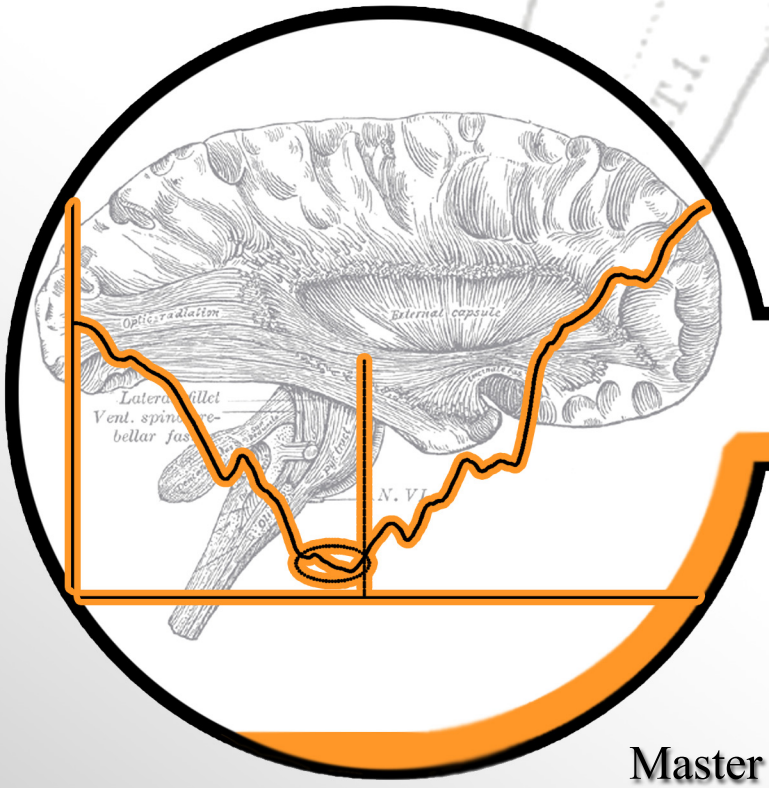
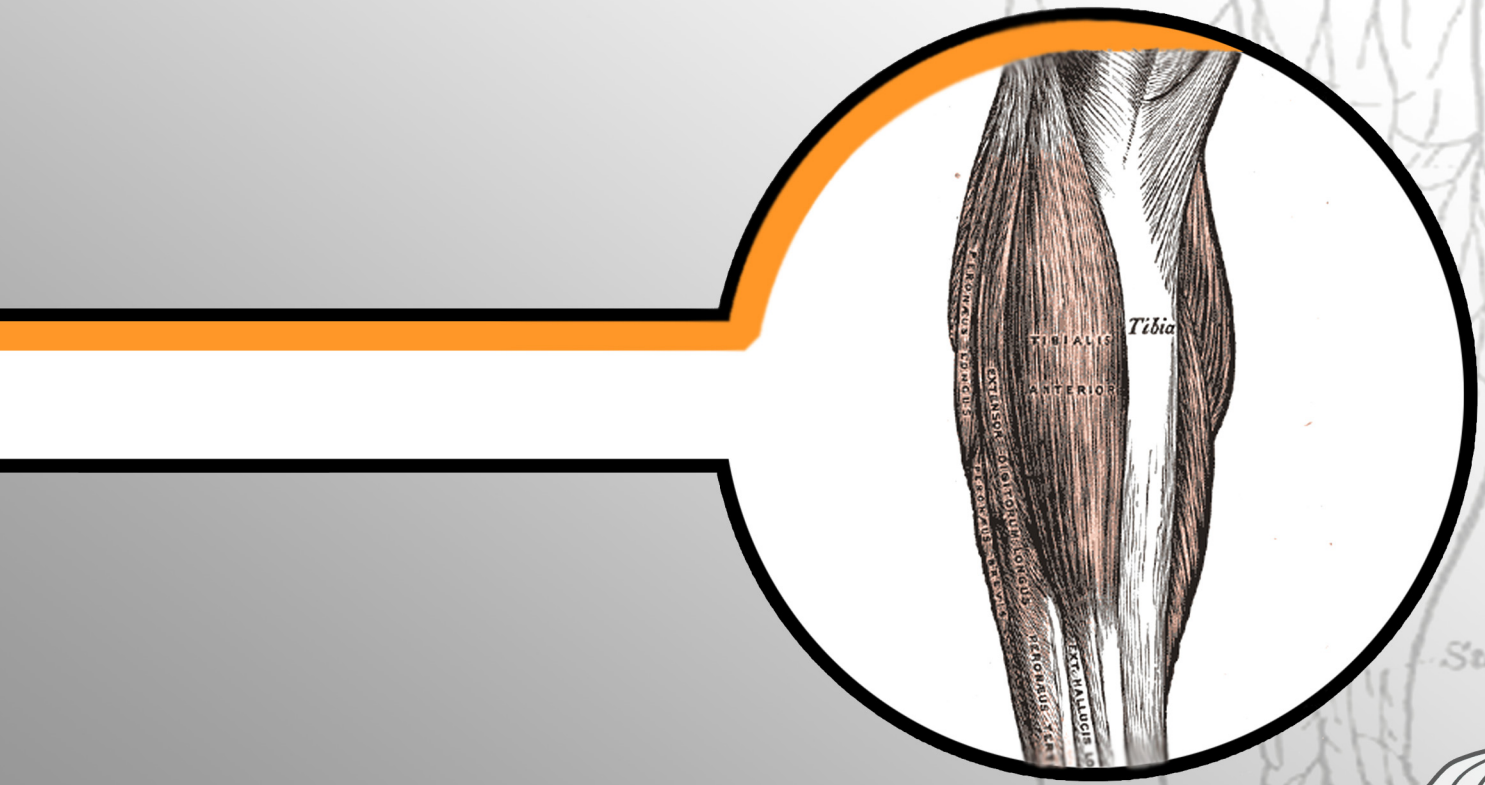
Changes in excitability of cortical projections to the tibialis anterior induced by concurrent motor imagination and peripheral electrical stimulation



Master Thesis for M.Sc.Biomed.Eng.

by

Johnny L. G Nielsen and Steffen Holmgaard



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Abstract:

Plasticity of the human motor cortex may play an important role in functional recovery after stroke. This study investigated a novel approach for changing excitability of the cortical projections to the tibialis anterior (TA), consisting of concurrent motor imagination and peripheral stimulation. It is hypothesized that changes in cortical excitability depend on when stimulation arrives during the cognitive process of movement. The movement-related cortical potential (MRCP) for each participating subject was measured. In three separate intervention sessions, repetitive pairings of an electrical stimulation applied to the common peroneal nerve was timed to arrive at the cortical level during an imaginary dorsiflexion in the preparation phase (INT1), in the execution phase (INT2) or after the execution phase (INT3) in relation to the individual MRCP. Motor-evoked potentials (MEPs) were elicited in the TA before and after each intervention, and the TA MEP size was extracted. Across subjects, the largest increase in the MEP size was observed in INT1 (143%), while the increase was less in INT2 (118%) and further reduced in INT3 (107%). This supports the hypothesis that the arrival of the stimulation depends on the cognitive state, although the variability in the data was large. Changes in the TA MEPs appeared not to be caused by spinal mechanisms. In addition, no significant changes in the antagonist MEP size were observed. The present results indicate that the rationale behind the approach is sound, opening opportunities for new rehabilitation strategies. However, further research on additional subjects is required to validate the hypothesis.

PREFACE

This report is the master thesis by Johnny L. G. Nielsen and Steffen Holmgaard as the conclusion of the M.Sc.Biomed.Eng. programme with specialization in Medical Systems. The work was carried out at the Department of Health Science and Technology (HST), Aalborg University, Denmark from February 2*nd* to June 4*th* 2009. The theme of the semester was *Applied Biomedical Engineering and Informatics*.

The project investigated a novel approach of inducing excitability of cortical projections to the tibialis anterior muscle using a combination of peripheral stimulation and motor imagination. After an extensive literature search, an experimental protocol was designed and pilot experiments were performed to confirm it. Experimental data was collected on four subjects and analyzed according to the protocol. It is anticipated that the reader has a fundamental knowledge of EEG, signal processing and neurophysiology. The project is primarily aimed at fellow students and others who share interests in applied neurophysiology and cortical plasticity.

The main report is structured as a scientific paper due to the significant degree of experimental focus and applied scientific work in the project. The Introduction outlines the background and rational behind the study leading up to the hypothesis. The Methods & Materials describes the experimental protocol and how the data was analyzed. The Results presents the main results from the experimental sessions and subsequent data analysis. In the Discussion, the implications and relevance of the results are discussed.
After the main report, three supplementary worksheets are presented in the appendix, intended to give the reader more in-depth information on some of the background topics relating to the project. They should not be considered as an integral part of the main report.

References for both the main report and the supplementary materials are listed at the very end of the report, beginning on page 38.

Steffen Holmgaard Johnny L. G. Nielsen

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Abstract

Plasticity of the human motor cortex may play an important role in functional recovery after stroke. This study investigated a novel approach for changing excitability of the cortical projections to the tibialis anterior (TA), consisting of concurrent motor imagination and peripheral stimulation. It is hypothesized that changes in cortical excitability depend on when stimulation arrives during the cognitive process of movement. The movement-related cortical potential (MRCP) for each participating subject was measured. In three separate intervention sessions, repetitive pairings of an electrical stimulation applied to the common peroneal nerve was timed to arrive at the cortical level during an imaginary dorsiflexion in the preparation phase (INT1), in the execution phase (INT2) or after the execution phase (INT3) in relation to the individual MRCP. Motor-evoked potentials (MEPs) were elicited in the TA before and after each intervention, and the TA MEP size was extracted. Across subjects, the largest increase in the MEP size was observed in INT1 (143%), while the increase was less in INT2 (118%) and further reduced in INT3 (107%). This supports the hypothesis that the arrival of the stimulation depends on the cognitive state, although the variability in the data was large. Changes in the TA MEPs appeared not to be caused by spinal mechanisms. In addition, no significant changes in the antagonist MEP size were observed. The present results indicate that the rationale behind the approach is sound, opening opportunities for new rehabilitation strategies. However, further research on additional subjects is required to validate the hypothesis.

I. Introduction

The human motor cortex is capable of reorganizing in response to natural changes such as voluntary motor exercise, injuries caused by limb amputation or spinal cord lesions, and stroke [1], [2]. Plastic changes might constitute the basis for learning and recovery of motor function following an injury such as stroke [3]. Despite novel interventional advances recently applied in the chronic stage of stroke [4], [5], significant functional recovery is still limited in the initial year after stroke [6]. However, these methods rely on the patients retaining a moderate degree of residual motor function, which is not the case for many stroke patients. At present, there is no treatment available for these patients. Therefore, it is important to continually expand our

understanding of the mechanisms underlying cortical plasticity and to investigate new ways to manipulate it [3].

One candidate mechanism for cortical plasticity was proposed by Hebb, called the law of coincident summation, who on theoretical grounds postulated that the temporal correlation of pre- and postsynaptic activity leads to synaptic strengthening [7]. This principle, termed associativity, has been confirmed experimentally in several animal studies, in which it was determined that the timing of correlated activity was vital for achieving the desired effect (for review, refer to [8]). An increase in synaptic output, known as long-term potentiation (LTP), is induced if an action potential arrives at the presynaptic neuron immediately before another action potential arrives at the postsynaptic neuron. In contrast, its counterpart long-term depression (LTD) is generated if the sequence of stimulation is reversed. Associative plasticity is considered the mechanism for persistent changes in synaptic efficacy underlying learning and memory [9].

Shaped after animal models of associative plasticity, a recently established protocol, termed paired associative stimulation (PAS), shows great promise to non-invasively induce lasting changes in the excitability of cortical structures in humans [10]–[12]. It employs the repetitive pairing of peripheral electrical stimulation applied to a nerve with pulses of transcranial magnetic stimulation (TMS) over the corresponding area of the motor cortex for the muscle innervated by the nerve. PAS induces a rapidly evolving (< 30 min), long lasting (> 60 min), yet reversible, and specific to the target muscle increase in cortical excitability when the interval between the two associative stimuli is timed to generate near-synchronous events in the motor cortex [10], [12]. It has been suggested that PAS relies on similar mechanisms to associative LTP studied at the cellular level in animals [13], [14]. PAS has been implemented to alter the excitability to various hand muscles [10], [12], [15], [16] and more recently to the tibialis anterior (TA) muscle of the lower limb muscle [17]–[19]. Nevertheless, the protocol is still not used in rehabilitation strategies, as it has not yet been proven that the effects carry over to functional benefits for patients [20]. Also, some patients are not suitable for TMS stimulation due to safety issues [21], [22]. Additionally, PAS may not be suitable for in-home rehabilitation due to the requirement for costly equipment (TMS stimulator), limiting its applicability as an extended rehabilitation approach, despite the promising therapeutic potential.

This study investigates a novel approach for inducing cortical plasticity, which does not rely on TMS stimulation or any residual motor function. One fundamental limitation of TMS is that

only the motor neurons of the target muscle in a limited area of the motor cortex can be excited during stimulation, which does not reflect the natural recruitment of motor neurons. A voluntary muscle contraction, termed motor execution, is accompanied by a change in the cortical activity over the area of the motor cortex controlling that muscle. This change, known as movement-related cortical potential (MRCP), can be detected in the electroencephalographic (EEG) signal as an increasing negative potential, reflecting cortical activity associated with the preparation and execution of a movement. The MRCP contains two major temporal components, a preparation phase (starting $1-2$ s before the onset of the movement) and an execution phase (starting approximately 500 ms before the onset). The preparation phase is primarily caused by cortical activity in the supplementary motor area (SMA), while the execution phase is thought to primarily originate from activity in the primary motor cortex. If a movement is only imagined, termed motor imagination, an MRCP is still generated, displaying similar temporal characteristics. For both motor execution and imagination, the cortical potential of the MRCP is at lowest, termed the peak-negativity, just prior to the planned onset of the movement [23]–[26]. During motor imagination, cortical motor neurons to the target muscle will therefore likely be activated in a similar fashion as during motor execution.

Therefore, it is conceivable that pairing peripheral stimulation to arrive at the level of the motor cortex during motor imagination can induce changes in the excitability of cortical projections to the target muscle. Additionally, the changes in cortical excitability might depend on the time of arrival of the stimulation during motor imagination, as different cortical areas are active during the preparation and execution phase. The hypothesis of this study is that change in cortical excitability depends on when the stimulation arrives at the cortical level during the cognitive process of movement. The aim of the study was to experimentally investigate the effect of concurrent imagination and peripheral nerve stimulation on cortical excitability.

II. Methods and Materials

A. Subjects

Six able-bodied individuals (5 males and 1 female; aged from 25 to 35 years) provided written and informed consent prior to participating in this study. The protocol was approved by the Scientific Ethics Committee Northern Jutland, Denmark (Case number: VN-20070015). At the time of the study, all subjects were free of any known neuromuscular disorders. Of the six subjects, two (both male) were unable to produce a clear peak-negativity in their MRCPs and were thus excluded from the study. The remaining subjects will be referenced as Sub1 - Sub4 in the following.

B. Stimulation

Peripheral electrical stimulation was applied to the deep branch of right common peroneal nerve (CPN) using an external stimulator (Noxitest IES 230, Aalborg, Denmark) with the cathode proximal. A suitable position for the stimulation electrodes (Pals Platinum, Axelgaard Manufacturing Co., Ltd, Denmark) was located, where a palpable response was produced in the distal tendon of the TA muscle with no activity from the synergistic peroneal muscles and no activity from the antagonist soleus (SOL). Palpation of SOL and peroneal muscles was performed during stimulation trials to find the optimal placement. 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 stimulation intensity was equal to the motor threshold.

A monophasic TMS stimulator (Magstim 200, Magstim Company, UK) with a focal figure of eight double coil (110 mm diameter) was used to apply single TMS pulses (inducing a posterior to anterior directed current in the brain) to elicit a motor-evoked potential (MEP) in the muscle of primary interest, which was the right TA muscle. The stimulation site was considered the point corresponding to the largest and most consistent TA MEPs generated in three consecutive TMS stimuli. For most subjects, this site was located $1-2$ cm to the left and posterior of the Cz position on the skull [27]. The position was marked and stored in a stereotactic 3D image guidance system (Brainsight TMS, Magstim Company Ltd, UK), which ensured that the TMS stimuli were consistently delivered over the same area of the motor cortex during one experimental session. Once the postition was identified, the resting motor threshold (rMT) for

the TA was found, defined as the lowest stimulation intensity that generated at least 5 of 10 consecutive TA MEPs with peak-to-peak amplitude higher than 50μV [28]. The coil was fixated by a mechanical arm at all times during TMS stimulation.

C. Recording

Electromyographic (EMG) signals were recorded from the TA and SOL muscles of the right leg. Surface electrodes (Ag/AgCl Neuroline 720, Ambu, Denmark) were placed in bipolar configuration (10 mm inter-electrode distance, electrode size 30×22 mm) following skin preparation by scrubbing with disposable alcohol swaps. In addition, a common reference electrode was placed on the tibia bone. The EMG signals were sampled at a frequency of 4 kHz, band-pass filtered at 0.5 Hz to 1 kHz by custom-made EMG amplifiers (SMI, Aalborg University, Denmark), digitized by a 16-bit data acquisition card and saved by custom made software (Mr. Kick II, Aalborg University, Denmark) for later offline analysis.

Electroencephalographic (EEG) signals were obtained from the skull using a 32-channel EEG-cap attached to an EEG-amplifier (NuAmps, Neuroscan, USA) and recorded in SCAN 4.3 software (Neuroscan, USA). According to the international $10-20$ system, four electrodes (Cz, Cpz, Fp1 and Fp2, impedances ¡ 5 kΩ) were prepared [27]. All channels were sampled at 1 kHz, band-pass filtered from 0.5 to 1 Hz and time-stamped for later offline analysis.

The somatosensory evoked potential (SEP) evoked by electrical stimulation of the CPN (stimulation every $200-220$ ms) were recorded with a single tin cup electrode placed on the scalp (Cz, band-pass filtered: 0.05 Hz - 1 kHz, sampling frequency: 10 kHz, referenced to Fz [27]). A common reference electrode was placed above the left eye. A total of 3000 traces in three 1000-trial sets were recorded and averaged on-line, according to the recommendations [29]. The SEP latency was measured as the time of occurrence of the first negative peak in relation to the peripheral stimulation, designated in the literature as the N34 peak [30].

D. Experimental procedures

All subjects went through one baseline session before proceeding to three intervention sessions. At least one full day elapsed between each of the four experimental sessions. Subjects were seated in a fixed chair (hip 90°, knees 130° and ankle 90° at all times) with their feet resting on equal height footplates.

E. Baseline session: measuring the MRCP following imaginary dorsiflexions

For each subject, the MRCP was recorded during imagination of dorsiflexion with their right foot. The purpose was to identify the point in time of the individual peak-negativity in order to time the peripheral stimulation in the subsequent intervention sessions. A custom-made LabView interface provided visual information (by a moving cursor in a dial) on when to mentally prepare, execute and release the movements, as shown in Fig. 1. The screen displaying the interface with the visual dial was placed, so that the view field of each subject included the right leg in order to facilitate attention on foot while observing the moving cursor. By verbal instructions, subjects were told to prepare for the movement from when the cursor began to move, to imagine executing a ballistic (as fast as possible) dorsiflexion at the transition between the blue and the orange phase (the visual cue), holding it through the orange phase and release this movement at some point in the yellow phase. The dial was moved randomly between three screen positions after each trial to increase subject attention. A new trial would start after a rest period of $3.5-4.5$ s. The time period of the blue phase was varied between $2-3$ s for each trial, while the orange phase was kept fixed at 1 s. The timing of these phases was chosen empirically. A total of 50 imaginary dorsiflexions were recorded in two 25-trial sets, with $1-2$ minute rest in between.
Prior to the MRCP recording, every subject was instructed to perform 25 actual ballistic dorsiflexions with the right foot following the same procedure as just described. The reason of this was to train the subjects in performing ballistic dorsiflexions uniformly in relation to the visual cue. Pilot tests showed that most subjects could not produce a clear peak-negativity in their MRCP during motor imagination, if they had not been familiarized with the interface at first (results not presented).
The dependent variable was the average peak-to-peak TA MEP amplitude evoked by TMS stimulation, while subjects were seated and at rest. A total of 16 stimuli (one every $7-10$ s) was applied at an intensity of 120% of rMT before (pre-measure), after (post-measure) and 30 min after (post30-measure) the imaginary movements. At the end of the baseline session, the subject's SEP latency was measured. The entire session lasted approximately 2 hours. All data was stored on the laboratory computer for later off-line analysis.

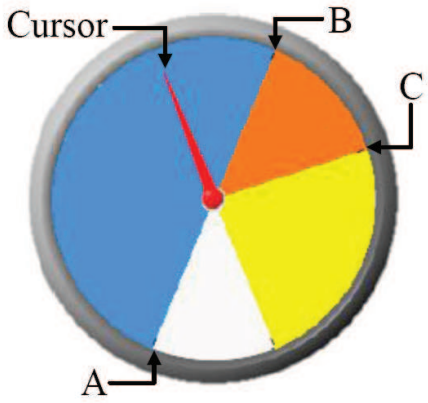

Fig. 1. The visual dial - the interface instructing the subjects to perform the imaginary movements. A moving cursor starts from the point **A** at the beginning of each trial. In the blue phase, subjects mentally prepares for performing the imaginary dorsiflexion, while the movement should be executed at the time instant when the cursor enters the orange phase at point **B**. The imaginary contraction is held throughout the orange phase and released after the point **C** in the yellow part. The time from **A** to **B** (blue phase) was randomly set to $2-3$ s for each trial, whereas the time from **B** to **C** was fixed at 1 s. A new trial would start every $3.5-4.5$ s.

F. Intervention sessions: effect of approach on cortical projections to the TA

The intervention sessions consisted of timing peripheral electrical stimulation to arrive at the cortical level at three different time instants of the cognitive processing of dorsiflexion. An overview of the experimental protocol is given in Fig. 2.

For each subject, the timing was set in relation to the individual mean peak-negativity of the MRCP measured during the baseline session. From the computer running the interface, a trigger signal was sent 2 s before the visual cue to the computer controlling the peripheral stimulation. A peripheral stimulation was given during each imaginary movement and timed to arrive either in the preparation phase (INT1, one standard deviation (SD) before mean peak-negativity), in the execution phase (INT2, on the mean peak-negativity) or after the execution phase (INT3, one SD after mean peak-negativity). The time delay (td_{stim}) for the stimulation in relation to the trigger signal was found using the following equation:

$$td_{stim} = trig - peakNeg_{mean} - SEP \pm delay \tag{1}$$

where $peakNeg_{mean}$ is the mean peak-negativity, SEP is the afferent conduction time of peripheral stimulation (SEP latency) and delay is either 0 for INT2 or $\pm$ $1\times$ the individual

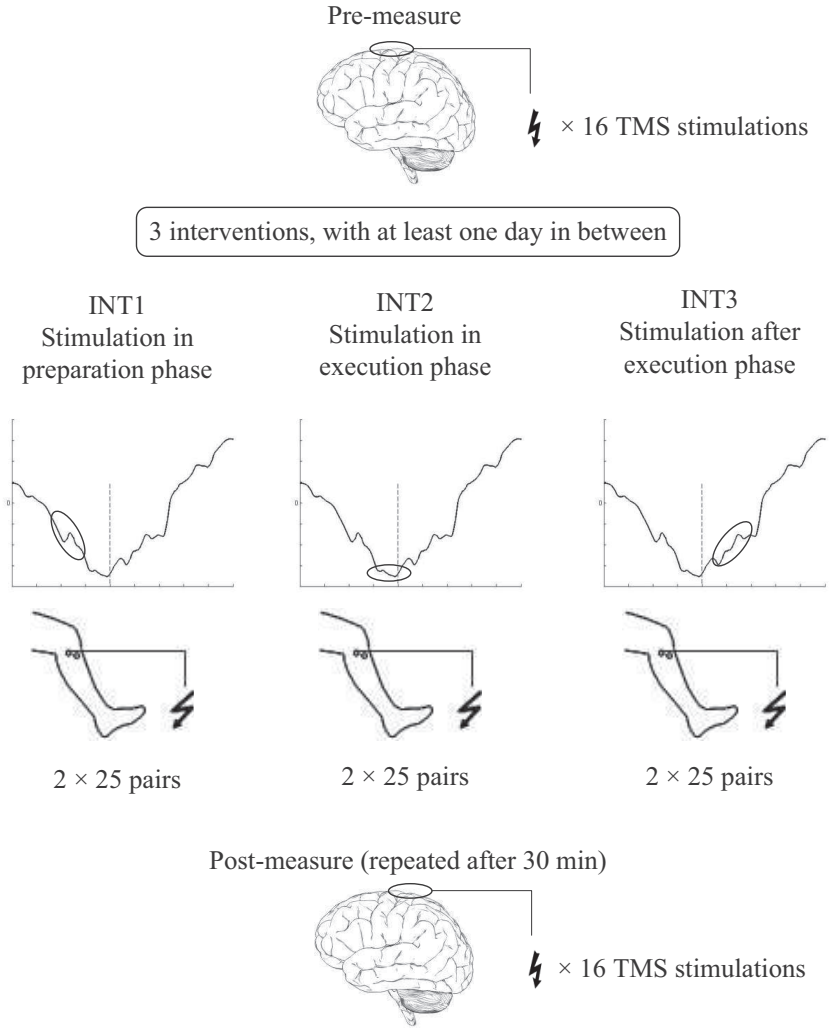

Fig. 2. Overview of the intervention sessions. Prior to the interventions 16 TMS stimuli were applied. The interventions consisted of concurrent motor imagination and peripheral stimulation. The timing of the stimulation was set in relation to the preparation and execution phase determined from the MRCP (INT1, INT2 or INT3). After the interventions, another 16 TMS stimuli were applied and repeated 30 min after.

SD of the mean peak-negativity for INT1 or INT3. The subject was instructed to disregard the sensation of the peripheral stimulation as much as possible, and focus on performing the imaginary movements in the same manner as during the baseline session. A total number of 50 pairings of peripheral stimulation and imaginary dorsiflexions were conducted in two 25-trial sets with $1-2$ minute rest in between. The sequence of the intervention sessions (INT1-3) was randomized for all subjects.

In the very beginning of each experimental session, a reinforcement contraction set of 25 ballistic dorsiflexions with the right foot was performed, as during the baseline session. Also the pre-,

post- and post30-measures was measured in the same way as previously described. Each of the intervention sessions lasted approximately 1.5 hours. All data was stored on the laboratory computer for later off-line analysis.

G. Effects on spinal excitability

In two subjects (Sub3 and Sub4), the TA stretch reflex was obtained in addition to the TMS evoked MEPs in order to investigate whether changes in peak-to-peak amplitudes of the MEPs were influenced by changes in spinal excitability. The right foot was fixated to a hydraulic controlled pedal (MTS systems Corporation 215.35), keeping the ankle joint in same angle as during the TMS procedure. First, the maximum voluntary contraction (MVC) for the TA muscle (maximum of three isometric contractions with 1 min rest interval) was found. Visual feedback of EMG activity in the TA was provided by a custom-built LabView-based program displaying a bar graph displaying percentages of MVC. While maintaining a level of contraction between $5-15\%$ of MVC, 30 perturbations of the right ankle joint was performed every $5-7$ s (amplitude of 8 deg; angular velocity of 300 deg/s). This was conducted as the first part of the pre-measure and the last part of the post-measure both during the baseline and the intervention sessions. All data was recorded, averaged and rectified on-line and stored for later off-line analysis.

H. Data analysis

The EEG signals containing MRCPs obtained in the baseline session were cut into epochs of 4.5 s (from 2 s before till 2.5 s after the visual cue) for each imaginary movement, using the EEGlab toolbox in MATLAB 7.7 (EEGlab v6.03b). The root-mean-square (RMS) values for all epochs were calculated. Any epoch containing more than $3\times$ the lowest RMS value from either the TA or SOL muscles were discarded, as were all epochs containing eye-movement artifacts. Wavelet denoising was applied (wavelet: 'db4') to smoothen the MRCP in each epoch. The time of occurrence for the minimum value of these epochs was plotted in a histogram and by visual inspection a suitable time window was chosen and outlying epochs were discarded. This was due to some epochs having a minimum value outside the plausible range, possibly due to artifacts or subject inattention. Based on the remaining epochs, the peak-negativity was designated as the time of occurrence for the minimum value of the averaged MRCP in relation to the visual cue. The mean peak-negativity and its SD were used to calculate the points in time for when to

apply the peripheral stimulation in the intervention session.

The TA MEPs obtained during pre-, post- and post30-measures were identified, and the peak-to-peak amplitudes of each measure were calculated for each experimental session. The changes in the cortical projections to the TA muscle was evaluated by normalizing the post- and post30-measures to the average pre-measure value in order to enable comparison between subjects due to the variability of MEP sizes. Changes were compared both within the same session (baseline and interventions across subjects) and between sessions (average across subjects). The same approach was done for the antagonist SOL MEPs.

A similar approach was applied to the TA stretch reflex, where the first of the three reflex peaks of the averaged and rectified waveform, designated as the M1 peak in the literature, was used as an indicator for spinal excitability [31]. The time instance of the averaged M1 peak was found for each of the pre- and post-measures, and a time window of ± 10 ms was identified. For each stretch reflex, the peak value within this time window was found and the RMS value of 100 ms pre-stretch EMG activity was subtracted to remove background activation. The peak value was normalized with respect to the pre-stretch RMS value.

III. RESULTS

A. MRCP and SEP

The average MRCP for each subject is shown in Fig. 3. On average, 20 out of 50 data epochs from each subject were discarded, leaving 30 valid for analysis (range: $20 - 38$).

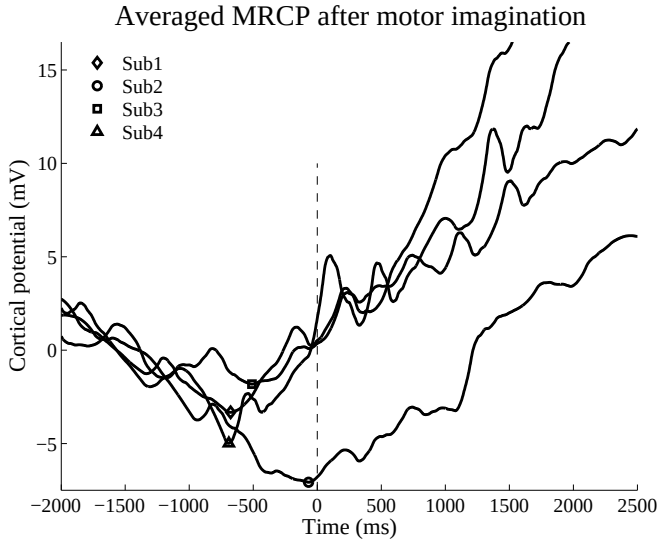

Fig. 3. Average MRCP obtained at the Cz position on the skull for each participating subject. The vertical dotted line is the temporal position of the visual cue, indicating the onset of imaginary motor execution. The mean peak-negativity for each subject is marked.

Across all subjects, the peak-negativity of the MRCPs occurred on average 487 ± 291 ms before the visual cue (mean peak-negativity indicated with dots in Fig. 3). The individual peak-negativity values for each subject, and the resulting stimulation times are listed in Table I.

Subject:	Sub1	Sub2	Sub3	Sub4
Mean peak-negativity for MRCP	677 ms	68 ms	510 ms	691 ms
SD for mean peak-negativity	334 ms	318 ms	418 ms	352 ms
SEP	35 ms	40 ms	37 ms	46 ms
INT1 (stimulation from visual cue)	−1046 ms	−426 ms	−965 ms	−737 ms
INT2 (stimulation from visual cue)	−712 ms	−108 ms	−547 ms	−1089 ms
INT3 (stimulation from visual cue)	−378 ms	210 ms	−129 ms	−385 ms

TABLE I
INDIVIDUAL MEAN PEAK-NEGATIVITY VALUES AND RESULTING STIMULATION TIME IN RELATION TO THE VISUAL CUE.

Fig. 4 shows the averaged (n=3000) SEP waveform for one subject (Sub3). Across all subjects, the mean SEP latency was found to be 39.5 ± 1.5 ms. According to Eq. 1 in II, the values obtained during the baseline session for each individual subject were used to calculate the stimulation delays for the intervention sessions.

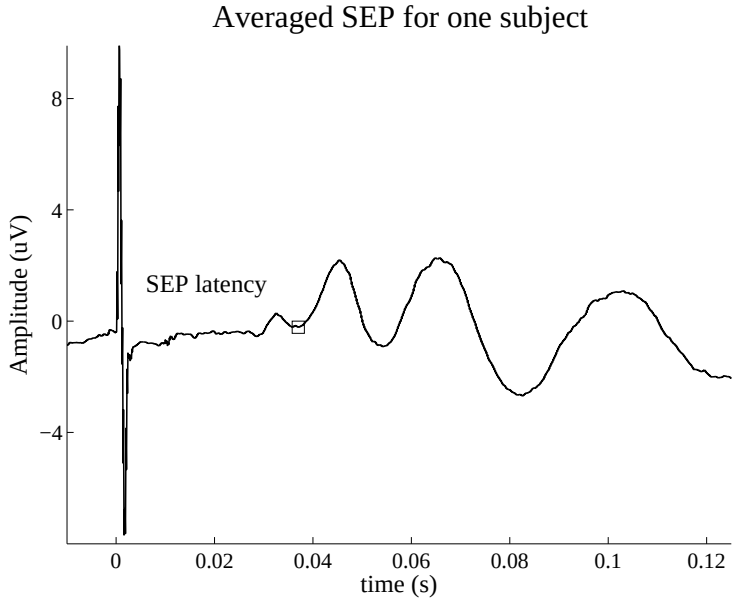

Fig. 4. Averaged (n=3000) somatosensory evoked potential (SEP) for one subject (Sub3) following electrical stimulation of the common peroneal nerve. The stimulation artifact is shown at time zero, and the SEP latency is equal to the time of occurrence of the first negative peak (N34 peak). The SEP latency for the subject shown was 37 ms, indicated by a square.

B. Excitability of cortical projections

Fig. 5 shows the averaged (n=16) raw TA MEP data before (pre-measure) and after (post-measure) each of the experimental sessions for one subject (Sub4). The increase in the raw MEP was 19μV for the baseline session, 29μV for INT1 and 9μV for INT3. In INT2, a decrease was observed on 24μV. Although this does not reflect the general picture of changes in TA MEP size.

Across all experimental sessions, the average (n=16) of the TA MEP peak-to-peak amplitudes during the pre-measures for each subject was for Sub1: $476 \pm 409\mu$V, for Sub2 $402 \pm 88\mu$V, for Sub3: $119 \pm 7\mu$V and for Sub4: $104 \pm 41\mu$V.

Fig. 6 shows average changes in TA MEP amplitude (n=16) for each of the experimental sessions

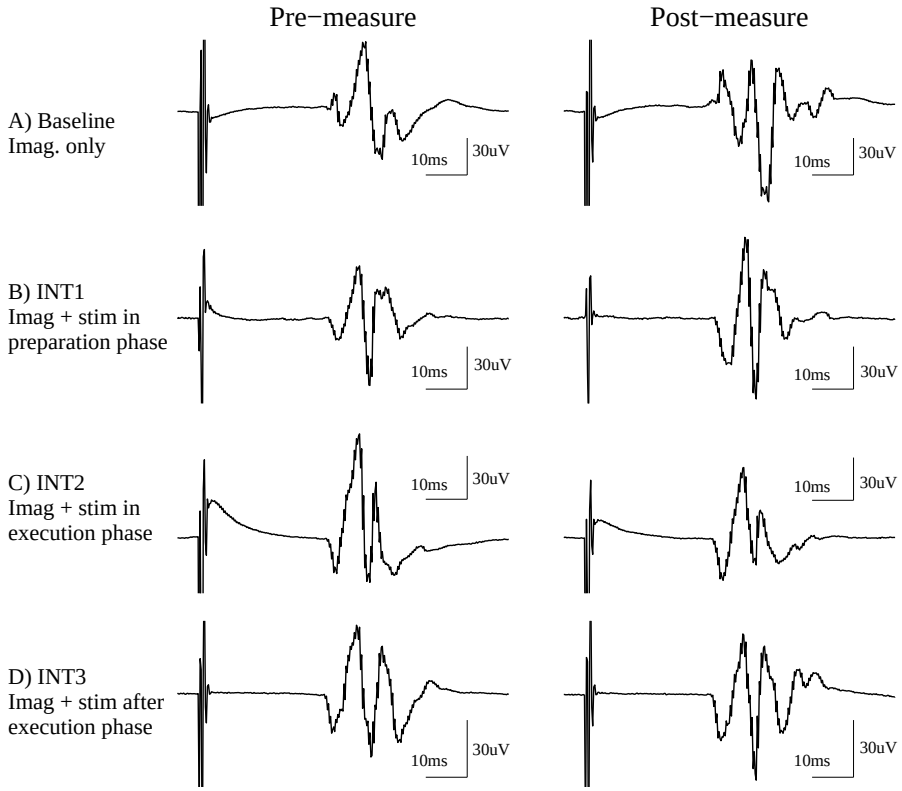

Fig. 5. Effects of each of the experimental sessions on the size of the TA MEP amplitude for one subject (Sub4). Each plot shows the averaged (n=16) TA MEP waveforms measured before (pre-measure) and after (post-measure) each session. **A:** TA MEP changes for the baseline session (only motor imagination). **B-D:** TA MEP changes for each of the three intervention sessions (pairing peripheral electrical stimulation with the motor imaginations); stimulation arriving at the cortical level in the preparation phase (**B, INT1**), in the execution phase (**C, INT2**) or after the execution phase (**D, INT3**). All MEPs were generated by TMS stimulation with the TA muscle in the resting condition.

as a percentage of the pre-measure values. It seems that there is a consistent increase in INT1 from pre- to post-measure, but the effect was not persisting after 30 mins for all subjects. In the baseline session, three subjects (Sub2-Sub4) showed similar increases in their TA MEPs, but for one subject (Sub1) the MEP size decreased quite dramatically. Regarding INT2, no consistent trend was detected between measurements, although two subjects showed decreases of TA MEPs following the intervention, which lasted till the post30-measure. In INT3, it appears that no significant change was present from pre- to post-measure, but two subjects showed increases in the post30-measure.

Across subjects, the relative changes in TA MEP amplitudes as a percentage of the pre-measures are shown in a bar plot in Fig. 7. On average, the MEP size increased by 112% from pre- to

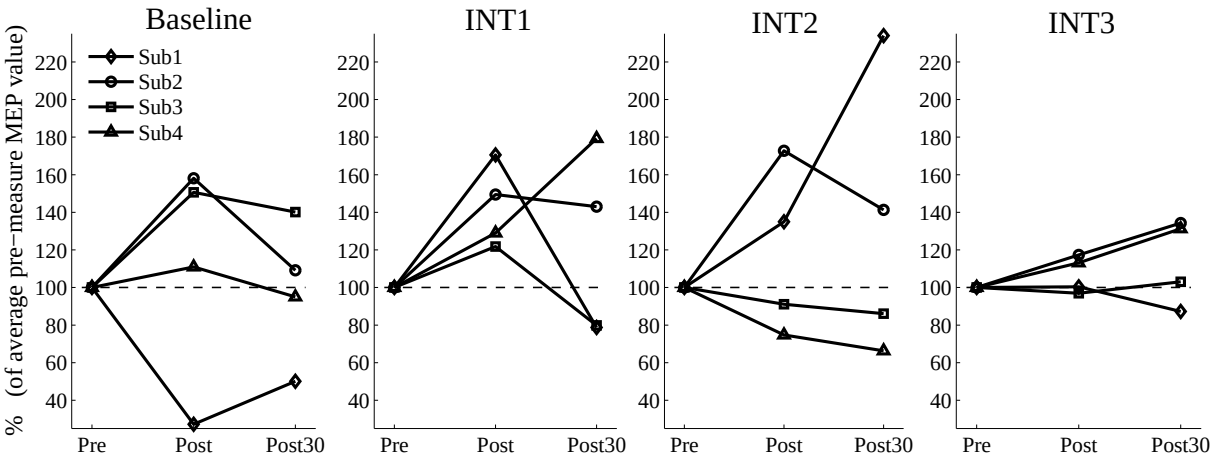

Fig. 6. Changes in average (n=16) peak-to-peak TA MEP amplitude normalized to 100% of the pre-measures for every subject in each of the experimental sessions.

post-measure in the baseline session and by 143%, 118% and 107% for the INT1-3, respectively. From this, it appears that there is a decrease in the effect of stimulation from INT1 to INT2 and a further decrease to INT3. Although after 30 mins, the change was relatively equal, as the TA MEPs increased in amplitude for the post30-measures in INT2 and INT3.

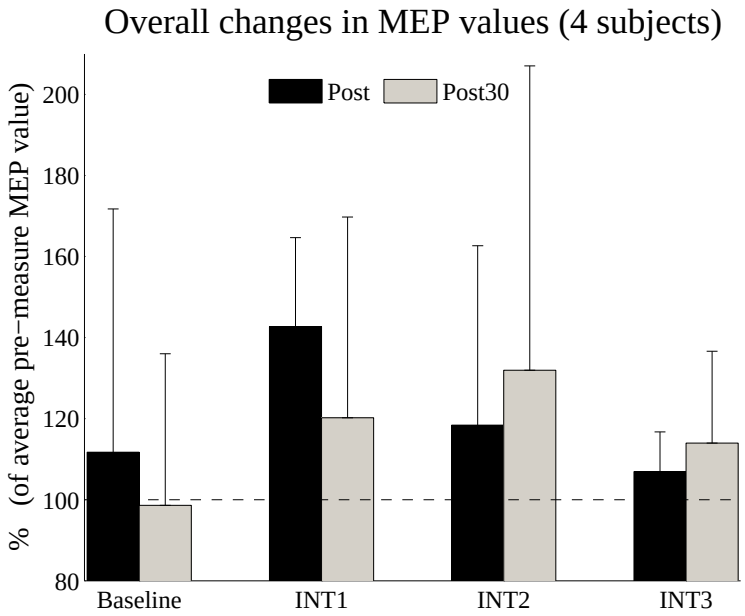

Fig. 7. Overall effect of the experimental sessions on the size of TA MEP amplitude across all subjects. Changes are indicated in percent of the pre-measure values. For each session, the mean value across subjects is plotted together with the SD.

The corresponding changes in the SOL MEP amplitude are also shown as a percentage of the pre-measures in Fig. 8. There was an increase in the MEP size for the baseline session from pre- to post-measure, whereas there was no significant increase during either of the interventions. From post- to post30-measure, the SOL MEPs decreased in size, except for INT2.

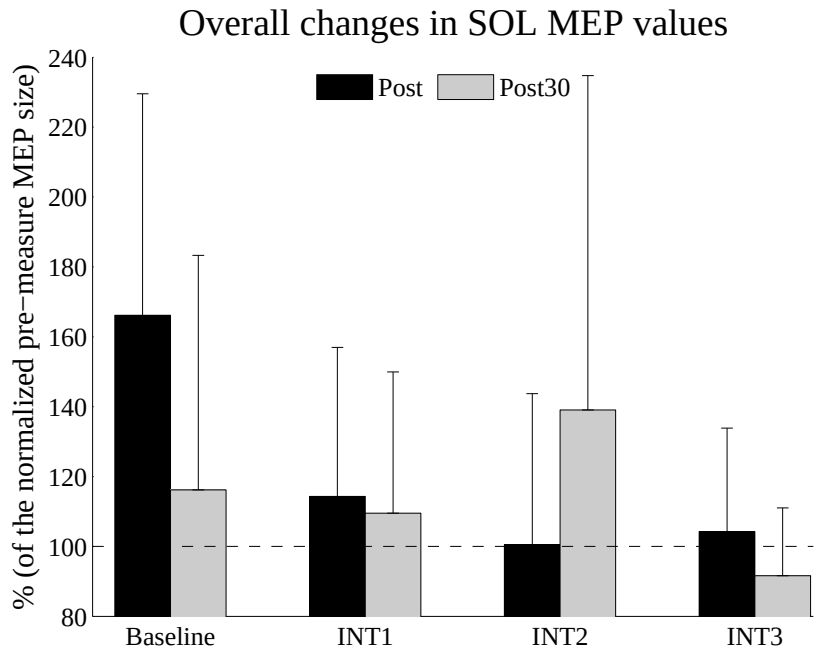

Fig. 8. Overall effect of the experimental sessions on the size of SOL MEP amplitude across all subjects. Changes are indicated in percent of the pre-measure values. For each session, the mean value across subjects is plotted together with the SD.

C. Changes in spinal excitability

Fig. 9 shows the changes in rectified M1 peak values for two subjects (Sub3 and Sub4). All values are normalized with respect to the level of pre-stretch activation. For neither subject, there appeared to be a significant change from pre- to post-measure, except for Sub4 in the baseline session.

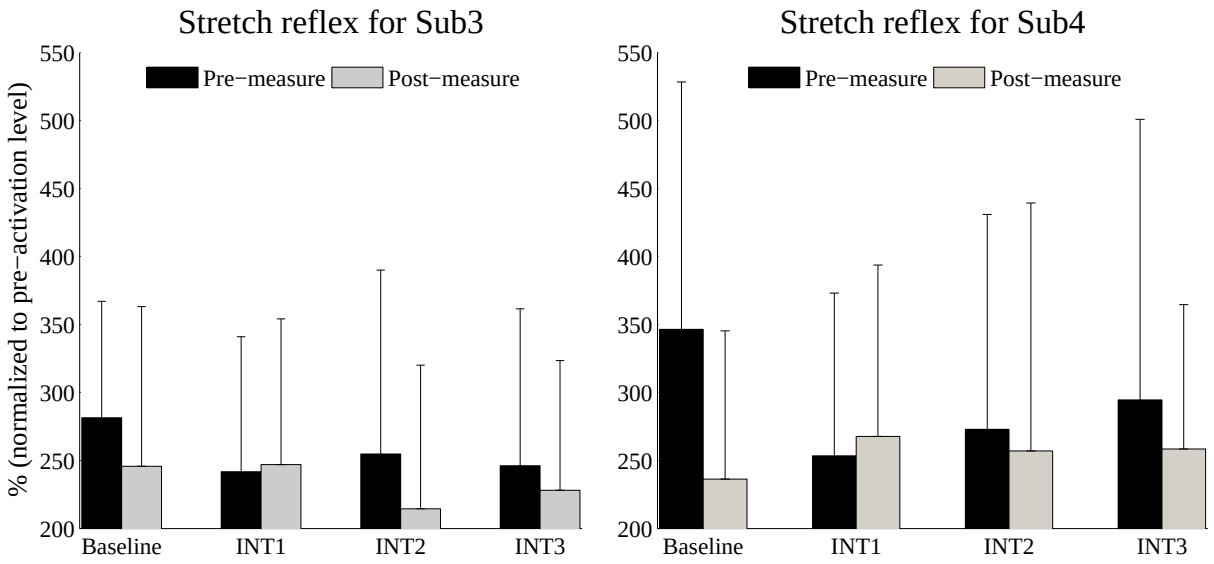

Fig. 9. Spinal excitability changes from pre- to post-measures in each of the sessions. Peak values for the M1 peak for the averaged (n=30) stretch reflexes was normalized with respect to the pre-stretch RMS value for EMG activity.

IV. Discussion

The aim of this study was to investigate the effect of a novel approach for changing the excitability of cortical projections to the TA. The approach was based on the repetitive pairing of a peripheral electrical stimulation timed to arrive at the motor cortex in various temporal phases of motor imagination of a dorsiflexion task. The present results indicate that cortical excitability can be enhanced in any of the experimental sessions. However, the largest and most consistent increase was observed when the stimulation arrived at the cortical level in the preparation phase (INT1). One possible reason for this could be that plasticity in the SMA is induced, which in turn affects the excitability of the primary motor cortex. The preparation phase is also longer compared to the execution phase, and associative activation during this phase is probably less susceptible to variations in subjects attention level. There is reason to believe that the changes observed is due to LTP-like mechanisms. When the stimulation was timed to arrive either in (INT2), or after (INT3) the execution phase, changes in the TA MEP size among subjects were quite inconsistent. This indicates that temporal correlation of peripheral stimulation and the cortical activation in these phases does not produce associative plasticity in the motor cortex.

Results also indicate that the changes in the TA MEPs was not due to alternations in the spinal excitability, as the M1 peak decreased in almost all of the experimental sessions. If an increase was observed, it was insignificant with respect to the pre-measure. Also, there did not appear to be a specific increase in any of the intervention sessions for the antagonist SOL MEPs. Although an increase was observed in the baseline post- and post30-measure as well as in the INT2 post30, the variability of these were also significantly larger than for the other measures, indicating that the approach is likely to be specific to the cortical projections of the TA muscle.

Despite the low number of subjects (n=4), and the fact that the variability of the results was quite large, the overall results of this study give reason to believe that the rationale behind the approach is sound, but further investigation is required to properly validate the hypothesis.

A. Methodological considerations

1) Measurement of the peak-negativity: In this study, the timing of the peripheral stimulus was based on an estimation for the temporal position of the peak-negativity of the average MRCP obtained during motor imaginations of ballistic dorsiflexions. For the four subjects, the mean peak-negativity occurred 487 ± 291 ms prior to the visual cue (shown in Fig. 3), and the large

variation of this result underlined the importance of measuring the individual MRCPs. However, even though the individual SEP latency was also measured and used in the stimulation timing, the precision of the SEP was likely not a significant factor (mean SEP: 39.5 ± 1.5 ms). One possible explanation for the large variability of the mean peak-negativity might be that subjects interpreted the movement to be imagined differently, which is known to change to shape of the averaged MRCP [32]. Also, since the estimation of the peak-negativity was done by averaging a relatively low number of epochs (on average n=30 in this study), changes in subject attention level combined with the natural variability of individual MRCPs during each epoch may have affected the position of the final peak-negativity.

One important issue when using the the peak-negativity of the MRCP to time the stimulation was that for some subjects (Sub2 and Sub3), the temporal position of the peak-negativity appeared to be very sensitive on the number of epochs; discarding a few epochs more or less would change the position of the averaged peak-negativity by several hundred milliseconds (results not shown), which would have a potentially significant influence on the timing of the peripheral stimulation. For Sub2, the ”true” mean peak-negativity might therefore have be located at -408 ms before the visual cue, which would actually have been more in line with the values for the other subjects (range: $510 - 691$ ms). Another important issue was the assumption that each subject in all experimental sessions performed the motor imaginations in a uniform manner and used the same timing with respect to the visual cue. By stimulating one SD before or after the mean peak-negativity, the majority (68%) of the stimuli was expected to arrive in the intended cognitive phase. As explained previously, the timing of the stimulations in the three subsequent sessions were performed based on the results of the baseline session (see Eq. 1). A deviation of how the subject performed the motor imagination in any of the subsequent intervention sessions might sufficiently shift the temporal position of the MRCP to cause the stimulation to arrive in a undesired phase, distorting the results. Therefore it might be advisable to investigate how the detection of the mean and SD for the peak-negativity can be improved. Also the minimum number of epochs necessary to ensure that the stimulation timing is based on a good estimate of the temporal position of the different phases of the MRCP. One possible approach would be to investigate other elements of the MRCP waveform than the peak-negativity, which might enable a more robust way to calculate the stimulation timing, and possibly an online adjustment of the stimulation timing.

2) Possible effect of voluntary motor executions prior to pre-measure: Before each experimental session the subjects performed 25 actual dorsiflexions with visual guidance from the interface, prior to the pre-measures. The question arises as to whether these contractions might have influenced the changes in excitability to the cortical projections to the TA muscle. In a recent study on PAS for the TA muscle, it was observed that 180 actual dorsiflexions, performed at a rate of 0.2 Hz non-significantly increased the TA MEPs by less than 10% from Pre to Post-measure (16 TMS stimuli at 120% of rMT, similar to this study) [19]. However, other studies indicate that motor learning might influence subsequent changes in plasticity, wherefore it cannot be ruled out that the 25 actual dorsiflexions just prior to the pre-measures have had an effect on the results [33]–[35].

3) Efficacy of the visual dial: One of the fundamental requirements of this study was the ability to time a peripheral stimulation with the various cognitive phases of motor imagination. To enable the subject to produce an imaginary MRCP at a specific time, the subject must be able to, ahead of time, to know how much time remains before the intended movement onset, and when exactly to perform the movement. The dial used in this study provides this information by showing a moving cursor, indicating at all times during an trial it's temporal position in relation to the intended movement onset. However, it requires the subject to be familiarized with it's speed and style of progression before it can be correctly interpreted, which was one of the main reasons for performing the 25 actual dorsiflexions prior to each session in this study. Other studies have used auditory beeps, moving oscilloscope cursors or blinking lights, but in all cases the subjects had to be familiarized with the cues first, and the studies did not report having investigated the efficacy of the cues used [23], [24], [26], [32]. In the current study, some subjects expressed that they found it hard to associate the moving dial with a ballistic dorsiflexion, but others did not report any misgivings. A moving oscilloscope cursor with an amplitude shift as a visual cue was investigated initially, but pilot tests showed that the amount of blinking and other eye-artifacts was high with this type of visual cue. However, the efficacy of various types of cue should be investigated further to ensure that subjects are presented with sufficient information, while not being distracted from the cognitive process of motor imaginations.

B. Functional relevance

The approach investigated in this study opens an opportunity for inducing cortical plasticity in stroke patients with sparse to no residual motor function left in their affected limbs. Potentially, this may promote a degree of function recovery of these patients, which is at present not possible using the existing rehabilitation strategies in the chronic phase of stroke. Furthermore, a system based on this approach might in the long term be expanded to an in-home rehabilitation strategy, as it only requires a peripheral stimulator, and the ability to perform motor imaginations.

APPENDIX
SUPPLEMENTARY MATERIALS

A. Worksheet on paired associative stimulation (PAS)

The intention of this worksheet is to describe the PAS protocol and to outline what the previous research has obtained using it. The theory behind associative plasticity will also shortly be explained. In addition, the factors important for protocol efficacy will be listed.

1) Introduction: Voluntary movements of the muscles of a limb is initiated and controlled by activity in areas of motor neurons specific to that limb in the motor cortex of the brain. The size of these areas differs and is, among other things, dependant of the degree of fine-control needed for a specific limb. This is known as the somatotopic representation of the motor cortex, sometimes also called the cortical homunculus. However, the size and shape of each area is not static but changes according to experience and physiological factors, a concept known as cortical plasticity [33], [36]. The adult human motor cortex thus undergoes a reorganization in response to natural changes such as voluntary motor exercise [33], [36]–[38], injury caused by spinal cord lesions [39] or amputation [40], and stroke [2]. If the size and excitability of an area of the motor cortex is changed, so is then the ability to generate a motor response in the muscles enervated by that area. In the recent years, there has been a great interest in clinical neurophysiology to understand the mechanisms underlying human brain plasticity, as it may be a necessary requirement for the development of strategies promoting recovery follow brain damage in human. It is well-known that the cortical excitability as well as the cortical representation of the affected muscles is reduced in stroke-survivors [2]. Cortical reorganization can also be induced artificially using repetitive electrical stimulation (rES) [41]–[43] and repetitive transcranial magnetic stimulation (rTMS) [44]–[46].

2) Associative plasticity: One candidate mechanism for cortical plasticity is one proposed by Hebb (1949), who postulated that the strength of a synapse may be modulated by correlated activity of a (weak) synaptic input to a postsynaptic cell with another (strong) input to the presynaptic cell. This principle (called associativity) has been confirmed in animal studies (for review see [8]), where strengthening of synaptic transmission, termed long-term potentiation (LTP), is observed if the postsynaptic neuron fires an action potential after an excitatory postsynaptic potential is induced by the presynaptic neuron. In contrast, long-term depression (LTD)

is generated, if the order of stimulation is reversed. Associative plasticity is considered the mechanism for persistent changes in synaptic efficacy underlying learning and memory [9], [13], and it may play an important role for cortical plasticity related to the acquisition and recovery of sensorimotor function [47].

3) PAS protocol: Shaped after models of associative plasticity in animals a protocol, termed paired associative stimulation (PAS), has been proposed to non-invasively induce lasting changes in excitability of the human cortex [10]. It employs repetitive pairing of a subthreshold peripheral electrical stimulus (most often applied to the median nerve), which precedes a single suprathreshold pulse of TMS applied over the hand area of the contralateral motor cortex by a distinct inter-stimulus interval (ISI). For hand muscles, this ISI is usually set to 25 ms, which approximately equals the time of the peripheral nerve volley to reach the motor cortex. If these pairs are repeated ¿90 times with an interval of approximately 20 s between each pairing (differs between protocols), then single TMS pulses evoke a larger electromyographic (EMG) response in the muscles, termed a motor evoked potential (MEP), than before the pairing. On the contrary, if the timing is changed, so that the TMS pulse is applied 10 ms before the peripheral stimulus, then the amplitude of the MEPs is reduced [12].

PAS is believed to induce cortical plasticity by the coincident activation of ”horizontal” intracortical fibers (presynaptic activation through TMS) and ”vertical” (thalamo-cortical or cortico-cortical) afferents (postsynaptic activation through peripheral electrical stimulation). This is supported in animal studies, where LTP can be induced in cortical slices when stimuli are paired in a similar manner [48]. By contrast, stimulation of neither horizontal, nor vertical pathways alone was sufficient to induce LTP when applied at low frequencies [48]. Although, the facilitatory effect of PAS can be blocked by administration of an N-methyl-D-aspartate (NMDA) receptor antagonist [11], which suggests that PAS relies on similar mechanisms to LTP studied at the cellular level in animals (for review see [14]). However, it has not yet been proven that the mechanisms are the same, wherefore associative plasticity induced by PAS is usually termed LTP/LTD-like plasticity [34]. A possible neural substrate for the effects induced by PAS may be LTP of horizontal cortico-cortical connections within the primary motor cortex [13], [38].

4) Spinal mechanisms: Changes in corticospinal excitability evoked by the PAS intervention are usually monitored at different delays (depending on the location of the target muscle) after

the intervention by measuring the amplitude of the MEPs following single pulse suprathreshold TMS. The MEP is a complex response, as it reflects the sum of activity from many groups of cortical cells, and also spinal cells, not only motor neurons, but also interneurons through different pathways (mono- and polysynaptic) [16]. As a consequence, it cannot be ruled out that subcortical or spinal mechanisms are involved in the measured change induced by the PAS intervention [49].

To address this issue, studies have compared PAS-induced changes of MEPs with those of F waves and occasionally with those of motor responses evoked by brain stem stimulation [10], [12], [50]. None of those studies found any change in spinal excitability using these methods. F-waves can be measured in the EMG signal during electrical stimulation of a peripheral nerve, and are thought to originate from a spinal response to the backfiring of a few antidromically activated (propagation of the action potential in the direction opposite to the normal) spinal motor neurons. One concern with employing F waves is that their sensitivity to short-term change in motor neuronal excitability is low and that MEPs and F waves might not be generated by the same population of spinal motor neurons [16]. In most subjects, F waves are easy to obtain, which may be the reason for their extensive use in monitoring spinal excitability. Generally, only a relatively small number of F waves (20) are measured in most studies [10], [12], [19], while others states that a sufficient number (50-100) of F waves must be averaged in order to approximate the F wave size [16].

Other studies have investigated H reflexes to asses if any change in spinal excitability has occurred following the PAS intervention for upper limb [16] and for lower limb [19]. An H-reflex is sometimes evoked during electrical stimulation of a peripheral nerve, where the stimulation generates both an EMG response in the target muscle (MEPs), but also stimulates the sensory nerve (1a afferent), which subsequently causes a postsynaptic depolarization of homonymous ? motor neuron. H reflexes are more sensitive than F waves to detect changes of the spinal motor neurons, but it is still unclear whether MEPs and H reflex reflects the activation of the same population of motor neurons. H reflexes can be difficult to obtain in most subjects, especially for distal hand and leg muscles, which might be the reason why some studies don’t find any changes in the H reflexes. Also, at least for lower limbs, evoked H-reflexes in the TA-muscle is attenuated compared to the soleus (SOL) muscle and generally only possible during a tonic contraction of the TA [51]. One study has found that increases in the MEP amplitude for upper

limb muscles were followed by a parallel increase in the H reflexes [16]. This demonstrates a long-term modification (¿20-40min) of the spinal excitability. Many reasons can be ascribed to this effect, but it was in Meunier et al. observed that an increase in the MEPs is always accompanied with an increase in the H reflex - never the opposite, while MEP facilitation was sometimes observed without any H reflex modification, indicating that the excitability change might be cortical ”in origin” [16].

Especially for the investigation of spinal excitability in lower limbs, the stretch reflex may hold advantages over F-waves or H-reflexes [31]. The stretch reflex is naturally evoked response when a muscle is ”unexpectedly” stretched, causing the 1a afferent fibers to increase their firing rate and excite the homonymous and heteronymous motor neurons to contract the muscle. This is seen as two or three peaks (M1, M2 and M3) in the EMG, depending on the muscle [31], [52]. The stretch reflex is thus a ”natural response” and it is generally possible to generate a stretch reflex in most subjects. In the TA-muscle, Petersen et al. determined that the first two peaks, M1 and M2, were primarily spinal in origin while it could not be ruled out that M3 contained a cortical component, both due to the late onset (95-99ms) which enabled a cortical response to the initial stretch, but also that M3 only occurs in healthy subjects when the muscle is stretched during a tonic contraction [31]. In fact, the size and the consistency of the peaks of the stretch reflex are very sensitive to the angle velocity, frequency of the perturbation, background muscle activity and limb position, making it very important to limit the fluctuations of these factors for the subjects during measurements of the stretch reflex [53].

5) PAS targeting lower limb muscles: PAS has also been used to change excitability of cortical projections to the tibialis anterior (TA) muscle of the lower limb [17]–[19]. When employing PAS on the lower limb, it may be important to individualize the ISI for each participant, since the afferent conduction time of peripheral nerve stimulation is known to vary greatly with height. By using the latency of the somatosensory-evoked potentials (first negative peak; N34 peak) following peripheral electrical stimulation of the common peroneal nerve (CPN) as the ISI (adding a central processing time of 6 ms), Mrachacz-Kersting et al. showed an significant increase in excitability for every subject, whereas this was not apparent when using a fixed ISI (55 ms for CPN stimulation), although when using a fixed ISI of 40 ms a significant decrease of the TA MEP amplitudes was observed, possibly due to spinal mechanisms [19]. These findings are in line with those found by Ziemann et al. for muscles of the hand [34].

6) Factors affecting PAS protocol efficacy: During PAS, the target muscle is usually relaxed. Mrachacz-Kersting et al. showed that activation of the TA muscle resulted in a significant increase in the MEPs, which was not the case, when PAS was delivered at rest [19]. Dorsiflexions alone at a frequency corresponding to the PAS frequency (0.2 Hz) did not change excitability significantly. Furthermore, no significant changes in the MEP amplitude of the antagonist soleus (SOL) were found, indicating that this technique of altering excitability is likely to be specific to the target muscle [19].

Attention towards the target limb when employing PAS has been shown to modulate the cortical excitability greatly [54]. In their first experiment, subjects were looking at the left hand, while PAS was targeted at the right hand, no effect was observed, which indicates that spatial manipulation can modulate the induction of plasticity. A second experiment aimed to manipulate the grade of attention. Maximal effect of PAS was found when subjects viewed their target hand, while it was a little reduced if they could only feel it. A complete blockade of plastic changes was observed when subject were given a mentally demanding cognitive task (mathematical calculation).

The after effects of PAS have shown to be rapidly evolving (¡30 min), long-lasting (¿60 min) and topographically specific, but yet reversible (return to baseline occurs within 24 hours) [10], [12].

B. Worksheet on movement-related cortical potential (MRCP)

The literature used in this worksheet is primarily found in these sources, and will therefore not be individually referenced; [23], [24], [26]. Information from other sources will be referenced where appropriate.

1) Movement-related cortical potentials: A voluntary movement of a muscle is accompanied by a change in the cortical activity over the area of the motor cortex that controls that muscle. This change is typically seen as an increasing negative potential, reflecting cortical activity associated with the planning and execution of the movement. The movement-related cortical potential (MRCP, or sometimes just MRP) is generally only visible by averaging electroencephalographic (EEG) signals recorded during several trials of the movement.

2) Phases of the MRCP: Although it may be a little difficult to distinguish them, the MCP is generally thought to contain two major temporal components. The early phase of the MRCP, sometimes called the preparation phase, typically begins 1-2 seconds before the execution of the movement, and can be measured bilaterally and symmetrically across the skull, but with a maximum amplitude over the vertex, termed Cz according to the international 10-20 system [27]. It is thought to be primarily produced by activity in the secondary motor area (SMA), and is seen as a slow increasing negative potential.

The second phase begins, sometimes called the execution phase, approximately 500ms before the onset of the movement and is visible in the MRCP as a rapidly increasing negativity. This activity is thought to primarily originate from activity in the contralateral primary motor cortex, following the somatotopic organization of the motor cortex. The cortical potential of the MRCP is lowest just before the planned onset of the execution of the movement, which is known as the peak-negativity of the MRCP.

Since the cortical area representing the limb of interest in our study, the right lower leg, is somatotopically located in the medial longitudinal fissure of the primary motor cortex, it seems reasonable that it should be possible to adequately measure both phases of MRCPs generated during lower limb movements from the vertex.

3) Imaginary MRCPs : If a movement is merely imagined, called motor imagery, an MRCP somewhat similar to that found during an actual movement is still generated, displaying the same temporal characteristics and for the preparation phase also the same slow increasing negative potential. However, the execution phase is less pronounced and displays no rapidly increasing

negative potential, making it more difficult to detect the transition from preparation to execution. This is due to a reduced activity in the primary motor cortex, a main contributor to the MRCP during motor execution, but little difference in the activity of the SMA, where movement planning takes place, during motor imagery.

However, for both motor imagery and motor execution, the negative potential of the MRCP does not decrease until after the movement had been initiated, meaning that the peak negativity of the MRCP is still located just prior to the temporal onset of the imaginary movement. Therefore, to be able to measure and detect the imaginary MRCP, it will be necessary to generate a time-synchronization between the recorded EEG-signals and the subject performing the motor imagery. This should be done in such a way that the subject is able to ”prepare” for the imaginary movement and then perform the imagined movement at a specific time-instant, around which the EEG-signals can be cut into epochs for later processing.

4) External cue and effect on precision: When a subject is instructed to anticipate an external cue, at which point the subject should then perform an imaginary movement, it is likely that factors such as subject attention, responsiveness and type of cue will affect the temporal precision of the MRCP. The peak negativity of the MRCP is expected to shift in time for individual trials of motor imagery, and the mean latency can therefore be viewed as a random variable, with a mean value and a probability density function. It can then be argued that, since the factors influencing the latency are primarily physiological and autonomous in nature, especially regarding the responsiveness and cortical processing time of the external cue, that the random variable is near-stochastic with a Gaussian distribution. In this case, the mean peak-negativity of a number of measured motor imaginary MRCPs, and the SD can be used as an estimator for the random variable.

C. Worksheet on brain-computer interface (BCI)

The intention of this worksheet is to define BCIs and describe the current status of BCI technology in relation to neurological rehabilitation for restoring motor function with focus on how BCIs can be used to induce brain plasticity.

1) Introduction: As the name implies, a brain-computer interface (BCI) establishes a communication link between brain signals and the outside world, in the form of a computer or another external device. The concept behind BCI technology is to analyze brain signals in order to classify and determine the output desired by the user, and to provide the result of the analysis to the user in a real-time and interactive manner, enabling the subject's acceptance or rejection of the result [55]. Such systems have found their use for people with severe motor disabilities (locked-in patients) enabling them once more to interact with their surroundings which clearly improves their quality of life. Another application for BCI technology is in neurological rehabilitation in order to restore lost motor function by inducing activity-dependent cortical plasticity in patients with progressive diseases, such as amyotrophic lateral sclerosis (ALS), multiple scleroses (MS) and Parkinson's, or in many patients with traumatic conditions after stroke, cerebral palsy or spinal cord injuries. Current rehabilitation methods do not restore near normal motor function, imposing the need for more effective alternatives for these patients [55].

2) Neurological rehabilitation: As mentioned, increasing attention is being put on using BCI techniques to assist in restoring and strengthening motor functions by targeting specific motor skills. The remainder of this worksheet will specifically focus on this issue.

Disease or traumatic damage to the central nervous system, especially stroke, is often followed by extensive changes in the cortical plasticity with severe consequences to surviving patients [55]. These changes may result in abnormal movement patterns, although some of the normal motor functions may be restored over time. However, if a repetitive abnormal movement exists, activity-dependent plasticity may result in these movements solidifying the changes in the motor cortex [55]. Therefore for any rehabilitation attempt of the motor functions to be successful, it must target the activity-dependent plasticity specifically and promote strengthening the normal functions over the abnormal. The underlying physiological basis for this training strategy is the Hebbian learning rule, which states that synapses that repeatedly act in a synergistic manner are strengthened [56]. If a BCI-system is designed to help the patient target and activate specific

areas of the motor cortex, by promoting normal movements, this may speed up the recovery and restoration of normal motor control [55], [56]. Patients like stroke survivors sometimes retain a degree of motor control, but have great difficulty initiating movements. This may be due to insufficient functional motor neurons remaining or due to central or peripheral fatigue [56]. However, if the remaining synaptic connections could be enhanced through dedicated Hebbian training and active neural feedback, the cortical plasticity of the motor cortex could lead to rapid improvements in motor control [34], [56], [57].

Paired associative stimulation (PAS) is a widely used protocol for inducing bi-directional activity-dependent plasticity in the human motor cortex. The technique employs the repetitive paring of peripheral electrical stimulation of a distal nerve (e.g. deep personal nerve of the lower limb) with transcranial magnetic stimulation (TMS) over the cortical representation of the muscle (corresponding to the tibialis anterior muscle) innervated by that nerve. If the stimuli are correctly timed to arrive at the motor cortex, an alternation in the cortical output to the muscle is observed (for more details consult the PAS worksheet).

However, TMS has a number of less desirable aspects with regards to patient comfort and economical issues. Therefore, it would be of clinical interest (beneficial), if an alternative approach could be developed for generating a cortical potential to coincide with the peripheral afferent nerve volley in a PAS-like protocol. When a person performs a voluntary movement of a limb, the cortical potential over the area representing that limb produces a characteristic pattern in the EEG potential, the so-called movement-related cortical potentials (MRCP).Previous studies have shown that the MRCPs can also be generated, even if the person only performs an imaginary movement (for more details consult the MRCP worksheet). Therefore, during the motor imaginary cortical motor neurons to the target muscle will be excited like when a voluntarily movement is being performed. Sometime during this phase, it could be interesting to combine the voluntarily induced cortical potential with a peripheral stimulation known from PAS, potentially achieving an effect comparable to that from PAS. This approach would clearly eliminate the negative aspects of TMS and also reduce the requirements for equipment in the clinical setting, as no TMS stimulator would be needed.

One potential problem with replacing the TMS stimulation with voluntarily generated MRCPs is in timing the arrival of the afferent stimulation in relation to cognitive excitation of cortical motor neurons. The firing pattern of the active motor neurons during an MRCP is asynchronous,

and its exact time thus cannot be known a-priori compared to the synchronous firing pattern during TMS stimulation, which is near-deterministic (externally triggered). As described in the worksheet on MRCP, a typical MRCP has a planning phase, a preparation phase and an execution phase, all timed in relation to the intended onset of the movement. It could be interesting to observe the change the cortical excitability, if the afferent stimulation was timed to arrive at the level of the motor cortex in each of the three phases of the MRCP. One possible solution for this could be to present the subject with a visual cue (a BCI-like system), indicating when to start preparing and when to initiate the imaginary movement. However it is important to take into account factors that might affect the timing of the MRCP, as each subject will likely interpret the planning of the movement differently, especially in relation to an external visual cue. The generation of the MRCP is a conscious cognitive process, and is greatly influenced by subject attention among other things, wherefore it can be viewed as a random process which has a mean value and a probability density function, in this case standard deviation (SD) (for more details consult the MRCP worksheet). Combined with adequate verbal instructions to ensure that the subject responds correctly to the cue, it is therefore necessary to measure the cortical potentials for a number of motor imaginary trials and calculate the average MRCP for each subject. . In relation to this, it would then be possible to calculate the average inter-stimulus interval (ISI) in order to make the afferent stimulation arrive in each of the three different cognitive phases of the MRCP, given that the afferent conduction time from peripheral electrical stimulation of on the distal nerve to the arrival at the motor cortex is taken into account (SEP latency).

In relation to the regular PAS-protocol, a recent study demonstrated that it was not irrelevant when the two signals coincide at the cortical level [19]. Even a small misalignment in time (5-10ms) of the two signals had a significant impact on the induced motor evoked potential (MEP) in the Tibialis anterior (TA) muscle. The timing of the stimuli must therefore be calculated as accurately as possible, preferably within the millisecond range, before the intervention (the application of PAS) is initiated. This will likely not be possible to achieve with the proposed approach due the variability in the average peak-negativity of the MRCP. But it is hoped that it will still be possible to show that a change in the cortical excitability is achievable, none the less.

If using motor imaginary in a PAS-like protocol is shown to provide an effect, it may open up for the treatment of patients suffering from a range of diseases, like survivors of stroke, cerebral

palsy or spinal cord injuries. These patients may still be able to imagine different movements, and by applying this new approach they may be able to recover some or most of their motor control, without having to undergo TMS stimulation. It may also be possible to develop rehabilitation systems where the patient is given a device with a built-in peripheral stimulator and BCI-display to take home and use it to train on a daily basis, thereby increasing the patient acceptance and comfort during the rehabilitation program, while at the same reducing costs by not requiring daily visits to the rehabilitation clinic. This may then also help the patient to more quickly reach a level of recovery, where the patient might be able to become self-sufficient and return to a more normal existence.

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