Changes in Excitability of the Cortical Projections to the Human Tibialis Anterior After Paired Associative Stimulation

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Mrachacz-Kersting N, Fong M, Murphy BA, Sinkjaer T. Changes in excitability of the cortical projections to the human tibialis anterior after paired associative stimulation. J Neurophysiol 97: 1951–1958, 2007. First published January 3, 2007; doi:10.1152/jn.01176.2006. Paired associative stimulation (PAS) based on Hebb’s law of association can induce plastic changes in the intact human. The optimal interstimulus interval (ISI) between the peripheral nerve and transcranial magnetic stimulation is not known for muscles of the lower leg. The aims of this study were to investigate the effect of PAS for a variety of ISIs and to explore the efficacy of PAS when applied during dynamic activation of the target muscle. PAS was applied at 0.2 Hz for 30 min with the tibialis anterior (TA) at rest. The ISI was varied randomly in seven sessions (n = 5). Subsequently, PAS was applied (n = 14, ISI = 55 ms) with the TA relaxed or dorsi-flexing. Finally, an optimized ISI based on the subject somatosensory evoked potential (SEP) latency plus a central processing delay (6 ms) was used (n = 13). Motor-evoked potentials (MEPs) were elicited in the TA before and after the intervention, and the size of the TA MEP was extracted. ISIs of 45, 50, and 55 ms increased and 40 ms decreased TA MEP size by 92% (P = 0.001). PAS delivered at rest resulted in a nonsignificant increase; however, when the ISI was optimized from SEP latency recordings, all subjects showed significant increases (P = 0.002). No changes in MEP size occurred in the antagonist. Results confirm that the excitability of the corticospinal projections to the TA but not the antagonist can be increased after PAS. This is strongly dependent on the individualized ISI and on the activation state of the muscle.

INTRODUCTION

Reorganization in the human adult cortex has been shown after natural changes such as voluntary motor exercise, injury caused by amputation, and stroke (Butefisch et al. 2000; Traversa et al. 1997). Cortical reorganization can also be induced artificially using techniques such as repetitive electrical peripheral nerve stimulation (rES) and repetitive transcranial magnetic stimulation (rTMS) (Khaslavskaia and Sinkjaer 2005; Khaslavskaia et al. 2002; Peinemann et al. 2004; Ridding et al. 2000).

Recently, a novel protocol was established based on Hebb’s law of coincident summation (Hebb 1949) where the presynaptic neuron is depolarized at the same time as the postsynaptic neuron (Stefan et al. 2000, 2006; Wolters et al. 2003) and more recently to the lower limb muscle tibialis anterior (TA) (Prior and Stinear 2006; Stinear and Hornby 2005). A number of these studies have reported that it was not possible to induce significant changes in all of the subjects tested (Stefan et al. 2000, 2006), whereas in some studies, the increases in the peak-to-peak size of the motor-evoked potential (MEP) amplitude after PAS were comparatively small (Stinear and Hornby 2005). A possible explanation is that the interstimulus interval (ISI) used was not optimal for all subjects, because most studies have implemented a standard ISI (~25 ms for the muscles of the hand). The afferent conduction time is known to vary with the height of the participant, although this is more pronounced for muscles of the lower limb compared with the upper limb. Prior and Stinear (2006) attempted to individualize the ISI for each participant using the latency of the MEP plus 5 ms. The average ISI implemented was 38.2 ms (range: 35–42 ms), and the authors argued that, based on previous literature on the afferent conduction velocity within the tibial nerve, this ISI is reasonable. However, the average latency for the arrival of the volley at the somatosensory cortex after tibial nerve stimulation is 38 ms (Cruse et al. 1982); thus at least for some of their subjects, the afferent volley arrived after the cortical stimulus and did not result in the associative plasticity at the level of the motor cortex. In their protocol, they stimulate the common peroneal nerve and the average time for the afferent volley to arrive at the somatosensory cortex has been reported.
to be between 34 and 46 ms (Petersen et al. 1998; Vas et al. 1981). The afferent signal then has to be relayed to the motor cortex for the pairing with the TMS-generated signal which requires a set central processing time. This time is currently not established but is thought to be $\sim 4$–10 ms. Based on these calculations, the shortest ISI that can reasonably result in an altered excitability of the cortical projections to the TA through coincident summation at the level of the motor cortex is 38 ms. However, Kujirai et al. (2006) provided data to suggest that the later (13) waves generated by the magnetic stimulus may play an important role in associative plasticity. If this is the case, it is possible that an ISI of 34 ms would suffice to alter the excitability of the cortical projections to the TA because the coincident summation of the peripheral input with the generated MEP does not have to occur at the onset of the MEP. A second explanation of why Stinear and Hornby (2005) observed such low increases in the TA MEP amplitude after PAS may be that the task itself may have interfered with the induction of plastic changes after PAS. Stefan et al. (2006) reported that dynamic motor training before PAS reduces the efficacy of PAS.

There were two aims of this study. Initially, the effect of PAS on the excitability of the cortical projections to the TA was explored for a variety of ISIs between the peripheral afferent volley and the TMS pulse. This was to determine if the effective time window is similar to what has previously been reported in rat hippocampal neurons (Bi and Poo 1998). A second aim was to explore the efficacy of PAS in altering the excitability of the cortical projections to the TA when it is applied during a dynamic activation of the target muscle TA. Preliminary results from this study have been reported previously in abstract form (Mrachacz-Kersting et al. 2005).

**Methods**

**Subjects**

Twenty-seven subjects (16 male and 11 female) provided written and informed consent to participate in this study. Approval was given by the University of Auckland Human Participants Ethics Committee (reference no. 2005/036). At the time of the study, all subjects were free of any known physical or neurological disorders and were 19–41 years old.

**Apparatus and instrumentation**

Surface electrodes (20 mm Blue Sensor Ag/AgCl, AMBU) were used to record the EMG activity of TA and soleus (SOL) of the right leg for all aspects of the experiments. The electrodes were placed in accordance with the recommendations of Garland et al. (1994). All data were sampled at a frequency of 4 kHz. The EMG signals were amplified and band-pass filtered at 20 Hz to 2 kHz and rectified.

**Stimulation**

A Magstim 200 (Magstim Company, Dyfed, UK) with a focal figure of eight double cone coil (110 mm diameter) was used to apply single pulses (with a posterior to anterior directed current) to elicit a MEP in the muscle of primary interest, which was the TA muscle. Stimulation of the common peroneal nerve (CPN) was applied using a peripheral stimulator with the cathode proximal. A suitable position for stimulation, defined as the site where a maximal M-wave was produced in the TA with no activity from the synergistic peroneal muscles and no activity from the antagonist SOL, was located.

Palpation of SOL and peroneal muscles was performed during stimulation trials to ensure that this was occurring. This site corresponded to a point just anterior to the level of the caput fibulae. The pulse width was set to 1 ms and the intensity to $1 \times$ motor threshold. Pilot studies revealed that these pulse settings produced the most consistent changes when combined with the transcranial magnetic stimulus.

**Somatosensory-evoked potentials**

In 19 subjects, the cortical potentials evoked by the imposed stimulation of the common peroneal nerve were recorded with surface electrodes (7 mm Ag/AgCl Hydrosport disposable adhesive electrodes, Physiometrix) placed on the scalp, according to the International 10–20 system (Yamada 2000). Somatosensory-evoked potentials (SEPs) were recorded with a single vertex electrode (Cpi; band-pass, 0.05–1,000 Hz; sampling rate, 10 kHz; referenced to the contralateral earlobe). A minimum of 800 (maximum, 3,000) traces were recorded and ensemble averaged on-line. The characteristics of the pulse were the same as those used during the application of PAS (width of 1 ms, intensity of $1 \times$ motor threshold). The arrival of the evoked potential was measured as the time of occurrence of the first negative peak, designated in the literature as the N34 peak.

**Procedures**

Subjects were seated in a fixed chair (hip, 90°; knee, 130°) with their right foot resting on a footplate and with a tight fitting cotton cap affixed to their head that had premarked coordinates in a 1-cm grid pattern. Initially, the stimulation intensity for the magnetic simulation was set at $\sim 50\%$ of stimulator output to find the optimal site for evoking a MEP in the TA. The best spot for stimulation (also termed the hot-spot) was taken as the coordinate where the peak-to-peak amplitudes of the recorded responses from the muscle (MEPs) was greater in the target muscle than amplitudes of adjacent coordinates for a given stimulus intensity. For all subjects, this site was $\sim 2$–3 cm anterior to the vertex, and a stimulation applied to this area also evoked a response in the SOL. Once the hot-spot was identified, the coil position was maintained by markings made on the cotton cap. This ensured that the stimulation was always applied over the same area of the motor cortex. Subsequently, the rest threshold (RTh), defined as the highest stimulus intensity that produced no more than 5 of 10 consecutive TA MEPs with an amplitude of $\sim 50 \mu$V while the muscle was at rest was identified. All subsequent stimuli were delivered at 120% of RTh to ensure a TA peak-to-peak MEP amplitude of $\sim 0.3$–$0.5 \text{ mV}$. However, because of the location of the TA representation on the motor cortex, it was not possible to attain a MEP amplitude of 0.5 mV in all subjects even when the intensity of the TMS pulse was increased. As a consequence, the amplitude value varied from 0.2 to 0.9 mV across all subjects.

**PAS protocol**

PAS consisted of a single electrical stimulation of the common peroneal nerve delivered at motor threshold (MT), followed by a single TMS pulse delivered to the motor cortex at an intensity of 120% RTh. A total of 360 pairs of stimuli were applied at a rate of 0.2 Hz. In some experiments and where indicated, these were applied in two sets of 180 paired stimuli. In all experiments, subjects were reminded every 5 min to concentrate on their TA muscle.

**Experiment 1: effect of ISI on PAS-induced changes**

In five subjects, a set of experiments was conducted where the ISI between the peripheral stimulus and the TMS was varied randomly, for each experiment. During these, the target muscle was maintained at rest while the peripheral stimulus was set at motor threshold and the TMS stimulus at 120% RTh. Seven ISIs were studied: 20, 30, 40, 45,
Experiment 2a: effect of target muscle activation on PAS-induced changes

Fourteen subjects partook in this part of the study that consisted of one of three interventions being applied to the subjects as outlined in Fig. 1. The order of these was varied randomly. The intervention termed PAS at rest consisted of a single electrical stimulation of the common peroneal nerve delivered at MT, followed by a single TMS pulse delivered to the motor cortex at an intensity of 120% RTh. The target muscle was at rest. A total of 360 pairs of stimuli were applied in two sets of 180 paired stimuli at a rate of 0.2 Hz. The timing between the two stimuli (ISI) was set at 55 ms. The second intervention was similar to the first intervention with the exception that subjects, instead of resting the TA muscle, were now asked to actively dorsiflex their right foot at 0.2 Hz in time to a metronome (PAS + DF). The peripheral and TMS stimuli were timed to start at the beginning of each dorsiflexion action when the TA activity was 5–10% maximum voluntary contraction (MVC). Custom build software provided feedback of the EMG activity in the TA to the subject to ensure that the level of the activity was maintained. The software did not trigger unless the activity of the TA was between 5 and 10% MVC. Most subjects required at least 20 repetitions to attain reproducible dorsiflexion movements. The third intervention consisted of the subjects dorsiflexing their right foot at a frequency of 0.2 Hz in time to a metronome (DF only). No stimuli were delivered during this last intervention.

The dependent variable was the average peak-to-peak MEP amplitude measured both before and after the intervention while the subjects were seated and at rest. In all experimental sessions, the MEP amplitude was also obtained halfway through the intervention. After the initial 15 min of the intervention (i.e., after 180 trials had been completed), the intervention was interrupted for ~5 min to record the dependent measure. The intervention was then continued for a further 15 min. A total of 16 stimuli were thus applied before the intervention (pre), halfway through the intervention (Int15min), and immediately after the intervention (post) at a stimulus intensity of 120% RTh. All data were stored on the laboratory computer for later off-line analysis.

50, 55, and 60 ms. A minimum of 2 days elapsed between any two experimental sessions. The mean peak-to-peak MEP amplitude measured both before and after the intervention was determined for 16 stimuli delivered 5–7 s apart.

Experiment 2b: effect of TMS intensity on PAS-induced changes

The TMS stimulus intensity was maintained at 120% RTh for all above experiments. In the PAS + DF intervention, however, the TMS stimulus acted on an already preactivated motor cortex, and it is known that this will result in a greater MEP amplitude compared with a resting condition (Kischka et al. 1993). In the control experiment, the stimulus intensity used during the intervention was therefore matched to produce a similar MEP amplitude during the intervention to that when the subject was at rest and the TMS intensity at 120% RTh. The condition is termed PAS + DFM

Five subjects who were known to show increases in the TA MEP amplitude after PAS at rest participated in this set of experiments. Initially, the average peak-to-peak MEP amplitude was determined for 16 TMS stimuli delivered at 120% RTh while the subjects were at rest. These measures served as the preintervention dependent variable. Subsequently, the subjects were asked to precontract the TA to 5–10% MVC and to hold this level of contraction. Subjects received continuous feedback of their TA EMG signal. The TMS stimulus intensity was now decreased initially in steps of 5% of stimulator output and in steps of 1% stimulator output, until the peak-to-peak MEP amplitude attained during the precontraction of the TA matched that during the rest condition. After this, the intervention was applied that consisted of PAS + DFM and was thus similar to the original PAS + DF condition with the exception that the cortical TMS stimulus consisted of the matched intensity setting.

The dependent variable (MEP amplitude) was measured in the same way as for experiment 2a. In addition to measuring the dependent variable pre, at Int15 min, and postintervention, measures were taken 15 and 30 min postintervention. All data were stored on the laboratory computer for later off-line analysis.

Experiment 3a: effect of optimizing ISI on PAS-induced changes

Thirteen subjects participated in this experiment. After the SEP measures for each individual subject, PAS was applied with the subjects at rest and an ISI of the SEP latency plus a set central processing delay of 6 ms. The 6-ms delay was chosen based on an average value from the literature, which indicates a central processing delay of 4–10 ms (Goldring et al. 1970; Petersen et al. 1998). Pilot studies from our laboratory (n = 3) using ISIs of SEP latencies plus 0–10 ms (in steps of 1 ms) confirmed that adding 6 ms to the individual SEP latency gave the greatest increases in excitability. The intensity of the TMS pulse was set at 120% RTh for pre and post measures as well as for the intervention. The peripheral stimulus was set as for all previous experiments at 1× MT. The dependent variable (MEP amplitude) was measured in the same way as for all previous experiments and stored on the laboratory computer for later off-line analysis.

Experiment 3b: effect of PAS on spinal excitability

To study the possibility that the changes in the TA MEP amplitude after PAS were caused by alterations in spinal excitability, H-reflexes were recorded in five subjects and F waves in seven subjects. PAS was applied as outlined in experiment 3a. To ensure that changes in spinal excitability could be linked with changes in the cortical projections to the TA, the peak-to-peak TA MEP was also recorded both before and after the intervention.

H reflex recording

The H reflex of the TA was obtained in five subjects by stimulating at the same site as used for the peripheral electrical stimulus for PAS. As stated previously, this site ensured that the peroneal muscles that
have inhibitory connections to the TA were not activated as confirmed by palpation. The stimulus width was set to 1 ms. Next, the stimulus intensity was increased until a maximum peak-to-peak M wave (M-max) was attained in the relaxed TA. This procedure was repeated three times to ensure repeatability of the measured M-max. The average of at least 20 maximum M waves was recorded and used as an indication of M-max. The stimulus intensity was now decreased until a peak-to-peak TA H reflex of 5–10% of M-max was observed that corresponded to ~50% of M-max. It was not possible to obtain H reflexes of larger size than ~10% of M-max in the TA. In total, 20–30 H reflexes were recorded both before and immediately after PAS, and the ISI varied between 5 and 10 s. The size of the average H reflexes was expressed as a percentage of M-max.

**F wave recording**

F waves were recorded from seven subjects because it is not possible to obtain a TA H reflex in most subjects. The stimulation site was maintained as for H reflex recordings while the stimulus width was set to 200 μs and the stimulus intensity to 130% of that required to generate M-max. A minimum of 20 F waves were recorded, and the peak-to-peak size was determined. To obtain 20 F waves, between 60 and 100 stimuli were applied, and the time between each stimulus varied between 5 and 10 s.

**Statistical analysis**

Repeated-measures ANOVA was used to determine if there was a significant difference between the different ISIs used in experiment 1 (within-subject factor: time). The effect of the three different interventions on TA MEP amplitude in experiment 2a was tested using a two-way repeated-measures ANOVA (within-subject factors: intervention, time). The effect of matching or not matching the TMS pulse intensity during the dorsiflexion condition (experiment 2b) was tested using a two-way repeated-measures ANOVA (within-subject factors: stimulus intensity, time). Paired t-test (2-tailed) was used to evaluate the effect of using individualized ISIs on TA MEP amplitude and to evaluate the changes in spinal excitability. Significance level was set at \( P < 0.05 \). If not stated otherwise, all data are given as mean ± SD.

**RESULTS**

**Effect of ISI on PAS-induced changes**

Across all subjects for whom SEP recordings were conducted, the afferent volley arrived at the somatosensory cortex at 44 ± 2 ms (range: 42–47 ms) after CPN stimulation. If a central processing of 4–10 ms is adapted (Petersen et al. 1998), the peripheral volley should reach the motor cortex at 46–57 ms poststimulation. During the PAS intervention, the TMS should be triggered at this time. To study if this timing is appropriate, five subjects were tested at various ISIs between the peripheral nerve stimulus and the TMS (Fig. 2). Repeated-measures ANOVA revealed a significant effect of the ISI on TA MEP amplitude \([F(6,24) = 3.69, P = 0.01]\). An ISI of 55 ms induced the greatest facilitation, and for experiments 2a and 2b, this was the ISI used. Interestingly, an ISI of 40 ms produced a significant decrease of the TA MEP amplitude in all subjects tested.

**Effect of target muscle activation on PAS-induced changes**

Figure 3 shows the averaged (16 sweeps) raw TA MEP data before and after the PAS only, DF only, and PAS + DF interventions for one subject. The increase in the raw MEP was 200 μV for both the PAS only and DF only interventions. For the PAS + DF intervention, the increase was 800 μV. PAS + DF produced the greatest increase, which is above and beyond the algebraic sum of the PAS and DF interventions when applied separately.

Across all subjects, the preintervention average TA MEP size was \( 0.29 ± 0.31 \) (DF only), \( 0.24 ± 0.13 \) (PAS only) and \( 0.26 ± 0.22 \) mV (PAS + DF). Postintervention values were \( 0.33 ± 0.18 \) (DF only), \( 0.37 ± 0.33 \) (PAS only), and \( 0.50 ± 0.39 \) mV (PAS + DF). Only eight subjects facilitated in the PAS-only condition and nine in the DF-only condition. The TA peak-to-peak MEP amplitude increased significantly for all subjects only for the PAS + DF intervention \([Fig. 4; F(2,26) = 6.59, P < 0.01]\). The TA MEP size increased on average by 92%.

**Effect of TMS pulse intensity on PAS-induced changes during the DF task**

Five subjects participated in experiment 2b. All subjects were chosen based on the fact that they had shown increases in

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Effect of 3 different interventions on size of raw TA MEP amplitude at rest for 1 subject. A: TA MEP changes for the paired associative stimulation (PAS)-only intervention. B: TA MEP changes for the dorsiflexion (DF)-only intervention. C: TA MEP changes for the PAS + DF intervention. Data are from 1 subject and at least 2 days elapsed between each test. Data are the average of 16 trials.
the peak-to-peak TA MEP amplitude after PAS delivered at rest during experiment 2a. Across all subjects, the TMS pulse intensity had to be reduced to 80–100% RTh, which corresponded to 34–42% stimulator output. Figure 5 shows the mean TA MEP group data with SD of the peak-to-peak TA MEP amplitude change expressed as a percentage of preintervention values. Repeated-measures ANOVA revealed a significant effect between pre- and post-TA MEP size \( F(1,4) = 11.61, P = 0.02 \) but not TMS pulse intensity \( F(1,4) = 0.02, P = 0.88 \).

Effect of optimizing ISI on PAS-induced changes

An ISI of 55 ms did not result in a significant change across all subjects in experiment 2a even though in experiment 1 it resulted in the greatest increases in TA MEP size. One possibility is that for some subjects, the ISI of 55 ms was not optimal, which may be reflected in the large SD observed in experiments 1 and 2a. In experiment 3, the ISI was therefore optimized after SEP recordings for all participants. The optimal ISI ranged from 46 to 55 ms across all subjects that participated in this part of the study. Figure 6 shows the average change in TA MEP amplitude as a percentage of preintervention values for all subjects \( n = 13 \) and the associated SD. In all subjects, the TA MEP amplitude increased significantly \( F(2,24) = 8.18, P = 0.002 \) by 96 and 88% for immediately before and 30 min after intervention, respectively.

Effect of PAS on spinal excitability

The average peak-to-peak size of the TA H reflex was 4.00 ± 1.53% of M-max \( n = 5 \) before the PAS intervention. This was not significantly different \( P = 0.9 \) from the peak-to-peak size of the H-reflex after PAS \( 4.05 ± 0.78\% \text{ of M-max} \). As indicated in METHODS, to link spinal changes to cortical changes, the peak-to-peak TA MEP amplitude was also recorded. This increased on average by 67%, which was significantly different from pre-PAS \( P = 0.008 \).

The peak-to-peak F wave recorded in seven subjects was not significantly different before \( 380 ± 180 \mu V \) or immediately after \( 450 ± 230 \mu V \) the PAS intervention \( P = 0.23 \), whereas the TA MEP size increased by 73% \( P = 0.01 \).

Effect of PAS on the antagonist SOL

After the DF-only condition, the SOL MEP size decreased nonsignificantly for all subjects. For the PAS-only and PAS + DF interventions, the peak-to-peak SOL MEP increased nonsignificantly \( F(2,26) = 0.82, P = 0.53 \).

DISCUSSION

The aim of this study was twofold. First, it aimed to determine the effective interval between the peripherally induced mixed nerve volley and the cortical stimulus for inducing significant changes in the excitability of the cortical projections to the TA muscle after PAS delivered at rest. This has not been reported before for the lower extremity. Results indicate that when the ISI is optimized for each participant according to individual SEP recordings, bi-directional changes in plasticity may be observed. This is in line with results presented by Ziemann et al. (2004) for muscles of the hand. The second aim was to study if PAS is more effective when applied to an activated muscle compared with a muscle at rest. Results showed that PAS can be induced selectively in the target muscles of the lower limb. At a set ISI across individ
uals, this is more effective when PAS is applied to an active muscle. PAS applied during rest or the dorsi-flexion task caused no significant changes in the MEP amplitude of the antagonist SOL, indicating that this technique of altering excitability is likely to be specific to the target muscle.

**Possible mechanisms**

PAS has been proposed to be a form of associative Hebbian learning. A number of conditions have to be satisfied in the classical model of associative Hebbian learning. These include 1) rapidly evolving effect (within 30 min), 2) persistence even on cessation of stimulation, 3) a specific temporal order of the two stimuli (i.e., up-regulated and down-regulated depending on the ISI), 4) reversibility, and 5) dependence on activation of N-methyl-D-aspartate (NMDA) receptors and involvement of L-type voltage-gated Ca$^{2+}$ channels. The effects of PAS, as applied in this study, evolved rapidly (<30 min), persisted after the cessation of the stimulation (effects were still present 30 min after the cessation of the stimulus, n = 13), and were strongly dependent on the order of the two successive stimuli. Although the dependence on NMDA receptors and the involvement of Ca$^{2+}$ channels were not addressed in this study, previous reports for the upper extremities, using similar protocols, have confirmed this to be the case (Stefan et al. 2002; Wolters et al. 2003).

The site where PAS-induced changes in excitability manifest remains to be elucidated. Stefan et al. (2000) and Wolters et al. (2003) implemented both F wave analysis and brain stem stimulation to show that the changes observed occur upstream from the brain stem. However, F waves are not necessarily generated by the same population of motoneurons as MEPs (Hultborn and Nielsen 1995), and the low subject numbers (n = 2) subjected to brain stem stimulation may not be representative of the population. A preferred measure of spinal excitability is the H-reflex (Hultborn and Nielsen 1995). In this study, it was possible to record H-reflexes from five subjects. The H-reflex amplitude did not change significantly. In addition, F waves were not altered after PAS applied at rest. However, because of the disadvantages of F wave data outlined above and the low subject numbers where it was possible to record H-reflexes, the possibility remains that at least some of the increases in excitability after PAS may be attributable to spinal mechanisms. Additionally, the decrease of the TA MEP amplitude at an ISI of 40 ms (experiment 1) may have been caused by recurrent inhibition—a spinal mechanism—of motoneurons after the peripheral stimulus. A similar decrease in MEP amplitude is observed at ISIs of 10 ms for upper limb hand muscles.

**Timing of TMS stimulus in relation to peripheral stimulus during the intervention**

The afferent volley arrived at the somatosensory cortex 42–47 ms after the peripheral electrical stimulus. This indicates that the TMS volley has to be elicited at ~46–57 ms later if a central processing delay of 4–10 ms is adopted. Results from experiment 1 showed that an ISI of 55 ms is optimal for inducing the greatest increases in the TA MEP amplitude when PAS is applied at rest. However, this timing is not consistent with the timing implemented by Stinear and Hornby (2005). These authors used an ISI equivalent to the average MEP latency plus 5 ms. The average TA MEP latency as observed in this study is ~30 ms, which is in agreement with previous reports (Petersen et al. 1998). Accordingly, this would mean that, in the previous study, the TMS stimulus was applied 35 ms after the peripheral stimulus, which is before the afferent volleys arrival at the somatosensory cortex. With this timing, it would therefore not be possible for the weaker afferent volley to have had an excitability effect on the postsynaptic cell at the time that the second suprathreshold TMS stimulus is given. Nevertheless, Stinear and Hornby (2005) reported a 21% increase in MEP size after their intervention. It is possible that the repetitive nature of the walking task itself caused the increase in the MEP size, similar to the increase observed after repetitive dorsi-flexion in this study. In this study, the dorsi-flexion only intervention produced a nonsignificant increase in TA MEP size of 28%.

In experiment 1, a decrease in the TA MEP amplitude was observed for an ISI of 40 ms. At this time, the afferent volley arising from the peripheral stimulus has not yet arrived at the somatosensory cortex, and thus the events triggered by the TMS precede those triggered by the common peroneal nerve stimulation. These results are similar to those reported by Wolters et al. (2003) for the abductor pollicis brevis (APB) muscle of the hand and conform to protocols established in rat hippocampus slice preparations (Bi and Poo 1998). Furthermore, these results confirm that bi-directional changes are produced in the TA MEP amplitude depending on the timing of the peripheral and central stimulus, one condition of Hebbian type learning.

**Effect of PAS on the TA MEP during rest compared with active dorsi-flexion**

Results presented here confirm previous findings (Stefan et al. 2000, 2006), which reported that PAS is not always effective when applied with the target muscle at rest (6 of the 14 subjects showed no effect or even a slight decrease in MEP amplitude in this study). However, when PAS was combined with a simple dorsi-flexion task, the TA MEP amplitude was increased for all subjects. This is in line with the results presented by Khaslavskaia and Sinkjaer (2005), who showed that the effectiveness of repetitive electrical stimulation is significantly increased when applied during voluntary activation of the target muscle; this was more recently presented by Kujirai et al. (2006). This may support the notion by Hulliger (1993) that muscle afferent feedback is of fundamental importance for motor plasticity, at least for the muscles of the lower limb. In a modified version of the PAS protocol, Stefan et al. (2000) studied the effect of digital nerve stimulation combined with TMS and found significant facilitation of the cortical projections to the APB muscle, whereas the same effect was not reported for the first dorsal interosseous muscle (Kujirai et al. 2006). Which afferents contribute to the facilitating effect of PAS remain to be elucidated.
argued that the TA MEP may differ to that during the phasic contraction during the DF + PAS condition. However, Capaday et al. (1999) collected data on the entire input–output relation and found no difference between MEP sizes elicited in the TA during tonic dorsiflexion or during the swing phase of walking at matched levels of background EMG activity.

An unexpected finding of experiment 2a was that, of the 14 subjects, 6 showed no evidence for an increased excitability of the cortical projections to the TA, leading to an overall nonsignificant effect of PAS when applied at rest. This contradicts the findings of experiment 1, where an ISI of 55 ms led to significant changes in TA MEP amplitude. However, only five subjects participated in that experiment, and the SD was large (Fig. 2). The results from experiment 2a confirm previous studies that have shown that PAS is not always effective when applied at rest (Charlton et al. 2003; Stefan et al. 2000, 2006). There are several reasons that may explain this effect: an inconsistent afferent stimulation across subjects (Charlton et al. 2003), different attention levels across subjects (Rosenkranz and Rothwell 2004, 2006; Stefan et al. 2004), and possibly different conduction times for the peripheral nerve volley across subjects. For the TA muscles, a fourth reason may be that the anatomical location of the cortical area representing the TA may differ to that during many cortical cells using TMS as for the hand area.

In experiment 2a, an ISI of 55 ms was used across subjects that resulted in an overall nonsignificant effect of PAS in increasing the excitability of the cortical projections to the TA. In that experiment, care was taken to ensure that CNP stimulation caused a slight but visible twitch in the TA without concomitant muscle activation from either the peroneals or the plantarflexors. It is thus unlikely that the afferent stimulus differed between subjects. All subjects were instructed to concentrate on their TA, however, it is possible that some subjects were able to do so more successfully than others, whereas for the PAS applied during the dorsi-flexion task, the subjects had to concentrate on contracting their TA in time to a metronome. Stefan et al. (2004) have shown that attention has a significant influence on the effectiveness of PAS. Furthermore, Rosenkranz and Rothwell (2004, 2006), using an intervention that only involved sensory input to alter the excitability of cortical projections to hand muscles, have shown that attention is critical for the effectiveness of interventional protocols that involve sensory stimulation. Attention was controlled in this experiment by reminding subjects every 5 min to think about their TA muscle. Arguably, this may not be as effective as the extraperipheral electrical stimulus administered by Stefan et al. (2004); however, the results from experiment 3a indicate that our method was effective in maintaining attention toward the target muscle. Thus the third possibility, that the conduction time for the peripheral nerve volley differed between subjects and that this caused the ISI to be optimized in only some of the subjects, was addressed in experiment 3a. The results highlight the importance of using an optimized ISI for each participant when applying PAS, because under these conditions it is effective in increasing the excitability of the cortical projections to the TA across all subjects.

The optimized ISI ranged from 46 to 55 ms, which differs from the average 38 ms reported by Prior and Stinear (2006) and Stinear and Hornby (2005). However, in the latter studies, the increases in MEP size were rather modest, and it is possible that the coincident summation of the peripheral volley and the cortical stimulus did not occur at the level of the motor cortex but rather downstream from it. Because in this study, we did not apply PAS during the gait cycle, our results are not directly comparable. It is possible that the task of walking itself suppressed the efficacy of PAS because it is mentally more demanding compared with the DF task in this study. Stefan et al. (2006) have shown that dynamic motor training performed before PAS leads to a temporary occlusion of associative motor cortical plasticity. It is not known if this phenomenon persists when PAS is applied during a functionally demanding task. However, if PAS and motor learning act on similar structures as suggested by Stefan et al. (2006), it may be likely that the voluntary movement overrides any artificially introduced stimulus, at least in the intact human. It may be argued that the DF task in this study is similar in complexity as the brisk thumb abduction task performed by participants in the study by Stefan et al. (2006). The question arises why the TA MEP was facilitated in this study. There are two important differences between the two tasks: first, the instructions to the participants differed. In this study, they were asked to perform the DF task once every 5 s compared with a rapid thumb abduction once every 2 s. Second, it is likely that the thumb abduction task also involved activity from the antagonist. In this study, it was ensured that the antagonist SOL remained inactive. Evidence that favors the former explanation was provided by Ziemann et al. (2004) who implemented a similar task to Stefan et al. (2006). In one of their control experiments, subjects were asked to perform the same thumb abduction task once every 2 seconds but at a slower pace. The results showed that, when the task is slowed, the same facilitation of APB MEP amplitudes was induced when subsequent PAS was applied compared with no prior motor task and PAS alone.

Functional significance

PAS as a possible therapeutic tool is able to increase (or decrease) cortical excitability that is specific to the target muscle. Furthermore, if combined with a simple dorsi-flexion movement, these increases are evident after only 15 min of the intervention. A number of other protocols such as rTMS (Di Lazzaro et al. 2002; Huang et al. 2005) and repetitive electrical stimulation (Khaslavskaia and Sinkjaer 2005; Khaslavskaia et al. 2002; Popovic et al. 2003) have been implemented in the past to artificially induce cortical plasticity. The former of these two methods has the disadvantage of a slight but significant risk of inducing epileptiform seizures (Anand and Hotson 2002; Wassermann 1998). Such risks are not reported with rES; however, rES requires a higher number of stimuli to be delivered for significant effects to be observed (Khaslavskaia and Sinkjaer 2005; Khaslavskaia et al. 2002; Popovic et al. 2003). With the reduced number of stimuli and its proven effectiveness, PAS combined with a simple task seems an attractive rehabilitative tool. McKay et al. (2002) studied the effects of repeatedly applying PAS, using a slightly modified version of the current protocol, over a 3-day period, and found that the effects persisted for at least 2 days. However, to date, it is not known if these effects carry over to functional benefits, and this should be further studied.
GRANTS
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