The efficacy of two protocols for inducing motor cortex plasticity in healthy humans – TMS study

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Abstract. Stimulation-induced plasticity represents an experimental model of motor cortex reorganization. It can be produced in awaked humans by combining the non-invasive electrical stimulation of somatosensory afferents *via* mixed peripheral nerves with the transcranial magnetic stimulation (TMS) of the motor cortex. Animal experiments indicate that an application of two converging inputs from various sources in a tightly coupled manner, following the so called Hebbian rule of learning, leads to an increase in motor cortical excitability.

The aim of our study was to compare the effects of two plasticity-inducing protocols by quantifying the motor cortex changes using TMS. Plasticity was induced by combining peripheral nerve stimulation with TMS (paired associative stimulation – PAS) and by peripheral motor point stimulation of two adjacent hand muscles (dual associative stimulation – DAS). The protocols were randomly applied in 12 right-handed healthy volunteers. The amplitudes of TMS-induced motor-evoked potentials (MEPs) in the right abductor pollicis brevis muscle were recorded before, immediately after PAS or DAS stimulation, and 10, 20 and 30 min later.

Both protocols led to significant and lasting changes in MEP amplitudes, however, a significantly larger increase in MEPs was observed after PAS than DAS. The results indicate that afferent input can differently affect cortical motor circuits and produce variable motor output. Thus, the efficacy of LTP-like mechanisms, presumably involved in Hebbian-like plasticity in humans, varies with the types/origin of the converging inputs. Our findings may be relevant when designing therapeutic interventions for improving motor function after neurological injury or disease.

Key words: Transcranial magnetic stimulation — Paired associative stimulation — Motor cortex plasticity — Humans

Introduction

In humans, plastic changes in the primary motor cortex (M1) have been explored in numerous studies by means of the non-invasive, safe, and painless technique of transcranial magnetic stimulation (TMS). Thus, TMS is well suited for transfering experimental concepts from the level of cellular physiology to the regional network level in humans. A magnetic coil placed on the scalp stimulates the motor cortex by electromagnetic induction, producing multiple descending potentials in the human cortico-spinal tract that can be recorded as short-latency motor-evoked potentials (MEPs) in the contralateral limb muscles. Since TMS induces electrical current flow parallel to the surface of the brain, horizontally oriented interneurones are preferentially excitated, causing transsynaptic activation of corticospinal tract neurones, at least at the threshold intensity (Hallett 2007).

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Early applications of TMS were primarily aimed at monitoring and detecting changes in motor cortex excitability and corticospinal tract integrity. It was subsequently found that the technique has a potential to interfere with an ongoing neuronal activity (Siebner and Rothwell 2003). This led to a series of studies aimed to detemine how to purposefully modulate motor cortex excitability in order to induce plastic changes. Low-frequency repetitive TMS (≤1 Hz) causes a consistent and lasting decrease in motor cortical excitability in healthy individuals in contrast to the "facilitatory" effects induced by a high-frequency repetitive TMS (5–20 Hz) (Pascual-Leone et al. 1994; Chen et al. 1997). Thus, TMS can induce a long-term reorganization of M1 since the effects outlast the period of stimulation.

Aside from the TMS, a long-term reorganization of M1 has also been reported after prolonged (~2 h) electrical stimulation of peripheral mixed nerve (Ridding et al. 2000) or motor point stimulation of adjacent small hand muscles (Ridding et al. 2003). The converging evidence led to combining central with peripheral stimulation to induce motor cortex plasticity in humans. This approach is analogous to the procedure used in neurophysiological model of associative stimulation in cortical slices. The concept is based on the principle of associative or Hebbian plasticity, which states that temporally correlated and convergent inputs from different sources result in increased synaptic strength of neurons that fire together. At a higher level of neuronal organization, Hebbian-based learning rules are related to responses of cortical neuronal networks, in a more temporally coherent manner, following behaviorally important inputs (Hebb 1949).

Hebbian-like principle may be employed in humans by pairing electric stimuli delivered to the median nerve with a single pulse TMS over the contralateral M1 at a precisely defined inter-stimulus interval (ISI; ~25 ms) to ensure a repetitive synchronous arrival of both inputs to M1 (Stefan et al. 2000). Plastic changes induced by this paired associative stimulation (PAS) protocol persist for at least 30–60 min, are topographically specific and critically time-dependent (Wolters et al. 2003).

The present study was designed to evaluate to what extent different stimulation-induced protocols can modulate motor cortex plasticity. Thus, we compared the effects of two frequently used protocols, namely PAS, that employs paired associative central and peripheral stimulation, and dual associative stimulation (DAS; Ridding et al. 2003) based on peripheral motor point stimulation of two adjacent hand muscles.

Materials and Methods

Experiments were performed on 12 right-handed healthy volunteers (4 women and 8 men) between 32 and 41 years of age (mean 34.1 ± 5.8 years). None had a history of neurological disease or was on CNS-active drugs at the time of the experiments. All subjects gave their written informed consent for participation in the study. The study was approved by the local Ethical Committee of the Military Medical Academy, Belgrade. The experiments conformed to the Declaration of Helsinki.

Timeline of experiment

Each subject participated in two experiments carried out in a pseudo-randomized order at least one week apart (Fig. 1).

EMG recording

During the experiment, subjects were comfortably seated in an armchair with their hands supported by armrests. Surface electromyographic (EMG) recordings in a belly-tendon montage were made from the right abductor pollicis brevis (APB) muscle using Ag-AgCl electrodes (diameter 9 mm). The raw EMG signal was amplified and filtered with a bandpass filter range of 20 Hz to 1 kHz (MS91; Medelec, UK). Signals were digitized at 5 kHz (CED 1401 plus; Cambridge Electronic Design, UK) and stored on a computer for subsequent off-line analysis.

Somatosensory evoked potentials

Median-nerve somatosensory-evoked potentials were recorded according to international guidelines (Cruccu et al. 2008) using surface electrodes. The active electrode was placed over the skull region overlying the primary somatosensory cortex (C3' using the international 10-20 system) while the reference electrode was placed over Fz. For each of a minimum of three reproductions, 1024 electrical stimuli (pulse width 300 μs, 3 Hz, 10–20 mA) were applied to the contralateral median nerve.

TMS

TMS was performed using a Magstim 200 stimulator with a monophasic current waveform (Magstim Co., Dyfed, UK) connected to a figure-of-eight-shape coil. The coil was held with a handle pointing backwards and laterally approximately 45 degrees to the inter-hemispheric line to induce an anteriorly directed current in the brain. This is the optimal orientation for activating the corticospinal system transsynaptically *via* horizontal cortical connections (Sakai et al. 1997). The coil was optimally positioned to evoke MEPs in the right APB muscle.

The resting motor threshold (RMT) was defined as a minimal stimulator output intensity that evoked a MEP

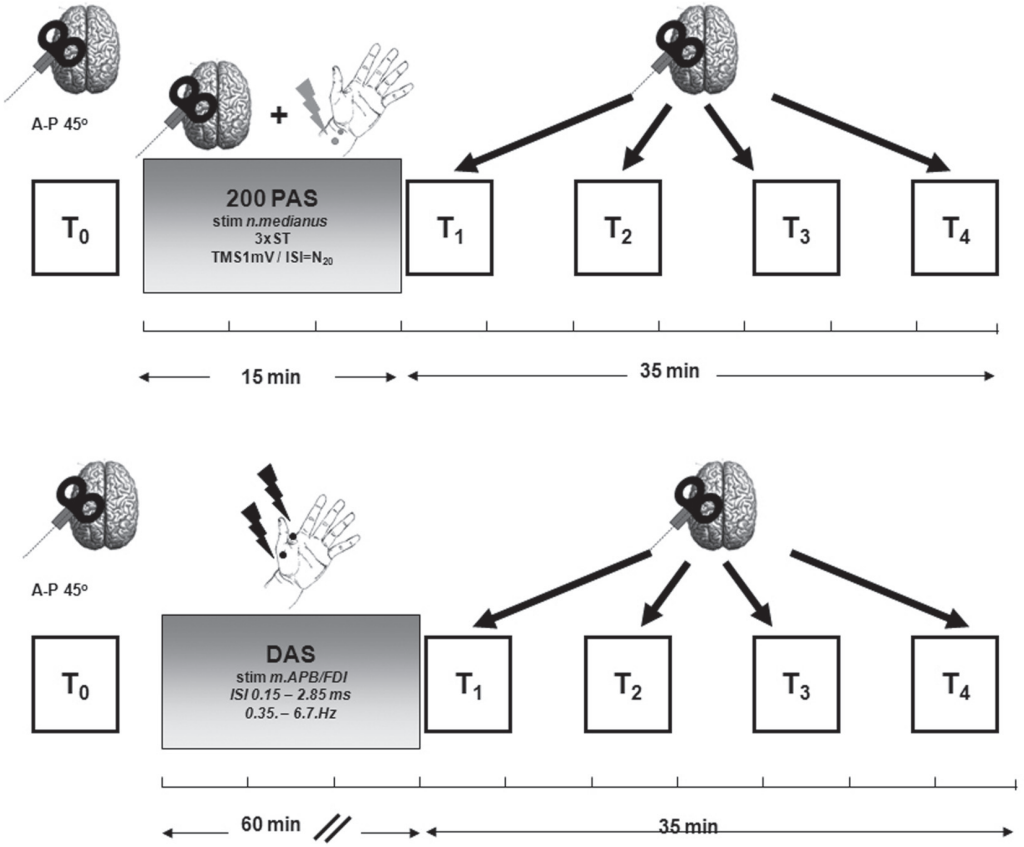

Figure 1. Time line of experiments (for details, see Materials and Methods). MEP amplitudes were measured before paired associative stimulation (PAS) or dual associative stimulation (DAS) (time point T_0), immediately after (T_1), and 10 min (T_2), 20 min (T3) and 30 min (T_4) later.

of ≥50 μV in five out of ten consecutive trials (Rossini et al. 1994). The intensity of magnetic stimulation was then adjusted to induce approximate peak-to-peak amplitude of 1 mV in the resting APB, when given without the preceding median nerve stimulus.

PAS

PAS consisted of 200 electrical stimuli of the right median nerve at the wrist, each paired with consecutive TMS over of the hand area of the left M1, at fixed ISI. The rate of paired stimulation was 0.25 Hz thus taking about 15 min to complete. Electrical stimulation was applied through a bipolar electrode (cathode proximal) using a constant current square wave pulse (duration 1 ms) at an intensity of 3 times perceptual threshold (range 0.9–3.6 mA). ISI between the median nerve stimulus and TMS were individually adjusted based on the N20 cortical component of the median nerve somatosensory evoked potential (Ziemann et al. 2004). Hence, ISIs for each subjects were equalling to the individual N20 cortical component of the median nerve somatosensory evoked potential (paired associative stimulation at ISI of individual N20 latency – PASN20) to induce a long-term potentiation-like increase in MEP amplitude in the APB. The values of N20 cortical latencies were in range 18.1–20.9 ms.

DAS

DAS paradigm was based on Ridding et al. (2003). Square-wave electrical stimuli of 1 ms duration (MS91; Medelec, UK) were applied to the motor points of the first dorsal interosseous and APB muscles simultaneously using surface electrodes (intensity of stimulation was in range from 12–28 mA). The timing between successive pairs of stimuli was randomised between 0.15 and 2.85 s in 8 steps (range 0.35–6.7 Hz). The timing of the inter-pulse intervals was controlled by Signal software (Cambridge Electronic Design Ltd., UK). The intensity of stimulation was adjusted for each muscle separately and set at a level just sufficient to evoke a minimal visible motor response. This intensity of stimulation was not painful. DAS paradigm was applied for a period of 1 h.

During the PAS and DAS procedures, the subjects were instructed to perform a task that demanded attention to the stimulated hand, because attention accentuates the LTP-like effect maximally (Stefan et al. 2004).

Quantification of PAS and DAS effects

MEP amplitudes were measured before PAS or DAS (time point T_0), immediately after (T_1), and 10 min (T_2), 20 min (T3) and 30 min (T_4) later (Fig. 1) in order to assess changes in left M1. The MEP amplitude reflects synaptic excitability in M1, which is regulated through various inhibitory and excitatory neurotransmitter systems (Boroojerdi et al. 2001).

RMT was measured immediately before T_0 and T_1 to check if PAS or DAS protocol induced changes in MEP amplitude that may confound their direct comparison. At each time point, 20 MEP were obtained at a mean inter-trial interval of 10 s and a random inter-trial interval variation of 25%. For each subject and time point, the single-trial peak-to-peak MEP amplitudes were averaged and normalized to the MEP amplitude measured at T_0.

Data analysis

Relaxation of the APB was monitored audio-visually with high gain EMG (50 μV/div.). Trials contaminated with voluntary EMG activity were discarded from analysis. Changes in MEPs induced by PAS and DAS were averaged over time points T_1 and T_4 and compared to MEPs before the intervention (T_0) using a two-tailed paired *t*-test. To test for the effect group, a two-way ANOVA was employed with time (T_1–T_4) as the within-subject factors and the induction protocol (PAS/DAS) as the between-subject factor. Paired two-tailed *t*-test was applied for post-hoc analyses (*p* value was adjusted for the number of comparisons during post-hoc analyses). Effects were considered significant, if $p < 0.05$. Results are given as means ± SD.

Results

Effects of stimulation protocols on RMT

RMTs were not affected by PAS or DAS, registered immediately after interventional procedure ($F_{2,12} = 0.97$, $p = 0.42$ and $F_{2,12} = 1.06$, $p = 0.41$, respectively). However, possible RMT changes later in the time course, could not be excluded totaly, although less probable. The results are shown in Fig. 2.

Effects of PAS and DAS on MEP amplitude

PAS resulted in an expected increase in MEP amplitude in the APB from 1.25 ± 0.33 mV at T_0 to an average of 1.97 ± 0.61 mV for time points T_1 to T_4 (T = 6.022, $p = 0.001$). The effect lasted for at least 30 min (Fig. 3, filled squares).

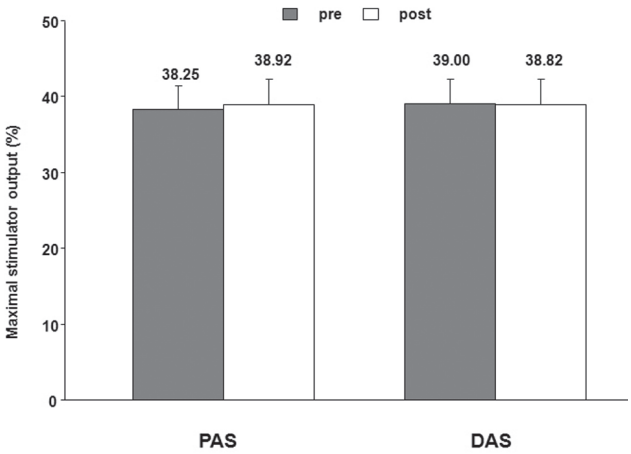

Figure 2. Effects of paired associative stimulation (PAS) and dual associative stimulation (DAS) on RMT ($n = 12$).

DAS also resulted in an expected increase in MEP amplitude in the APB (1.18 ± 0.22 mV at T_0 to 1.49 ± 0.43 mV at T_1–T_4, T = 5.02, $p = 0.01$) that lasted for at least 20 min (Fig. 3, empty circles).

The difference in MEP modulation between the PAS and DAS group was highly significant ($F_{1,11} = 43.2$, $p < 0.001$). The peak increase relative to baseline was 42 ± 13 % for PAS and 23 ± 6 % for DAS and occurred at 20 and 10 min after the stimulation period, respectively.

In summary, both protocols lead to significant and lasting changes of MEP amplitudes, however, the magnitude and duration of MEP increase was significantly greater after PAS than DAS protocol.

Correlations between motor cortex excitability and after-effects of two protocols for inducing motor cortex plasticity

To test whether the the RMT might be related to peak change after PAS and DAS, we examined the relationship between RMT and magnitude of peak amplitude changes for each protocol, separately. Simple regression analysis between the resting MEP threshold measured expresssed in percentages of maximal magnetic stimulator output (with the peak MEP amplitudes changes (normalized data) has shown weak positive associations for PAS and no association for DAS (Pearson's rho $\rho = 0.368$ PAS and $\rho = 0.042$ DAS, $p < 0.05$; Fig. 4).

Correlation between peak changes in PAS and DAS across subjects has revealed weak positive association, too ($\rho = 0.51$, $p < 0.05$), but without possibility to establish relevant corela-

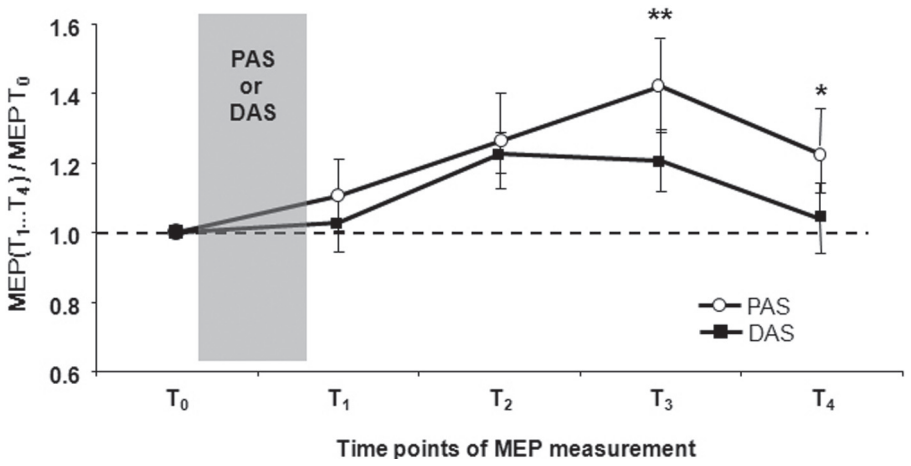

Figure 3. Lasting increase in motor-evoked potential (MEP) amplitude in the resting APB muscle induced by paired associative stimulation (PAS, empty circles) and dual associative stimulation (DAS, filled squares). Times of MEP testing are denoted on the x-axis (see Fig. 1). MEPs at different time points (MEP $T_1 \ldots T_4$) are normalized to MEP amplitude measured at T_0 (MEP T_0). Each subject was tested twice. All data are means ± SEM from 12 subjects.

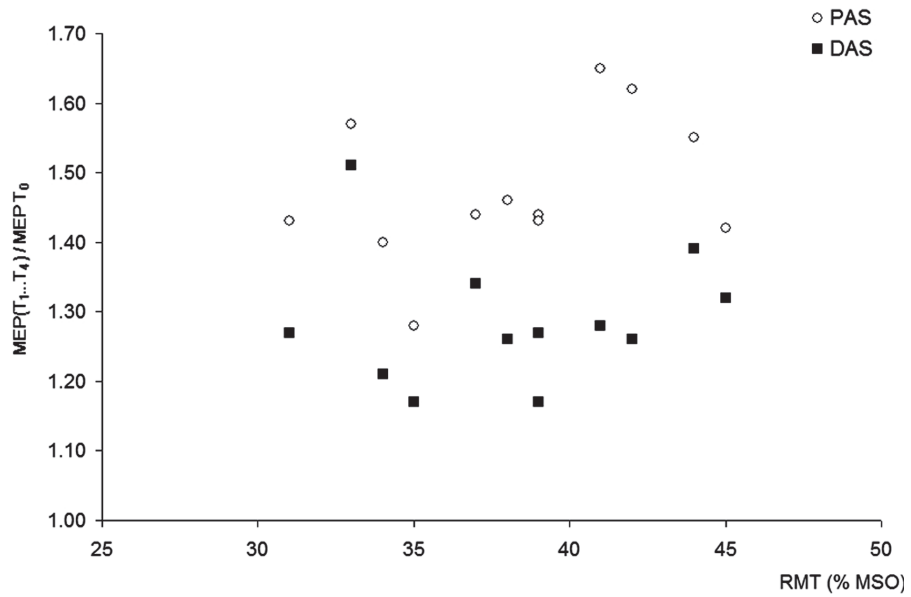

Figure 4. Individual peak changes of motor-evoked potential (MEP) amplitudes (y-axis) are plotted against the resting motor threshold (RMT, x-axis) for induced by paired associative stimulation (PAS, empty circles) and dual associative stimulation (DAS, filled squares) across subjects. MEPs at different time points (MEP $T_1 \ldots T_4$) are normalized to MEP amplitude measured at T_0 (MEP T_0). MSO, maximal stimulator output.

tion between after-effect magnitude for different protocols at same subjects.

Discussion

The most relevant finding of our study is the induction of significant and lasting MEP changes with both interventional procedures, with a maximal increase in motor cortex excitability at 20 min thereafter. However, an increase in motor excitability was significantly larger after PAS compared to DAS protocol.

A number of studies have shown that different experimental manipulations, such as application of repetitive TMS over M1, somatosensory afferent input modulation, or administration of CNS-active pharmacological agents lead to

a modulation of M1 output (for review see Ilić and Ziemann 2005). Hence, it is generally accepted that by changing the level of motor cortex excitability it is possible to open key permissive mechanisms of plasticity (Sanes and Donoghue 2000). In particular, experimental animal models have shown that reducing motor cortex inhibitory tone, such as after the cortical lesions, promotes functional plasticity of representational maps in the cerebral cortex (Nudo 1997). According to this concept, reduction of GABA-ergic inhibitory tone could be efficacious in promoting motor recovery in persons with chronic motor deficits (Hallett 2002).

This view is in keeping with the well-established analogy between the LTP, as shown in animal models of learning-induced cortical plasticity (Rioult-Pedotti et al. 2000) and the LTP-like plasticity in humans (Ziemann et al. 2004) that is considered a basic mechanism for acquisition of new motor skills.

To the best of our knowledge, this is the first report comparing the effects of two non-invasive inductive protocols on motor cortex plasticity in the same group of healthy subjects. Similar concept was applied previously in an attempt to compare three plasticity inducing protocols on the excitability of motor cortex as well as sensorimotor organization (Rosenkranz and Rothwell 2006). Their findings have shown that different protocols have been tested shown a different distribution of effects on various neurophysiological parameters. Several reasons have been discussed to explain changes that have been described. It is suggested that different protocols can have effects on different subsets of cortical neurones, that is high probable. However, the main difference between this study and our approach is that we have used two models of convergent impulses to sensori-motor cortex. Although, there are several differences between applied protocols that are not balanced, like shorter duration of PAS comparing to DAS. However, in spite the fact that each associative stimulation protocols were claimed highly effective to induce prolonged after-effect MEP changes, there was no head-to-head comparision.

The non-invasive plasticity inducing TMS protocols applied in our study reflect different mechanisms of M1 modulation. PAS is based on the original concept of Hebbian rule of learning, wherein employing two stimuli originating from different sources in a strict temporally coherent manner increases the synaptic strength of the two related inputs. On the other hand, DAS relies on synergistic effects on the M1 of paired afferent inputs from similar peripheral afferents (Ridding et al. 2003).

Correlation analyses that have been performed showed no significant associations between resting MEP threshold and peak MEP changes after PAS and DAS across subjects. As a matter of fact, that finding was not surprising because original papers had not reported any threshold modulation in spite of robust effects on induced MEP amplitude arround or above 1 mV (Stefan et al. 2000; Ridding et al. 2003). Following to widely accepted hypothesis of motor cortex excitability, RMT assesses excitation of the first few neurons recruited by the lowest intensity of cortical stimulation (so called "core motor neurons"), which have the highest excitability (Ridding and Rothwel 1997). Further to magnetic stimulation intensity increase, additional neural elements are excitated as reveals the recruitment curve. Obviously, effects of plastic modulation of motor cortex, at least by the way as we estimate, are prominent at sigmoid part of input-output curve.

Finally, we have performed an additional correlation analysis between peak MEP changes in PAS and DAS across subjects, that have shown only weak positive association without acceptable statistical significance. Nevertheless, similar mode of afferent stimulation still opens a possibility that PAS and DAS, at least partially could share some common mechanisms (e.g. cortical neuronal subsets) through which observed effects are realized.

In summary, the present findings show that PAS compared to DAS induces a greater and longer lasting MEP facilitation in a shorter period of time. Our results favour the use of PAS when the goal is to increase motor cortex excitability, as it may be advantageous when combining various rehabilitation interventions aimed at improving motor recovery. Further experiments in neurologic population are necessary for refining plasticity inducing TMS protocols and for testing the potential of PAS protocol in combination therapies.

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