Inducing Homeostatic-Like Plasticity in Human Motor Cortex Through Converging Corticocortical Inputs

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Abstract

Inducing homeostatic-like plasticity in human motor cortex through converging corticocortical inputs. J Neurophysiol 102: 3180–3190, 2009. First published September 2, 2009; doi:10.1152/jn.91046.2008. Transcranial stimulation techniques have revealed homeostatic-like metaplasticity in the hand area of the human primary motor cortex (M1HAND) that controls stimulation-induced changes in corticospinal excitability. Here we combined two interventional protocols that induce long-term depression (LTD)-like or long-term potentiation (LTP)-like plasticity in left M1HAND through different afferents. We hypothesized that the left M1HAND would integrate LTP- and LTD-like plasticity in a homeostatic fashion. In ten healthy volunteers, low-intensity repetitive transcranial magnetic stimulation (rTMS) of the left dorsal premotor cortex (PMD) was first applied to produce an LTP-like increase (5 Hz rTMS) or LTD-like decrease (1 Hz rTMS) in corticospinal excitability in left M1HAND via premotor-to-motor inputs. Following PMD rTMS, paired-associative stimulation (PAS) was applied to the right median nerve and left M1HAND to induce spike-time-dependent plasticity in sensory-to-motor inputs to left M1HAND. We adjusted the interstimulus interval to the N20 latency of the median nerve somatosensory-evoked cortical potential to produce an LTP-like increase (PAS, 5 ms) or an LTD-like decrease (PAS, 2 ms) in corticospinal excitability. The amplitude of motor-evoked potentials was recorded from intrinsic hand muscles to assess stimulation-induced changes in corticospinal excitability. Premotor-to-motor preconditioning triggered a homeostatic response to subsequent sensory-to-motor PAS. After facilitatory 5 Hz rTMS, “facilitatory” PAS, 2 ms suppressed corticospinal excitability. Likewise, “inhibitory” PAS, 5 ms facilitated corticospinal excitability after “inhibitory” 1 Hz rTMS. There was a negative linear relationship between the excitability changes induced by PMD rTMS and those elicited by subsequent PAS. Excitability changes were not paralleled by changes in performance during a finger-tapping task. These results provide evidence for a homeostatic response pattern in the human M1HAND that integrates acute plastic changes evoked through different “input channels.”

INTRODUCTION

Long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy play a crucial role for learning and memory (Bergmann et al. 2008; Malenka 1994; Ziemann and Siebner 2007; Siebner et al. 2004; Ziemann et al. 2008). In these studies, a first interventional protocol was applied to facilitate or inhibit corticospinal excitability in the primary motor hand area (M1HAND). If the first intervention facilitated motor cortex excitability, homeostatic mechanisms would strongly favor LTD-like plasticity in response to the second intervention. Conversely, if the first intervention resulted in an inhibition of corticospinal excitability, the second intervention would become very efficient at inducing LTP-like plasticity in the preconditioned M1HAND. For instance, excitability-enhancing preconditioning of M1 increased the efficacy of a subsequent excitability-reducing session of low-frequency repetitive transcranial magnetic stimulation (rTMS) (Iyer et al. 2003; Siebner et al. 2004). Although these studies provided converging evidence that corticospinal excitability within M1 is controlled by a BCM-like homeostatic mechanism, they were not designed to answer the question whether homeostatic plasticity is also expressed by incoming converging corticocortical inputs into M1.

The present study combined two conditioning protocols—premotor rTMS and paired-associative stimulation (PAS)—to investigate whether a homeostatic response pattern can be provoked in the left M1HAND through distinct afferent projections.
We first applied low-intensity rTMS over the left dorsal premotor cortex (PMD), which is thought to produce its activity-driven changes in corticospinal excitability through specific premotor-to-motor connections (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). Following PMD rTMS, we paired electrical stimulation of the right median nerve with single-pulse TMS of the left M1\textsubscript{HAND} (Stefan et al. 2000; Ziemann et al. 2004). It has previously been shown that PAS can produce LTP- or LTD-like effects in specific sensory-to-motor inputs to the corticospinal motoneurons of the M1\textsubscript{HAND}, depending on the interval between the peripheral and cortical stimulus (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004). We hypothesized that the M1\textsubscript{HAND} would integrate the conditioning effects of both interventional protocols in a homeostatic fashion.

METHODS

Participants

Eleven healthy male individuals (mean age: 28.8 yr; range: 23–44 yr) took part in the study. Ten participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield 1971). One participant was a consistent left-hander. Written informed consent was obtained from all subjects before the experiments. All subjects completed a safety screen questionnaire prior to the study (Paulus and Siebner 2007). The study was approved by the ethics committee of the Christian-Albrechts-University Kiel. All experiments conformed to the Declaration of Helsinki.

Experimental design

The main experiment consisted of two sessions, performed ≥1 week apart because, in healthy subjects, repeated rTMS of the left PMD can prolong the aftereffects on M1 excitability when given on consecutive days but not when applied with an interval of 1 week (Baumer et al. 2003). The order of sessions was counterbalanced across subjects. In both sessions, participants underwent two interventions (Fig. 1). First, they received rTMS to the left PMD. After rTMS preconditioning, PAS was applied to the right median nerve and left M1\textsubscript{HAND}. In session A, we combined two protocols that usually induce a lasting suppression of corticospinal excitability in the ipsilateral M1\textsubscript{HAND}, when given alone. We first applied “inhibitory” low-frequency (1 Hz) rTMS to the left PMD (Rizzo et al. 2004). After 1 Hz rTMS, we performed “inhibitory” PAS using an interstimulus interval (ISI) that equaled the individual N20 latency of the median nerve. In session B, we combined two protocols that usually produce a lasting potentiation of corticospinal excitability in left M1\textsubscript{HAND} (PMD 5 Hz rTMS of left PMD followed by PAS\textsubscript{N20–2ms} of the right median nerve and left M1\textsubscript{HAND}). Seven subjects participated in 2 control experiments in which PAS\textsubscript{N20–5ms} (session C) or PAS\textsubscript{N20–2ms} (session D) were given alone without a preceding rTMS session. Seven subjects were investigated in another 2 control experiments with 1 Hz rTMS (session E) or 5 Hz rTMS (session F) applied to the PMD without following PAS. Changes in corticospinal excitability were assessed at the beginning of the experiment (T\textsubscript{baseline}), immediately after PMD rTMS (T\textsubscript{post-rTMS}), immediately after PAS (T\textsubscript{post-PAS}), and 15 min after PAS (T\textsubscript{post-PAS}2). rTMS, repetitive transcranial magnetic stimulation; PMD, dorsal premotor cortex; PAS, paired-associative stimulation; M1\textsubscript{HAND}, hand area of human primary motor cortex; LTD, long-term depression; LTP, long-term potentiation.
measurements of corticospinal excitability were carried out in blocks immediately before \( T_{\text{baseline}} \) and after \( T_{\text{post-TMS1}} \) rTMS to PMD as well as immediately \( T_{\text{post-TMS1}} \) and 15 min \( T_{\text{post-TMS2}} \) after the end of PAS. In each block, we applied suprathreshold single-pulse TMS to the left M1HAND to quantify changes in corticospinal excitability after rTMS of PMD and PAS. Motor responses were recorded from the right first dorsal interosseus (FDI) muscle, abductor pollicis brevis (APB) muscle, and abductor digiti minimi (ADM) muscle at rest and during slight tonic contraction. After each block of TMS measurement, participants performed a tapping task to probe the effects of rTMS and PAS on motor function.

Seven individuals who had already participated in the main experiment (mean age and SE: 33 ± 3.19 yr) took part in two additional experimental sessions in which PAS\(_{\text{N20-5ms}}\) (session C) and PAS\(_{\text{N20+2ms}}\) (session D) were applied without preconditioning the left PMD with rTMS (Fig. 1). These experiments were necessary to probe the time course of changes in motor-evoked potential (MEP) amplitude induced by the PAS protocol alone. The timing of postinterventional MEP measurements corresponded to \( T_{\text{post-PAS1}} \) and \( T_{\text{post-PAS2}} \) of the main experiment (Fig. 1).

Besides, in seven individuals we examined the aftereffects of 1-Hz rTMS at the motor hot spots of the APB or ADM muscle. Third, the motor task to probe clinical effects of rTMS involved predominantly activity of the FDI muscle. Corticospinal excitability was measured in four blocks before and after rTMS of the left PMD as well as twice after PAS conditioning. To each block, we first recorded 40 MEPs with the hand muscles completely relaxed. Before the first block of measurements \( T_{\text{baseline}} \), stimulus intensity was adjusted to elicit MEPs in the right FDI muscle with mean peak-to-peak amplitude of about 1 mV (SI\(_{\text{1mv at rest}}\)). After MEP recordings at rest, we measured 20 MEP amplitudes and the cortical silent period (CSP) while participants made a tonic adduction of the index finger at about 10–15% of maximum force. The CSP is a period of electromyographic silence that can be evoked by a transcranial stimulus in the tonically preactivated target muscle (Siebner and Rothwell 2003). At baseline \( T_{\text{baseline}} \), stimulus intensity was adjusted to evoke an MEP with an amplitude of 1 mV (SI\(_{\text{1mv active}}\)) or a CSP that lasted about 100 ms in the tonically preactivated right FDI muscle. SI\(_{\text{1mv at rest}}\), SI\(_{\text{1mv active}}\), and SI\(_{\text{CSP 100ms}}\) were kept constant throughout the session. MEPs in the active muscle were recorded in all experimental sessions to match the amount of voluntary motor activity across sessions.

Surface electromyographic (EMG) activity of the right FDI muscle was continuously monitored with high-gain EMG (50 \( \mu \)V/division). Auditory feedback of EMG activity was provided to help participants to completely relax or to maintain a constant level of tonic contraction. Silver/silver chloride cup electrodes were placed over the belly and tendon of the right FDI, APB, and ADM muscles. EMG responses to single TMS pulses were recorded from these muscles on a trial-by-trial basis (Neurology System, Digitimer, Welwyn Garden City, Herts, UK). EMG signals were amplified (X1,000), band-pass filtered, digitized at a rate of 5 kHz using an analog–digital interface, and stored on a personal computer for off-line analysis (CED 1401 interface and Signal software, Cambridge Electronic Design, Cambridge, UK). The high-pass filter was set at 80 Hz and the low-pass filter at 2 kHz.

Peak-to-peak MEP amplitudes of the right FDI, APB, and ADM muscles were measured off-line on a trial-by-trial basis and mean MEP amplitudes at rest and during tonic contraction were calculated for each block of measurement (Nu Cursor software, Sobell Research Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College of London, UK). The trials that had been recorded during tonic contraction at SI\(_{\text{1mv active}}\) served to calculate the duration of the CSP. In each trial the CSP duration was measured from the onset of the MEP to the data point at which EMG activity reached a third of the mean prestimulus EMG level.

**Finger-tapping task**

After each block of TMS measurements participants performed a finger-tapping task. Two buttons were attached onto a tapping board at a distance of 3 cm. Subjects were instructed to perform alternating button presses as quickly as possible with the tip of the right index finger (alternating adduction–abduction movements) for 15 s. Before subjects started the tapping task, the tip of the index finger was placed...
between two buttons; the wrist of the right hand was fixed on the table to prevent movements with the forearm. The task was repeated twice. The mean number of taps per trial was used as a measure of motor function. Participants performed the finger-tapping task in all experimental sessions to match voluntary motor activity across experimental sessions.

**Repetitive TMS of left PMD**

For rTMS of the left PMD, we used the protocol reported by Rizzo et al. (2004) to induce long-lasting bidirectional changes in corticospinal excitability in the ipsilateral M1\_HAND. The PMD was functionally defined using the motor hot spot of the right FDI muscle as anchor point. The site of PMD stimulation was located 2.5 cm anterior to the motor hot spot of the right FDI muscle. PMD rTMS was performed with the same figure-of-eight–shaped coil “MC-B70” and MagPro-100 stimulator that was used for MEP measurements over the M1\_HAND (Medtronic-neuromuscular). PMD rTMS consisted of 1,500 biphasic stimuli. The coil was held tangentially to the skull, with the handle pointing 45° posterolaterally. Stimuli had a biphasic waveform with a pulse width of about 280 μs. The second phase of the stimulus induced a posterior-to-anterior current flow in the left PMD. The intensity of PMD rTMS was set at 90% of the MT, as determined over the motor hot spot of the left FDI muscle using a biphasic pulse. In sessions A and E, rTMS was given at a rate of 1 Hz to produce an LTP-like increase in motor thresholds of the relaxed FDI muscle. PMD rTMS followed by PAS used an ISI that was 5 ms shorter than the individual N20 latency (presumably GABA\_ergic) interneurons (Siebner et al. 1998; Werhahn et al. 1999). The mean tapping rate was used to assess motor function. For each measure, separate two-factorial repeated-measures ANOVAs were performed with the factors INTERVENTION (two levels: 1 Hz PMD rTMS followed by PAS and PMD 5 Hz rTMS followed by PAS) and TIME of measurements (four or three levels, respectively: T\_baseline, T\_post-PAS1, T\_post-PAS2). If there was a significant effect for the factor MUSIC in the three-factorial ANOVA, two-factorial follow-up ANOVAs were computed for each muscle alone, with the factors INTERVENTION and TIME.

The duration of the CSP and the mean tapping rate of the right index finger during the abduction–adduction tapping task were used as complementary outcome measures. The duration of the CSP in the tonically contracting FDI muscle probled the excitability of cortical (presumably GABA\_ergic) interneurons (Siebner et al. 1998; Werhahn et al. 1999). The mean tapping rate was used to assess motor function. Between session A and session B. The three-factorial repeated-measures ANOVA included the factors INTERVENTION (two levels: 1 Hz PMD rTMS vs. 5 Hz PMD rTMS and PAS vs. PAS) respectively, MUSCLE (three levels: APB, FDI, ADM muscles), and TIME of measurements (four levels: T\_baseline, T\_post-PAS1, T\_post-PAS2).

The Huynh–Feldt method was used to correct for nonsphericity in all ANOVAs. Depending on a significant \( P \) value, we performed post hoc paired \( t \)-tests to characterize the differences among experimental conditions that produced significant main effects or interactions in the ANOVA. We also performed a linear regression analysis to test whether the individual changes in MEP amplitudes of the relaxed FDI muscle that were induced by PMD rTMS predicted individual changes in MEP amplitudes produced by subsequent PAS. Paired Student’s \( t \)-tests were performed to test for baseline differences in stimulus intensity, MT, MEP amplitude, duration of CSP, and tapping rate between session A and session B. In all analyses, the level of statistical significance was set to \( P < 0.05 \).

**RESULTS**

**Effects of PMD rTMS and PAS on corticospinal excitability**

One subject developed a transient loss of consciousness during single-pulse TMS at the beginning of the first experimental session. Experimental procedures were stopped and the individual was excluded from the study. Statistical analysis was based on the data of the remaining 10 volunteers. The motor thresholds and stimulus intensities used for TMS of the M1\_HAND or PMD were comparable between session A and session B (Table 1).

Figure 2 illustrates relative changes in mean MEP amplitudes of the relaxed FDI, APB, and ADM muscles during sessions A and B. The three-factorial repeated-measures ANOVA showed a main effect of MUSCLE \( [F_{(2,18)} = 6.28; P = 0.009] \) but no main effect of INTERVENTION or TIME. The main effect of MUSCLE was caused by smaller MEP amplitudes in the ADM muscle relative to the FDI and APB muscles. However, the experimental interventions that were applied during sessions A and B had different effects on corticospinal excitability at rest (Table 2, Fig. 2). This was confirmed by an interaction between INTERVENTION and TIME \( [F_{(3,27)} = 3.96; P = 0.018] \). In accordance with Rizzo et al. (2004), mean MEP amplitudes of the relaxed right FDI muscle decreased after 1 Hz rTMS to the left PMD in session A relative to baseline \( [t_{(9)} = −2.65; P = 0.026] \), whereas PMD 5 Hz rTMS induced an increase in MEP amplitude in session...
TABLE 1. Motor threshold and stimulus intensities used for transcranial stimulation to evoke MEP in the right FDI target muscle in session A and session B

<table>
<thead>
<tr>
<th>Measure</th>
<th>Session A (PMD 1-Hz rTMS Followed by PASN20−5ms)</th>
<th>Session B (PMD 5-Hz rTMS Followed by PASN20+2ms)</th>
<th>P-Value (t-Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rMT</td>
<td>41.8 ± 2.4</td>
<td>40.8 ± 2.1</td>
<td>0.348</td>
</tr>
<tr>
<td>aMT</td>
<td>34.6 ± 2.6</td>
<td>32.4 ± 1.6</td>
<td>0.254</td>
</tr>
<tr>
<td>SI1mV at rest</td>
<td>47.6 ± 3.1</td>
<td>48.3 ± 2.9</td>
<td>0.621</td>
</tr>
<tr>
<td>SI1mV active</td>
<td>41.6 ± 3.3</td>
<td>39.9 ± 2.7</td>
<td>0.175</td>
</tr>
<tr>
<td>SICSP 100ms</td>
<td>45.6 ± 3.5</td>
<td>44.9 ± 3.4</td>
<td>0.741</td>
</tr>
<tr>
<td>SIpwmotor rTMS</td>
<td>32.3 ± 2.8</td>
<td>31.2 ± 1.8</td>
<td>0.534</td>
</tr>
</tbody>
</table>

Values are means ± SE, with respect to percentage of maximal stimulator output. The motor threshold to evoke peak-to-peak MEP amplitude was measured at rest (rMT) and during slight tonic contraction (aMT). SIpwmotor rTMS refers to the stimulus intensity that evoked an MEP of 1 mV amplitude in the resting FDI muscle. This stimulus intensity was also used for TMS of the left M1HAND during PAS. Likewise, SIpwmotor rTMS means the stimulus intensity to evoke an MEP of 1 mV amplitude during active conditions. SICSP 100ms means the stimulus intensity that evoked a cortical silent period (CSP) with duration of about 100 ms. Monophasic TMS pulses were used throughout the whole session except for SIpwmotor rTMS. Corresponding to 90% of individual aMT values for any of the measures obtained in sessions A and B, so it was ensured that both sessions were conducted in equivalent baseline conditions (corresponding P-values in right column).

B [t(9) = 2.768, P = 0.022]. Due to this bidirectional modulation of cortical excitability, mean MEP amplitudes of the FDI muscle were significantly smaller after PMD 1 Hz rTMS compared with MEPs after PMD 5 Hz rTMS [mean MEP amplitudes at Tpost-TMS: session A vs. session B: t(9) = −3.06, P = 0.014]. Subsequent PAS did not further enhance the effect on corticospinal excitability that had been induced by rTMS over PMD. On the contrary, PAS reversed excitability changes that had been induced by PMD rTMS (Fig. 2). In session A, “inhibitory” PASN20−5ms, which would normally suppress corticospinal excitability (Wolters et al. 2003; Ziemann et al. 2004), facilitated the mean MEP amplitude of the relaxed FDI muscle when given after “inhibitory” PMD 1 Hz rTMS [t(9) = 3.182; P = 0.011]. In session B, “facilitatory” PASN20+2ms, which typically increases mean MEP amplitude (Stefan et al. 2000; Ziemann et al. 2004), tended to decrease MEP amplitudes in the relaxed FDI muscle when given after “facilitatory” 5 Hz rTMS of left PMD [t(9) = −1.9; P = 0.09]. For MEP measurements after PAS, pairwise comparisons revealed no differences in mean MEP amplitudes between session A and session B [Tpost-PAS1: t(9) = −0.42; P = 0.682; Tpost-PAS2: t(9) = 1.41; P = 0.193]. These findings show that the excitability shift induced by PMD rTMS activated a BCM-like homeostatic mechanism in the left M1HAND that reversed the sign of corticospinal plasticity induced by subsequent PAS.

Regression analysis provided further evidence that PMD preconditioning triggered BCM-like homeostatic mechanism in the left M1HAND, showing that the reversal of the “normal” PAS effect increased with the shift in corticospinal excitability induced by PMD rTMS (Fig. 3). When we pooled the data of sessions A and B, we found a negative linear relation between the preconditioning effects of PMD rTMS and the conditioning effects of subsequent PAS on mean MEP amplitude in the relaxed FDI muscle (beta = −0.701; t = −4.741; P = 0.001).

The initial change in MEP amplitude that was induced by PMD rTMS accounted for roughly 50% of the variance of the aftereffect induced by subsequent PAS (R² = 0.492). The expression of the homeostatic response pattern induced by the consecutive application of two “LTP”-inducing protocols (i.e., PMD 5 Hz rTMS followed by PASN20+2ms) or two “LTD”-inducing protocols (i.e., PMD 1 Hz rTMS followed by PASN20−5ms) showed muscle-specific differences (Table 2, Fig. 2). This was indicated by the three-factorial ANOVA, showing an interaction between INTERVENTION, MUSCLE, and TIME [F(6,54) = 3.15; P = 0.011]. To explore this difference in more detail, we computed separate two-factorial ANOVAs for each hand muscle. Follow-up ANOVAs showed an interaction of INTERVENTION and TIME for the mean MEP amplitude for the relaxed FDI muscle [F(3,27) = 5.20; P = 0.006] and ADM muscle [F(3,27) = 5.38; P = 0.005]. An interaction between INTERVENTION and TIME was absent in the APB muscle [F(3,27) = 0.89; P = 0.46]. Taken together, these results show that a homeostatic response pattern was consistently expressed in the relaxed FDI and ADM muscles.

![FIG. 2. Relative changes in MEP amplitude of the relaxed right FDI muscle (top), APB muscle (middle), and ADM muscle (bottom) induced by PMD rTMS and PAS of the right median nerve and left M1HAND. Black rectangles denote relative changes in MEP amplitude induced by PMD 1 Hz rTMS and subsequent PASN20−5ms (session A), open rectangles give relative changes in MEP amplitude caused by PMD 5 Hz rTMS followed by PASN20+2ms (session B). Mean MEP amplitudes were normalized and expressed as percentage of MEP baseline amplitude. Error bars show the SE. Asterisks indicate significant differences in non-normalized mean MEP amplitudes as revealed by post hoc Student’s paired t-test (*P < 0.05; **P < 0.01). FDI, first dorsal interosseus; APB, abductor pollicis brevis; ADM, abductor digiti minimi; MEP, motor-evoked potential; rTMS, repetitive transcranial stimulation; PAS, paired associative stimulation.](http://jn.physiology.org/)

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which are innervated by the ulnar nerve, but not in the APB muscle innervated by the median nerve.

In contrast to MEP amplitudes at rest, rTMS of the left PMd followed by PAS did not have specific effects on MEP amplitudes in the active muscle. Three-factorial ANOVA showed only unspecific facilitatory MEP changes during the repeated measurements [TIME: \(F_{(3,27)} = 4.82, P = 0.008\), but no interaction between interventional protocol, muscle, and time of measurement (Table 3).

Neither PMD rTMS nor PAS significantly modified the duration of the CSP in the tonically contracting FDI muscle (Table 3). Two-factorial repeated-measures ANOVA revealed no main effect of INTERVENTION or TIME and no interaction between the two factors. The interventional protocols also had no specific consistent effects on mean tapping rate (Table 3). Although the tapping rate tended to increase with time during both sessions [\(F_{(3,27)} = 2.93; P = 0.052\)], the type of intervention had no differential effects on mean tapping rate. In particular, the changes in corticospinal excitability induced by PMD rTMS or PAS were not paralleled by changes in tapping rate.

**Control experiments**

In seven individuals we investigated the aftereffects of PMD rTMS alone on corticospinal excitability (sessions E and F, Fig. 4A, Table 4; Supplemental Fig. S1A). The time course of excitability changes was examined by measuring MEP at baseline, directly after PMD rTMS and, additionally, at time points that matched Tpost-PAS1 and Tpost-PAS2 in sessions A and B. In accordance with previous studies (Gerschlager et al. 2001; Rizzo et al. 2004), 1 Hz PMD rTMS induced a persistent suppression of MEP at rest, whereas 5 Hz PMD rTMS resulted in a lasting MEP facilitation. This was reflected in the three-factorial ANOVA, which showed an interaction between INTERVENTION and TIME [\(F_{(2,3,11,6)} = 5.08, P = 0.022\)] for MEP at rest. There was also a main effect of MUSCLE [\(F_{(1,9,9,7)} = 8.4, P = 0.008\)]. A two-factorial ANOVA, which considered only the mean MEP amplitudes of the relaxed FDI target muscle, also showed an interaction between INTERVENTION and TIME [\(F_{(1,9,13,8)} = 3.3, P = 0.043\)], whereas the same two-factorial ANOVAs for the relaxed ADM and APB muscles failed to show a significant interaction between INTERVENTION and TIME (Supplemental Fig. S1A). We also tested for electrophysiological changes in the active muscle. ANOVA showed a difference in mean MEP amplitude of the active muscle [\(F_{(1,03,4,1)} = 12.3, P = 0.024\)] and duration of the silent period [\(F_{(1,3,15)} = 11.8, P = 0.041\)] between the hand muscles, but no effect of INTERVENTION or TIME and no significant interaction.

In seven individuals we examined the conditioning effects of PAS without a preceding session of PMD rTMS (sessions C and D, Fig. 4B, Table 4; Supplemental Fig. S1B). In agreement with previous studies that had used slightly different PAS protocols (Stefan et al. 2000; Wolters et al. 2003; Ziemann et

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**Table 2.** Mean peak-to-peak (mV) of MEPs recorded from the right relaxed FDI, APB, and ADM muscles during session A and session B

<table>
<thead>
<tr>
<th></th>
<th>Session A (PMD 1 Hz rTMS Followed by PAS&lt;sub&gt;N20–5ms&lt;/sub&gt;)</th>
<th>Session B (PMD 5 Hz rTMS Followed by PAS&lt;sub&gt;N20+2ms&lt;/sub&gt;)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FDI</td>
<td>APB</td>
</tr>
<tr>
<td>MEP amplitude at T&lt;sub&gt;baseline&lt;/sub&gt;</td>
<td>1.36 ± 0.16</td>
<td>1.09 ± 0.19</td>
</tr>
<tr>
<td>MEP amplitude at T&lt;sub&gt;post-rTMS&lt;/sub&gt;</td>
<td>0.84 ± 0.10</td>
<td>0.85 ± 0.22</td>
</tr>
<tr>
<td>MEP amplitude at T&lt;sub&gt;post-PAS1&lt;/sub&gt;</td>
<td>1.21 ± 0.15</td>
<td>0.96 ± 0.18</td>
</tr>
<tr>
<td>MEP amplitude at T&lt;sub&gt;post-PAS2&lt;/sub&gt;</td>
<td>1.42 ± 0.15</td>
<td>1.12 ± 0.20</td>
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</table>

Values are means ± SE. Mean MEP amplitude was measured before (T<sub>baseline</sub>) and after PMD rTMS (T<sub>post-rTMS</sub>), as well as twice after PAS (T<sub>post-PAS1</sub>, T<sub>post-PAS2</sub>). The lower part of the table summarizes the results of pairwise comparisons (Student’s t-test, contrasting two measurements (T<sub>baseline</sub> vs. T<sub>post-rTMS</sub> vs. T<sub>post-PAS2</sub>) vs. T<sub>baseline</sub> vs. T<sub>post-PAS2</sub>).
The main finding of this study is that the “normal” direction of PAS-induced plasticity in the human M1HAND can be flipped by preconditioning the ipsilateral PMD with rTMS. When corticospinal excitability in the left M1HAND was suppressed by a preceding session of PMD 1 Hz rTMS, the “normal” inhibitory effect of PASN20−5ms on MEP amplitude was reversed into facilitation. Enhancing corticospinal excitability with 5 Hz rTMS blocked the facilitatory effect of PASN20+2ms on MEP amplitude. Indeed, PASN20+2ms tended to induce a decrease in MEP amplitude in the context of an increased level of M1 excitability. Reversal of the “normal” PAS effect on corticospinal excitability correlated with the previous shift in corticospinal excitability induced by rTMS of the ipsilateral PMD. Together, these results indicate that the change in motor cortex excitability after PMD rTMS triggered a BCM-like homeostatic mechanism in the left M1HAND, that reversed the sign of corticospinal plasticity induced by subsequent PAS.

Control experiments confirmed previous work showing that PMD rTMS without subsequent PAS induced a suppression (1 Hz PMD rTMS) or facilitation (5 Hz PMD rTMS) of corticospinal excitability for >30 min beyond the time of rTMS (Gerschlager et al. 2001; Rizzo et al. 2004). The stable after-effects after PMD rTMS alone indicate that the excitability changes found in the main experiment are not simply due to a spontaneous wearing off of the conditioning effects induced by PMD rTMS. Control experiments using PAS alone demonstrated that PASN20−5ms induced a lasting suppression of corticospinal excitability, whereas PASN20+2ms resulted in a sustained facilitation. These findings confirmed that our slightly modified PAS protocol was as effective as other PAS protocols in inducing bidirectional spike-time–dependent plasticity (STDP)–like effects on corticospinal excitability (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004). Together, the experimental results provide converging evidence for a homeostatic mechanism that effectively counteracted the excitability changes induced by a preceding PMD rTMS session.

The present results confirm and extend previous work that sequentially applied two interventional protocols over the M1HAND to probe a homeostatic BCM-like mechanism in the human primary motor cortex (Iyer et al. 2003; Lang et al. 2004; Muller et al. 2007; Siebner et al. 2004). Two studies used transcranial direct current stimulation (tDCS) to induce homeostatic BCM-like plasticity in the M1HAND (Lang et al. 2004; Siebner et al. 2004). When preceded by excitability-enhancing anodal tDCS, a subsequent session of 1 or 5 Hz PMD rTMS caused a depression of corticospinal excitability. Conversely, the same 1 or 5 Hz rTMS protocols induced an increase of corticospinal excitability if preceded by excitability-depressing cathodal tDCS (Lang et al. 2004; Siebner et al. 2004). The transcortical application of DC causes lasting shifts in intrinsic excitability of the corticospinal pyramidal cells, which have a strong impact on postsynaptic activity (Bindman et al. 1962).
FIG. 4. Control experiments. A: the impact of PMD rTMS on M1 activity without following PAS in the relaxed right FDI muscle. Black circles represent relative changes in MEP amplitude induced by 1 Hz rTMS (session E). Open circles represent relative changes in MEP amplitude induced by 5 Hz rTMS (session F). B: the effect of PAS on MEP amplitudes of the relaxed right FDI muscle without a preconditioning session of PMD rTMS. Black circles represent relative changes in MEP amplitude induced by PASN20−5ms (session C). Open circles represent relative changes in MEP amplitude induced by PASN20+2ms (session D). MEP amplitudes were normalized and expressed as percentage of MEP amplitudes recorded at baseline; level of significance was added from post hoc paired t-test performed on raw data. rTMS, repetitive transcranial magnetic stimulation; PAS, paired-associative stimulation.

Since tDCS triggered homeostatic plasticity by changing the postsynaptic activity of corticospinal neurons, rather than by changing the activity of specific presynaptic inputs, preconditioning the M1_HAND with tDCS was not suited to test the integrative properties of homeostatic metaplasticity. In addition, the studies that used a rTMS–rTMS paradigm to reveal homeostatic plasticity chose rTMS protocols that produced no or only minor effects on corticospinal excitability when given alone (Lang et al. 2004; Siebner et al. 2004). Thus these studies did not test whether homeostatic plasticity can flip the sign of stimulation-induced plasticity, turning an efficient protocol that would usually induce LTD-like plasticity into an LTD-inducing protocol and vice versa.

The potential of homeostatic plasticity to reverse the sign of stimulation-induced plasticity was recently demonstrated in a study that used PAS of the median nerve and contralateral M1_HAND to trigger and probe homeostatic plasticity (Muller et al. 2007). PAS was first applied to produce a bidirectional, LTD-like (PASN20−5ms) or LTP-like (PASN20+2ms) shift in motor cortex excitability. At 30 min after the end of the first PAS session, a second PAS protocol was applied. The second PAS session always used PASN20+2ms that would usually have an LTP-like effect on corticospinal excitability. PASN20+2ms increased corticospinal excitability when given after LTD-inducing PASN20−5ms. The aftereffect of PASN20+2ms was switched from facilitation to depression, if conditioned by LTD-inducing PASN20−5ms. In contrast to the study by Muller et al. (2007), in which the second PAS was applied after the effect of the first intervention had faded off, we applied PAS to the M1_HAND while neuronal excitability was still altered by the preceding PMD rTMS. However, this did not affect the homeostatic response pattern. Premotor rTMS shifted corticospinal excitability in the M1_HAND toward the margins of the modification range. This did not enhance the normal response pattern to subsequent PAS, but reversed it according to a homeostatic rule.

The present findings provide evidence that homeostatic metaplasticity can be induced by combining two interventional protocols that change excitability in the M1_HAND through different “input channels.” We used low-intensity rTMS of the left PMD to trigger homeostatic BCM-like mechanisms in the left M1_HAND. It has been assumed that this low-intensity PMD rTMS induces M1 changes via premotor–motor fiber connections and not by the spread of the stimulus pulse to M1 (Munchau et al. 2002). Focal rTMS of the PMD produces its conditioning effects on corticospinal excitability in the ipsilateral M1_HAND through specific premotor-to-motor connections that provide synaptic inputs onto the corticospinal neurons (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). In contrast, the conditioning effects of PAS on corticospinal excitability are mediated through specific sensory-to-motor inputs onto corticospinal output neurons in the M1_HAND. Together, the results show that the intracortical circuits in the human M1_HAND controlling corticospinal excitability effectively integrate the plasticity-inducing effects elicited through sensory-to-motor inputs (PAS) and premotor-to-motor inputs (PMD rTMS) in a homeostatic fashion.

Since TMS excites corticospinal neurons mainly through excitation of interneurons, which synapse onto corticospinal neurons, PMd rTMS and PAS may have modified the excitability of interneurons or corticospinal neurons. Therefore we cannot determine where in the M1_HAND the integrative homeostatic response was generated. Integration might have taken place at the level of the corticospinal output neurons triggering homeostatic regulation of synaptic efficacy. Another possibility would be that the homeostatic control of plasticity occurred more up-stream in the M1_HAND, for instance within interneuronal pools receiving converging sensory-to-motor and premotor-to-motor inputs. Likewise, we cannot infer whether the observed priming effects reflect a homosynaptic or heterosynaptic expression of homeostatic metaplasticity in the M1_HAND. Our in vivo measurements at a regional level also provide no clues regarding the underlying cellular mechanisms.

Focal rTMS of the PMD and PAS involve different physiological mechanisms to induce LTP- or LTD-like plasticity in the motor cortex (Thickbroom 2007). PMD rTMS elicits cooperative activity in specific premotor-to-motor inputs to the M1_HAND and its bidirectional effects on corticospinal excitability depend on rate-dependent activation of these presynaptic inputs (Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). In contrast to the activity-dependent mechanisms mediating premotor-to-motor plasticity, the bidirectional ef-
TABLE 4. Peak-to-peak MEP amplitudes (mV) of recorded from the right relaxed FDI, APB, and ADM muscles during sessions C, D, E, and F

<table>
<thead>
<tr>
<th></th>
<th>FDI 1-Hz rTMS</th>
<th>FDI 5-Hz rTMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session E</td>
<td>Session F</td>
</tr>
<tr>
<td></td>
<td>FDI</td>
<td>APB</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{baseline}}$</td>
<td>0.96 ± 0.11</td>
<td>0.60 ± 0.11</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post-rTMS1}}$</td>
<td>0.79 ± 0.10</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post-rTMS2}}$</td>
<td>0.57 ± 0.04</td>
<td>0.40 ± 0.09</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post-PAS1}}$</td>
<td>0.60 ± 0.11</td>
<td>0.47 ± 0.13</td>
</tr>
<tr>
<td>r-test ($T_{\text{baseline}}$ vs. $T_{\text{post-rTMS1}}$)</td>
<td>$t = 1.4$</td>
<td>$t = 2.8$</td>
</tr>
<tr>
<td>r-test ($T_{\text{baseline}}$ vs. $T_{\text{post-PAS1}}$)</td>
<td>$P = 0.22$</td>
<td>$P = 0.033$</td>
</tr>
<tr>
<td>r-test ($T_{\text{baseline}}$ vs. $T_{\text{post-PAS2}}$)</td>
<td>$P = 0.011$</td>
<td>$P = 0.003$</td>
</tr>
<tr>
<td>Session C (PAS$_{N20}$−5ms)</td>
<td>$P = 0.009$</td>
<td>$P = 0.039$</td>
</tr>
</tbody>
</table>

Values are means ± SE. Mean MEP amplitude was measured before ($T_{\text{baseline}}$) and after rTMS (top part of the table) or PAS (bottom part of the table). Pairwise comparisons (Student’s t-test) contrast $T_{\text{baseline}}$ versus the different $T_{\text{post-rTMS}}$ or $T_{\text{post-PAS}}$ for each intervention.

Effects of PAS critically depend on the timing between the peripheral and cortical stimulus (Stefan et al. 2000; Zieman et al. 2004) and thus can induce STDP-like effects in the human cortex (Thickbroom 2007). The reversal of rate-dependent plasticity (induced by PMD rTMS) by timing-dependent plasticity shows that the effects of these types of TMS-induced plasticity interact in a homeostatic fashion.

Learning a motor skill can also modify the response of the $M^1_{\text{HAND}}$ to subsequent PAS conditioning (Rosenkranz et al. 2007; Stefan et al. 2006; Zieman et al. 2004). Compatible with a BCM-like homeostatic mechanism, a single learning session during which participants learned ballistic thumb movements occurred subsequent PAS$_{N20+2ms}$-induced LTP-like plasticity, but enhanced PAS$_{N20-5ms}$-induced LTD-like plasticity (Stefan et al. 2006; Zieman et al. 2004). Accordingly, early motor skill training attenuated stimulation-induced LTD while enhancing stimulation-induced LTD in the motor cortex of adult rats (Rioult-Pedotti et al. 2000). The observation that motor learning can effectively trigger homeostatic BCM-like mechanisms underscores the physiological relevance of homeostatic plasticity in regulating the level of cortical excitability.

In the present study, the homeostatic response to PAS was more strongly expressed in the relaxed FDI and ADM muscles, which are innervated by the ulnar nerve, than in the APB muscle innervated by the median nerve. This was unexpected because previous studies reported that in healthy subjects, PAS of the right median nerve and the left $M^1_{\text{HAND}}$ produce somatotopically specific changes in MEP amplitudes in the APB muscle innervated by the median nerve without spread of the excitability changes to the ADM or FDI muscle (Quartarone et al. 2003; Stefan et al. 2000; Weise et al. 2006; Wolters et al. 2003). The difference in the somatotopic expression of the PAS effect may be related to our modified PAS protocol, which applied TMS over the motor hot spot of the right FDI muscle rather than using the motor hot spot of the APB muscle. The FDI muscle and the ADM muscle are functionally linked because they are synergistically activated when spreading the fingers. This hypothesis is supported by the control experiment in which only PAS was applied. Here the FDI muscle displayed a clear bidirectional change in MEP amplitude in the measurement 15 min after PAS with only a minor bidirectional shift in excitability in the APB and ADM muscles (see Supplemental figure).

Neither PAS$_{N20+2ms}$ nor PAS$_{N20-5ms}$ modulated the duration of the CSP when given after rTMS. This is different from previous studies that reported a prolongation of the CSP after PAS at an ISI of 25 ms (Quartarone et al. 2003; Stefan et al. 2000; Wolters et al. 2003). Again, slight differences in the PAS protocol may account for this discrepancy. Alternatively, the failure of PAS to modify the excitability of the intracortical circuits mediating the CSP may reflect a priming effect of rTMS on subsequent PAS. However, this negative result must be interpreted with caution as a different picture might have emerged if we had used a wider range of stimulus intensities, probing the relationship between stimulus intensity and SP duration.

The tapping rate showed an unspecific increase during the course of the experiment, but the interventions and the resulting changes in corticospinal excitability had no specific effect on tapping rates. Although this shows that basic motor behavior was not affected by the changes in excitability, it does not exclude a functional relevance of the observed excitability changes. The sensitivity to detect behavioral changes that parallel the excitability changes may depend on the type of motor task. The functional impact of the observed excitability changes may be clinically relevant in the context of motor learning. In fact, a recent study provided evidence that the conditioning effect of PAS influences the ability to learn a
Experimental interventions that induce acute shifts in corticospinal excitability do not always trigger a BCM-like homeostatic response in the human M1 HAND. Motor skill learning revealed a homeostatic pattern in response to PAS only after the first session of motor practice but not after 5 days of motor practice (Rosenkranz et al. 2007). In addition, the facilitatory effect of PAS on corticospinal excitability in the M1 HAND was enhanced when preconditioned by excitability-enhancing anodal tDCS, whereas excitability-reducing cathodal tDCS reversed the aftereffect of subsequent PAS from facilitation into inhibition (Nitsche et al. 2007). The failure of tDCS to trigger a BCM-like homeostatic response to PAS-induced plasticity is surprising because tDCS effectively triggered homeostatic plasticity when given before rTMS (Lang et al. 2004; Siebner et al. 2004). In addition, PAS-induced plasticity was influenced homeostatically by a preconditioning session of PAS (Muller et al. 2007), PMD rTMS (present study), or motor skill learning (Stefan et al. 2006; Ziemann et al. 2004). Together, these studies indicate that homeostatic plasticity is not triggered automatically by interventions that produce acute shifts in motor cortex excitability. Transcranial stimulation and motor learning rather seem to trigger an array of multiplastic responses of which only some are homeostatic in nature (Huang et al. 2008).

In conclusion, we demonstrate a BCM-like homeostatic response in the human M1 HAND, which effectively integrates stimulation-induced shifts in corticospinal excitability that are elicited through different input channels. This homeostatic integration within the M1 HAND might help to stabilize activity-driven changes in corticospinal excitability within a physiologicrange.

G R A N T S

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R E F E R E N C E S

Ballistic movement in a homeostatic fashion (Jung and Ziemann 2009).


