

Modulation of Preparatory Volitional Motor Cortical Activity by Paired Associative Transcranial Magnetic Stimulation

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Abstract: Paired associative transcranial magnetic stimulation (PAS) has been shown to induce long-term potentiation (LTP)-like or long-term depression (LTD)-like change in excitability of human primary motor cortex (M1), as probed by motor evoked potential (MEP) amplitude. In contrast, little is known about PAS effects on volitional motor cortical activity. In 10 healthy subjects, movement related cortical potentials (MRCP) were recorded to index volitional motor cortical activity during preparation of simple thumb abduction (prime mover: abductor pollicis brevis, APB) or wrist extension movements (prime mover: extensor carpi radialis, ECR). PAS_{LTP} increased, PAS_{LTD} decreased, and PAS_{control} did not change MEP_{APB}, while MEP_{ECR}, not targeted by PAS, remained unchanged in all PAS conditions. PAS_{LTP} decreased MRCP negativity during the late Bereitschaftspotential (–500 to 0 ms before movement onset), only in the APB task, and predominantly over central scalp electrodes contralateral to the thumb movements. This effect correlated negatively with the PAS_{LTP} induced increase in MEP_{APB}. PAS_{LTD} and PAS_{control} did not affect MRCP amplitude. Findings indicate a specific interference of PAS with preparatory volitional motor cortical activity, suggestive of a net result caused by increased M1 excitability and disrupted effective connectivity between premotor areas and M1. *Hum Brain Mapp* 30:3645–3656, 2009. © 2009 Wiley-Liss, Inc.

Key words: movement related cortical potential; Bereitschaftspotential (BP); long-term potentiation-like plasticity; transcranial magnetic stimulation; paired associative stimulation; human motor cortex

INTRODUCTION

Transcranial magnetic stimulation (TMS) can induce long-term changes in excitability of the human primary motor cortex (M1) [Fitzgerald et al., 2006; Ziemann et al., 2008]. Usually, these effects are demonstrated by single-pulse or paired-pulse TMS protocols. The most commonly used measure is motor evoked potential (MEP) amplitude, an estimate of excitability of the corticomotoneuronal projection [Hallett, 2007]. A particularly well investigated TMS protocol to induce MEP change is paired associative

Contract grant sponsor: German Research Foundation; Contract grant number: ZI 542/4-2.

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Received for publication 21 December 2008; Revised 16 February 2009; Accepted 2 March 2009

DOI: 10.1002/hbm.20793

Published online 21 April 2009 in Wiley InterScience (www.interscience.wiley.com).

stimulation (PAS), in which pairs of electrical peripheral nerve stimulation and focal TMS of contralateral M1 are applied [Stefan et al., 2000; Ziemann et al., 2008]. Depending on the interval between the two stimuli, PAS induces a long-term potentiation (LTP)-like increase in MEP amplitude if the afferent pulse from peripheral nerve stimulation reaches M1 at the same time or a few milliseconds before TMS (PAS_{LTP}), or a long-term depression (LTD)-like decrease in MEP amplitude in the case of a reversed order of these events (PAS_{LTD}) [Müller et al., 2007; Wolters et al., 2003; Ziemann et al., 2004]. The changes in MEP amplitude originate at the motor cortical rather than a subcortical or spinal site [Di Lazzaro et al., 2009; Stefan et al., 2000], and the properties of this MEP change (cooperativity, duration >30 min, dependence on NMDA receptor activation) are akin to LTP and LTD as defined at the cellular level in slice or neuronal culture experiments [Cooke and Bliss, 2006; Ziemann et al., 2008]. Furthermore, the critical dependence of the direction of PAS effects on timing of electrical peripheral nerve stimulation relative to TMS is reminiscent of bidirectional spike timing-dependent plasticity, where LTP occurs if action potentials consistently follow excitatory postsynaptic potentials, whereas LTD occurs with the reversed order of events [Caporale and Dan, 2008; Dan and Poo, 2004; Markram et al., 1997].

While the consequences of PAS and other TMS plasticity protocols on M1 excitability have been studied in detail, remarkably little is known about their effects on volitional motor cortical activity and motor behavior. The majority of studies showed, with few exceptions [Jäncke et al., 2004; Schlaghecken et al., 2003; Yoo et al., 2008], that TMS plasticity protocols do not affect simple motor behavior, such as the maximum tapping rate, in the hand contralateral to M1 stimulation [Agostino et al., 2007; Chen et al., 1997; Lee et al., 2003; Muellbacher et al., 2000; Rossi et al., 2000; Rounis et al., 2005; Sommer et al., 2002; Wassermann et al., 1996]. A likely more sensitive route of detecting subtle TMS effects despite the absence of overt changes in motor behavior is to assess volitional motor cortical activity by EEG measures such as movement related cortical potentials (MRCP) [Deecke, 2000; Deecke et al., 1969; Kornhuber and Deecke, 1965; Shibasaki and Hallett, 2006]. A 5 Hz rTMS protocol, which typically increases MEP amplitude, increased contingent negative variation amplitude during movement preparation in a warned choice reaction time task [Holler et al., 2006]. In contrast, a 1 Hz repetitive TMS (rTMS) protocol, which typically decreases MEP amplitude, decreased MRCP amplitude during preparation of thumb opposition movements [Rossi et al., 2000]. These data suggest that PAS or rTMS induced changes in MEP amplitude are coupled with changes into the same direction in volitional motor cortical activity.

However, rTMS of M1 does not only alter local excitability at the site of stimulation but also changes effective connectivity between M1 and other motor areas, in particular the dorsolateral premotor cortex (PMd) [Lee et al., 2003; Oliviero et al., 2003; Rounis et al., 2005; Strens et al., 2002],

an area crucially important for preparation of voluntary movement [Chouinard and Paus, 2006; Hoshi and Tanji, 2007; Wise, 1985]. Effective connectivity analysis showed that, after 1 Hz rTMS, movement-related coupling of PMd and supplementary motor area (SMA) with the stimulated M1 increased [Lee et al., 2003; Rounis et al., 2005], while, after 5 Hz rTMS, this coupling decreased [Rounis et al., 2005]. Therefore, effects of TMS plasticity protocols on preparatory volitional motor cortical activity may be more complex than suggested by the previous data.

Here we investigated the effects of PAS_{LTP} vs. PAS_{LTD} on MRCP amplitude. We focused particularly on the late Bereitschaftspotential (late BP), which is thought to be generated largely in the PMd and M1 contralateral to the movement, and to reflect executive preparatory motor activity closely linked to the subsequent motor action [Shibasaki and Hallett, 2006]. PAS-induced LTP-like and LTD-like effects on MEP amplitude are topographically specific to the motor representation activated by TMS and median nerve stimulation [Stefan et al., 2000; Weise et al., 2006]. One aim of this study was to test if a similar representational specificity applies to the effects of PAS on MRCP amplitude. To this end, we tested MRCPs elicited by two different movement tasks, thumb abduction (targeted by TMS and median nerve stimulation) vs. wrist extension (not targeted by the median nerve stimulus). Given the so far very limited amount of knowledge of effects of TMS plasticity protocols on volitional motor cortical activity, this study is exploratory in nature. Nevertheless, very likely this work will improve our understanding of preparatory volitional motor activity in motor cortico-cortical networks and its modification by brain stimulation. This knowledge will pertain to clinical applications for treatment of patients in whom this activity is disordered, such as in dystonia [Deuschl et al., 1995], Parkinson's disease [Touge et al., 1995], spino-cerebellar ataxia [Lu et al., 2008], traumatic brain injury [Wiese et al., 2004], or cerebral stroke [Green et al., 1999].

METHODS

Subjects

Ten healthy subjects participated in the experiments (five men; mean age \pm SD, 27.3 ± 6.7 years; range, 19–38 years). All subjects were right-handed according to the Edinburgh Handedness Inventory [Oldfield, 1971]. All gave their written informed consent prior to the study. The experimental procedures were in accord with the Declaration of Helsinki and approved by the local Ethics Committee.

Experimental Design

Subjects were seated on a comfortable reclining chair with both arms relaxed. They participated in three different PAS protocols in separate sessions at least 1 week apart in a single-blinded, randomized, controlled crossover

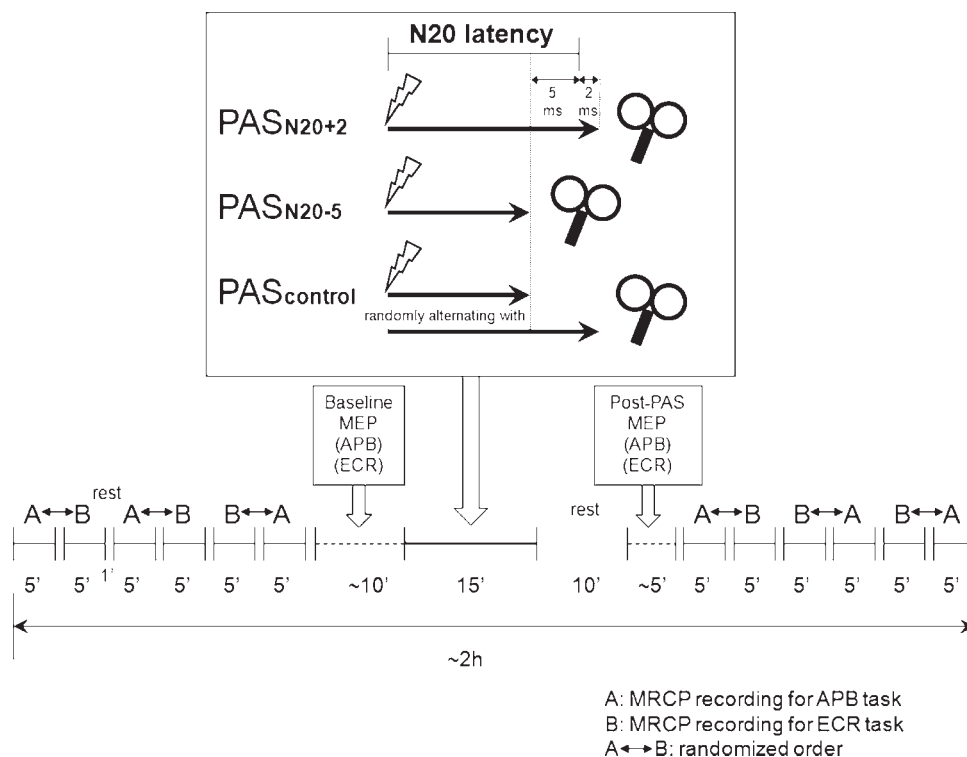


Figure 1.

Experimental design and time line of MRCP and MEP measurements before and after one of three different paired associative stimulation protocols (PAS_{LTP} , PAS_{LTD} , $PAS_{control}$). For details, see Methods section.

design. MRCP recordings were obtained before and after the PAS intervention (see Fig. 1). Pre- and post-PAS MRCP recordings consisted of six 5-min recording blocks with a 1-min break in between (see Fig. 1). In three of these blocks, subjects were requested to contract their right abductor pollicis brevis (APB) muscle by performing repeatedly brief thumb abduction movements, and in the other three blocks they were requested to contract their right extensor carpi radialis (ECR) muscle by performing brief wrist extension movements. The order of blocks was randomized with the constraint that each pair of blocks had to include one APB and one ECR task (labeled “A” and “B”, respectively, in Fig. 1). Movement rate was self-paced (approximately one movement every 6 seconds). In addition, corticomotoneuronal excitability was measured by 20 single-pulse MEPs recorded from the right APB and ECR immediately before and 10 min after the PAS intervention (see Fig. 1).

EEG and EMG Recordings

Fifty-six Ag/AgCl scalp electrodes were used for EEG recording. One electrode was placed below the left outer canthus to record the electrooculogram (EOG). Impedance of all electrodes was kept below 5 k Ω . All electrodes were

referenced to linked earlobe electrodes and raw signals were filtered with a bandpass of 0.05–70 Hz (NeuroScan[®] SynAmps, Neurosoft, Sterling, VA). Pairs of electrodes were taped bilaterally over the APB and ECR for surface electromyography (EMG) recording. The EMG signals were rectified and filtered at a bandpass of 30–200 Hz. EOG, EEG and EMG signals were sampled simultaneously, digitized at a rate of 2 kHz and stored for offline analysis. During the recordings, subjects were requested to fix a red spot on a screen 1.5 m in front of them and to perform the motor task with their right APB or ECR as described above.

TMS and PAS

TMS and PAS were applied while the EEG cap remained on the scalp and electrode leads disconnected from the EEG amplifiers. TMS was delivered through a focal figure-of-eight stimulating coil (diameter of each wing, 70 mm) connected to a Magstim 200 magnetic stimulator (Magstim Co., Carmarthenshire, Wales, UK) with a nearly monophasic current waveform. The coil was held tangential to the cap-on-scalp over the presumed hand area of the left M1 with the handle pointing backwards and $\sim 45^\circ$ away from the midline. This way the induced

current in the brain was directed from lateral-posterior to medial-anterior, an optimal current direction for exciting corticospinal neurons in M1 transsynaptically [Di Lazzaro et al., 2004]. The optimal coil position ('hot spot') was determined as the site where TMS at a slightly suprathreshold intensity produced consistently the largest MEPs in right APB. This site was marked on the scalp by a soft tip pen to assure a constant placement of the coil throughout the session. The intensity of TMS was adjusted to produce MEPs of on average 1 mV in peak-to-peak amplitude (MEP_{1mV}) in the resting APB. Twenty trials were obtained with a randomly varying intertrial interval ranging from 7.5–12.5 s, to minimize anticipation of the next trial. MEPs were recorded simultaneously in the right APB and right ECR (bandpass filter 20–2 kHz; Counterpoint Mk2 Electromyograph; Dantec, Skovlunde, Denmark). The signals were then digitized at a rate of 5 kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK) and stored in a laboratory computer for offline-analysis (Spike2 for Windows, Version 3.05, CED).

PAS was applied according to a protocol established previously by our group [Müller-Dahlhaus et al., 2008b; Müller et al., 2007; Ziemann et al., 2004]. It consisted of 225 pairs of electrical stimulation of the right median nerve at the wrist followed by a single TMS pulse over the hot spot of the APB motor representation of the left M1. Pairs were delivered at a rate of 0.25 Hz (i.e. duration of PAS, 15 min). Electrical stimulation was applied through a bipolar electrode (cathode proximal), using constant current square wave pulses (duration, 1 ms) at an intensity of three times the perceptual threshold. The intensity of TMS was adjusted to produce MEP_{1mV} when given without conditioning median nerve stimulation. Three PAS protocols were applied, which differed with respect to the interstimulus interval (ISI) between median nerve and M1 stimulation (see Fig. 1): In PAS_{LTP} , the ISI equaled the individual N20-latency of the median nerve somatosensory-evoked cortical potential plus 2 ms. In PAS_{LTD} , the ISI was set to the individual N20-latency minus 5 ms. In $PAS_{control}$, ISIs randomly alternated between PAS_{LTP} and PAS_{LTD} intervals. These PAS conditions were chosen because they induced LTP-like (PAS_{LTP}), LTD-like (PAS_{LTD}) or no change ($PAS_{control}$) of the stimulated M1 representation of the APB in previous studies [Müller et al., 2007]. As motor attention may significantly affect the magnitude of these PAS effects [Stefan et al., 2004], the level of attention was controlled by using a light emitting diode (LED) attached to the right wrist which flashed randomly (0.2–1 Hz) during the PAS intervention with subjects requested to count the total number of flashes and report it as correctly as possible at the end of PAS.

Data Analysis and Statistics

Statistics were performed with StatView for Windows (Version 5.0.1, SAS Institute).

MEP amplitude

Averages of MEP amplitude (in mV) were calculated in each subject from the twenty trials of each recording block in APB and ECR, and before and after PAS. Effects of PAS on MEP amplitude were then analyzed by a three-way repeated measures analysis of variance (rmANOVA) with the within subject factors of MUSCLE (APB, ECR), PAS PROTOCOL (PAS_{LTP} , PAS_{LTD} , $PAS_{control}$) and TIME (pre-PAS, post-PAS). Conditional on a significant *F* value, post hoc comparisons were performed using paired-sample *t*-tests with Bonferroni correction for multiple comparisons.

Motor performance

Performance of volitional movements during the MRCP recordings was assessed by the movement rate, and quantitative analysis of the rectified EMG of the voluntary EMG burst (peak amplitude and onset-to-peak time). These three measures were analyzed by separate three-way rmANOVAs with the within subject effects of MUSCLE (APB, ECR), PAS PROTOCOL (PAS_{LTP} , PAS_{LTD} and $PAS_{control}$) and TIME (pre-PAS, post-PAS). Conditional on a significant *F* value, post hoc comparisons were conducted by paired-samples *t*-tests with Bonferroni correction for multiple comparisons.

MRCP amplitude

For MRCP analysis, epochs from 2000 ms before the onset of the voluntary EMG burst (–2000 ms) to 1000 ms after EMG onset (1000 ms) were segmented. The initial 250 ms (–2000 to –1750 ms) were assigned as baseline. Onset of the voluntary EMG burst (0 ms) was marked visually in each sweep. All EEG segments were inspected for artifacts according to a preset algorithm. Forty three percent of all EEG segments were contaminated by blink artifacts in the EOG, muscle artifacts or slow potential shifts $>40 \mu V$ suggestive of movement or sweating artifacts, and were discarded from further analysis. In each subject, on average 79 artifact-free EEG segments were rectified, aligned to EMG onset, and averaged according to motor task (APB vs. ECR), PAS condition (PAS_{LTP} vs. PAS_{LTD} vs. $PAS_{control}$) and time (pre-PAS vs. post-PAS). Two subcomponents of the MRCP were analyzed: the early BP from –1500 ms to –500 ms, and the late BP from –500 ms to EMG onset [Shibasaki et al., 1980; Shibasaki and Hallett, 2006]. BP amplitude was quantified by taking the mean amplitude of the EEG signal (in μV) in these two epochs. Since the major MRCP activity localizes to the central scalp [Shibasaki et al., 1980], MRCP statistics focused to the 21 electrodes covering this area (C5A, C3A, C1A, CZA, C2A, C4A, C6A, C5, C3, C1, CZ, C2, C4, C6, TCP1, C3P, C1P, PZA, C2P, C4P, TCP2, nomenclature according to the International 10–20 EEG system). Four MRCP data sets (early BP and late BP, APB task and ECR task) were analyzed separately by three-way rmANOVAs with the within-subject

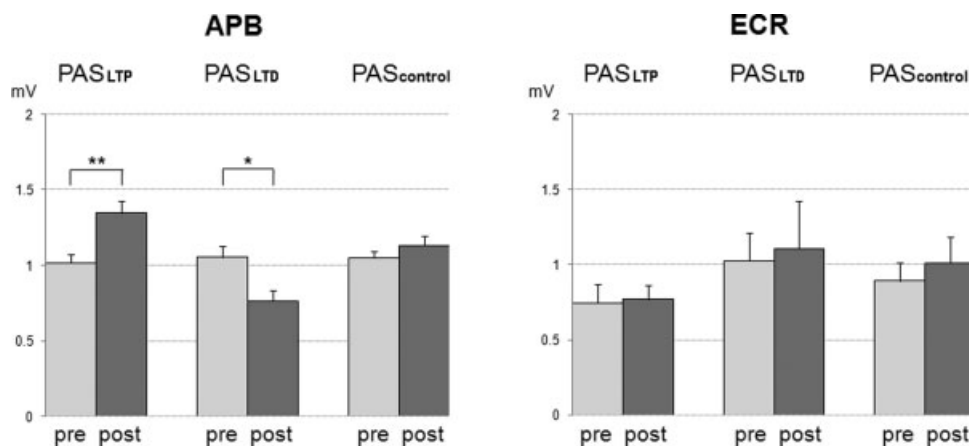


Figure 2.

MEP amplitudes (in mV) pre-PAS (light gray columns) vs. post-PAS (dark gray columns, mean \pm S.E.M) in the APB (left diagram) and ECR (right diagram). MEP amplitudes in the APB increased after PAS_{LTP} and decreased after PAS_{LTD} (* $P < 0.05$; ** $P < 0.01$) while there were no changes in MEP amplitude in the ECR.

factors of ELECTRODE POSITION (21 levels), PAS PROTOCOL (PAS_{LTP}, PAS_{LTD} and PAS_{control}) and TIME (pre-PAS, post-PAS). Conditional on a significant F value, post hoc paired-sample t -tests were performed using Bonferroni correction for multiple comparisons.

Mapping of PAS effects on MRCP amplitude

For each PAS protocol and motor task, and separately for the early BP and the late BP a scalp current source density (CSD) map was calculated based on the voltage difference post-PAS vs. pre-PAS at each of the 56 electrode positions (Brain Vision Analyzer Version 2.0.0.3031, Brain Products GmbH, Germany).

Relation of PAS induced change in MEP and BP amplitude

Finally, possible correlations between PAS induced changes (post-PAS minus pre-PAS) in BP amplitude (dependent variable) vs. changes in MEP amplitude (independent variable) were explored by linear regression analyses at those electrode locations where PAS had a significant effect on BP amplitude.

Throughout the paper, data are presented as means \pm SD if not stated otherwise. For all tests a p value of <0.05 was considered significant.

RESULTS

None of the subjects experienced any noticeable adverse effects during or after the study. All subjects were highly cooperative throughout the experimental procedures.

PAS Effects on MEP Amplitude

RmANOVA showed no significant main effects but significant interactions of MUSCLE and PAS PROTOCOL ($F_{2,18} = 5.45$, $P = 0.014$) and MUSCLE, PAS PROTOCOL and TIME ($F_{2,18} = 7.80$, $P = 0.0036$). These interactions were explained by the post hoc comparisons, which showed an increase in MEP amplitude in the APB after PAS_{LTP} ($P < 0.01$), a decrease after PAS_{LTD} ($P < 0.05$), and no change after PAS_{control}, whereas MEP amplitudes in the ECR did not change by any of the PAS protocols (all $P > 0.05$) (see Fig. 2). There were no differences in MEP amplitude in the APB prior to PAS between PAS conditions (one-way rmANOVA: $F_{2,9} = 0.15$, $P = 0.86$; PAS_{LTP}: 1.02 ± 0.18 mV; PAS_{LTD}: 1.06 ± 0.24 mV; PAS_{control}: 1.05 ± 0.13 mV, light gray columns in Fig. 2) which could have accounted for these differential PAS effects on MEP amplitude of the APB. In addition, there were also no significant differences in the pre-PAS measurements of MEP amplitude in the ECR between PAS conditions (one-way rmANOVA: $F_{2,9} = 2.45$, $P = 0.11$; PAS_{LTP}: 0.74 ± 0.41 mV; PAS_{LTD}: 1.03 ± 0.59 mV; PAS_{control}: 0.89 ± 0.39 mV, light gray columns in Fig. 2). This absence of pre-PAS differences supports the validity of the observed lack of differential PAS effects on MEP amplitude in the ECR.

PAS Effects on Motor Performance

The mean movement rate across all conditions was 0.155 ± 0.03 s⁻¹. Three-way rmANOVA of movement rate revealed no significant main effect of MUSCLE ($P = 0.054$), PAS PROTOCOL ($P = 0.18$) or TIME ($P = 0.22$) or any of their interactions (all $P > 0.40$).

Three-way rmANOVA of the amplitude of the voluntary EMG burst showed a main effect of TIME ($F_{1,9} = 19.5$, $P =$

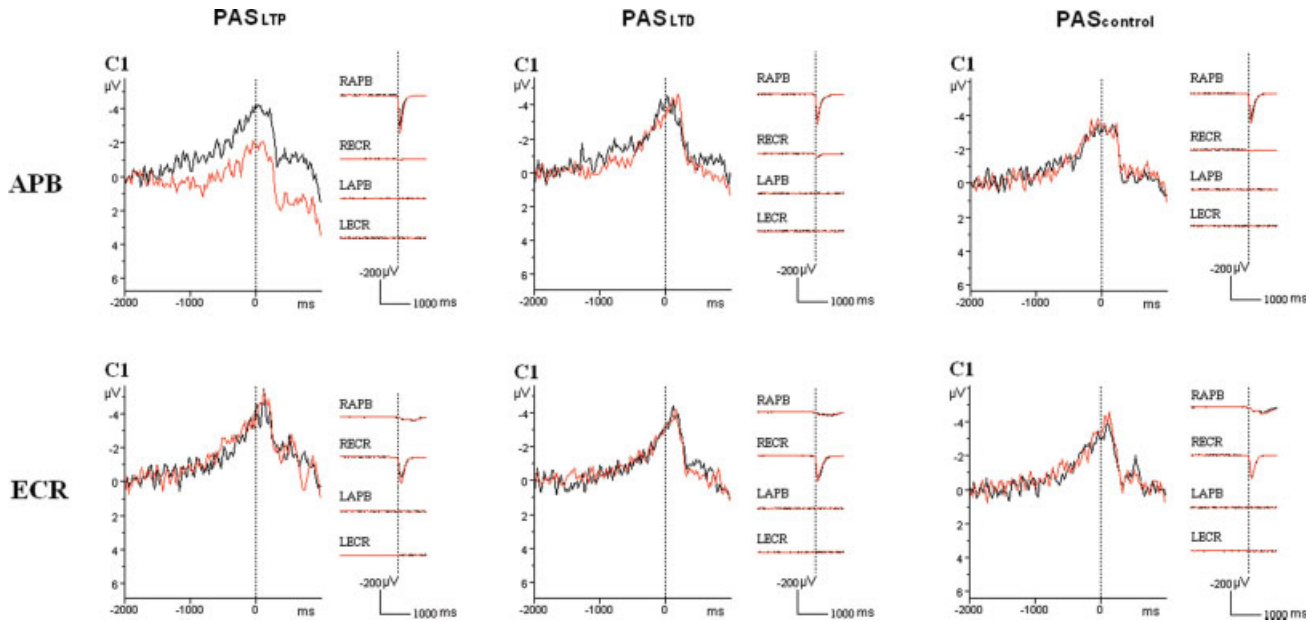


Figure 3.

Superimposition of the grand average ($n = 10$ subjects) MRCP waveforms (in μV) recorded from the C1 electrode for the APB task (upper row) and the ECR task (lower row) before (black curves) and after (red curves) PAS_{LTP} (left column), or PAS_{LTD} (middle column), or $\text{PAS}_{\text{control}}$ (right column). Vertical dotted lines mark the onset of the voluntary EMG burst in the task

muscle. Note the reduction of the MRCP negativity specifically after PAS_{LTP} in the APB task, but no MRCP change after PAS_{LTD} or $\text{PAS}_{\text{control}}$ or in the ECR task. In addition, the averaged rectified EMG from right and left APB (RAPB, LAPB) and ECR (RECR, LECR) are shown. Calibrations, 1000 ms and 200 μV .

0.0017) but no significant effects of MUSCLE ($P = 0.48$), or PAS PROTOCOL ($P = 0.15$), or any of their interactions (all $P > 0.1$). The effect of TIME was explained by a slight increase in EMG burst amplitude post-PAS irrespective of MUSCLE or PAS PROTOCOL (pre-PAS: $261.7 \pm 119.3 \mu\text{V}$; post-PAS: $305.3 \pm 145.7 \mu\text{V}$) (see Fig. 3). RMANOVA of the onset-to-peak time of the EMG burst revealed a significant main effect of MUSCLE ($F_{1,9} = 9.22$, $P = 0.014$) but no other significant main effects (PAS PROTOCOL: $P = 0.26$; TIME: $P = 0.69$) or interactions thereof (all $P > 0.05$). The effect of MUSCLE was explained by longer onset-to-peak times in ECR compared to APB, irrespective of PAS PROTOCOL or TIME (APB: 91.4 ± 45.6 ms; ECR: 122.1 ± 42.7 ms) (see Fig. 3). The lack of specific PAS effects on movement rate, or amplitude or onset-to-peak time of the voluntary EMG burst is an important null finding, which largely excludes a significant role of variation in motor performance in the observed specific PAS effects on MRCP amplitude (see below).

PAS Effects on MRCP Amplitude

The three-way rMANOVAs of all four MRCP data sets (early BP and late BP in APB task, early BP and late BP in ECR task) all showed a significant main effect of ELEC-

TRODE POSITION (all $P < 0.01$, Table I), reflecting the central and for the late BP predominantly left hemispheric MRCP distribution. In addition, the three-way rMANOVA of the early BP in the APB task revealed a main effect of TIME ($F_{1,9} = 23.7$, $P = 0.0009$, Table I) but no other main effects or interactions thereof. The effect of TIME was explained by a decrease of the early BP negativity after PAS, irrespective of ELECTRODE POSITION or PAS PROTOCOL (pre-PAS: $-0.70 \pm 0.78 \mu\text{V}$; post-PAS: $-0.10 \pm 0.78 \mu\text{V}$). Furthermore, the three-way rMANOVA of the late BP in the APB task demonstrated a significant interaction between PAS PROTOCOL and TIME ($F_{2,18} = 5.53$, $P = 0.013$, Table I) and between ELECTRODE POSITION, PAS PROTOCOL and TIME ($F_{40,360} = 1.59$, $P = 0.016$, Table I). Post hoc testing showed that a significant effect of TIME occurred only in the PAS_{LTP} protocol ($F_{1,9} = 14.0$, $P = 0.0046$) but not in the PAS_{LTD} and $\text{PAS}_{\text{control}}$ protocols (all $P > 0.7$). The effect of TIME in the PAS_{LTP} protocol was reflected by a decrease of the late BP negativity after PAS (pre-PAS: $-2.15 \pm 1.0 \mu\text{V}$, post-PAS: $-0.85 \pm 1.15 \mu\text{V}$). Further exploration of the triple interaction between ELECTRODE POSITION, PAS PROTOCOL and TIME by post hoc testing revealed that the late BP was significantly reduced only after PAS_{LTP} and only at the following electrodes: CZA (pre-PAS: $-2.27 \pm 1.33 \mu\text{V}$, post-PAS: $-0.72 \pm 1.37 \mu\text{V}$, $P = 0.0022$), C1 (pre-PAS: $-2.86 \pm 1.01 \mu\text{V}$,

TABLE I. Repeated measures analysis of variance (rmANOVA) of the PAS effects on the early BP and the late BP

	df	Early BP: APB		Late BP: APB		Early BP: ECR		Late BP: ECR	
		F	P	F	P	F	P	F	P
Electrode position ^a	20,180	2.61	0.0004**	6.25	<0.001**	2.43	0.001**	7.01	<0.001**
PAS protocol ^b	2,18	1.62	0.22	1.19	0.33	0.45	0.64	0.50	0.62
Time ^c	1,9	23.74	0.0009**	4.25	0.069	0.44	0.52	0.37	0.56
Electrode position × PAS protocol	40,360	1.39	0.07	1.07	0.37	0.75	0.86	0.85	0.73
PAS protocol × time	2,18	2.24	0.14	5.53	0.013*	0.14	0.87	2.06	0.16
Electrode position × time	20,180	0.20	0.39	0.90	0.59	0.62	0.89	0.80	0.72
Electrode position × PAS protocol × time	40,360	1.01	0.46	1.59	0.016*	0.91	0.63	0.99	0.50

* $P < 0.05$, ** $P < 0.01$; df, degrees of freedom.

^a21 levels (C5A, C3A, C1A, CZA, C2A, C4A, C6A, C5, C3, C1, CZ, C2, C4, C6, TCP1, C3P, C1P, PZA, C2P, C4P, TCP2).

^bThree levels (PAS_{LTP}, PAS_{LTD}, and PAS_{control}).

^cTwo levels (pre-PAS and post-PAS).

post-PAS: $-0.98 \pm 1.01 \mu\text{V}$, $P = 0.0017$), and C1P (pre-PAS: $-2.81 \pm 1.26 \mu\text{V}$, post-PAS: $-1.07 \pm 1.65 \mu\text{V}$, $P = 0.0014$). This PAS protocol and task specific effect is illustrated for the representative electrode C1 in Figure 3. Finally, rmANOVAs of early and late BP in the ECR task revealed no significant main effects (except on ELECTRODE POSITION) and no interactions thereof (Table I).

of the voltage difference post-PAS minus pre-PAS). The only conspicuous change was a reduction in MRCP negativity post-PAS_{LTP}, only in the APB task, and only for the late BP. This effect was localized over the central scalp area with slight predominance in the left hemisphere. The electrode locations with statistically significant voltages differences (CZA, C1, C1P) are indicated by blue dots.

Mapping of PAS Effects on MRCP Amplitude

Figure 4 shows the topographic distribution of the PAS induced scalp voltage change (current source density map

Relation Between PAS Effects on MEP and MRCP

Regression analysis between the PAS_{LTP}-induced MEP change and significant PAS_{LTP}-induced late BP change in

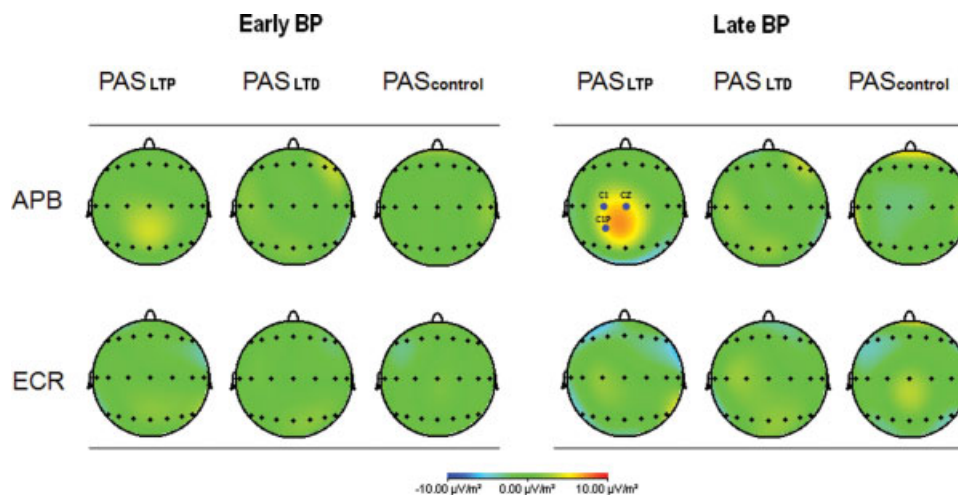


Figure 4.

Current source density map of the PAS induced MRCP voltage change (difference of post-PAS minus pre-PAS). Left part of the diagram: early BP; right part of the diagram: late BP; upper row: APB task; lower row: ECR task. A PAS effect was noted only after PAS_{LTP} only for the late BP (reduction of BP negativity) and

only in the APB task. This effect was localized over the central scalp area, predominantly in the left hemisphere. Those electrodes with a statistically significant PAS_{LTP} effect are indicated by blue dots (CZA, C1, and C1P).

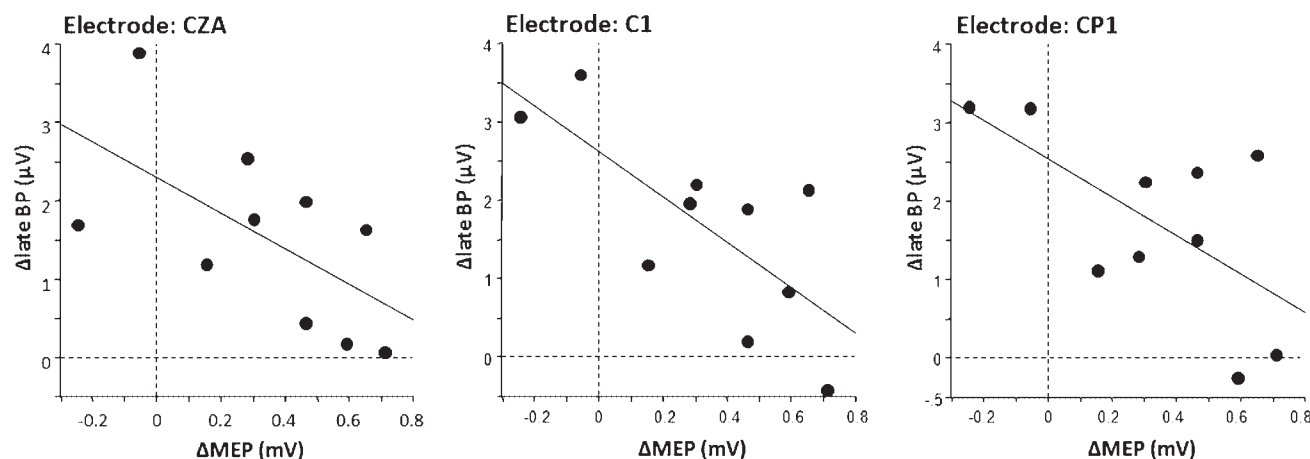


Figure 5.

Linear regression analyses between PAS_{LTP} induced late BP change in the APB task (dependent variable, y -axis, in μV) and PAS_{LTP} induced MEP change in the APB (independent variable, x -axis, in mV) at those electrodes that showed a significant PAS_{LTP} effect on the late BP amplitude (cf. Fig. 4). A significant negative correlation was found at the C1 electrode ($r = -0.73$, $P = 0.016$).

the APB task revealed a significant negative correlation at the C1 electrode ($r = -0.73$, $P = 0.016$), but only nonsignificant trends for negative correlations at the two other electrode positions (CZA, C1P, Fig. 5). Individuals with strong MEP increase had weak change in the late BP at the C1 electrode, while those with weak MEP change or even slight MEP decrease had a strong decrease in late BP negativity (see Fig. 5).

DISCUSSION

PAS Effects on MEP Amplitude

The present findings are in agreement with previous reports [Kujirai et al., 2006; Kuo et al., 2007; Müller et al., 2007; Rosenkranz et al., 2007; Stefan et al., 2002; Stefan et al., 2000; Stefan et al., 2006; Weise et al., 2006; Wolters et al., 2003; Ziemann et al., 2004] by demonstrating spike-timing dependent plasticity-like bidirectional changes in MEP amplitude (see Fig. 2) depending on the interstimulus interval between median nerve stimulus and TMS pulse used in the PAS_{LTP} vs. PAS_{LTD} protocols. In addition, this study replicated two other important features of PAS-induced plasticity: first, no change in MEP amplitude occurred if the interval between median nerve stimulus and TMS pulse alternated randomly between N20 + 2 and N20-5 [Müller et al., 2007]. Therefore, this PAS protocol can be considered an ideal control condition because identical physical stimuli as in the PAS_{LTP} and PAS_{LTD} protocols are applied, and because it is impossible for the subjects to distinguish between these protocols. Second,

the PAS_{LTP} and PAS_{LTD} effects were topographically specific [Stefan et al., 2000; Weise et al., 2006] because they occurred only in the APB, a hand muscle innervated by the stimulated median nerve, but not in the ECR, a more proximal muscle innervated by the nonstimulated radial nerve. This topographical specificity allowed exploring to which extent PAS effects on MRCP amplitude show similar specificity, i.e. manifestation in the APB task but not in the ECR task.

PAS Effects on Late BP Amplitude

The early BP surface negativity preceding a unimanual movement is thought to arise from increasing depolarization of the superficial layer of the apical dendrites of pyramidal neurons in a distributed cortical network consisting of the SMA and the PMd bilaterally, while the late BP close to movement onset can be largely explained by activity predominantly in the PMd and M1 contralateral to the movement [Ikeda et al., 1992; Shibasaki and Hallett, 2006; Toma et al., 2002]. While the exact relationship of the early and late BP to the intention and preparation to move is not fully understood, convergent evidence supports the view that the early BP is closely related to preparatory aspects of the forthcoming movement such as motor intention, selection or preparatory set whereas the late BP is particularly related to its executive aspects, such as amplitude, precision or complexity (for review, [Shibasaki and Hallett, 2006]).

The major novel finding of this study is a specific PAS effect on the late BP: PAS_{LTP} resulted in a decrease of the

late BP negativity, only at central scalp electrodes (CZA) and electrodes over the sensorimotor cortex contralateral to the movement (C1, C1P), and only for the APB task (Table I, Figs. 3 and 4). A meaningful interpretation of this finding should take into account that PAS_{LTP} increases corticomotoneuronal excitability in M1, as reflected by increased MEP amplitude (see findings of this study, and for review [Ziemann et al., 2008]), and that a corticomotoneuronal excitability increasing 5 Hz rTMS protocol decreases movement-related effective connectivity of PMd with M1 [Rounis et al., 2005]. In other words, output neurons in M1 became less responsive to volitional motor input from PMd. Volitional motor cortical activation engages the same or at least a significantly overlapping corticomotoneuronal system as TMS [Bawa and Lemon, 1993; Chen and Hallett, 1999; Di Lazzaro et al., 1998]. Therefore, it can be assumed that preparatory volitional motor cortical activity, if of similar magnitude before and after PAS_{LTP}, would result in an increase in the late BP negativity after PAS_{LTP} because an increased number of the more excitable M1 output neurons would become activated by the volitional motor command. In contrast, if PAS_{LTP} decreases movement-related effective connectivity between PMd and M1 to a similar extent as 5 Hz rTMS, it can be assumed that PAS_{LTP} results in a decrease of the late BP negativity because less M1 output neurons will respond to the volitional motor command. The PAS_{LTP} induced change in the late BP negativity correlated inversely with the change in MEP amplitude, with a large positive Y-intercept, indicating strong decrease of BP negativity with zero change in MEP amplitude (see Fig. 5). While it is clearly speculative at this stage how to best explain this relation, it should reflect a net result caused by a superimposition of the single effects on late BP amplitude. One possibility is that decreased movement-related effective connectivity between PMd and M1 is the dominating effect (already in the absence of MEP amplitude change) and increased M1 excitability comes into play only with increasing MEP amplitude. Two previous studies support the PAS_{LTP} induced reduction in MRCP negativity: One recent near-infrared spectroscopy demonstrated that PAS_{LTP} decreased the movement-related oxygenated hemoglobin response in M1 (T. Murakami, personal communication), and a PET study showed that 5 Hz rTMS also decreased movement-related regional cerebral blood flow in M1 [Rounis et al., 2005]. The present findings seem to differ from those of one other study, which reported an increase in contingent negative variation amplitude after 5 Hz rTMS [Holler et al., 2006]. However, these data cannot be directly compared to the present study as movements were made in a precued Go/No-Go reaction time task rather than self-paced. Finally, it is known that the BP is also generated in subcortical structures such as the basal ganglia [Rektor, 2003; Rektor et al., 2001] and the ventrolateral thalamus [Paradiso et al., 2004]. As TMS plasticity protocols applied to M1 can affect neuronal activity in these subcortical structures [Strafella et al., 2003], it is pos-

sible that the observed PAS_{LTP}-induced decrease of the late BP negativity was mediated, at least in part, by actions at these subcortical sites.

Extracellular single cell recording experiments in monkeys showed that facilitatory input from premotor cortices to M1 is controlled by the level of inhibition in M1 and can be muted or strongly reduced by local administration of the GABAA receptor agonist muscimol into M1 [Shimazu et al., 2004]. Therefore, one could speculate that enhanced excitability of inhibitory interneurons in M1 after PAS_{LTP} has contributed to the reduction of the late BP. This, however, is unlikely because PAS_{LTP} does not alter short-interval intracortical inhibition (SICI) [Rosenkranz and Rothwell, 2006; Stefan et al., 2002], an accepted measure of GABAergic inhibition in M1 [Di Lazzaro et al., 2000; Ilic et al., 2002; Müller-Dahlhaus et al., 2008a; Paulus et al., 2008; Ziemann et al., 1996].

The peak amplitude of the voluntary EMG burst was slightly larger post-PAS, irrespective of PAS protocol and task (see Fig. 3). Likely, this reflects an unspecific practice effect. This does not impinge on the interpretation of the PAS_{LTP} induced decrease of the late BP negativity because stronger volitional motor output increases rather than decreases the BP negativity [Slobounov et al., 2004]. Therefore, if anything, the PAS_{LTP} induced decrease of the late BP has been underestimated.

It is not clear why PAS_{LTD} did not enhance but rather had no effect on the late BP. One possibility is that PAS_{LTD} increased movement-related effective connectivity from SMA and PMd to M1, similar to previous observations after 1 Hz rTMS [Lee et al., 2003; Rounis et al., 2005], and that this increased responsiveness of M1 output neurons to volitional motor input largely matched the PAS_{LTD} induced decrease in the excitability of these neurons.

The present findings are partly different from those of the only other available study on interference of a TMS plasticity protocol (1 Hz rTMS) with MRCP amplitude [Rossi et al., 2000]. Those authors found a generalized, i.e. topographically nonspecific decrease of the BP after real rTMS but not after sham rTMS. It is difficult to compare those data with the present ones because BP analysis had not been segregated into early vs. late BP, and the effect of rTMS on MEP amplitude was not explored. However, there is close similarity of those findings with the present observation that the early BP in the APB task was depressed after PAS, irrespective of PAS protocol and electrode position (Table I). As this effect occurred across PAS protocols and was topographically nonspecific, it can be concluded that it was independent of PAS induced modulation of excitability in M1. This is in line with the generator sources of the early BP bilaterally in the pre-SMA and the SMA proper that do not include M1 [Cui and Deecke, 1999; Shibasaki and Hallett, 2006]. It is therefore possible that the low-frequency rTMS (0.25 Hz in all PAS conditions) per se was responsible for the early BP depression, while a simple order effect is unlikely because the BP

remained unchanged after sham rTMS in the previous study [Rossi et al., 2000].

CONCLUSIONS

PAS of M1 can modulate volitional motor cortical processes in the late stages of movement preparation as indexed by changes in the late BP negativity. This effect is specific as it occurred only in the PAS_{LTP} protocol (not in the PAS_{LTD} and PAS_{control} protocols), only in the APB task (not in the ECR task not targeted by PAS), only for the late BP (not the early BP) and only at electrode sites over or close to M1 contralateral to the hand movement. Sculpturing of MRCP by means of TMS plasticity protocols may be a potential future therapeutic application in neurological disorders with abnormal MRCPs, such as dystonia, Parkinson's disease, or stroke [Shibasaki and Hallett, 2006].

ACKNOWLEDGMENTS

The authors thank Mr. Robert Liao for his assistance in manufacturing the LED device used in this study.

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