Homeostatic modulation of stimulation-dependent plasticity in human motor cortex

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Abstract

Objective:
Since recently, it is possible, using noninvasive cortical stimulation, such as the protocol of paired associative stimulation (PAS), to induce the plastic changes in the motor cortex, in humans that mimic Hebb's model of learning. Application of TMS conjugated with peripheral electrical stimulation at strictly coherent temporal manner lead to convergence of inputs in the sensory-motor cortex, with the consequent synaptic potentiation or weakening, if applied repetitively. However, when optimal interstimulus interval (ISI) for induction of LTP-like effects is applied as a single pair, Motor evoked potential (MEP) amplitude inhibition is observed, the paradigm known as short-latency afferent inhibition (SLAI).

Methods: Aiming to resolve this paradox, PAS protocols were applied, with 200 repetitions of TMS pulses paired with median nerve electrical stimulation, at ISI equal to individual latencies of evoked response of somatosensory cortex (N_{20}) (PAS_{LTP}), and at ISI of N_{20} shortened for 5 msec (PAS_{LTD}) - protocols that mimic LTP-like changes in the human motor cortex. MEP amplitudes before, during and after interventions were measured as an indicator based on output signals originating from the motor system.

Results: Post-intervention MEP amplitudes following the TMS protocols of PAS_{LTP} and PAS_{LTD} were facilitated and depressed, respectively, contrary to MEP amplitudes during intervention. During PAS_{LTP} MEP amplitudes were significantly decreased in case of PAS_{LTP}, while in the case of PAS_{LTD} an upward trend was observed.

Conclusion: A possible explanation for the seemingly paradoxical effect of PAS can be found in the mechanism of homeostatic modulation of plasticity. Those findings indicate the existence of

complex relationships in the development of plasticity induced by stimulation, depending on the level of the previous motor cortex excitability.

Introduction

Neural plasticity represents the ability of neural circuits to undergo changes in function or organization due to previous activity. A modification of presynaptic inputs, can cause extended activity-dependent changes of efficacy of excitatory or inhibitory synaptic connections between neurons, known as synaptic plasticity (Citri & Malenka*, 2008*). As it was shown in animal models, long-term potentiation (LTP) of synaptic transmission is a long-lasting, activity-dependent form of synaptic plasticity that can be induced experimentally by applying a sequence of short, high-frequency stimulations to presynaptic neurons (Bliss & Collinridge*, 1993*). Long-term depression (LTD), contrary to LTP, is characterized by a decrease in synaptic efficacy (Rothwell, 1997). According to the learning rule introduced by Hebb, the synaptic connection between two neurons is strengthened if the firing of the presynaptic neuron is repeatedly and persistently paired with firing of the postsynaptic neuron, the process known as associative plasticity (Hebb, 1949). However, prolonged periods of synaptic activity tend to drive networks into either a maximized or a minimized state of neuronal firing, that needs a sort of "gain control" in order to maintain physiological functions within a set narrow range (homeostatic plasticity). Since the introduction of transcranial magnetic stimulation (TMS), in the mid 1980s it became possible to stimulate cortical regions with repetitive pulses of TMS that provide us an opportunity to replicate animal findings through noninvasive, neurophysiological studies on humans. The introduction of a paradigm of paired associative stimulation (PAS) that couples TMS over motor cortex preceded by electrical stimuli delivered on a peripheral nerve in strict temporal coherence led us to the possibility of exploring LTP- or LTD-like phenomena in the

human motor cortex (Stefan et al, 2000). When afferent sensory input from peripheral nerve electrical stimulation reaches the motor cortex at the time of magnetic pulse delivery, PAS induces LTP-like facilitation of MEP amplitudes in the target muscle. Contrary, if the magnetic pulse is delivered over scalp prior to arrival of sensory input to the cortex, PAS changes the direction of synaptic modification, inducing LTD-like plasticity (Stefan et al, 2002).

However, contrary to repeated stimulation with two coherent stimuli in PAS paradigm (200 pairs), when electrical shock to the median nerve at wrist was given at ISIs between 19 and 21 ms before TMS, in single pair, MEP amplitudes were suppressed, a phenomenon called short latency afferent inhibition (SLAI) (Tokimura et al, 2000). At the same ISI, PAS paradigm with repetitive associative stimulation produces opposite effect, post-intervention MEP amplitude augmentation. To our knowledge, previous studies were focused mainly on the difference between the MEP amplitude before and after the intervention, but tracking changes in MEP amplitude during the interventional period, has not been analyzed (Stefan et al, 2000, Ziemann et al, 2004).. In accordance with known effects of a single pair of stimuli in SLAI paradigm, we have performed this study aiming to evaluate the possibility that PAS interventional protocols move the threshold of neuronal activation in the opposite direction of the changes which are observed during post-intervention follow-up period.

Methods

Experiments were performed on 14 right-handed healthy volunteers (5 female) between 24 and 47 years of age (mean 36.9 ± 8.1 years). None had a history of neurological disease or were 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.

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 20Hz to 1 kHz (MS91 Medelec, Medelec, UK). Signals were digitized at 5 kHz (CED 1401 plus, Cambridge Electronic Design, Cambridge, 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 frontal midline position (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.

Transcranial magnetic stimulation (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 over the presumed scalp projection of primary motor cortex (M-1) 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 of ≥50 μV in five out of ten consecutive trials (Chen et al., 2008). 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.

Paired associative stimulation (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 M-1, at fixed ISI. The rate of paired stimulation was 0.25 Hz thus taking about 15 minutes 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 the perceptual threshold (range 0.75 - 3.45 mA). Inter-stimulus interval between the median nerve stimulus and TMS were individually adjusted based on the N_{20} cortical component of the median nerve somatosensory evoked potential (Ziemann et al., 2004). For this reason, the ISIs for each subject were adapted to the individual N_{20} latency, in order to enhance LTP- like changes in MEP amplitude in the target muscle

(PAS_{LTP}). In order to induce LTD-like effects ISI of N_{20}–5 msec was used (PAS_{LTD}). The values of N_{20} cortical latencies were in range 18.7 – 21.0 ms.

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.

Resting motor threshold (RMT) was measured before and (baseline, time point B) immediately after (post-interventional time 1, P_1) to check if any of two PAS protocols induced changes in MEP amplitude that may confound further analysis. 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 baseline.

MEP amplitudes were measured before PAS (baseline, time point B), immediately after (P_1), and 5 min (P_2), 10 min (P_3), 15 min (P_4), 20 min (P_5) and 30 min later (P_6) in order to assess changes in left M1 (Fig. 1). Furthermore MEP amplitudes that were registered during interventional protocols were split in bins of 10 MEPs (total of 20 bins) in order to study the time changes. The MEP amplitude reflects synaptic excitability in M-1, which is regulated through various inhibitory and excitatory neurotransmitter systems (Boroojerdi *et al., 2001*).

MEP amplitude means were calculated for each time bin, including baseline values. The MEPs during interventions as well as post-intervention MEPs were normalized and are given as ratios of the baseline determined immediately before intervention.

Place Fig 1. near here !

Regarding the criteria for successful intervention, we have accepted the criteria derived from

animal studies that marked LTP or LTD respectively as a facilitation or depression of the amplitude of postsynaptic response of at least 10%, which is also represented by more than 2 SD as compared to the baseline amplitude (Hess and Donoghue, 1999).

Changes in MEPs induced by PAS protocols were averaged over time points P_1 and P_6 and compared to MEPs before the intervention (B) using a two-tailed paired t-test. To test for effects of group, a repeated measures ANOVA was run with the within-subject effects TIME (P_1-P_6)and the between-subject effect group (LTP, LTD).

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.

Considering a potential differences in MEP amplitudes during interventional protocols Friedman two-way analysis of variance by ranks was performed, followed by Wilcoxon Signed Ranks tests for post-hoc testing.

Results

Effects of interventional procedures on RMT

RMTs were not affected by PAS_{LTP} or PAS_{LTD}, registered immediately after intervention (ANOVA: p = 0.42 and p = 0.41, respectively).

Effects of PAS_{LTP} and PAS_{LTD} on post-intervention MEP amplitude

PAS_{LTP} resulted in an expected increase in MEP amplitude in the APB from 0.984 ± 0.257 mV at baseline to an average of 1.401 ± 0.389 mV for time points P_1 to P_6 (p <0.01), while PAS_{LTD} induced MEP amplitude inhibition starting from 0.859 ± 0.251 mV at baseline, to an average of 0.676 ± 0.256 mV for time points $P_1$to P_6 (p <0.01). Both induced effects lasted for at least 30 min (Figs 2. and 3).The overal MEP amplitude modulation for PAS_{LTP} and PAS_{LTD}, were 41.45±17.76% and 22.39±7.39%, respectively.

<u>Interventions</u>

The Friedmann test showed a statistically significant global decrease for the MEP amplitudes during PAS_{LTP} protocol (p<0.05) (Fig.2), while suggestive trend of MEP amplitude increase during PAS_{LTD} protocol did not reach the level of statistical significance (p=0.169) (Fig.3). Further analysis by the paired Wilcoxon test showed a significant decrease at specific time points as assigned with asterixis (Fig.2).

Place Fig 2. and 3. near here !

Discussion

The knowledge of cortical plasticity in humans is limited and mainly related to indirect data originated from non-invasive experiments based on different protocols involving TMS. Among several models of inducing LTP- or LTD-like plasticity in the intact human brain, PAS protocol, based on the principle of Hebbian-like associative plasticity, has attracted most attention. The reason is that PAS protocol resembles invasive stimulation protocols applied at hippocampal tissue sections or those stimulation patterns, similar to bidirectional spike-timing dependent plasticity, that have been performed in animal models (Caporale and Day, 2008). However, previous neurophysiological studies in humans have also confirmed that LTP- or LTD-like plasticity in humans can be counterbalanced depending on the previous history of synaptic activityIt was implied that such a compensatory effect is intended to provide adjustment of overall synaptic weight and firing rate within the neural network, and within the physiological range. Those findings were confirmed if PAS_{LTP} and PAS_{LTD} protocols are previously primed with motor training, since in the former motor practice caused the cancellation of the effect of stimulation, while in the latter it was the opposite (Ziemann et al, 2004, Rosenkranz et al, 2007). Contrary to the above, if the sequence of motor activity and PAS was exchanged for each other replaced, homeostatic intervention was observed only in the case of LTD-like, but not in LTP-like plasticity (Jung and Ziemann, 2009). However, these studies have analyzed the modulation of MEP amplitude in the post-intervention period, because the potential benefits in rehabilitation processes related precisely to this period. Conversely, if the research focus was directed towards the understanding of physiological processes that underlie the modulation of MEP amplitude, intervention period should be thoroughly analyzed, too.

Therefore, we focused our attention on MEP amplitude changes during PAS protocol, in order to resolve the paradox of the inverse MEP amplitude modulation during vs. after intervention. Our results have shown that PAS_{LTP} protocol leads to MEP amplitude inhibition during the intervention protocol, opposite to subsequent typical MEP amplitude facilitation in post-intervention period. Considering the PAS_{LTD} protocol effects, despite the trend of MEP amplitude increase during intervention protocol, the statistically significant difference was not achieved, which may reflect the relatively small number of stimulation sessions. One possible explanation could be found in the mechanism of homeostatic plasticity, which is aimed to limit synaptic plasticity within the desired physiological range (Abraham and Bear, 1996; Turrigiano and Nelson, 2004). In this way, the history of previous activities of the motor system enables bidirectional modulation, or in other words, if the pre-induced MEP amplitude caused inhibition, the output from the system will subsequently meet with the facilitation, as it was destined to act within the framework of optimal values. These results indicate the complex nature of the interaction of activity-dependent plasticity in relation to the temporal sequence of subsequent forms of activation, requiring further detailed experiments to explore cortical plasticity modulation under different conditions.

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Legends

Figure 1. Time line of experiments (for details, *see* Methods).

Figure 2. Effects of PAS_{LTP} on MEPs amplitude during intervention and post intervention in the resting *abductor pollicis b*revis (APB) muscle. The PAS intervention took about 15 minutes to complete. All interventional data are binned in 10 block steps and are means of 14 subjects; error bars are ± 1 SEM. Times of MEP testing are denoted on the x-axis. MEPs are normalized to MEP amplitude measured at B. Conditioned MEP amplitudes were divided by test MEPs. Significant supression was evoked at time points of intervention (I_2, I_{5-7}, I_{11-13}, I_{15-17} and I_{19-20}) in APB muscle, contrary to post intervention effect those consistenly differed from baseline. The asterixes marks a significant difference ($P < 0.05$; Friedmann test, followed by the paired Wilcoxon test, *post hoc analysis*). All data are means ± 1 SEM from 14 subjects.

Figure 3. . Effects of PAS_{LTD} on MEPs amplitude during intervention and post intervention in the resting abductor pollicis brevis (APB) muscle. Conventions and arrangement are the same as in Fig. 2.

Fig.1.

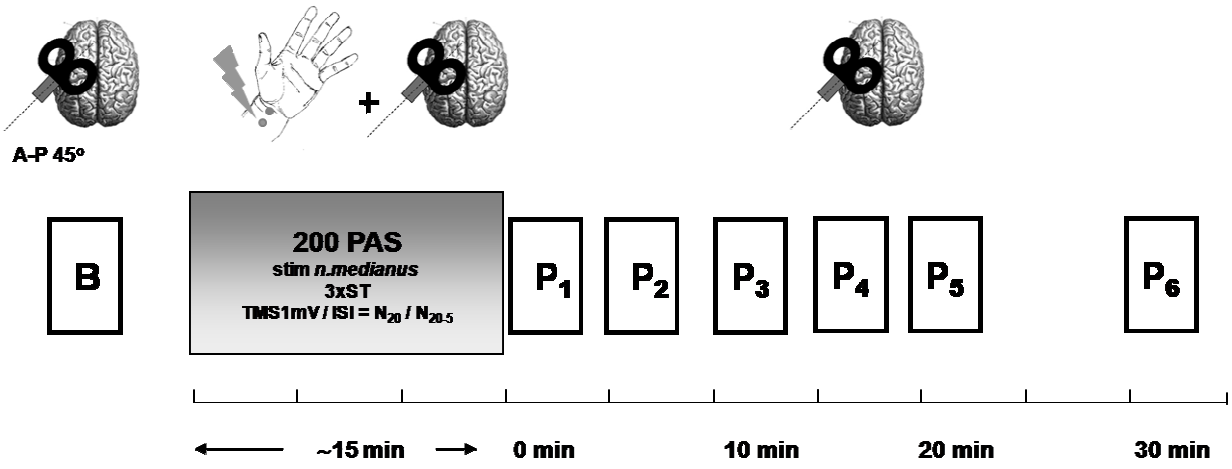

A-P 45°
B
200 PAS
stim n.medianus
3xST
TMS1mV / ISI = N20 / N20-5
P1
P2
P3
P4
P5
P6
~15 min
0 min
10 min
20 min
30 min

Fig.2.

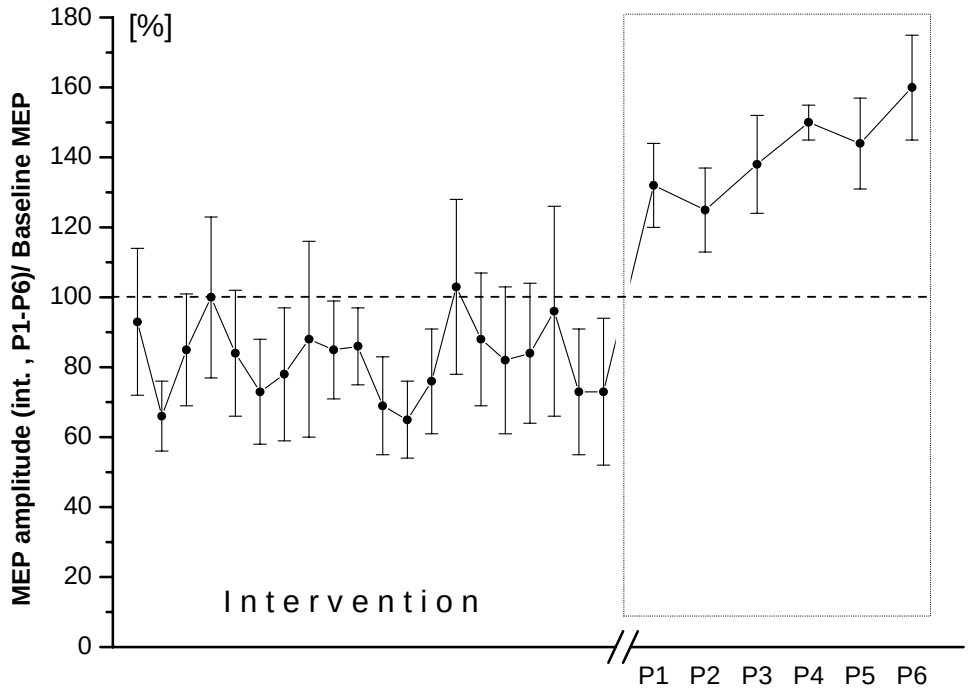
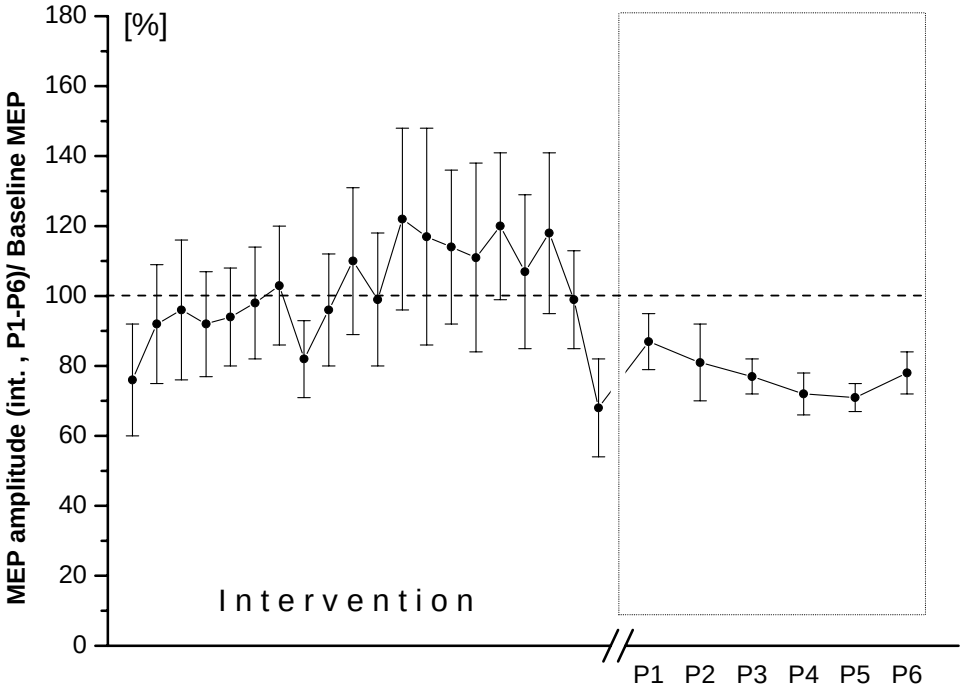

[%]
MEP amplitude (int. , P1-P6)/ Baseline MEP
180
160
140
120
100
80
60
40
20
0
Intervention
P1
P2
P3
P4
P5
P6

Fig.3.


[%]
180
160
140
120
100
80
60
40
20
0
MEP amplitude (int. , P1-P6)/ Baseline MEP
Intervention
P1
P2
P3
P4
P5
P6