing the largest MEPs from the right FDI muscle (the motor hotspot). The position and orientation of the coil for the motor hotspot were marked according to magnetic resonance imaging (MRI) via Visor2 TMS Neuronavigation (eemagine Medical Imaging Solutions GmbH, Berlin, Germany), and the coil was held in place to maintain its position. T1-weighted images were obtained using a 1.5 T MRI scanner before the experiment (SIGNA HD, GE Healthcare, Milwaukee, WI, USA). The TMS intensity was set to evoke a MEP of approximately 1 mV in the FDI muscle. Consistent with previous studies [18-20], we delivered TMS at a rate of 0.2 Hz during data collection.

7. Experimental procedure

MEPs were measured before (Pre), immediately after (Post 0), 5 min after (Post 5), 10 min after (Post 10), 15 min after (Post 15), and 20 min after (Post 20) PES (Figure 2). All subjects underwent 15 single-pulse TMS trials to measure MEP in each measurement block.

In Experiment 1, subjects received the following stimulus conditions: (i) sES-motor_30 Hz, (ii) sES-sensory_30 Hz, and (iii) ppES-sensory_30 Hz. In Experiment 2, subjects received (i) sES-sensory_15 Hz, (ii) sES-sensory_60 Hz, (iii) ppES-sensory_15 Hz, and (iv) ppES-sensory_60 Hz. In Experiment 3, subjects received (i) ppES-sensory_15 Hz (10 ms) and (ii) ppES-sensory_15 Hz (15 ms). For all subjects, experiments using the different stimulation conditions were performed at least three days apart.

8. Data analysis

The peak-to-peak amplitudes of MEP were measured in each measurement block in each experiment. The maximum and minimum values were removed from the MEP amplitudes, and the remaining MEP amplitudes were averaged.

All statistical analyses were performed using SPSS 21.0 for Windows. The normal distribution of the data was assessed using Shapiro–Wilk's test; the statistical significance was defined as $P < 0.05$. Because the data were not normally distributed, the Friedman test was used instead of the ANOVA test to assess the effect of each stimulus condition on MEP amplitude in all experiments.

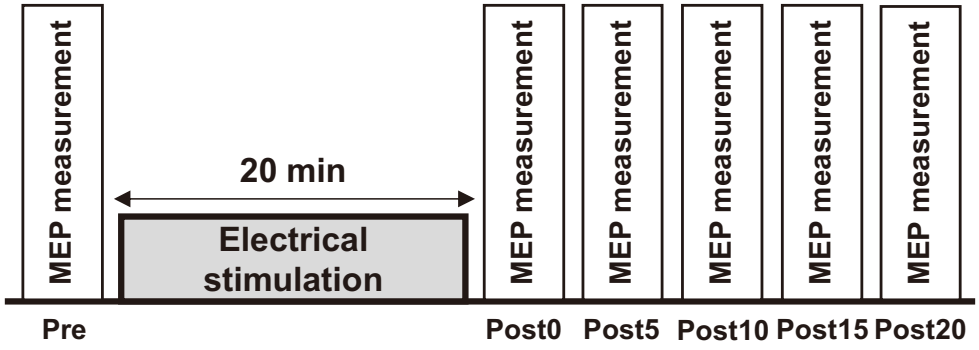

Figure 2. Experimental protocol.
Peripheral electrical stimulation was applied for 20 min. MEP was measured before stimulation (Pre), immediately after stimulation (Post 0), 5 min after stimulation (Post 5), 10 min after stimulation (Post 10), 15 min after stimulation (Post 15), and 20 min after stimulation (Post 20).

To compare MEP amplitudes among times, the statistical significance of the Friedman test was defined as $P < 0.05$. In addition, we compared MEP amplitudes before and immediately after, 5, 10, 15, and 20 min after PES for each stimulus condition using the Wilcoxon signed-rank test with Bonferroni correction; the statistical significance was defined as $P < 0.05/15 = 0.0033$. Effect sizes were calculated using the following formula: $r = Z/\sqrt{N}$ (r, effect size; Z, z value; N: Observation number), according to a previous study [21].

Results

1. Effect of sES and ppES on corticospinal excitability (Experiment 1)

The typical MEP waveforms in the FDI muscle from one participant are shown in Figure 3, while the effects of sES and ppES on mean MEP amplitudes are shown in Figure 4 and Table 1. In the sES-motor_30 Hz condition, the Friedman test indicated significant differences in terms of MEP amplitudes among times ($\chi^2_{(14)} = 26.653$, $P < 0.001$). MEP was significantly increased 10 min after sES-motor_30 Hz compared to baseline. In the sES-sensory_30 Hz condition, the Friedman test indicated significant differences in terms of MEP amplitudes among times ($\chi^2_{(14)} = 20.571$, $P = 0.001$). MEP was significantly decreased immediately after sES-sensory_30 Hz compared to baseline. In the ppES-sensory_30 Hz condition, the Friedman test revealed significant differences in terms of MEP amplitudes among times ($\chi^2_{(14)} = 21.878$, $P = 0.001$). MEP was significantly increased 15 min after ppES-sensory_30 Hz compared to baseline.

2. Effect of stimulation frequency of sES and ppES on corticospinal excitability (Experiment 2)

The typical MEP waveforms in the FDI muscle from one participant are shown in Figure 5, while the effects of sES and ppES on mean MEP amplitudes are shown in Figure 6 and Table 2. In the sES-sensory_15 Hz and 60 Hz conditions, the Friedman test showed significant differences in terms of MEP amplitudes among times (sES-sensory_15 Hz, $\chi^2_{(13)} = 32.868$, $P < 0.001$; sES-sensory_60 Hz, $\chi^2_{(13)} = 34.451$, $P < 0.001$). MEP was significantly decreased immediately after, 5 min after, 10 min after, 15 min after, and 20 min after both sES-sensory_15 Hz and 60 Hz compared with baseline MEP before delivering PES. In the ppES-sensory_15 Hz and 60 Hz conditions, the Friedman test showed significant differences in

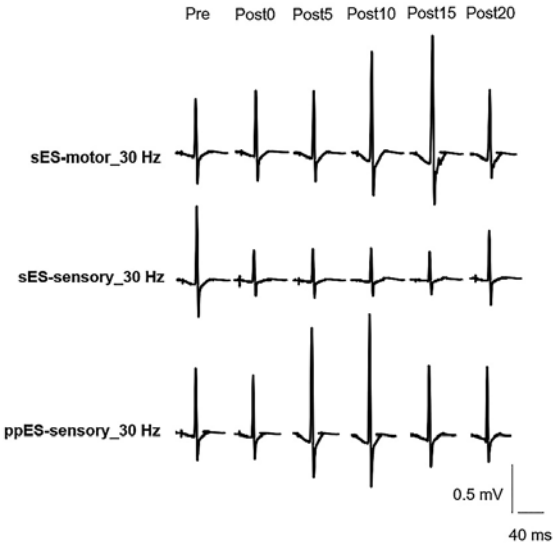

Figure 3. Typical MEP waveform (Experiment 1).
Typical MEP waveform of the first dorsal interosseous (FDI) at sES-motor_30 Hz and sES-sensory_30 Hz, ppES-sensory_30 Hz.

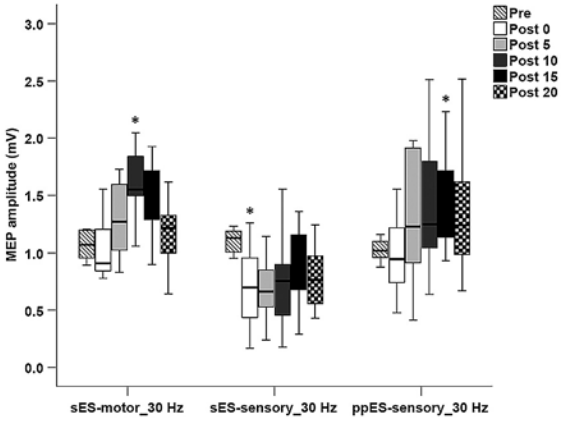

Figure 4. Effect of sES and ppES on MEP (Experiment 1).
At sES-motor_30 Hz, MEP increased significantly 10 min after stimulation compared with before stimulation. At ppES-sensory_30 Hz, MEP increased significantly 15 min after stimulation compared with before stimulation. By contrast, at sES-sensory_30 Hz, MEP was significantly decreased immediately after stimulation compared with before stimulation. When comparing sES-sensory_30 Hz and ppES-sensory_30 Hz and sES-motor_30 Hz and sES-sensory_30 Hz, differences were observed from 5 min until 20 min after stimulation.
$^{*}P < 0.05$. Error bars indicate SE.

Table 1. Statistical results of Experiment 1 (comparison before and after peripheral electrical stimulation).

		Pre vs. Post 0	Pre vs. Post 5	Pre vs. Post 10	Pre vs. Post 15	Pre vs. Post 20
sES-motor_30 Hz	*P* value	0.3	0.016	0.001	0.004	0.14
	Effect size	0.28	0.65	0.86	0.78	0.39
sES-sensory_30 Hz	*P* value	0.002	0.006	0.016	0.011	0.004
	Effect size	0.85	0.73	0.65	0.68	0.76
ppES-sensory_30 Hz	*P* value	0.683	0.124	0.009	0.002	0.026
	Effect size	0.11	0.41	0.70	0.81	0.60

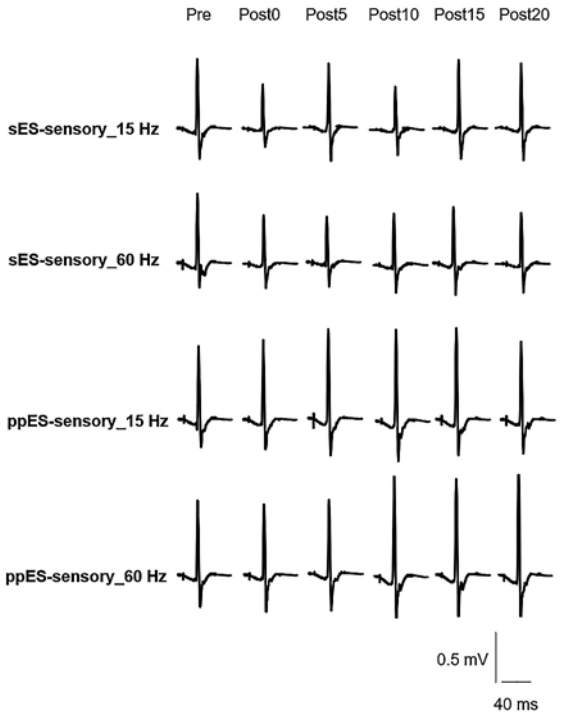

Figure 5. Typical MEP waveform (Experiment 2). Typical MEP waveform of the FDI at sES-sensory_15 Hz, sES-sensory_60 Hz, ppES-sensory_15 Hz, and ppES-sensory_60 Hz.

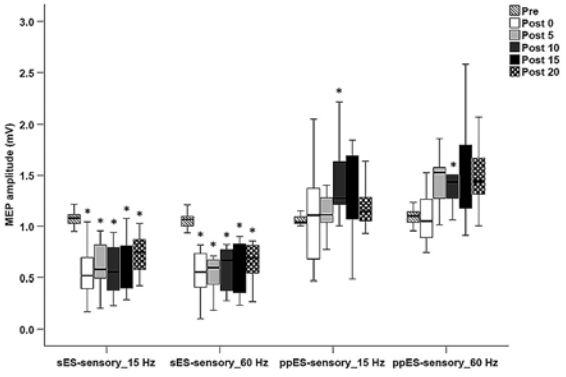

Figure 6. Effect of stimulation frequency of sES and ppES on MEP (Experiment 2).
At sES-sensory_15 Hz and sES-sensory_60 Hz, MEP declined immediately after stimulation until 20 min after stimulation. By contrast, compared to before stimulation, both ppES-sensory_15Hz 10 min and ppES-sensory_60Hz 10 min after stimulation showed MEP increases. Moreover, at ppES-sensory_15 Hz and ppES-sensory_60 Hz, MEP was greater than at sES-sensory_15 Hz and sES-sensory_60 Hz from immediately after stimulation until 20 min after stimulation.
$^{*}P < 0.05$. Error bars indicate SE.

Table 2. Statistical results of Experiment 2 (comparison before and after peripheral electrical stimulation).

		Pre vs. Post 0	Pre vs. Post 5	Pre vs. Post 10	Pre vs. Post 15	Pre vs. Post 20
sES-sensory_15 Hz	*P* value	0.001	0.001	0.001	0.001	0.001
	Effect size	0.88	0.88	0.88	0.88	0.88
sES-sensory_60 Hz	*P* value	0.001	0.001	0.001	0.001	0.001
	Effect size	0.88	0.88	0.88	0.88	0.88
ppES-sensory_15 Hz	*P* value	0.807	0.152	0.003	0.019	0.064
	Effect size	0.07	0.40	0.82	0.65	0.51
ppES-sensory_60 Hz	*P* value	0.917	0.006	0.002	0.019	0.006
	Effect size	0.03	0.77	0.86	0.65	0.77

terms of MEP amplitude among times (ppES-sensory_15 Hz, $\chi^2_{(13)} = 12.736$, $P = 0.026$; ppES-sensory_60 Hz, $\chi^2_{(13)} = 21.835$, $P = 0.001$). MEP was significantly increased 10 min after ppES-sensory_15 Hz and 60 Hz compared to baseline.

3. Effect of stimulation pulse interval of ppES on corticospinal excitability (Experiment 3)

The typical MEP waveforms in the FDI muscle from one participant are illustrated in Figure 7, while the effects of ppES on MEP amplitudes are shown in Figure 8 and Table 3. In the ppES-sensory_15 Hz (10 ms) condition, the Friedman test showed no significant differences in terms of MEP amplitude among times ($\chi^2_{(15)} = 1.210$, $P = 0.944$). By contrast, in the ppES-sensory_15 Hz (15 ms) condition, the Friedman test showed significant differences in terms of MEP amplitude among times ($\chi^2_{(15)} = 28.448$, $P < 0.001$). The Wilcoxon signed-rank test with Bonferroni correction revealed that ppES-sensory_15 Hz (10 ms) did not show a significant difference in MEP amplitudes. By contrast, MEP significantly decreased immediately after, 5 min after, 10 min after, 15 min after, and 20 min after ppES-sensory_15 Hz (15 ms) compared with baseline MEP before delivering PES.

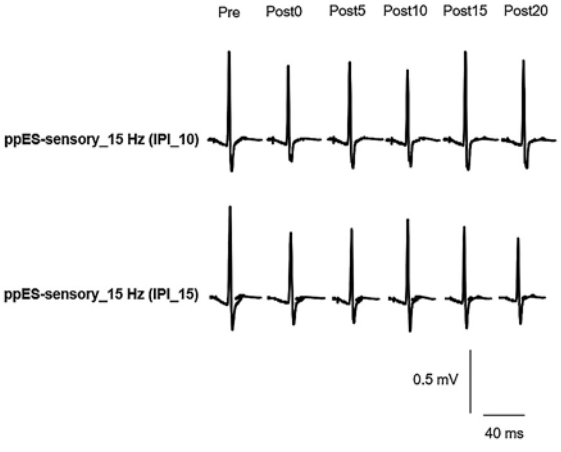

Figure 7. Representative MEP waveform (Experiment 3).
Typical MEP waveform of the FDI at ppES-sensory_15 Hz (10 ms) and ppES-sensory_15 Hz (15 ms).

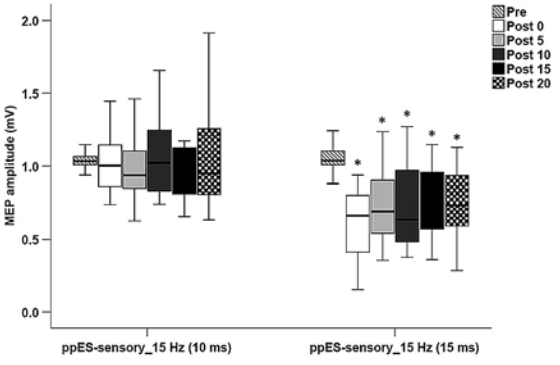

Figure 8. Effect of stimulation pulse interval of ppES on MEP (Experiment 3).
At ppES-sensory_15 Hz (10 ms), no changes in MEP were observed before or after stimulation. By contrast, at ppES-sensory_15 Hz (15 ms), a significant decrease in MEP was observed from immediately after stimulation until 20 min after stimulation compared to before stimulation. When comparing ppES-sensory_15 Hz (10 ms) and ppES-sensory_15 Hz (15 ms), a significant difference was observed in MEP from immediately after stimulation until 20 min after stimulation.
*$P < 0.05$. Error bars indicate SE.

Table 3. Statistical results of Experiment 3 (comparison before and after peripheral electrical stimulation).

		Pre vs. Post 0	Pre vs. Post 5	Pre vs. Post 10	Pre vs. Post 15	Pre vs. Post 20
ppES-sensory_15 Hz (10 ms)	*P* value	0.910	0.650	0.955	0.496	0.427
	Effect size	0.117	0.176	0.102	0.073	0.029
ppES-sensory_15 Hz (15 ms)	*P* value	0.001	0.002	0.003	0.002	0.001
	Effect size	0.88	0.79	0.78	0.81	0.84

Discussion

This study reported three important findings. First, ppES with an interval of 5 ms between two doublets increased MEP like sES above the motor threshold. Second, even if the total number of pulses of ppES was the same as that of PES-sensory, ppES with an interval of 5 ms between two doublets increased corticospinal excitability, whereas sES-sensory decreased corticospinal excitability. Finally, ppES with an interval of 10 ms between two doublets had no effect on MEP, whereas ppES with an interval of 15 ms between two doublets decreased MEP.

The application of long-duration ppES at 30 Hz significantly increased corticospinal excitability. By contrast, a previous study found that long-duration ppES at 10 Hz had no effect on corticospinal excitability [3]. However, the effect of ppES on corticospinal excitability appears to depend on the stimulation frequency. For example, PES at 10 Hz has been reported to increase [5-10] as well as decrease [17] corticospinal excitability, suggesting variation in the effect of PES at 10 Hz. Conversely, several previous studies have reported that PES at 30 Hz significantly increases the corticospinal excitability [12-14]. As per Andrews et al. [14], we used 30 Hz as the stimulation frequency of ppES, and the observed effect was consistent with their findings. Furthermore, previous work revealed that long-duration ppES with a 5 ms interval between two doublets effectively reduced short-latency afferent inhibition (SAI) [3]. Considering that SAI is related to the activity of cholinergic inhibitory interneurons [22], ppES with a 5 ms interval between two doublets might decrease interneuronal activity. Thus, ppES with a 5 ms interval between two doublets might reduce the inhibitory effect from the periphery to the primary motor cortex via the primary somatosensory cortex, resulting in increased corticospinal excitability. Furthermore, ppES-sensory_30 Hz increased MEP similarly to sES-motor_30 Hz in this study. The reason why ppES-sensory_30 Hz increased MEP similarly to sES-motor_30 Hz remains unknown. One possible examination for ppES and sES-motor induced MEP facilitation is the specific timing of direct Ia fiber activation by PES and somatosensory input from contracting muscle. sES-motor induces afferent input evoked by electrical stimulation as well as somatosensory input evoked by contracting muscle, as sES-motor activates both Ia fibers and axons of motor neurons. Considering that afferent input by PES and somatosensory input induced by contracting muscle reaches the cerebral cortex in very short intervals, a pair of somatosensory afferent inputs at a very short interval might be an important factor for increasing primary motor cortex activity. On the other hand, ppES above the sensory threshold with an interval between two doublets of 5 ms stimulated Ia fibers twice without activation of motor neurons, so that the pair of afferent

inputs induced by ppES reaches the cerebral cortex in a very short interval. Thus, ppES above the sensory threshold with a 5 ms interval between two doublets could increase corticospinal excitability, including primary motor cortex, above the motor threshold to the same extent as sES. On the other hand, it is possible that the modulation of MEP induced by ppES and sES related to spinal excitability because spinal excitability can lead to changes in MEP. Several previous studies have reported that PES has no effect on M-waves [6, 23, 24] or F-waves [4, 6, 15]. Thus, long-duration ppES might have no influence on spinal excitability. However, Chipchase et al. [16] reported that PES modulates spinal excitability. Additional studies are warranted to reveal the effect of long-duration ppES on spinal excitability.

In Experiments 1 and 2, the effect of long-duration ppES-sensory on corticospinal excitability was inconsistent with that of long-duration sES-sensory, even when the total number of pulses was the same as that of sES-sensory. Similarly, a previous study found that short-duration (5s) ppES-sensory significantly increased corticospinal excitability while short-duration sES had no effect on corticospinal excitability, even when the total number of pulses was the same as that of sES-sensory. These results indicate that corticospinal excitability is increased by long-duration ppES irrespective of total number of pulses.

Although long-duration ppES with an interval of 5 ms between two doublets significantly increased corticospinal excitability, long-duration ppES with an interval of 10 ms between two doublets had no effect on MEP and long-duration ppES with an interval of 15 ms between two doublets decreased MEP. Similarly, a previous study reported that short-duration ppES with an interval of 10 ms between two doublets had no effect on MEP, while short-duration ppES with an interval of 15 ms interval between two doublets tended to decrease MEP [3]. The reasons why modulation of corticospinal excitability following ppES depends on the interval between two doublets are yet to be investigated. However, they might be associated with excitability in the primary somatosensory cortex (S1). Hoshiyama et al. [25] found that a single electrical pulse could induce N20m as an indicator of the S1 excitability, but N20m was not identified using a paired-pulse paradigm with an inter-pulse interval of < 9 ms [25]. These results suggest that excitability in the S1 can be reduced further by a paired-pulse paradigm than by a single electrical pulse. Furthermore, a previous study has revealed that decreased S1 activity induced by continuous theta-burst stimulation increases corticospinal excitability [26]. Considering that the ppES used in this study comprised a train of two single electrical pulses with an inter-pulse interval < 9 ms, long-duration ppES with an interval of 5 ms between two doublets might decrease S1 excitability, thereby resulting in increased corticospinal excitability. On the other hand, the longer the interval between the two doublets of ppES is, the shorter the interval between the two doublets was to the inter-train interval. For instance, an interval between two doublets of 15 ms is approximately equal to an inter-train interval of sES-sensory_60 Hz (16.6 ms). Considering that sES-sensory_60 Hz significantly decreased corticospinal excitability in the present study, long-duration ppES with an interval between two doublets of 15 ms might decrease corticospinal excitability to the same extent as sES below the sensory threshold.

This study has one limitation. Corticospinal excitability is modulated by altered spinal excitability and / or corticomotor excitability, suggesting that altered spinal excitability and/or corticomotor excitability is involved in increased corticospinal excitability induced by long-duration ppES. In further examinations, the measurements of spinal excitability using H-reflex or F-reflex are required to investigate the mechanism underlying the modulatory effect of long-duration ppES on corticospinal excitability.

In conclusion, application of ppES of 20 min with an interval of 5 ms between two doublets over ulnar nerve significantly increased the corticospinal excitability in the FDI muscle 10-15 min after the intervention, regardless of stimulation frequency. Conversely, ppES of 20 min with an interval between two doublets of 15 ms significantly decreased the corticospinal excitability in the FDI muscle, and the effect persisted for at least 20 min after the intervention. Given that the muscles in the paralyzed limbs of some patients with stroke tend to become fatigued, long-duration pass comprising two afferent inputs from Ia fibers (without muscle contraction) may be applied for movement disorders after stroke.

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Conflicts of Interest

There are no conflicts of interest to declare.

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