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Modulation of excitability in human primary somatosensory and motor cortex by paired associative stimulation targeting the primary somatosensory cortex

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Abstract

Input from primary somatosensory cortex (S1) to primary motor cortex (M1) is important for high-level motor performance, motor skill learning and motor recovery after brain lesion. This study tested the effects of manipulating S1 excitability with paired associative transcranial stimulation (S1-PAS) on M1 excitability. Given the important role of S1 in sensorimotor integration, we hypothesized that changes in S1 excitability would be directly paralleled by changes in M1 excitability. We applied two established protocols (S1-PAS_{LTP} and S1-PAS_{LTD}) to the left S1 to induce long-term potentiation (LTP)-like or long-term depression (LTD)-like plasticity. S1 excitability was assessed by the early cortical components (N20–P25) of the median nerve somatosensory-evoked potential. M1 excitability was assessed by motor-evoked potential amplitude and short-interval intracortical inhibition. Effects of S1-PAS_{LTP} were compared with those of a PAS_{LTP} protocol targeting the left M1 (M1-PAS_{LTP}). S1-PAS_{LTP} and S1-PAS_{LTD} did not result in significant modifications of S1 or M1 excitability at the group level due to substantial interindividual variability. The individual S1-PAS-induced changes in S1 and M1 excitability showed no correlation. Furthermore, the individual changes in S1 and M1 excitability induced by S1-PAS_{LTP} did not correlate with changes in M1 excitability induced by M1-PAS_{LTP}. This demonstrates that the effects of S1-PAS in S1 are variable across individuals and, within a given individual, unrelated to those induced by S1-PAS or M1-PAS in M1. Potentially, this extends the opportunities of therapeutic PAS applications because M1-PAS 'non-responders' may well respond to S1-PAS.

Introduction

The somatosensory and motor cortices are anatomically and functionally highly connected to enable the successful accomplishment of behavior through sensorimotor integration (Jones *et al.*, 1978; Ghosh *et al.*, 1987; Huerta & Pons, 1990; Porter & Lemon, 1993; Stepniewska *et al.*, 1993). Somatosensory input is essential for accurate motor performance (Pearson, 2000) and for learning new motor skills (Pavlides *et al.*, 1993). Stroke patients with somatosensory deficits compared with those without somatosensory deficits show a delayed recovery of motor function (Reding & Potes, 1988). Functional magnetic resonance imaging studies further support the notion of a specific role of the primary somatosensory cortex (S1) in motor recovery from stroke (Pineiro *et al.*, 2001; Schaechter *et al.*, 2006). Proof-of-principle studies demonstrate that somatosensory afferent input by repetitive peripheral nerve stimulation of the paretic hand enhances the effectiveness of neurorehabilitation in stroke patients (Conforto *et al.*, 2002; Sawaki *et al.*, 2006; Celnik *et al.*, 2007, 2009).

In healthy subjects, non-invasive brain stimulation protocols such as repetitive transcranial magnetic stimulation, theta burst stimulation, paired associative stimulation (PAS) and transcranial direct current stimulation can induce long-term potentiation (LTP)-like and long-term depression (LTD)-like changes of the stimulated cortical neuronal network (Cooke & Bliss, 2006; Ziemann *et al.*, 2008; Müller-Dahlhaus *et al.*, 2010). Stimulation of S1 may result in local changes in S1 excitability as measured by somatosensory-evoked potential (SEP) amplitude (Wolters *et al.*, 2005) but these changes are weak and inconsistent between studies (Litvak *et al.*, 2007; Bliem *et al.*, 2008; Murakami *et al.*, 2008; Pellicciari *et al.*, 2009; Tamura *et al.*, 2009). Furthermore, stimulation of S1 can also lead to behavioral changes in tactile perception ability (Knecht *et al.*, 2003; Tegenthoff *et al.*, 2005; Bliem *et al.*, 2008).

Much less is known about the effects of S1 stimulation on excitability of the adjacent M1 although this would be a primary aim when following up on the behavioral and clinical evidence on the importance of sensorimotor integration for motor skill learning and motor recovery after brain lesions. To our knowledge, only two

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studies have addressed this question: continuous theta-burst stimulation of S1 (Ishikawa *et al.*, 2007; Katayama *et al.*, 2010) and intermittent theta-burst stimulation of S1 (Katayama *et al.*, 2010), although producing significant local changes in the excitability of the stimulated S1, leave M1 excitability unaltered as indexed by unchanged motor-evoked potential (MEP) amplitudes.

Here we applied two S1-PAS protocols to induce bidirectional LTP/D-like plasticity in S1 of healthy subjects (Wolters *et al.*, 2005) and evaluated concurrent changes in the excitability of the adjacent M1 by measuring MEP amplitude and short-interval intracortical inhibition (SICI), indexes of corticospinal excitability and GABA-Aergic motor cortical inhibition, respectively. Given the tight anatomical connections between S1 and M1, our hypothesis was that LTP/D-like plasticity in S1 would be associated with similar plasticity in M1.

Materials and methods

Subjects

Eleven healthy, right-handed subjects participated in the study [mean age, 24 ± 0.75 (SEM) years, five women]. Subjects fulfilled all inclusion and exclusion criteria of a transcranial magnetic stimulation safety checklist (Keel *et al.*, 2001). No subject had a history of neurological or psychiatric disease. Right-handedness was verified by Edinburgh handedness questionnaire (Oldfield, 1971) with a mean laterality score of 0.82 ± 0.05. Only non-smokers were included because chronic nicotine intake alters motor cortical excitability (Lang *et al.*, 2008) and PAS-induced plasticity (Thirugnanasambandam *et al.*, 2011). Measurements were performed in the afternoon to maximize neuroplasticity effects of PAS (Sale *et al.*, 2007). To avoid possible effects of menstrual cycle on cortical excitability or plasticity (Smith *et al.*, 2002; Inghilleri *et al.*, 2004), female subjects were included only if they took a hormonal method of contraception. Written informed consent was obtained from all subjects prior to their participation in the study. The study conformed to the latest version of the Declaration of Helsinki and was approved by the ethics committee of the hospital of the Goethe-University of Frankfurt.

General study design

The study was performed in a pseudorandomized controlled crossover design. Sessions in a given participant were separated by at least 4 days to avoid carryover effects. In each session, the early components (N20–P25) of median nerve somatosensory-evoked potentials (MN-SEP), an index of S1 excitability, motor-evoked potential input–output (IO-MEP) curves, an index of corticospinal excitability (Hallett, 2007), and short-interval intracortical inhibition input–output (IO-SICI) curves, an index of motor cortical GABAA receptor-dependent inhibition (Ziemann *et al.*, 1996; Peurala *et al.*, 2008), were determined immediately before (*pre*), immediately after (*post1*) and 30 min after the PAS intervention (*post2*).

In the main experiment, all 11 subjects participated in five experimental sessions (Table 1). According to previous studies (Wolters *et al.*, 2005; Litvak *et al.*, 2007; Bliem *et al.*, 2008) the PAS protocols were set up to induce LTP- or LTD-like plasticity in S1 (conditions A and B) or to induce no change in excitability of S1 (control, condition C). In addition, two PAS protocols targeted M1 (conditions D and E in Table 1) with adjusted transcranial magnetic stimulation (TMS) intensity but otherwise the same settings as conditions A and B to control for current spread from S1 to M1 (see below for details).

TABLE 1. Experimental conditions

Condition	PAS interval	Site of TMS	Expected effect	Measures
A	N20–2.5 ms	S1	LTP in S1 and M1	SEP, IO-MEP, IO-SICI
A′				IO-SEP
B	N20–15 ms	S1	LTD in S1 and M1	SEP, IO-MEP, IO-SICI
B′				IO-SEP
C	N20 + 20 ms	S1	Control	SEP, IO-MEP, IO-SICI
D	N20–2.5 ms	M1	Control	SEP, IO-MEP, IO-SICI
E	N20–15 ms	M1	Control	SEP, IO-MEP, IO-SICI
F	N20 + 2 ms	M1	LTP in M1	$MEP_{1\ mV}$

After completion of the main experiment, two additional experimental conditions A′ and B′, corresponding to PAS stimulation of conditions A and B, were performed in a subset of five subjects (mean age, 23.4 ± 0.5 years, four women) to obtain input–output curves of the MN-SEP (IO-SEP) at the three time points (*pre* PAS, *post1*, *post2*) to increase sensitivity of detection of subtle PAS effects that might have been missed in the main experiment. IO-SEP was measured by variation in the intensity of electrical MN stimulation (see below for details).

Finally, all subjects were tested with a well-established M1-PAS protocol (Müller-Dahlhaus *et al.*, 2008; Jung & Ziemann, 2009) to induce LTP-like plasticity in M1 (condition F in Table 1) for comparison with S1-PAS-induced LTP-like plasticity in S1 (condition A in Table 1) in the same individuals. Twenty MEPs were recorded at two time points (*pre*, *post1*).

Transcranial magnetic stimulation

Subjects were seated in a comfortable reclining chair with a mounted headrest. Focal TMS was delivered through a figure-of-eight coil (diameter of each wing, 70 mm) connected through a Bistim module to two Magstim 200 magnetic stimulators (Magstim Company, Whitland, Wales, UK) with a monophasic current waveform. The coil was held tangential to the scalp with the handle pointing backwards and 45° away from the midline to induce currents in the brain from posterior-lateral to anterior-medial. The site of left M1 stimulation was determined as the coil position from where TMS slightly above motor threshold elicited consistently the largest MEP amplitudes in a contralateral hand muscle, the right abductor pollicis brevis (APB). This optimal position (M1 'hot spot') was marked with a soft-tipped pen to ensure the same coil position over the whole experiment. Given the slightly oblique orientation of the central sulcus and the close vicinity of M1 and S1 hand representations on both sides of the central sulcus, a position about 2 cm posterior and 1 cm lateral to the M1 hot spot was chosen as the approximate S1 stimulation site. The exact target position of TMS during PAS over the left S1 (conditions A–C in Table 1) was verified and, if necessary, adjusted to the individual brain anatomy using a frameless TMS navigation system (Localite TMS Navigator; Localite GmbH, Sankt Augustin, Germany) (Bliem *et al.*, 2008). The coil orientation was the same as described for M1 stimulation above. The S1 stimulation site was on average 1.52 ± 0.1 cm posterior and 0.78 ± 0.1 cm lateral from the M1 hot spot.

MEP recordings

MEPs were recorded from the right APB by surface electromyography using Ag–AgCl cup electrodes in a belly-tendon montage. The raw

electromyography (EMG) signal was amplified, band-passed filtered (20 Hz–2 kHz; Counterpoint Mk2 electromyograph, Dantec Skovlunde, Denmark), digitized at an analog/digital (A/D) rate of 5 kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK) and stored in a lab computer. Customized software (SPIKE2 for Windows, version 3.05; Cambridge Electronic Design) was used for visual online display and offline analysis.

Experimental procedures

The resting motor threshold (RMT) was determined to the nearest 1% of maximum stimulator output as the minimum intensity eliciting an MEP > 0.05 mV in at least five of 10 consecutive trials. In addition, $MEP_{1\ mV}$ was determined as the intensity inducing peak-to-peak amplitude of approximately 1 mV in the resting APB. The active motor threshold (AMT) was determined during approximately 10% of the maximum isometric contraction of the APB (monitored by audio-visual feedback of the EMG signal) as the lowest intensity to elicit an MEP of at least 0.1 mV in the curve average of five consecutive trials. In sessions D and E (Table 1), RMT was also determined with the stimulation coil placed over S1 (S1-RMT).

IO-MEP reflects corticospinal excitability over a significant range of stimulus intensities (Ridding & Rothwell, 1995; Möller *et al.*, 2009). IO-MEP was measured over intensities of 80–120% $MEP_{1\ mV}$ in 10% steps (i.e. five levels of stimulus intensity), and eight trials were recorded at each intensity in randomized order to avoid hysteresis effects (Möller *et al.*, 2009). This intensity range was chosen because it covers the steepest part of the IO-MEP curve (Rosenkranz *et al.*, 2007). Averages of the single-trial peak-to-peak MEP amplitudes were calculated at each of the intensities. In addition, IO-MEP slope was calculated from the steepest part of the IO-MEP curve by taking the linear fit of the values 90–110% $MEP_{1\ mV}$ (Rosenkranz *et al.*, 2007).

IO-SICI was measured with paired-pulse TMS, using a conditioning pulse intensity of 60–90% AMT (four different steps), a test pulse that was adjusted to elicit $MEP_{1\ mV}$ when given alone, and an interstimulus interval of 2.0 ms to avoid contamination by short-interval intracortical facilitation (Peurala *et al.*, 2008). The four paired-pulse conditions and the test pulse alone condition were each repeated eight times in pseudo-randomized order. SICI at all intensities of the conditioning pulse was expressed as a ratio of the mean conditioned over mean unconditioned MEP (Kujirai *et al.*, 1993). IO-MEP and IO-SICI were measured while the subjects voluntarily relaxed the target muscle. This was monitored by providing online audio-visual feedback of the EMG signal at high amplification (50 μV per division).

MN-SEP recordings

MN-SEPs were recorded while the subjects voluntarily relaxed with eyes closed. The active electroencephalography electrode was placed at C3′, 2 cm posterior to C3 according to the International 10–20 system, corresponding to the putative site of the left S1, while the reference electrode was placed over the frontal midline (Fz). Resistance was < 5 kΩ. The right median nerve was stimulated through a bipolar electrode (cathode proximal) with a constant current square pulse of 0.2 ms duration at rate of 3.2 Hz (Counterpoint Mk2 electromyograph). Stimulus intensity was adjusted before each measurement to 110% of twitching threshold in the thenar muscle. *Pre*-PAS 2 × 300 trials, and at time points *post1* and *post2* 1 × 300 trials with a sweep time from 50 ms before to 100 ms after the stimulus were averaged. The raw SEP signal was amplified, band-passed filtered (2 Hz–2 kHz (Counterpoint Mk2 Electromyograph), digitized (A/D rate 5 kHz, CED Micro 1401) and stored for offline analysis.

In the extra sessions A′ and B′, MN-SEP were recorded in the same way as above but seven different stimulus intensities were applied based on the individual perceptual sensory threshold (ST) and motor threshold (MT), i.e. the minimal intensities inducing a sensory perception beneath the electrode or a twitch in the thenar muscle, respectively. The applied intensities were: ST, MT/2, MT−ST, MT−ST/2, MT, MT + ST/2 and MT + ST. A Digitimer Constant Current Stimulator DS7A (Digitimer Limited, Letchworth Garden City, UK) with constant current square pulse of 0.2 ms duration at a voltage 300 μV and a rate of 4 Hz was used. For all intensities, one block of 1 × 300 trials was recorded. The order of blocks was pseudo-randomized.

Paired associative stimulation

PAS consisted of 225 pairs of electrical stimulation of the median nerve of the right hand and focal TMS stimulation of the left S1 (conditions A–C and A′–B′ in Table 1) or M1 (conditions D–F in Table 1) at a rate of 0.25 Hz (i.e. total PAS duration, 15 min). Electrical stimulation was delivered through a bipolar electrode (cathode proximal) using constant current square wave pulses of 1.0 ms duration. Stimulus intensity was set to elicit a small M-wave of approximately 200 μV in peak-to-peak amplitude in the right APB. The M-wave was monitored and stimulus intensity was adjusted online if necessary to maintain the size of this motor response throughout PAS. The intensity of TMS over S1 (conditions A–C and A′–B′ in Table 1) was set to 120% RMT of the right APB when determined over the M1 hot spot. In contrast, TMS over M1 (conditions D and E in Table 1) was adjusted to a lower intensity to mimic possible TMS effects in M1 when targeting S1 in conditions A–C. The adjustment was performed according to $120\% \times (\text{M1-RMT}^2/\text{S1-RMT})$ (Gerschlager *et al.*, 2001). For instance, if S1-RMT was 50% of maximum stimulator output, and M1-RMT was 40%, then TMS intensity in conditions D and E was set to $120\% \times (40\%^2/50\%) \approx 38\%$ of maximum stimulator output. For comparison, TMS intensity in conditions A–C was then $120 \times 40\% = 48\%$ of maximum stimulator output. In condition F, TMS intensity was set to elicit $MEP_{1\ mV}$. The interstimulus interval between the median nerve stimulus and TMS was always related to the individual N20 latency of the MN-SEP, and the values in the different experimental conditions are given in Table 1. They are in accordance with previous PAS studies (Wolters *et al.*, 2005; Müller *et al.*, 2007; Bliem *et al.*, 2008) to elicit LTP-like (condition A), LTD-like (condition B) or no plasticity (condition C) in S1, or LTP-like plasticity in M1 (condition F).

Because the level of attention directed to the stimulated hand may influence the magnitude of PAS effects (Stefan *et al.*, 2004), subjects were instructed to count the number of flashes which were emitted at random intervals (range, 1–5 s) from an LED mounted on the right wrist.

Data and statistical analysis

All raw data were exported offline with customized software SPIKE2 and further processed and analysed by MATLAB Version 7.2. Statistical analysis was performed using SPSS version 17.0.

For SEP analysis, the signal baseline was determined as the mean value of the period 50–0 ms prior to stimulation. The first negative

peak in an expected time window of 17–23 ms was determined as the N20, followed by the positivity P25. N20–P25 amplitude was computed peak-to-peak. In addition, N20 and P25 amplitudes were calculated as peak differences from pre-stimulus baseline. A two-way repeated-measures analysis of variance (rmANOVA) with the within-subject factors Condition (five levels: A–E) and Time (three levels: *pre*, *post1*, *post2*) was carried out separately for N20–P25, N20 and P25. For evaluation of IO-SEP, a three-way rmANOVA with the additional within-subject factor Intensity (seven levels) was calculated. For IO-MEP and IO-SICI, three-way rmANOVAs with the main within-subjects effects Condition (five levels), Time (three levels) and the Intensity (IO-MEP: five levels; IO-SICI: four levels) were calculated, and for IO-MEP slope a two-way rmANOVA with the effects of Condition and Time as above. In case of significant main effects, *post-hoc* testing was performed with Fisher's protected least significant difference test. Data are reported as means ± SEM.

Linear regression analyses were performed to correlate the individual PAS-induced SEP and MEP changes. All *post*-PAS data were normalized to the *pre*-PAS data. SEP data were entered as N20–P25 amplitudes, while MEP data (conditions A and B, Table 1) were entered as means of 80–120% $MEP_{1\ mV}$ to obtain a single MEP value representative of the whole IO-MEP curve, or as $MEP_{1\ mV}$ (condition F, Table 1). Multiple comparisons were corrected for by the Bonferroni method. Results were considered significant at $P < 0.05$.

Results

S1 excitability changes measured by MN-SEP

The N20–P25 amplitude of the MN-SEP was affected by Time ($F_{2,20} = 3.64$, $P = 0.045$), but not Condition or the interaction Condition*Time (Fig. 1, Table 2). This was explained by a significant increase of N20–P25 amplitude at time point *post1* ($P = 0.001$) and *post2* ($P = 0.003$) when compared with *pre*, while *post1* and *post2* were not different from each other ($P = 0.95$). The effect of Time was no longer significant when tested in the single Conditions (all $P > 0.05$). N20 amplitude and P25 amplitude were not affected by Time or Condition. N20–P25 (Fig. 1, Supporting Information Table S1), N20 and P25 at time point *pre* were not different across Conditions (all $P > 0.05$) and thus pre-PAS differences did not explain the observed findings.

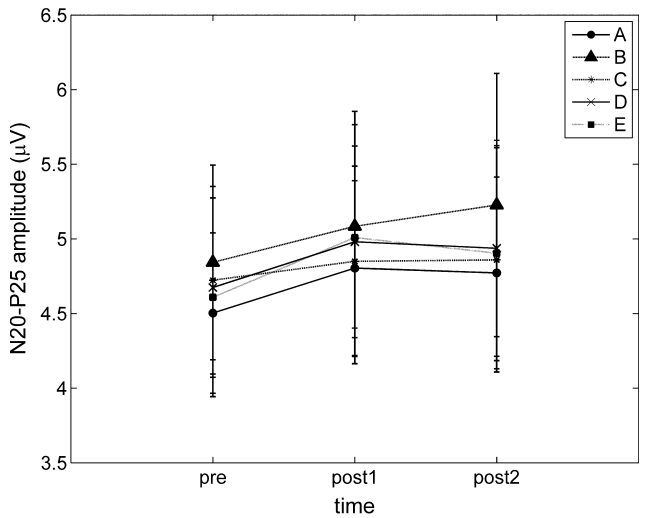

FIG. 1. N20–P25 amplitudes of the MN-SEP at the three time points (*pre, post1, post2*) for the five PAS conditions (A–E, cf. Table 1). All data are means ± SEM from 11 subjects. Note the overall slight increase in N20–P25 post-PAS but absence of differential effects between PAS conditions.

IO-SEP (analysis of N20–P25 amplitudes) depended on Intensity ($F_{6,24} = 11.78$, $P < 0.0001$) (Fig. 2, Tables 2 and S2) but not on Time or Condition, or any of their interactions. Therefore, the lack of a main effect of Condition in sessions A–E cannot be attributed to a ceiling effect because the IO-SEP experiments revealed no interaction of Condition and Intensity, i.e. S1-PAS did also not exert any differential effects in the low-intensity range of the IO-SEP curves (Fig. 2, Supporting Information Table S2). *Pre*-PAS values in conditions A′ and B′ did not differ significantly ($P = 0.15$) and therefore cannot account for the observed lack of PAS effects.

M1 excitability changes measured by IO-MEP

IO-MEP was significantly affected by Time ($F_{2,20} = 8.96$, $P = 0.002$) and Intensity ($F_{4,40} = 50.84$, $P < 0.0001$) and their interaction Time*Intensity ($F_{8,80} = 3.58$, $P = 0.001$) while the main effect of Condition and all other interactions were not significant (Fig. 3, Tables 2 and S3). The effect of Time was explained by a significant increase of IO-MEP *post1* vs. *pre* ($P < 0.001$), *post2* vs. *pre* ($P < 0.001$) and *post2* vs. *post1* ($P = 0.015$). The interaction Time*Intensity was explained by the stronger increase in the high-intensity range of the IO-MEP curve (Fig. 3, Supporting Information Table S3). However, there was no significant main effect of Time or of the interaction Time*Intensity for any of the single conditions (all $P > 0.05$). Finally, IO-MEP slope was not affected by Condition, Time or their interaction (all $P > 0.05$). *Pre*-PAS IO-MEP curves were not different across Conditions ($P = 0.68$) (Fig. 3, Supporting Information Table S3). Therefore, baseline differences cannot explain the observed lack of differential PAS effects on IO-MEP.

M1 excitability changes measured by IO-SICI

IO-SICI depended on Intensity ($F_{3,30} = 34.69$, $P < 0.0001$) but not on Condition or Time or their interactions (Fig. 4, Tables 2 and S4). The *pre*-PAS IO-SICI did not show any difference across Conditions ($P = 0.73$) (Supporting Information Table S4). Therefore, baseline differences cannot explain the observed lack of differential PAS effects on IO-SICI. Furthermore, Supporting Information Table S4 shows that the unconditioned test MEP amplitudes closely matched the target value of $MEP_{1\ mV}$ across all conditions and time points. Accordingly, unconditioned test MEP amplitude was unaffected by Condition, Time or their interaction (all $P > 0.4$), indicating that variation in test MEP amplitude cannot explain the observed lack of different PAS effects on IO-SICI.

Correlation of MN-SEP and MEP changes

Although we failed to demonstrate significant effects of S1-PAS on S1 excitability (measured by N20–P25 amplitude) and M1 excitability (measured by IO-MEP and IO-SICI) at the group level, there was large interindividual variability (Supporting Information Tables S1–S4) that formed an adequate basis for the correlation analyses to address the primary aim of this study, i.e. the effects of individual S1-PAS induced excitability change in S1 on concurrent excitability change in M1.

Changes in N20–P25 amplitude induced by S1-PAS (analysis pooled over conditions A and B, Table 1) did not correlate with changes in MEP amplitude induced in the same S1-PAS protocols, either at time point *post1* ($r = -0.36$, $P = 0.10$) or at time point *post2*

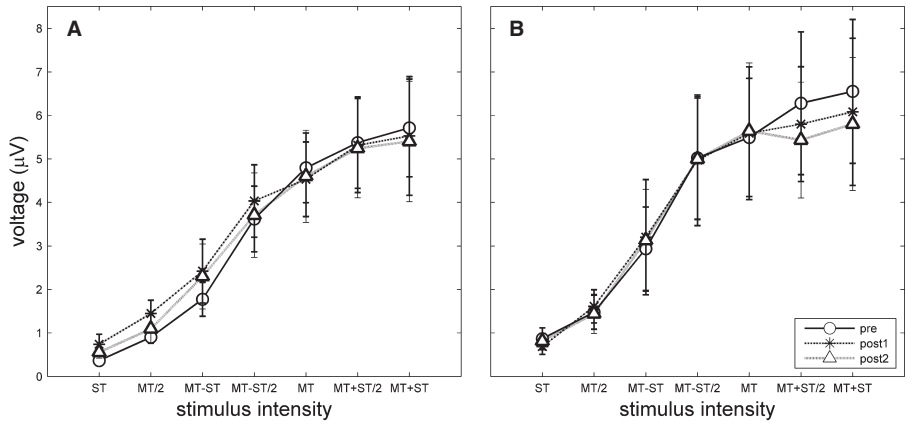

FIG. 2. N20–P25 input–output curves (IO-SEP) at the three time points (*pre*, *post1*, *post2*) separated for the PAS conditions A′ (A) and B′ (B) (cf. Table 1). All data are means ± SEM from five subjects. Note the absence of significant PAS effects on IO-SEP.

TABLE 2. Results of the rmANOVAs (significant effects are highlighted in bold)

	SEP (N20–P25)			IO-SEP			IO-MEP			IO-SICI		
Effects	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Condition	4	0.63	0.65	1	2.89	0.17	4	0.64	0.64	4	0.48	0.75
Time	2	3.64	**0.045**	2	0.25	0.79	2	8.96	**0.002**	2	0.63	0.54
Intensity		–		6	11.78	**0.0001**	4	50.84	**0.0001**	3	34.69	**0.0001**
Condition*Time	8	0.26	0.98	2	0.73	0.51	8	0.35	0.94	8	0.32	0.95
Condition*Intensity		–		6	1.25	0.32	16	0.95	0.51	12	0.69	0.76
Time*Intensity		–		12	1.54	0.15	8	3.58	**0.001**	6	0.94	0.47
Condition*Time*Intensity		–		12	0.71	0.74	32	0.43	0.99	24	0.39	0.99

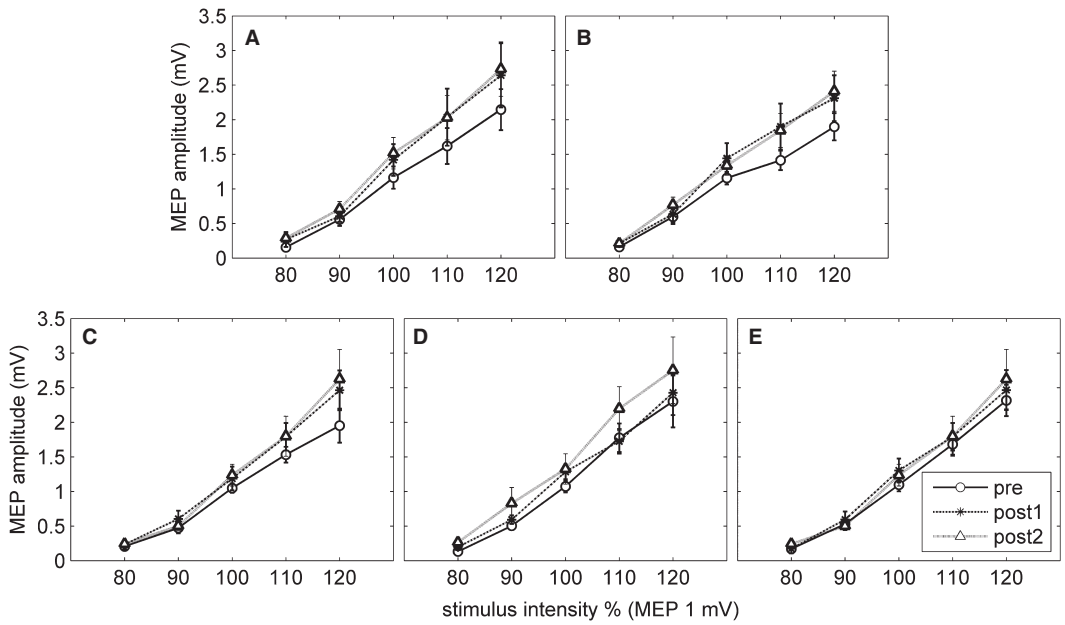

FIG. 3. MEP input–output curves (IO-MEP) at the three time points (*pre*, *post1*, *post2*) separated for the PAS conditions A–E (A–E) (cf. Table 1). All data are means ± SEM from 11 subjects. Note the non-specific MEP increase in all PAS conditions in the high-intensity part of the IO-MEP curve. These effects were weak and no longer significant within the single PAS conditions.

($r = -0.11$, $P = 0.61$) (Fig. 5A). Regression analyses performed separately for condition A and B also did not result in significant correlations (all $P > 0.05$).

The changes in N20–P25 amplitude induced by S1-PAS$_{LTP}$ (condition A, Table 1) did not correlate with the MEP$_{1\ mV}$ changes induced by M1-PAS$_{LTP}$ (condition F, Table 1), either at time point

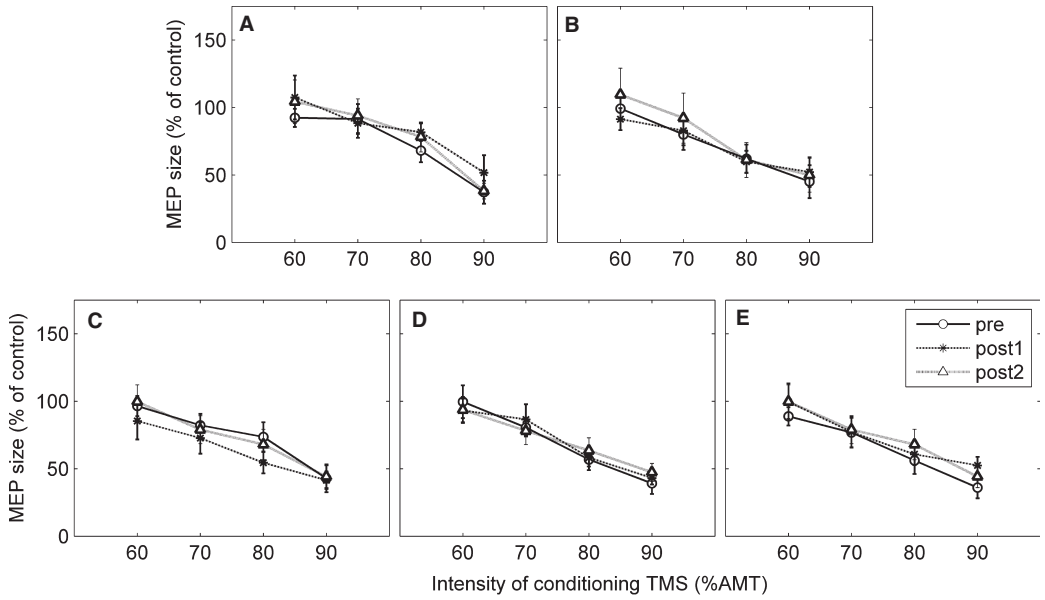

FIG. 4. SICI input–output curves (IO-SICI) at the three time points (*pre*, *post1*, *post2*) separated for the PAS conditions A–E (A–E) (cf. Table 1). All data are means ± SEM from 11 subjects. Note the absence of PAS effects on IO-SICI.

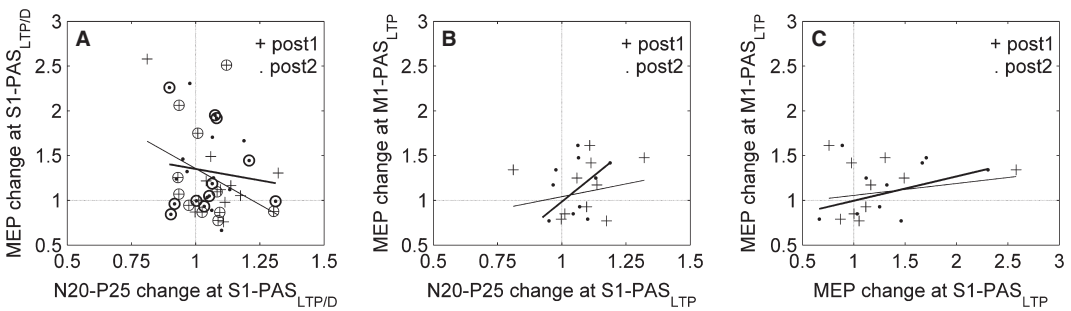

FIG. 5. (A) Correlation plot of individual N20–P25 amplitude changes (*x*-axis) vs. MEP changes (*y*-axis, all data normalized to *pre*-PAS values) induced by S1-PAS [conditions A (symbols without circles) and B (symbols with circles) in Table 1] at time points *post1* and *post 2*. (B) Correlation plot of individual N20–P25 amplitude changes (*x*-axis) induced by S1-PAS$_{LTP}$ (condition A in Table 1) vs. MEP changes (*y*-axis, all data normalized to *pre*-PAS values) induced by M1-PAS$_{LTP}$ (condition F in Table 1) at time points *post1* and *post 2*. (C) Correlation plot of individual MEP change (*x*-axis) induced by S1-PAS$_{LTP}$ (condition A in Table 1) vs. MEP change (*y*-axis, all data normalized to *pre*-PAS values) induced by M1-PAS$_{LTP}$ (condition F in Table 1) at time points *post1* and *post 2*. Regression lines (thin line – *post1*; thick line – *post 2*) are shown in all plots. Dotted vertical and horizontal lines at 1.0 indicate no change. Note that there is substantial interindividual variability of all N20–P25 and MEP changes but no correlation between them.

post1 ($r = 0.19$, $P = 0.58$) or at time point *post2* ($r = 0.49$, $P = 0.12$) (Fig. 5b). The changes in MEP amplitude induced by S1-PAS$_{LTP}$ (condition A, Table 1) did not correlate with the changes in MEP amplitude induced by M1-PAS$_{LTP}$ (condition F, Table 1), either at *post1* ($r = 0.17$, $P = 0.62$) or at *post2* ($r = 0.32$, $P = 0.34$) (Fig. 5C).

Finally, changes in N20–P25 amplitude induced by S1-PAS$_{LTP}$ (condition A, Table 1) vs. changes in N20–P25 amplitude induced by M1-PAS$_{N20–2.5}$ (control condition D), or S1-PAS$_{LTD}$ (condition B) vs. M1-PAS$_{N20–15}$ (control condition E), or pooled analysis of S1-PAS$_{LTP}$ and S1-PAS$_{LTD}$ (conditions A and B) vs. M1-PAS (conditions D and E) did not correlate with each other (all $P > 0.05$). These nil results strongly suggest that the individual SEP changes induced by S1-PAS cannot be attributed to current spread to M1, which then in turn may have caused these SEP changes.

Discussion

The main novel findings of this study are that S1-PAS had no significant effects on M1 excitability at the level of group means and that the individual changes in S1 excitability induced by S1-PAS did not correlate with changes in M1 excitability induced by S1-PAS or M1-PAS. Furthermore, S1-PAS$_{LTP}$ and S1-PAS$_{LTD}$ did not induce significant LTP/D-like change in S1 excitability at the level of group means due to substantial interindividual variability. The single findings are discussed in detail below.

Absence of MEP and SICI changes after S1-PAS

The influence of S1 plasticity-inducing non-invasive brain stimulation protocols on excitability of the anteriorly adjacent M1 has been evaluated in only two studies. In one, continuous theta-burst stimulation of S1 resulted in significant but short-lasting decreases of early cortical components of the MN-SEP amplitude but no changes in MEP amplitude (Ishikawa *et al.*, 2007). In a similar study, continuous theta-burst stimulation of S1 led to no change of N20–P25 amplitude, while intermittent theta-burst stimulation resulted in a long-lasting increase of N20–P25 amplitude, but neither experimental condition was associated with any change in MEP amplitude

(Katayama *et al.*, 2010). Possible reasons for this lack of effect on M1 excitability were not discussed. The present data are in agreement with those previous findings. Furthermore, linear regression analysis revealed no correlation of individual SEP changes with concomitant MEP changes induced by the same S1-PAS protocol (Fig. 5A). The lack of such a correlation may be explained by insufficient stimulus intensity reaching M1 or, more likely, by the fact that the optimal interstimulus intervals for producing LTP/D-like effects in M1 are some 7 ms longer than those in S1 (Wolters *et al.*, 2005). In particular, the interval N20–2.5 ms, which was found to be optimal to produce LTP-like effects in S1 by S1-PAS (Wolters *et al.*, 2005), has little effect on M1 excitability by M1-PAS (Wolters *et al.*, 2003). The absence of significant effects of S1-PAS on SICI may be due to the same reasons. In addition, these data are in accordance with several M1-PAS studies which did not find changes in SICI despite significant LTP/D-like changes in MEP amplitude (Stefan *et al.*, 2002; Rosenkranz & Rothwell, 2006; Cirillo *et al.*, 2009).

Absence of correlation of individual S1 vs. M1 plasticity

To the best of our knowledge, the present study is the first to compare PAS-induced plasticity in S1 vs. M1 within the same individuals. We found that the individual N20–P25 amplitude change induced by S1-PAS_{LTP} does not correlate with the MEP amplitude change induced by M1-PAS_{LTP} (Fig. 5B), although the two PAS protocols were optimized with respect to interstimulus interval for induction of LTP-like effects (S1-PAS: N20–2.5 ms; M1-PAS: N20 + 2 ms). Therefore, a 'PAS responder' in M1 often is not a PAS responder in S1 and *vice versa*. The findings point to the existence of regional specificity of the responsiveness of cortical areas to LTP/D induction, a phenomenon noted earlier in slice preparations of rat neocortex where identical LTP-induction protocols resulted in LTP in S1 but not in M1 (Castro-Alamancos *et al.*, 1995). Therefore, it is highly likely that regional specificity forms another determinant of brain stimulation-induced plasticity, adding complexity to the long list of known factors, such as age, gender, previous activity in the stimulated network or genetic polymorphisms (Ridding & Ziemann, 2010). The present results also show that S1-PAS_{LTP} may induce quite substantial individual changes in MEP amplitude which are also independent of those induced by M1-PAS_{LTP} (Fig. 5C). This individual regional specificity in modifying M1 excitability by either S1-PAS_{LTP} or M1-PAS_{LTP} may extend opportunities in therapeutic PAS applications, for instance after cerebral stroke, where it might be intended to enhance excitability of the ipsilesional M1 to facilitate motor recovery (Ward & Cohen, 2004; Hummel & Cohen, 2006).

Absence of MN-SEP changes after S1-PAS

LTP/D-like changes in N20–P25 amplitude after S1-PAS were first described by Wolters *et al.* (2005). LTP-like increases in N20–P25 occurred at intervals between MN electrical stimulation and TMS of S1 of N20–5, N20–2.5 ms and N20, while an LTD-like decrease of N20–P25 amplitude was observed at the interval N20–20 ms. The effect size was small (approximately 10%) and relatively large numbers of subjects had to be tested to achieve significant results (n = 15–40) (Wolters *et al.*, 2005). The same group of authors were unable to replicate the LTD-like change (Litvak *et al.*, 2007) and several subsequent studies also showed inconsistent results – only one study confirmed the original LTP-like increase (Pellicciari *et al.*, 2009) whereas several others failed to demonstrate significant LTP-like (Bliem *et al.*, 2008; Murakami *et al.*, 2008; Tamura *et al.*, 2009) and LTD-like changes (Bliem *et al.*, 2008; Murakami *et al.*, 2008) with S1-PAS protocols similar or identical to the original protocol of Wolters *et al.* (2005). This survey suggests that the S1-PAS effects on S1 excitability are weak and inconsistent. The present non-significant findings are therefore in accordance with the literature. They are also in agreement with LTP-like plasticity induced in M1 by M1-PAS_{LTP}. It was demonstrated that this effect is highly variable in an unselected population of healthy subjects, with approximately 50% showing MEP increase and the other 50% showing no change or even MEP decrease (Müller-Dahlhaus *et al.*, 2008). Multiple determinants, such as age, gender, menstrual cycle, time of day, attention and genetic polymorphisms, may contribute to this substantial interindividual variability (Ridding & Ziemann, 2010), which was also found in the present experiments for changes in S1 excitability after S1-PAS (cf. Fig. 5A and B). Most of these determinants were controlled for (see Materials and methods) but substantial variability of S1 plasticity remains. In recent M1-PAS experiments, participants were screened and only included if they showed significant M1-PAS induced LTP- or LTD-like plasticity (Ziemann *et al.*, 2004; Heidegger *et al.*, 2010; Korchounov & Ziemann, 2011). Selective inclusion of 'PAS responders' may be important if testing modification of a definitive PAS effect by another (e.g. pharmacological) intervention is the primary aim of the study.

A concern of the present nil findings was that saturation of N20–P25 amplitudes with the conventional MN-SEP recordings may have concealed S1-PAS effects that might have been present in the non-saturated range of N20–P25 amplitudes (Gerber & Meinck, 2000). To address this concern, additional IO-SEP recordings were performed (conditions A′ and B′ in Table 1). The nil findings were confirmed (Fig. 2). Therefore, it can be concluded that N20–P25 amplitude saturation does not explain why S1-PAS did not result in significant LTP/D-like effects.

One limitation of this study is the relatively small number of subjects tested. To demonstrate at the group level and at a type II error of ≤ 10% and a type I error ≤ 2.5% (for a two-sided test) that S1-PAS does not result in S1 excitability change would have required testing of approximately 380 subjects. However, it was not the aim of this study to demonstrate such a nil finding and therefore in this respect the small number of subjects is irrelevant.

It cannot be entirely excluded that a larger number of tested subjects would have resulted in significant correlations of the individual S1-PAS-induced changes in S1 excitability with those in M1 excitability induced by S1-PAS or M1-PAS (Fig. 5). Pooling of the S1-PAS_{LTP} and S1-PAS_{LTD} data (22 tests from 11 subjects, Fig. 5a) did not reveal such a correlation, strongly suggesting that correlations between PAS-induced S1 and M1 plasticity are weak, should they exist.

These findings contrast with one previous M1-$PAS_{LTP/D}$ study which resulted in an increase/decrease of MEP amplitude and, concomitantly, in an increase/decrease of N20–P25 amplitude (Murakami *et al.*, 2008). The reason for this disparity of plasticity in S1 by M1-PAS but not S1-PAS, which was also noted for low-frequency repetitive TMS (Enomoto *et al.*, 2001), is not entirely clear. It may be speculated that stimulation of M1 excites long-range cortico-cortical fibers connecting Brodmann area 3b of S1 with M1 where they are bending from the gray–white matter border into M1 (DeFelipe *et al.*, 1986). This may result in antidromic excitation of the projection neurons in Brodmann area 3b and could contribute to the observed changes in the N20–P25 potential which is generated in the posterior bank of the central sulcus, corresponding to Brodmann area 3b (Allison *et al.*, 1989; Urbano *et al.*, 1997; Huang *et al.*, 2000).

Finally, this study was motivated by previous observations that input from S1 to M1 is important for accurate motor performance

(Pearson, 2000) and for learning new motor skills (Pavlides *et al.*, 1993). The present data do not challenge this view because the behavioral consequences of manipulating S1 excitability by S1-PAS on motor skill performance or motor learning were not explored. This is a relevant topic to be tested in future studies.

In conclusion, our findings demonstrate that S1-PAS effects in S1 are variable between individuals and unrelated to those induced by S1-PAS or M1-PAS in M1 in the same individuals.

Supporting Information

Additional supporting information can be found in the online version of this article:

Table S1. Means (±SEM) of the N20–P25 amplitudes of the MN-SEP prior to and immediately and 30 min after PAS.

Table S2. Means (±SEM) of the N20–P25 amplitudes of the MN-SEP input–output curves prior to and immediately and 30 min after PAS.

Table S3. Means (±SEM) of the MEP amplitude input–output curves prior to and immediately and 30 min after PAS.

Table S4. Means (±SEM) of the unconditioned test MEP amplitudes and SICI input–output curves (expressed as ratios of conditioned/unconditioned MEP amplitudes) prior to and immediately and 30 min after PAS.

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Abbreviations

AMT, active motor threshold; APB, abductor pollicis brevis; EMG, electromyography; IO-MEP, input–output curve of motor-evoked potential; IO-SEP, input–output curve of somatosensory evoked potential; IO-SICI, input–output curve of short-interval intracortical inhibition; LTD, long-term depression; LTP, long-term potentiation; M1, primary motor cortex; M1-$PAS_{LTP,}$ PAS protocol applied over M1 inducing LTP-like plasticity; MEP, motor-evoked potential; $MEP_{1\ mV,}$ motor-evoked potential of amplitude 1 mV; MN-SEP, median nerve somatosensory-evoked potential; MT, motor threshold; PAS, paired associative stimulation; RMT, resting motor threshold; S1, primary somatosensory cortex; S1-$PAS_{LTD,}$ PAS protocol applied over S1 inducing LTD-like plasticity; S1-$PAS_{LTP,}$ PAS protocol applied over S1 inducing LTP-like plasticity; SEP, somatosensory-evoked potential; SICI, short-interval intracortical inhibition; ST, perceptual sensory threshold; TMS, transcranial magnetic stimulation.

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