Inducing homeostatic-like plasticity in human motor cortex through converging cortico-cortical inputs

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Abstract

Transcranial stimulation techniques have revealed homeostatic-like metaplasticity in the hand area of the human primary motor cortex (M1\textsubscript{HAND}) that controls stimulation induced changes in corticospinal excitability. Here we combined two interventional protocols which induce long-term depression (LTD)-like or long-term potentiation (LTP)-like plasticity in left M1\textsubscript{HAND} through different afferents. We hypothesized that the left M1\textsubscript{HAND} would integrate LTP- and LTD-like plasticity in a homeostatic fashion. In ten healthy volunteers, low-intensity repetitive transcranial magnetic stimulation (rTMS) of left dorsal premotor cortex (PMD) was first applied to produce an LTP-like increase (5Hz rTMS) or LTD-like decrease (1Hz rTMS) in corticospinal excitability in left M1\textsubscript{HAND} via premotor-to-motor inputs. Following PMD rTMS, paired-associative stimulation (PAS) was applied to the right median nerve and left M1\textsubscript{HAND} to induce spike-time dependent plasticity (STDP) in sensory-to-motor inputs to left M1\textsubscript{HAND}. We adjusted the interstimulus interval to the N20-latency of the median nerve somatosensory-evoked cortical potential to produce an LTP-like increase (PAS\textsubscript{N20+2ms}) or an LTD-like decrease (PAS\textsubscript{N20-5ms}) in corticospinal excitability. The amplitude of motor-evoked potentials (MEPs) 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 5Hz rTMS, “facilitatory” PAS\textsubscript{N20+2ms} suppressed corticospinal excitability. Likewise, “inhibitory” PAS\textsubscript{N20-5ms} facilitated corticospinal excitability after “inhibitory” 1Hz 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 M1\textsubscript{HAND} that integrates acute plastic changes evoked through different “input channels”.
Keywords (optional)

Homeostatic plasticity, motor evoked potential, paired associative stimulation, premotor cortex, primary motor cortex, repetitive transcranial magnetic stimulation
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 2008). Because of its positive feed-back nature, synaptic plasticity tends to destabilize the level of activity in neuronal networks (Abbott and Nelson 2000; Turrigiano 1999; Turrigiano and Nelson 2004). Multiple homeostatic regulatory mechanisms have been identified that control synaptic plasticity at the cellular level (Abbott and Nelson 2000; Davis 2006; Marder and Prinz 2002; Perez-Otano and Ehlers 2005; Turrigiano and Nelson 2000). A theoretical framework of homeostatic plasticity was provided by the Bienenstock–Cooper–Munro (BCM) theory of synaptic modification (Bienenstock et al. 1982). According to the BCM theory, postsynaptic neuronal activity is stabilized by a modification threshold for induction of LTP or LTD which is dynamically adjusted according to the time-averaged postsynaptic firing rate. A prolonged reduction in postsynaptic activity would lower the LTP threshold and raise the LTD threshold. Conversely, a prolonged increase in postsynaptic activity would lower the LTD threshold and raise the LTP threshold. A key feature of the BCM modification threshold is its heterosynaptic expression (Bienenstock et al. 1982). This implies that any activity-driven change in the modification threshold will alter the changeability of all synapses regardless of which synaptic inputs have been active or silent (Abraham et al. 2001).

Using a preconditioning-conditioning approach, transcranial stimulation techniques have been successfully combined to demonstrate homeostatic BCM-like mechanisms in the human motor cortex which control the threshold for inducing LTP-like or LTD-like plasticity (Iyer et al. 2003; Lang et al. 2004; Muller et al. 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 (M1\_HAND). If the first intervention facilitated motor cortex excitability,
homeostatic mechanisms would strongly favour 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 M1\textsubscript{HAND}. For instance, excitability enhancing preconditioning of M1
increased the efficacy of a subsequent excitability-reducing session of low-frequency rTMS (Iyer
et al. 2003; Siebner et al. 2004). While 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 cortico-cortical inputs into M1.

The present study combined two conditioning protocols, premotor rTMS and paired associiative
stimulation (PAS), to investigate whether a homeostatic response pattern can be provoked in the
left M1\textsubscript{HAND} 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 paired associative stimulation
(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

11 healthy male individuals (mean age: 28.8 years, range 23-44 years) took part in the study. 10 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 were conforming to the Declaration of Helsinki.

Experimental design

The main experiment consisted of two sessions which were performed at least one week apart because in healthy subjects repeated rTMS of the left PMD can prolong the after effects on M1 excitability when given on consecutive days but not when applied with an interval of one 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 pre-conditioning, PAS was applied to the right median nerve and left M1HAND. In session A, we combined two protocols which usually induce a lasting suppression of corticospinal excitability in the ipsilateral M1HAND when given alone. We first applied “inhibitory” low-frequency (1Hz) rTMS to left PMD (Rizzo et al. 2004). After 1Hz rTMS, we performed “inhibitory” PAS using an ISI that equalled the individual N20-latency of the median nerve somatosensory-evoked cortical potential (SSEP) minus 5ms (PAS$_{N20-5ms}$) (Muller et al. 2007; Ziemann et al. 2004). In session B, two conditioning protocols were used that normally
facilitate corticospinal excitability in the ipsilateral $M1_{\text{HAND}}$. We first conducted facilitatory high-frequency (5Hz) rTMS over the left PMD (Rizzo et al. 2004) followed by facilitatory PAS at an ISI that matched the individual N20-latency plus 2ms ($PAS_{N20+2ms}$) (Muller et al. 2007; Ziemann et al. 2004). We deliberately combined two interventions that normally would induce LTP-like effects (session A: 5Hz PMD rTMS followed by $PAS_{N20+2ms}$) or two interventions that normally would induce LTD-like effects (session B: 1Hz PMD rTMS followed by $PAS_{N20-5ms}$) because we considered these combination most indicative of homeostatic interactions.

All interventional protocols were in accordance with published safety recommendations (Wassermann 1998). Experimental sessions were performed between 2.00 and 7.00 pm to minimize the influence of circadian changes in cortical responsiveness to the interventional protocols.

Measurements of corticospinal excitability were carried out in blocks immediately before ($T_{\text{baseline}}$) and after ($T_{\text{post-rTMS}}$) rTMS to PMD as well as immediately ($T_{\text{post-PAS 1}}$) and 15 minutes ($T_{\text{post-PAS 2}}$) after the end of PAS. In each block, we applied suprathreshold single-pulse TMS to the left $M1_{\text{HAND}}$ 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 years) took part in two additional experimental sessions in which $PAS_{N20-5ms}$ (session C) and $PAS_{N20+2ms}$ (session D) was applied without pre-conditioning the left PMd with rTMS (Fig.1). These experiments were necessary to probe the time course of changes in MEP amplitude.
induced by the PAS protocol alone. The timing of postinterventional MEP measurements corresponded to $T_{\text{post-PAS 1}}$ and $T_{\text{post-PAS 2}}$ of the main experiment (Fig.1).

Besides, in seven individuals we examined the after effects of 1Hz rTMS (session E) and 5Hz rTMS (session F) of left PMD without subsequent PAS of left M1HAND (mean age and SE: 30±2.25 years). Four out of the seven subjects had participated in session A and B. The timing of MEP measurements corresponded to the timing of MEP measurements in the main experiment ($T_{\text{baseline}}$, $T_{\text{post-rTMS 1}}$, $T_{\text{post-rTMS 2}}$= $T_{\text{post-PAS 1}}$ and $T_{\text{post-rTMS 3}}$= $T_{\text{post-PAS 2}}$, Fig.1).

**Measurements of corticospinal excitability**

Participants were seated comfortably in a reclining chair. Measurements were performed with a figure-of-eight- shaped coil “MC-B70” and a MagPro-100 stimulator (Medtronic-neuromuscular, Skovlunde, Denmark). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. Single monophasic pulses with duration of approximately 100µs were applied to the motor “hot spot” of the right FDI muscle. The monophasic pulse induced a posterior-to-anterior current in the left M1HAND with a current flow approximately perpendicular to the central sulcus. Single transcranial pulses were given at an inter-stimulus interval of 8s with a jitter of 20%.

At the beginning of each experiment, we first established the optimal position for activating the contralateral FDI muscle by moving the coil in 0.5 cm steps around the presumed M1HAND. The coil position at which a slightly suprathreshold stimulus elicited a maximal MEP was marked with a pen as the "motor hot spot" and used for TMS of the M1HAND. We then determined the motor threshold (MT) of the right FDI muscle at rest and during slight tonic contraction. The resting MT was defined as the minimum intensity that evoked a motor response of 50µV peak-to-
peak amplitude in five out of ten consecutive trials in the relaxed FDI muscle. Active MT was defined as the minimum intensity that elicited a reproducible MEP of at least 200µV in the tonically contracting FDI muscle in at least 5 out of 10 consecutive trials. Participants maintained a force level of approximately 10–15% of maximum force during measurements of the active MT. We used the motor hot spot of the FDI muscle rather than that of the APB muscle for TMS of the left M1\textsubscript{HAND} for three reasons. First, the FDI hot spot was used in all previous studies that investigated the conditioning effects of PMD rTMS on corticospinal excitability in ipsilateral M1\textsubscript{HAND} (Baumer et al. 2003; Gerschlager et al. 2001; Munchau et al. 2002; Rizzo et al. 2004). Second, MEP amplitudes recorded from the FDI muscle were usually larger or at least matched the MEP amplitudes recorded from the APB and ADM muscle when TMS was applied to 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 left PMD as well as twice after PAS conditioning. In each block, we first recorded 40 MEPs with the hand muscles completely relaxed. Before the first block of measurements (T\textsubscript{baseline}), stimulus intensity was adjusted to elicit MEPs in the right FDI muscle with mean peak-to-peak amplitude of approximately 1mV (SI\textsubscript{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 approximately 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\textsubscript{baseline}), stimulus intensity was adjusted to evoke a MEP with an amplitude of 1mV (SI\textsubscript{1mV active}) or a CSP that lasted approximately 100 ms in the tonically pre-activated right FDI muscle (SI\textsubscript{CSP 100ms}). SI\textsubscript{1mV at rest}, SI\textsubscript{1mV active} and SI\textsubscript{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 EMG activity of the right FDI muscle was continuously monitored with high-gain EMG (50µV/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 Ltd., Welwyn Garden City, Herts, UK). EMG signals were amplified (1000x), band-pass filtered, and digitized at a rate of 5kHz using an analogue-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 muscle 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 Dept. of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College of London, United Kingdom). The trials that had been recorded during tonic contraction at SI_{CSP}^{100ms} 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 where EMG activity reached a third of the mean pre-stimulus 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 15s. 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. to induce long-lasting bidirectional changes in corticospinal excitability in the ipsilateral M1\textsubscript{HAND} (Rizzo et al. 2004). 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 2.5cm 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\textsubscript{HAND} (Medtronic-neuromuscular, Skovlunde, Denmark). PMD rTMS consisted of 1500 biphasic stimuli. The coil was held tangentially to the skull with the handle pointing 45° postero-laterally. Stimuli had a biphasic waveform with a pulse width of approximately 280µs. 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 active MT as determined over the motor hot spot of the left FDI muscle using a biphasic pulse. In session A and E, rTMS was given at a rate of 1 Hz to produce an LTD-like decrease in MEP amplitude (Fig.1). The rTMS protocol consisted of two continuous trains (2x
750 stimuli) separated by an inter-train interval of 1 min. In session B and F, we applied high-frequency rTMS at a rate of 5 Hz to induce an LTP-like increase in MEP amplitude (Fig. 1). The 1500 stimuli were split into five trains of 300 stimuli using an inter-train interval of about 1 min.

**Paired associative stimulation**

The PAS protocol consisted of 200 paired pulses given at a rapid rate of 0.25 Hz as used in previous studies (Muller et al. 2007; Ziemann et al. 2004). Each stimulus pair consisted of a conditioning electrical stimulus to the right median nerve and a second stimulus to the left M1 HAND. The ISI between the peripheral and transcranial stimulus was individually adjusted to the N20 peak of the SSEP elicited by electrical stimulation of the right median nerve. The mean N20 latency in all subjects was 19.8 ± 0.2 ms. In session A and C, PAS used an ISI that was 5 ms shorter than the individual N20 latency (PAS N20-5 ms), while the ISI was 2 ms longer than the N20 latency in session B and D (PAS N20+2 ms). At these ISIs, PAS can induce bidirectional Hebbian changes in corticospinal excitability according to a temporal rule that is characteristic of spike timing dependent plasticity (Wolters et al. 2003; Wolters et al. 2005).

Electrical stimulation of the right median nerve was delivered at the wrist through bipolar surface electrodes (cathode proximal). The stimuli had a square-wave configuration with duration of 1 ms, and the intensity was set at 300% of the sensory perception threshold, that was defined as the first sensation while increasing electrical stimulation strength. The stimulus intensity of the TMS pulse corresponded to SI1mV at rest.

**Statistical analysis**
Statistical analysis focused primarily on excitability changes of the corticospinal output neurons. The effects of the experimental interventions on corticospinal excitability were tested using a three-factorial repeated-measures analysis of variance (ANOVA) for MEP at rest and in active conditions. The non-normalized mean MEP amplitude of the relaxed hand muscles was defined as dependent variable. For the main experiments (session A, B) the ANOVA model included the factors INTERVENTION (two levels: PMD 1Hz rTMS followed by PAS_{N20-5ms} and PMD 5Hz rTMS followed by PAS_{N20+2ms}), MUSCLE (three levels: APB, FDI, ADM muscles), and TIME of measurements (four levels: T_{baseline}, T_{post-rTMS}, T_{post-PAS 1}, T_{post-PAS 2}).

The same ANOVA model was applied to analyze changes in non-normalized MEP amplitudes in the control experiments (session C-F). The three-factorial ANOVA included the factors INTERVENTION (two levels: 1Hz PMD rTMS vs. 5Hz PMD rTMS and PAS_{N20+2ms} vs. PAS_{N20+2ms}, respectively), MUSCLE (three levels: APB, FDI, ADM muscles), and TIME of measurements (four or three levels respectively: T_{baseline}, T_{post-rTMS 1}, T_{post-rTMS 2}, T_{post-rTMS 3} or T_{baseline}, T_{post-PAS 1}, T_{post-PAS 2}). If there was a significant effect for the factor MUSCLE 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 probed the excitability of cortical (presumably GABAergic) 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: PMD 1Hz rTMS followed by PAS_{N20-5ms} and PMD 5Hz rTMS followed by PAS_{N20+2ms}), and TIME of measurements (four levels: T_{baseline}, T_{post-rTMS}, T_{post-PAS 1}, T_{post-PAS 2}).
The Huynh-Feldt method was used to correct for non-sphericity 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 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\textsubscript{HAND} or PMD were comparable between session A and B (Table 1).

Fig.2 illustrates relative changes in mean MEP amplitudes of the relaxed FDI, APB, and ADM muscles during session A and B. The three-factorial repeated-measures ANOVA showed a main effect of MUSCLE (F\textsubscript{2,0.18}= 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 ($F_{3,0.27} = 3.96; P = 0.018$). In accordance with Rizzo et al. (2004), mean MEP amplitudes of the relaxed right FDI muscle decreased after 1Hz rTMS to left PMD in session A relative to baseline ($T_9 = -2.65; P = 0.026$), while PMD 5Hz rTMS induced an increase in MEP amplitude in session 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 1Hz rTMS compared to MEPs after PMD 5Hz rTMS (mean MEP amplitudes at $T_{\text{post-rTMS}}$: Session A versus 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” PAS$_{N20-5ms}$ 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 1Hz rTMS ($T_9 = 3.182; P = 0.011$). In session B, “facilitatory” PAS$_{N20+2ms}$ 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” 5Hz rTMS of left PMD ($T_9 = -1.9; P = 0.09$).

For MEP measurements after PAS, pair-wise comparisons revealed no differences in mean MEP amplitudes between session A and B ($T_{\text{post-PAS 1}}$: $T_9 = -0.42; P = 0.682$; $T_{\text{post-PAS 2}}$: $T_9 = 1.41; P = 0.193$). These findings show that the excitability shift induced by PMD rTMS activated a BCM-like homeostatic mechanism in left $\text{M1}_{\text{HAND}}$ that reversed the sign of corticospinal plasticity induced by subsequent PAS.

Regression analysis provided further evidence that PMD pre-conditioning triggered BCM-like homeostatic mechanism in the left $\text{M1}_{\text{HAND}}$, 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 session A and B, we found a negative linear relation between the pre-
conditioning 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 approximately 50% of
the variance of the after effect 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 5Hz rTMS followed by PASN20+2ms) or two “LTD”-
inducing protocols (i.e., PMD 1Hz 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 (F6.0; 54.0 = 3.15; P = 0.01). 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 (F3.0; 27 = 5.20; P = 0.006) and ADM muscle
(F3.0; 27.0 = 5.38; P = 0.005). An interaction between INTERVENTION and TIME was absent in
the APB muscle (F3.0; 27.0 = 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 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 left PMd followed by PAS had no specific effects
on MEP amplitudes in the active muscle. Three-factorial ANOVA only showed unspecific
facilitatory MEP changes during the repeated measurements (TIME: F3, 27=4.82, p=0.008), but no
interaction between interventional protocol, muscle and time of measurement (Table 3).

Neither PMD rTMS nor PAS modified significantly 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 ($F_{3.0, 27.0} = 2.93; P = 0.052$), 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 after effects of PMD rTMS alone on corticospinal excitability (session E, F, Fig. 4A, Table 4, supplementary Fig. A). The time course of excitability changes was examined by measuring MEP at baseline, directly after PMD rTMS and additionally, at time points which matched $T_{\text{post-PAS 1}}$ and $T_{\text{post-PAS 2}}$ in session A and B. In accordance with previous studies (Gerschlager et al. 2001; Rizzo et al. 2004), 1Hz PMD rTMS induced a persistent suppression of MEP at rest, whereas 5Hz 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 only considered the mean MEP amplitudes of the relaxed FDI target muscle also showed an interaction between INTERVENTION and TIME ($F_{3.0, 18} = 3.3, p=0.043$), whereas the same two-factorial ANOVAS for the relaxed ADM and APB muscles failed to show a significant interaction between INTERVENTION and TIME (supplementary Fig. A). 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 ($F_{1, 3}$
=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 (session C, D, Fig.4 B, Table 4, supplementary Fig. B). In agreement with previous studies that had used slightly different PAS protocols (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004) our “inhibitory” PAS_{\text{N20-5ms}} protocol caused a lasting suppression of mean MEP amplitudes at rest when given alone, likewise, the “facilitatory” PAS_{\text{N20+2ms}} protocol resulted in a lasting increase in mean MEP amplitudes at rest when given alone (Fig. 4B, Table 4).

The three-factorial ANOVA confirmed a differential effect of the two different PAS interventions on MEP at rest. There was an interaction among the three experimental factors intervention, muscle and time (F_{3,8, 22.5}=5.96, p=0.002). MEP amplitudes differed among the three muscles (main effect of muscle: F_{1,5, 9.3}=5.48, p=0.033). A follow-up two-factorial ANOVA demonstrated an interaction between INTERVENTION and TIME (F_{1,2, 7.4}=7.13, p=0.027) for the MEPs recorded from the FDI target muscle, demonstrating a difference in the conditioning effects of the two PAS interventions. There were no significant interactions between INTERVENTION and TIME for MEP at rest in the APB and ADM muscle. Visual inspection of the group data confirms that the differential effects of the ISI on post-interventional MEP changes were most pronounced in the FDI muscle, yet the ADM and APB muscles also showed a similar trend (supplementary Fig. B).

For MEPs recorded from the active muscle the same three-factorial ANOVA showed no significant effect of INTERVENTION, TIME and no interaction among the experimental factors, there was only a significant effect of MUSCLE (F_{1,4; 8.4}= 60.71, p<0.001). Both PAS protocols
had no consistent effects on the duration of the cortical silent period in the FDI muscle in the two-factorial ANOVA model.

Discussion

The main finding of this study is that the “normal” direction of PAS-induced plasticity in the human M1\textsubscript{HAND} can be flipped by pre-conditioning the ipsilateral PMD with rTMS. When corticospinal excitability in the left M1\textsubscript{HAND} was suppressed by a preceding session of PMD 1Hz rTMS, the “normal” inhibitory effect of PAS\textsubscript{N20-5ms} on MEP amplitude was reversed into facilitation. Enhancing corticospinal excitability with PMD 5Hz rTMS blocked the facilitatory effect of PAS\textsubscript{N20+2ms} on MEP amplitude. Indeed, PAS\textsubscript{N20+2ms} tended to induce a decrease in MEP amplitude in the context of an increased level of M1 excitability. The 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 left M1\textsubscript{HAND} 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 (1Hz PMD rTMS) or facilitation (5Hz PMD rTMS) of corticospinal excitability for more than 30 minutes 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 PAS\textsubscript{N20-5ms} induced a lasting suppression of corticospinal excitability whereas PAS\textsubscript{N20+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 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 which 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 M1\textsubscript{HAND} to probe 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 employed tDCS to induce homeostatic BCM-like plasticity in the M1\textsubscript{HAND} (Lang et al. 2004; Siebner et al. 2004). When preceded by excitability-enhancing anodal tDCS, a subsequent session of 1Hz or 5Hz PMD rTMS caused a depression of corticospinal excitability. Conversely, the same 1Hz or 5Hz 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). Since tDCS triggered homeostatic plasticity by changing the postsynaptic activity of corticospinal neurons rather than by changing the activity of specific pre-synaptic inputs, pre-conditioning the M1\textsubscript{HAND} with tDCS was not suited to test the integrative properties of homeostatic metaplasticity. In addition, the studies which used a tDCS-rTMS paradigm to reveal homeostatic plasticity chose rTMS protocols which produced no or only minor effects on corticospinal excitability when given alone (Lang et al. 2004; Siebner et al. 2004). Hence these studies did not test whether homeostatic plasticity can flip the sign of stimulation-induced plasticity, turning an efficient protocol that would usually induce LTP-like plasticity into a 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 M1HAND to trigger and probe homeostatic plasticity (Muller et al. 2007). PAS was first applied to produce bi-directional, LTD-like (PASN20-5ms) or LTP-like (PASN20+2ms) shift in motor cortex excitability. 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 a LTP-like effect on corticospinal excitability. PASN20+2ms increased corticospinal excitability when given after LTD-inducing PASN20-5ms. The after effect of PASN20+2ms was switched from facilitation to depression, if conditioned by LTP-inducing PASN20+2ms. In contrast to the study by Muller et al. (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 M1HAND 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 M1HAND towards 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 M1HAND through different “input channels”. We used low-intensity rTMS of the left PMD to trigger homeostatic BCM-like mechanisms in the left M1HAND. It has been assumed that this low-intensity PMD rTMS induces M1 changes via premotor-motor fibre 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 ipsilateral M1HAND 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 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 can not 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 can not 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 effects of PAS critically depend on the timing between the peripheral and cortical stimulus (Stefan et al. 2000;
Ziemann et al. 2004), and thus can induce spike-time dependent plasticity (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 M1\textsubscript{HAND} to subsequent PAS
conditioning (Rosenkranz et al. 2007; Stefan et al. 2006; Ziemann et al. 2004). Compatible with a
BCM-like homeostatic mechanism, a single learning session during which participants learned
ballistic thumb movements occluded subsequent PAS\textsubscript{N20+2ms} -induced LTP-like plasticity but
enhanced PAS\textsubscript{N20–5ms}-induced LTD-like plasticity (Stefan et al. 2006; Ziemann et al. 2004).
Accordingly, early motor skill training attenuated stimulation-induced LTP 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 M1\textsubscript{HAND} produce a 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 as 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 muscle.

Neither PAS\textsubscript{N20+2ms} nor PAS\textsubscript{N20-5ms} modulated the duration of the CSP when given after PMD rTMS. This is different to previous studies which 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 PMD 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. While this shows that basic motor behaviour was not affected by the changes in excitability, it does not exclude a functional relevance of the observed excitability changes. The sensitivity to detect behavioural 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 ballistic movement in a homeostatic fashion (Jung and Ziemann 2009).
Experimental interventions that induce acute shifts in corticospinal excitability do not always trigger a BCM-like homeostatic response in the human M1\textsubscript{HAND}. Motor skill learning only revealed a homeostatic pattern in response to PAS after the first session of motor practice but not after five days of motor practice (Rosenkranz et al. 2007). In addition, the facilitatory effect of PAS on corticospinal excitability in the M1\textsubscript{HAND} was enhanced when pre-conditioned by excitability-enhancing anodal tDCS, whereas excitability-reducing cathodal tDCS reversed the after effect 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 pre-conditioning 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 metaplastic responses of which only some are homeostatic in nature (Huang et al. 2007).

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

\textbf{Acknowledgements}

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Table 1. Motor threshold and stimulus intensities that were used for transcranial stimulation to evoke MEP in the right FDI target muscle in session A (PMD 1Hz rTMS followed by PAS_{N20-5ms}) and session B (PMD 5Hz rTMS followed by PAS_{N20+2ms}). All values are given as 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). SI_{1mv at rest} refers to the stimulus intensity that evoked a MEP of 1mV amplitude in the resting FDI muscle. This stimulus intensity was also used for TMS of the left M1_{HAND} during PAS. Likewise SI_{1mv active} means the stimulus intensity to evoke a MEP of 1mV amplitude during active conditions. SI_{CSP 100ms} means the stimulus intensity that evoked a cortical silent period (CSP) with duration of approximately 100ms. Monophasic TMS pulses were used throughout the whole session except for SI_{premotor rTMS} corresponding to 90% of individual aMT using biphasic pulses. This stimulation intensity was used for rTMS of the left PMD. Group data are given as mean ± one fold standard error. Student’s paired T-test revealed no significant differences between mean values for any of the measures obtained in session A and B, so it was ensured that both sessions were conducted in equivalent baseline conditions. (corresponding P-values in right column).

Table 2. Mean peak-to-peak amplitudes (mV) of MEPs recorded from the right relaxed FDI, APB, and ADM muscle during session A (PMD 1Hz rTMS followed by PAS_{N20-5ms}) and session B (PMD 5Hz rTMS followed by PAS_{N20+2ms}). Data are presented as mean ± one fold standard error. Mean MEP amplitude was measured before (T_{baseline}) and after PMD rTMS (T_{post-rTMS}), as well as twice after PAS (T_{post-PAS 1}, T_{post-PAS 2}). The lower part of the table summarizes the results of pair-wise comparisons (Student’s T-test) contrasting two measurements (T_{baseline} versus T_{post-rTMS}, T_{post-rTMS} versus T_{post-PAS 2}, T_{baseline} versus T_{post-PAS 2}).
Table 3. Peak-to-peak MEP amplitudes (MEP Ampl.) and duration of the cortical silent period (CSP) in the active FDI muscle as well as the total numbers of taps made during the 15s period for session A (PMD 1Hz rTMS followed by PAS \( N_{20-5ms} \)) and session B (PMD 5Hz rTMS followed by PAS \( N_{20+2ms} \)). Data are presented as mean ± one fold standard error. Mean MEP amplitude was measured before (\( T_{\text{baseline}} \)) and after PMD rTMS (\( T_{\text{post-rTMS}} \)), as well as twice after PAS (\( T_{\text{post-PAS 1}}, T_{\text{post-PAS 2}} \)). The lower part of the table summarizes the results of pair-wise comparisons (Student’s T-test) contrasting two measurements (\( T_{\text{baseline}} \) versus \( T_{\text{post-rTMS}} \), \( T_{\text{post-rTMS}} \) versus \( T_{\text{post-PAS 2}}, T_{\text{baseline}} \) versus \( T_{\text{post-PAS 2}} \)).

Table 4. Peak-to-peak amplitudes of MEPs recorded from the right relaxed FDI, APB, and ADM muscle during session C (PAS \( N_{20-5ms} \)), D (PAS \( N_{20+2ms} \)), E (PMD 1Hz PMD rTMS), F (PMD 5Hz PMD rTMS). Data are presented as mean ± one fold standard error. Mean MEP amplitude was measured before (\( T_{\text{baseline}} \)) and after PMD rTMS (upper part of the table) or PAS (lower part of the table). Pair-wise comparisons (Student’s T-test) contrast \( T_{\text{baseline}} \) versus the different \( T_{\text{post-rTMS}} \) or \( T_{\text{post-PAS}} \) for each intervention.

Figure 1. Experimental design. Ten subjects participated in the main experiment (session A and B) in which we first applied rTMS to the left PMD followed by PAS of the right median nerve and left M1_{HAND}. In session A, we applied two interventional protocols that usually would elicit a lasting LTD-like suppression of corticospinal excitability in left M1_{HAND} (PMD 1Hz rTMS of left PMD followed by PAS_{N20-5ms} of the right median nerve and left M1_{HAND}). Session B consisted of
two interventional protocols that normally would produce a lasting LTP-like potentiation of corticospinal excitability in left M1\textsubscript{HAND} (PMD 5Hz rTMS of left PMD followed by PAS\textsubscript{N20+2ms} of the right median nerve and left M1\textsubscript{HAND}). Seven subjects participated in two 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 two experiments with 1Hz rTMS (session E) or 5Hz 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 1}) and 15min after PAS (T\textsubscript{post-PAS 2}) PAS.

**Figure 2.** Relative changes in MEP amplitude of the relaxed right FDI muscle (upper panel), APB muscle (middle panel), and ADM muscle (lower panel) induced by PMD rTMS and PAS of the right median nerve and left M1\textsubscript{HAND}. Black rectangles denote relative changes in MEP amplitude induced by PMD 1Hz rTMS and subsequent PAS\textsubscript{N20-5ms} (session A), open rectangles give relative changes in MEP amplitude caused by PMD 5Hz rTMS followed by PAS\textsubscript{N20+2ms} (session B). Mean MEP amplitudes were normalized and expressed as percentage of MEP baseline amplitude. Error bars show the standard error of the mean. Asterisks indicate significant differences in non normalized mean MEP amplitudes as revealed by post-hoc Student’s paired T-test (* \(p < 0.05\); ** = \(p < 0.01\)).

**Figure 3.** The graph plots the individual changes in MEP amplitude of the relaxed FDI muscle after PMD rTMS (x-axis) against individual changes in MEP amplitude produced by subsequent
PAS (y-axis). The conditioning effects of PMD rTMS predicted the direction and strength of the PAS effect on corticospinal excitability. The dotted line gives the regression slope showing an inverse linear relationship between the effects of PMD rTMS and the subsequent PAS effect on mean MEP amplitude in the relaxed FDI muscle (Beta = -0.701; T = -4.741; P = 0.001; r² = 0.492). Squares denote relative MEP changes in session A (PMD 1Hz rTMS and subsequent PAS_N20-5ms). Circles indicate relative MEP changes in session B (PMD 5Hz rTMS followed by PAS_N20+2ms).

**Figure 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 1Hz rTMS (session E). Open circles represent relative changes in MEP amplitude induced by 5Hz 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 PAS_N20-5ms (session C). Open circles represent relative changes in MEP amplitude induced by PAS_N20+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-tests performed on raw data.

**Supplementary Figure.** Control experiments in more detail. **A.** Relative changes in MEP amplitude of the relaxed right FDI muscle (upper left panel), APB muscle (middle left panel), and ADM muscle (lower left panel) induced by PMD rTMS alone. Black circles denote relative changes in MEP amplitude induced by 1Hz rTMS, open circles reflect relative changes in MEP amplitude caused by 5Hz rTMS. **B.** Relative changes in MEP amplitude of the relaxed right FDI
muscle (upper right panel), APB muscle (middle right panel), and ADM muscle (lower right panel) induced by PAS of the right median nerve and left M1\textsubscript{HAND}. Black circles indicate relative changes in MEP amplitude induced by \textit{PAS}_{N20-5ms}, open circles correspond to relative changes in MEP amplitude after \textit{PAS}_{N20+2ms}. Mean MEP amplitudes were normalized and expressed as percentage of MEP baseline amplitude. Error bars show the standard error of the mean. Asterisks indicate significant differences in non normalized mean MEP amplitudes as revealed by post-hoc Student’s paired T-test (* \( p < 0.05; \) ** = \( p < 0.01 \)).
### Table 1

<table>
<thead>
<tr>
<th>Measure</th>
<th>Session A (PMD 1Hz rTMS followed by PAS\textsubscript{(N20-5ms)}}</th>
<th>Session B (PMD 5Hz rTMS followed by PAS\textsubscript{(N20+2ms)}}</th>
<th>p-value (T-Test)</th>
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<td>rMT</td>
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<td>aMT</td>
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### Table 2

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<th>Session A (PMD 1Hz rTMS followed by PAS&lt;sub&gt;N20-5ms&lt;/sub&gt;)</th>
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<th>Session B (PMD 5Hz rTMS followed by PAS&lt;sub&gt;N20+2ms&lt;/sub&gt;)</th>
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<td></td>
<td>FDI</td>
<td>APB</td>
<td>ADM</td>
<td>FDI</td>
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<td>MEP amplitude (mV) at T&lt;sub&gt;baseline&lt;/sub&gt;</td>
<td>1.36 ± 0.16</td>
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<td>MEP amplitude (mV) at T&lt;sub&gt;post-PAS 1&lt;/sub&gt;</td>
<td>1.21 ± 0.15</td>
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<td>MEP amplitude (mV) at T&lt;sub&gt;post-PAS 2&lt;/sub&gt;</td>
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<td>1.09 ± 0.17</td>
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<td>T = 2.65</td>
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### Table 3

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<td>(PMD 1Hz rTMS</td>
<td>(PMD 5Hz rTMS</td>
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<tr>
<td></td>
<td>followed by PAS</td>
<td>followed by PAS</td>
</tr>
<tr>
<td></td>
<td>N(_{20-5ms})</td>
<td>N(_{20+2ms})</td>
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<td>120.79±8.93</td>
<td>99.24±8.82</td>
</tr>
<tr>
<td><strong>Number of taps</strong></td>
<td>70.3±3.1</td>
<td>68.8±2.4</td>
</tr>
<tr>
<td><strong>T post-rTMS</strong></td>
<td>2.31±0.35</td>
<td>1.81±0.33</td>
</tr>
<tr>
<td><strong>CSP (ms)</strong></td>
<td>128.68±11.63</td>
<td>101.54±14.1</td>
</tr>
<tr>
<td><strong>Number of taps</strong></td>
<td>68.5±3.5</td>
<td>69.2±2.5</td>
</tr>
<tr>
<td><strong>T post-PAS1</strong></td>
<td>2.02±0.31</td>
<td>2.06±0.31</td>
</tr>
<tr>
<td><strong>CSP (ms)</strong></td>
<td>122.89±13.05</td>
<td>105.45±13.2</td>
</tr>
<tr>
<td><strong>Number of taps</strong></td>
<td>69±3.3</td>
<td>69.9±2.9</td>
</tr>
</tbody>
</table>

**t-test (T baseline versus T post-rTMS)**

<table>
<thead>
<tr>
<th></th>
<th><strong>T</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T baseline</strong></td>
<td>1.34</td>
<td>0.212</td>
</tr>
<tr>
<td><strong>T post-rTMS</strong></td>
<td>-0.944</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>t = -2.77</strong></td>
<td><strong>p = 0.022</strong></td>
<td></td>
</tr>
</tbody>
</table>

**t-test (T post-rTMS versus T post-PAS 2)**

<table>
<thead>
<tr>
<th></th>
<th><strong>T</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T post-rTMS</strong></td>
<td>-0.58</td>
<td>0.185</td>
</tr>
<tr>
<td><strong>T post-PAS 2</strong></td>
<td>0.70</td>
<td>0.334</td>
</tr>
<tr>
<td><strong>t = -0.58</strong></td>
<td><strong>p = 0.334</strong></td>
<td></td>
</tr>
</tbody>
</table>

**t-test (T baseline versus T post-PAS 2)**

<table>
<thead>
<tr>
<th></th>
<th><strong>T</strong></th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T baseline</strong></td>
<td>-0.93</td>
<td>0.375</td>
</tr>
<tr>
<td><strong>T post-PAS 2</strong></td>
<td>-0.49</td>
<td>0.636</td>
</tr>
<tr>
<td><strong>t = -2.43</strong></td>
<td><strong>p = 0.038</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>FDI</th>
<th>APB</th>
<th>ADM</th>
<th>FDI</th>
<th>APB</th>
<th>ADM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEP amplitude at $T_{\text{baseline}}$ (mV)</td>
<td>0.96±0.11</td>
<td>0.60±0.11</td>
<td>0.41±0.14</td>
<td>0.84±0.12</td>
<td>0.66±0.19</td>
<td>0.37±0.13</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post rTMS 1}}$ (mV)</td>
<td>0.79±0.10</td>
<td>0.45±0.07</td>
<td>0.26±0.05</td>
<td>1.22±0.26</td>
<td>0.83±0.30</td>
<td>0.45±0.19</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post rTMS 2}}$ (mV)</td>
<td>0.57±0.04</td>
<td>0.40±0.09</td>
<td>0.20±0.03</td>
<td>1.10±0.19</td>
<td>0.51±0.14</td>
<td>0.41±0.15</td>
</tr>
<tr>
<td>MEP amplitude at $T_{\text{post rTMS 3}}$ (mV)</td>
<td>0.60±0.11</td>
<td>0.47±0.13</td>
<td>0.20±0.03</td>
<td>1.47±0.41</td>
<td>0.63±0.13</td>
<td>0.40±0.10</td>
</tr>
<tr>
<td>t-test ($T_{\text{baseline}}$ versus $T_{\text{post rTMS 1}}$)</td>
<td>$T=1.4$</td>
<td>$T=2.8$</td>
<td>$T=1.2$</td>
<td>$T=-2.1$</td>
<td>$T=-1.0$</td>
<td>$T=-1.0$</td>
</tr>
<tr>
<td></td>
<td>p=0.22</td>
<td>p=0.033</td>
<td>p=0.26</td>
<td>p=0.081</td>
<td>p=0.36</td>
<td>p=0.38</td>
</tr>
<tr>
<td>t-test ($T_{\text{baseline}}$ versus $T_{\text{post rTMS 2}}$)</td>
<td>$T=3.6$</td>
<td>$T=4.7$</td>
<td>$T=1.55$</td>
<td>$T=-1.7$</td>
<td>$T=1.3$</td>
<td>$T=-0.7$</td>
</tr>
<tr>
<td></td>
<td>p=0.011</td>
<td>p=0.003</td>
<td>p=0.17</td>
<td>p=0.14</td>
<td>p=0.23</td>
<td>p=0.50</td>
</tr>
<tr>
<td>t-test ($T_{\text{baseline}}$ versus $T_{\text{post rTMS 3}}$)</td>
<td>$T=3.8$</td>
<td>$T=2.6$</td>
<td>$T=1.45$</td>
<td>$T=-1.7$</td>
<td>$T=0.3$</td>
<td>$T=-0.4$</td>
</tr>
<tr>
<td></td>
<td>p=0.009</td>
<td>p=0.039</td>
<td>p=0.19</td>
<td>p=0.15</td>
<td>p=0.81</td>
<td>p=0.74</td>
</tr>
<tr>
<td></td>
<td>Session C</td>
<td></td>
<td>Session D</td>
<td></td>
<td></td>
<td></td>
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<td>------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>MEP amplitude at T_baseline (mV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{PAS}_{N20-5\text{ms}} )</td>
<td>1.08±0.08</td>
<td>0.91±0.19</td>
<td>0.47±0.09</td>
<td>0.97±0.10</td>
<td>0.95±0.16</td>
<td>0.5±0.12</td>
</tr>
<tr>
<td>( \text{PAS}_{N20+2\text{ms}} )</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>MEP amplitude at T_post-PAS _1 (mV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{PAS}_{N20-5\text{ms}} )</td>
<td>0.71±0.09</td>
<td>0.69±0.15</td>
<td>0.49±0.10</td>
<td>1.18±0.14</td>
<td>0.97±0.19</td>
<td>0.46±0.08</td>
</tr>
<tr>
<td>( \text{PAS}_{N20+2\text{ms}} )</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>MEP amplitude at T_post-PAS _2 (mV)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{PAS}_{N20-5\text{ms}} )</td>
<td>0.83±0.09</td>
<td>0.79±0.17</td>
<td>0.43±0.13</td>
<td>1.42±0.20</td>
<td>1.04±0.12</td>
<td>0.57±0.16</td>
</tr>
<tr>
<td>( \text{PAS}_{N20+2\text{ms}} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>t-test (T_baseline versus T_post-PAS _1)</strong></td>
<td>T= 4.3</td>
<td>T= 3.6</td>
<td>T= -0.3</td>
<td>T= -1.2</td>
<td>T= -0.4</td>
<td>T= 0.3</td>
</tr>
<tr>
<td></td>
<td>p= 0.005</td>
<td>p= 0.011</td>
<td>p= 0.76</td>
<td>p=0.26</td>
<td>p=0.72</td>
<td>p=0.79</td>
</tr>
<tr>
<td><strong>t-test (T_baseline versus T_post-PAS _2)</strong></td>
<td>T= 3.1</td>
<td>T= 1.6</td>
<td>T=0.7</td>
<td>T= -2.5</td>
<td>T= -0.9</td>
<td>T= -0.4</td>
</tr>
<tr>
<td></td>
<td>p= 0.021</td>
<td>p= 0.15</td>
<td>p= 0.49</td>
<td>p=0.05</td>
<td>p= 0.42</td>
<td>p=0.72</td>
</tr>
</tbody>
</table>