

Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation

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Abstract Paired associative stimulation (PAS) can increase motor cortical excitability, possibly by long-term potentiation (LTP)-like mechanisms. As the capability of the cortex for plasticity decreases with age, we were interested here in testing interindividual variability and age-dependency of the PAS effect. Motor-evoked potentials (MEPs) were recorded from the resting right *abductor pollicis brevis* muscle before and for 30 min after PAS in 27 healthy subjects (22–71 years of age). PAS consisted of 225 pairs (rate, 0.25 Hz) of right median nerve stimulation followed at an interval equaling the individual N20-latency of the median nerve somatosensory-evoked cortical potential plus 2 ms by transcranial magnetic stimulation of the hand area of left primary motor cortex (PAS_{N20+2}). The PAS_{N20+2}-induced changes in MEP amplitude (ratio post PAS/pre PAS) were highly variable (1.00 ± 0.07 , range 0.36–1.68). Fourteen subjects showed the expected LTP-like MEP increase (responders) while 13 subjects showed a long-term depression (LTD)-like MEP decrease (non-responders). Responders had a significantly lower resting motor threshold (RMT) and minimum stimulus intensity to elicit MEPs of 1 mV (MEP_{1 mV}) than non-responders. RMT

and MEP_{1 mV} correlated significantly negatively with the PAS_{N20+2} effect. The absolute PAS_{N20+2} effect size irrespective of its direction decreased with age ($r = -0.57$, $P = 0.002$), i.e., LTP-like and LTD-like plasticity were large in young subjects but substantially smaller in elderly subjects. In conclusion, measures of motor cortical excitability (RMT, MEP_{1 mV}) and age determine direction and magnitude of PAS effects in individual subjects.

Keywords Age · Motor cortex · Paired associative stimulation · Plasticity · Transcranial magnetic stimulation

Introduction

Paired associative stimulation (PAS) is a now widely used protocol to non-invasively induce lasting changes in the excitability of the human motor cortex. First described in 2000 (Stefan et al. 2000), it employs repetitive pairing of a peripheral electrical stimulus (most often applied to the median nerve), which precedes a single pulse of transcranial magnetic stimulation (TMS) applied to the hand area of the contralateral primary motor cortex (M1) by a distinct interstimulus interval. Shaped after models of associative plasticity in animal neocortical slices (Markram et al. 1997), PAS induces a rapidly evolving (<30 min), long-lasting (>60 min), yet reversible, and input-specific increase in corticomotor excitability when the interval between the two associative stimuli is timed to generate near-synchronous events in the motor cortex (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004; Morgante et al. 2006; Weise et al. 2006; Meunier et al. 2007; Muller et al. 2007). The site of this action has been located at a cortical level (Stefan et al. 2002; Wolters et al. 2003), but recently it was shown that spinal mechanisms might also be

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involved (Meunier et al. 2007). The facilitatory effect of PAS on corticomotor excitability could be blocked by administration of an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Stefan et al. 2002). Given these properties, it was proposed (Stefan et al. 2002) that PAS relies on mechanisms similar to long-term potentiation (LTP) as studied at a cellular level (Bliss and Collingridge 1993; Cooke and Bliss 2006). A possible neural substrate for the effects induced by PAS may be LTP of horizontal cortico-cortical connections within M1 (Rioutl-Pedotti et al. 1998; Rioutl-Pedotti et al. 2000).

Several PAS studies from different groups are now reported in the literature (Wolters et al. 2003; Stefan et al. 2004; Ziemann et al. 2004; Stinear and Hornby 2005; Fratello et al. 2006; Morgante et al. 2006; Quartarone et al. 2006; Rosenkranz and Rothwell 2006; Ueki et al. 2006; Meunier et al. 2007; Muller et al. 2007; Sale et al. 2007). It emerges from these studies that PAS-induced changes in corticomotor excitability seem to be quite variable across subjects. Screening data from the work of Stefan and colleagues (2004) suggested that only ~75% of naïve subjects show an LTP-like increase in motor-evoked potential (MEP) amplitude after PAS. Similar results were reported for effects induced by a PAS protocol on lower limb muscles (Fig. 3a in Stinear and Hornby 2005). In addition, Fratello and colleagues (2006) showed low *intraindividual* reproducibility of PAS effects across sessions with some subjects showing a PAS-induced decrease rather than increase in MEP amplitude in single sessions (Fig. 2 in Fratello et al. 2006). Although several studies have now reported high levels of interindividual variability (Stefan et al. 2000; Quartarone et al. 2003; Stinear and Hornby 2005; Bagnato et al. 2006), individual data were most often not provided.

There is considerable evidence for age-dependency of other forms of plastic changes within human motor cortex (Sawaki et al. 2003; Ward et al. 2007). Practice-dependent plasticity declines as a function of age with subjects older than ~50 years experiencing only minor gains (Sawaki et al. 2003). Moreover, this age-related decline in motor performance may be due to a reduced ability to *modulate* activity appropriately (i.e., task-specifically) in motor networks (Ward et al. 2007). Furthermore, normal aging is associated with a relative decrease in the excitability of inhibitory circuits within M1 (Peinemann et al. 2001), which may have a role in gating stimulation-induced motor cortical plasticity (Ziemann et al. 1998).

In the present study, we specifically addressed interindividual variability and age-dependency of PAS-induced effects on MEP amplitude in a small hand muscle. Given previous evidence for an age-dependency of other forms of motor cortical plasticity (Sawaki et al. 2003; Ward et al. 2007), we hypothesized that PAS-induced effects on MEP amplitude decline with age.

Methods

Subjects

Twenty-seven healthy subjects aged 22–71 years, participated in this study (16 females; mean age, 38.2 ± 3.1 years; median, 31 years). All subjects were consistent right-handers according to the Edinburgh Handedness Inventory (Oldfield 1971). No subject had a history of neurological disease or was on CNS-active drugs at the time of the experiments. All subjects completed the adult safety screen questionnaire (Keel et al. 2001) and written informed consent was obtained prior to participation. The experiments conformed to the Declaration of Helsinki and were approved by the ethics committee of Johann Wolfgang Goethe University Hospital, Frankfurt am Main, Germany.

Electromyography

Subjects were seated comfortably in a reclining chair with their eyes open. Motor-evoked potentials (MEPs) were recorded from the right *abductor pollicis brevis* (APB) muscle by surface electromyography (EMG), using 1-cm Ag–AgCl cup electrodes in a belly-tendon montage. The EMG raw signal was amplified and band-pass filtered (20 Hz to 2 kHz; Counterpoint Mk2 electromyograph; Dantec, Skovlunde, Denmark), digitized at an A/D rate of 5 kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK) and stored in a laboratory computer for online visual display and later offline-analysis using customized data collection and conditional averaging software (Spike 2 for Windows, Version 3.05, CED).

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) was delivered through a figure-of-eight coil (diameter of each wing, 70 mm) connected to a Magstim 200 magnetic stimulator (Magstim Co., Carmarthenshire, 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 activate the corticospinal system preferentially transsynaptically via horizontal cortico-cortical connections (Di Lazzaro et al. 2004). The optimal coil position over the hand area of the left primary motor cortex (M1) for eliciting MEPs in the right APB ('hot spot') was determined as the site where TMS at a slightly suprathreshold intensity consistently produced the largest MEPs. This site was marked with a soft-tipped pen in order to assure a constant placement of the coil throughout the session. Resting motor threshold (RMT) was defined to the nearest 1% of maximum stimulator output (MSO) as the lowest stimulus intensity which elicited MEPs > 50 μ V in at least five out of ten consecutive trials.

Paired associative stimulation

Paired associative stimulation (PAS) was applied according to a protocol previously used by our group inducing LTP-like increases in MEP amplitude (Ziemann et al. 2004; McDonnell et al. 2007; Muller et al. 2007). It consisted of 225 pairs of electrical stimulation of the right median nerve at the level of the wrist and a single TMS pulse over the hot spot of the APB motor representation of the left M1 (see above) at a rate of 0.25 Hz. 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 MEPs of, on average, 1 mV peak-to-peak amplitude ($MEP_{1\text{ mV}}$) in the resting APB when given without the preceding median nerve stimulus. The interstimulus interval (ISI) between median nerve and M1 stimulation equaled the individual N20-latency of the median nerve somatosensory-evoked cortical potential plus 2 ms (PAS_{N20+2}). In order to maintain a standardized level of attention during the PAS intervention, subjects were instructed to stay alert, voluntarily relax the right APB and count the number of median nerve stimuli.

Time line of experiments

The time points of MEP measurements are shown in Fig. 1 (B_0, B_1 – B_6). At time point B_0 , the TMS intensity was adjusted to $MEP_{1\text{ mV}}$. After 15 min of PAS_{N20+2} (see above) MEPs were recorded at time points B_1 – B_6 using exactly the same stimulus intensity as at baseline. At each time point (B_0, B_1 – B_6), 20 MEPs were obtained in the resting right APB muscle at a rate of 0.1 Hz with a random intertrial interval variation of 25% to limit anticipation of the next trial. Complete voluntary relaxation of the APB muscle was monitored audio-visually with high-gain EMG (50 $\mu\text{V}/\text{division}$). Trials contaminated with voluntary EMG activity were discarded from the analysis. MEP measurements at

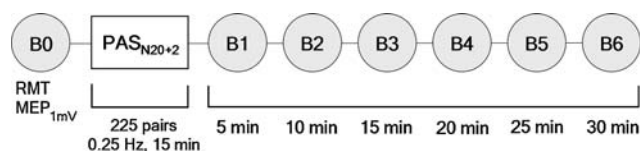


Fig. 1 Time line of experiments. B_0 and B_1 – B_6 denote time points of motor-evoked potential (MEP) measurements recorded in the resting right *abductor pollicis brevis* muscle. At time point B_0 , transcranial magnetic stimulation (TMS) intensity was adjusted to produce MEPs of, on average, 1 mV peak-to-peak amplitude ($MEP_{1\text{ mV}}$). Paired associative stimulation (PAS) was applied at an interstimulus interval of the individual N20-latency of the median nerve somatosensory-evoked cortical potential plus 2 ms (PAS_{N20+2}). Measurements of MEP amplitudes at time points B_1 – B_6 , using the same TMS intensity as at time point B_0 , covered a period of 30 min after the end of PAS_{N20+2} . RMT, resting motor threshold

time points B_1 – B_6 were conducted every 5 min; thus, measurements at time points B_1 – B_6 covered a period of 30 min after PAS_{N20+2} .

Data analysis and statistics

For each subject and time point, the single-trial peak-to-peak MEP amplitudes were averaged and normalized to the MEP amplitude measured at baseline (time point B_0). PAS-induced plasticity was assessed by the grand average of normalized MEP amplitudes measured at time points B_1 – B_6 . Values > 1.0 indicate responders to PAS_{N20+2} , whereas values ≤ 1.0 indicate non-responders to PAS_{N20+2} .

To test for the effects of PAS_{N20+2} we conducted a repeated measures analysis of variance (rmANOVA) with the within-subjects factor *TIME* ($B_0, B_1, B_2, B_3, B_4, B_5, B_6$). Sphericity was tested by Mauchly's test.

To quantify the effect size of PAS_{N20+2} -induced changes in MEP amplitudes irrespective of their direction (absolute PAS_{N20+2} effect) we calculated $|1 - \Sigma B_i/6B_0|$ with $i = 1, \dots, 6$, that is the absolute value of 1 minus the grand average of MEPs at time points B_1 – B_6 normalized to the baseline at time point B_0 .

In order to evaluate the age-dependency of PAS_{N20+2} -induced motor cortical plasticity, a linear regression analysis was computed with the absolute PAS_{N20+2} effect as the dependent and age as the independent variable. To evaluate the dependency of the PAS_{N20+2} effect on motor cortical excitability, the same analysis was performed with the PAS_{N20+2} effect as the dependent variable and RMT and $MEP_{1\text{ mV}}$, respectively, as the independent variables.

In all tests, the level of statistical significance was set to $P < 0.05$. All data are expressed as the mean \pm 1 standard error of the mean (SEM).

Results

Interindividual variability

RMANOVA did not show a significant main effect of *TIME* ($F_{6,156} = 0.47, P = 0.83$). This result was due to substantial interindividual variability of the PAS_{N20+2} -induced changes in MEP amplitude (1.00 ± 0.07 , range 0.36–1.68). Fourteen of the 27 subjects (52%) showed the expected increase in MEP amplitude after PAS_{N20+2} (responders), while 13 subjects (48%) showed a decrease (non-responders; see Fig. 2a).

Responders (PAS_{N20+2} effect, 1.26 ± 0.06) had a significantly lower RMT ($39.3 \pm 1.3\%$ MSO) than non-responders (PAS_{N20+2} effect, 0.73 ± 0.06 ; RMT, $47.6 \pm 2.1\%$ MSO; unpaired two-tailed Student's *t* test; $T = 3.45, P = 0.002$). RMT correlated negatively with the PAS_{N20+2}

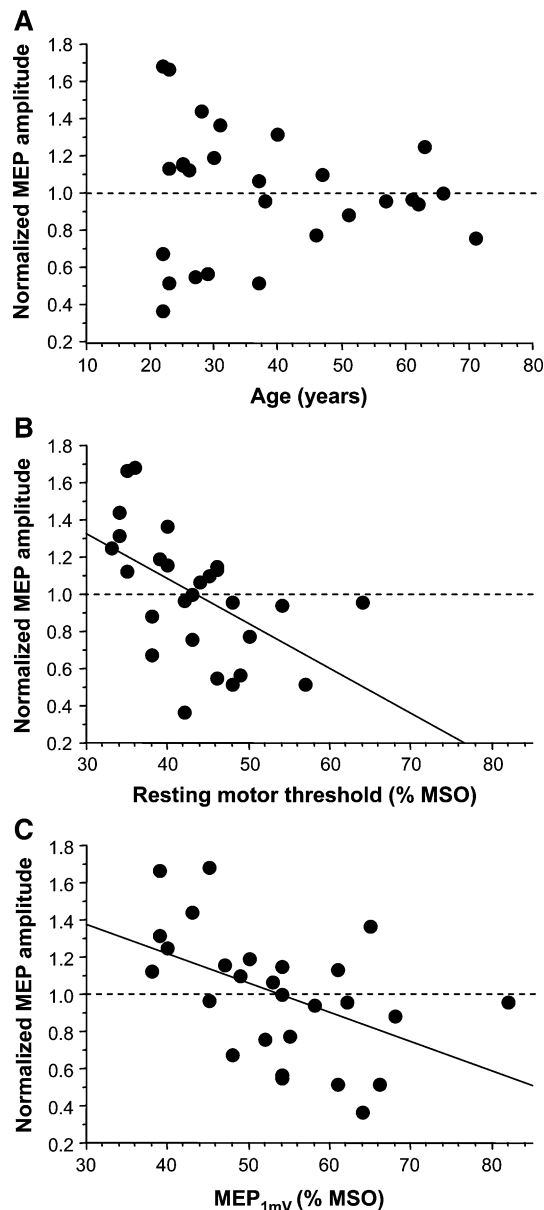


Fig. 2 Interindividual variability of paired associative stimulation (PAS_{N20+2}) effects. **a** PAS_{N20+2} effects were assessed by the grand average of motor evoked potential (MEP) amplitudes at time points B₁ to B₆ normalized to the baseline at time point B₀. Values > 1.0 indicate responders to PAS_{N20+2}, whereas values ≤ 1.0 indicate non-responders to PAS_{N20+2}. PAS_{N20+2} induced motor cortical plasticity showed substantial interindividual variability (PAS_{N20+2} effect 1.00 ± 0.07, range 0.36–1.68) with 14 of 27 subjects (52%) being responders (1.26 ± 0.06), and the remaining 13 subjects (48%) being non-responders to PAS_{N20+2} (0.73 ± 0.06). The PAS_{N20+2} effects from two subjects with the same age (25 years) were virtually identical and, therefore, their data points map to the same spot. Note the decreasing magnitude of PAS_{N20+2} induced MEP changes with age. **b** Resting motor threshold (RMT) was measured at baseline and expressed as percentage of maximum stimulator output (% MSO). RMT correlated negatively with the PAS_{N20+2} effect ($r = -0.53$, $P = 0.005$). **c** MEP_{1 mV} denotes the transcranial magnetic stimulation intensity at baseline adjusted to produce MEPs of, on average, 1 mV peak-to-peak amplitude in the resting right *abductor pollicis brevis* muscle (in % MSO). MEP_{1 mV} correlated negatively with the PAS_{N20+2} effect ($r = -0.48$, $P = 0.01$)

effect, i.e., a low RMT was associated with a strong LTP-like increase in MEP amplitude, whereas a high RMT was associated with a long-term depression (LTD)-like decrease in MEP amplitude ($r = -0.53$, $P = 0.005$; Fig. 2b). Likewise, the stimulus intensity needed to elicit MEP_{1 mV} was significantly lower in responders ($48.4 \pm 2.2\%$ MSO) than in non-responders ($59.2 \pm 2.7\%$ MSO; unpaired two-tailed Student's t test; $T = 3.10$, $P = 0.005$), while these intensities produced MEPs of comparable size in responders (0.93 ± 0.06 mV) and non-responders (1.06 ± 0.1 mV) at baseline (time point B₀; unpaired two-tailed Student's t test; $T = 1.12$, $P > 0.2$). MEP_{1 mV} correlated negatively with the PAS_{N20+2} effect, i.e., a low MEP_{1 mV} was associated with an LTP-like increase in MEP amplitude, whereas a high MEP_{1 mV} was associated with an LTD-like decrease in MEP amplitude ($r = -0.48$, $P = 0.01$; Fig. 2c).

Age-dependency

The magnitude of the absolute PAS_{N20+2} effect, that is the effect size of PAS_{N20+2}-induced changes in MEP amplitude irrespective of their direction, decreased linearly as a function of age ($r = -0.57$, $P = 0.002$; Fig. 3). This indicates that PAS_{N20+2} induced large-size LTP- and LTD-like changes in MEP amplitude in young subjects whereas these effects were substantially decreased in elderly subjects. This result was confirmed by dividing our study sample into two groups (young vs. elderly subjects). The cut-off between groups was either set to the median of age of our study sample (31 years) or to a biologically more meaningful age of 50 years, which according to previous work revealed an age-dependent group effect on other forms of motor cortical plasticity (Sawaki et al. 2003; Floel et al. 2005). The young groups showed a significantly larger absolute PAS_{N20+2} effect than the elderly groups for both cut-off ages (Table 1). Importantly, neither RMT nor MEP_{1 mV} were correlated with age (RMT, $r = 0.19$, $P > 0.3$; MEP_{1 mV}, $r = 0.11$, $P > 0.5$; see also Table 1), and therefore cannot explain the age-related decline in PAS_{N20+2} induced motor cortical plasticity.

Discussion

The results of our study show that there is substantial interindividual variability of the after-effects induced by paired associative stimulation (PAS_{N20+2} protocol) (Ziemann et al. 2004; McDonnell et al. 2007; Muller et al. 2007). Fourteen of the 27 subjects (52%) showed the expected increase in MEP amplitude after PAS_{N20+2} (responders) while thirteen subjects (48%) showed a decrease (non-responders). Responders had a lower resting motor threshold (RMT) and transcranial magnetic stimulation intensity needed to elicit

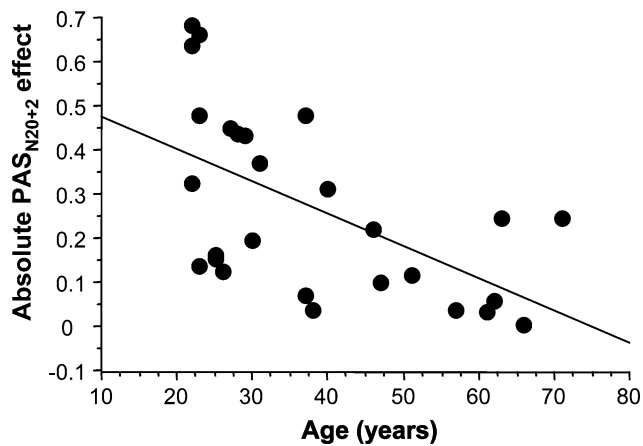


Fig. 3 Age-dependency of paired associative stimulation (PAS_{N20+2}) effects. The absolute PAS_{N20+2} effect was calculated as $|1 - \sum B_i/6B_0|$ with $i = 1, \dots, 6$, that is the absolute value of 1 minus the grand average of MEPs at time points B_1 to B_6 normalized to the baseline at time point B_0 . The magnitude of the absolute PAS_{N20+2} effect decreased as a function of age ($r = -0.57$, $P = 0.002$)

motor-evoked potentials (MEPs) of 1 mV amplitude ($MEP_{1\text{ mV}}$) than non-responders. Both RMT and $MEP_{1\text{ mV}}$ were correlated negatively with the PAS_{N20+2} effect on MEP amplitude. Moreover, findings revealed that the effect size of motor cortical plasticity induced by PAS_{N20+2} decreased linearly with age: while the effect size was large in young subjects, PAS_{N20+2} failed to induce motor cortical plasticity in elderly subjects.

Interindividual variability of PAS effects

Several studies reported considerable interindividual variability to PAS protocols both for upper and lower limb muscles (Stefan et al. 2000; Stinear and Hornby 2005; Bagnato et al. 2006; Fratello et al. 2006). Our findings suggest that only about two-thirds (9/14) of young healthy subjects [mean age, 25.4 ± 0.8 (SEM) years; see Table 1;

Fig. 2a] respond with an LTP-like increase in MEP amplitude to PAS_{N20+2} . The rate of responders to PAS_{N20+2} in our study is in line with prior work by Stefan and colleagues (2004), where 76% of naïve young healthy subjects [mean age, 24.6 ± 3.8 (SD) years] showed an increase in MEP amplitude after PAS in a screening session. Comparable results also came from a study by Fratello and colleagues (2006) where a rate of $\sim 78\%$ of naïve young subjects [mean age, 26.1 ± 0.6 (SEM) years] was reported as responders to PAS (cf. Fig. 2, first session in Fratello et al. 2006). Also, previous work from our group reported the occurrence of LTD-like MEP depression in single subjects after the PAS_{N20+2} protocol (cf. Fig. 2, placebo condition in McDonnell et al. 2007; cf. Fig. 4 in Muller et al. 2007). Furthermore, it emerges that a number of protocol parameters may influence PAS-induced changes in MEP amplitude; among them the frequency, intensity, number and interstimulus interval of the two associative stimuli (Wolters et al. 2003), attention (Stefan et al. 2004), the activation level of the target muscle (Kujirai et al. 2006) and the time of day (Sale et al. 2007).

A high interindividual variability of MEP changes induced by other repetitive TMS protocols (rTMS) was also noted previously: most young subjects respond by an MEP decrease to low-frequency (1 Hz) rTMS and an increase to high-frequency (>5 Hz) rTMS, but a smaller subgroup ($\sim 25\%$ of the subjects) showed the opposite effects, i.e., an MEP increase to low-frequency rTMS and a decrease to high-frequency rTMS (Maeda et al. 2000; Gangitano et al. 2002). Although those authors already speculated on individual anatomical differences in the orientation of sulci and gyri or differences in the orientation of motor cortical interneurons, the reason for the observed substantial interindividual variation in stimulation-induced motor cortical plasticity remained unclear.

There is strong evidence that the neuronal mechanisms underlying PAS involve those of synaptic plasticity (Bliss

Table 1 Measures of motor cortical excitability and plasticity in different age groups

Age group	≤ 31 years	>31 years	≤ 50 years	>50 years
Number of subjects	14	13	20	7
Mean age (years)	$25.4 \pm 0.8^{**}$	$52.0 \pm 3.3^{**}$	$30.1 \pm 1.8^{**}$	$61.6 \pm 2.4^{**}$
PAS_{N20+2} effect	1.04 ± 0.12	0.96 ± 0.06	1.02 ± 0.09	0.97 ± 0.06
Absolute PAS_{N20+2} effect	$0.38 \pm 0.05^{**}$	$0.15 \pm 0.04^{**}$	$0.32 \pm 0.05^*$	$0.11 \pm 0.04^*$
RMT (%MSO)	41.6 ± 1.7	45.1 ± 2.3	42.6 ± 1.4	45.3 ± 3.9
$MEP_{1\text{ mV}}$ (%MSO)	52.0 ± 2.5	55.2 ± 3.2	52.4 ± 2.0	57.0 ± 5.4

Age cut-offs were set as the median of age of the study sample (31 years) and according to previous work on the age-dependency of other forms of motor cortical plasticity (50 years; Sawaki et al. 2003; Floel et al. 2005). Note that paired associative stimulation (PAS_{N20+2}) induced changes in motor-evoked potential (MEP) amplitude irrespective of their direction (absolute PAS_{N20+2} effect) were significantly larger in the younger as compared to the older age group for both age cut-offs. RMT, resting motor threshold. $MEP_{1\text{ mV}}$, transcranial magnetic stimulation intensity to elicit MEPs of, on average, 1 mV peak-to-peak amplitude. MSO, maximum stimulator output

* $P < 0.05$, ** $P < 0.01$, Student's unpaired two-tailed t test. All values are given as mean \pm 1 SEM

and Collingridge 1993; Cooke and Bliss 2006). PAS-induced effects on corticomotor excitability evolve rapidly (<30 min), are long-lasting (>60 min), yet reversible, input-specific (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004; Morgante et al. 2006; Weise et al. 2006; Meunier et al. 2007; Muller et al. 2007) and can be blocked by administration of an NMDA receptor antagonist (Stefan et al. 2002; Wolters et al. 2003). Accordingly, one possible explanation for the observed substantial interindividual variability to PAS may be constituted by genetic polymorphisms of neural signals involved in synaptic plasticity. For example, a single-nucleotide missense polymorphism in the gene coding for the brain-derived neurotrophic factor (BDNF), one of the key neural signals orchestrating synaptic plasticity, is associated with modified experience-dependent motor cortical plasticity (Kleim et al. 2006). It is intriguing to assume that polymorphisms of key neural signals for synaptic plasticity are also associated with the broad variation of PAS-induced motor cortical plasticity among subjects observed in this and previous studies. This idea gains support from our findings that responders to PAS_{N20+2} showed a more excitable motor cortex (lower values for RMT and MEP_{1 mV}) than non-responders, and that these measures correlated negatively with the PAS_{N20+2} effect (Fig. 2b–c). RMT and MEP_{1 mV} may thus reflect part of a genetically determined endophenotype (Wassermann 2002), which is involved in determining magnitude and direction of stimulation-induced motor cortical plasticity in a given individual.

In contrast, there was no difference between the sensory perception threshold of electrical stimulation of the median nerve between responders (0.80 ± 0.07 mA) and non-responders (0.92 ± 0.07 mA; $P > 0.2$), and sensory perception threshold did not correlate with the PAS_{N20+2} effect ($r = -0.30$, $P = 0.13$). These findings point to a network-specific conjunction between motor cortical excitability measures and PAS effects. RMT is thought to reflect excitability of cortico-cortical axons projecting onto corticospinal neurons (Ziemann et al. 1996), and this can be directly linked to the notion that the same cortico-cortical connections serve as the neural substrate of LTP (Rioul-Pedotti et al. 1998, 2000) and PAS-induced LTP-like plasticity (Ziemann et al. 2004) in motor cortex.

Recently, it was shown that musicians regulate motor cortical excitability and plasticity with a higher gain than non-musicians (Rosenkranz et al. 2007). Musicians showed steeper recruitment curves for excitatory corticospinal and intracortical inhibitory projections as compared to non-musicians and their range of PAS-induced changes of MEP amplitudes and corticospinal projection recruitment was wider. As these neurophysiological characteristics partly depended on the age at which instrumental playing was commenced and on practice intensity, the authors hypothesized

that the motor cortical excitability measures may reflect neural adaptations to long-term musical practice such as an increase in the number and modifiability of synapses. Thus, it is well possible that responders as opposed to non-responders to PAS show different anatomical and physiological characteristics, partly as a consequence of endophenotypic traits, and partly reflecting experience-/training-induced neural plasticity. These traits likely allow responders to recruit corticospinal projections and mechanisms of synaptic plasticity more efficiently than non-responders. Musicians then constitute a special and long-term practice-trained group of strong responders.

Fratello and colleagues (2006) reported high *intraindividual* test–retest variability of PAS effects, with some subjects even showing a decrease in MEP amplitude in one and an increase in another PAS session (Fig. 2 in Fratello et al. 2006). Therefore, an alternative explanation to account for the interindividual variability of PAS effects may be homeostatic metaplasticity. This is currently thought to constitute a fundamental stabilizing mechanism to enable both selective modification and maintenance of synaptic strength in a physiological range. The PAS effect on motor cortical excitability critically depends on the recent history of neuronal activity in M1. The LTP-like effect of PAS_{N20+2} could be suppressed or, in some instances, even switched to an LTD-like effect, if it was preconditioned by another PAS_{N20+2} protocol (Muller et al. 2007). However, homeostatic metaplasticity is unlikely to explain the present findings, as only one PAS protocol was performed per subject under standardized conditions, largely excluding systematic variation of motor activity prior to PAS.

Age-dependency of PAS effects

We found that the magnitude of PAS_{N20+2}-induced motor cortical plasticity substantially decreases with age: while the effect size of PAS_{N20+2}-induced motor cortical plasticity was large in young subjects, PAS_{N20+2} failed to induce motor cortical plasticity in elderly subjects (Fig. 3; Table 1). There is considerable evidence for age-dependency of other forms of plastic changes within human motor cortex (Sawaki et al. 2003; Ward et al. 2007). Practice-dependent plasticity declines as a function of age with subjects older than ~50 years experiencing only minor gains (Sawaki et al. 2003). Moreover, this age-related decline in motor performance may be due to a reduced ability to *modulate* activity appropriately (i.e., task-specifically) in motor networks (Ward et al. 2007). Our findings are in line with these reports of an age-related decline in the capacity of the human motor cortex to undergo plastic change. It is important to note that, consistent with previous findings (Wassermann 2002; Pitcher et al. 2003), there was no effect of age on RMT, and therefore variation of motor

cortical excitability per se cannot explain the age-related decline in PAS induced plasticity.

In contrast, other studies showed a preserved LTP-like response to PAS in elderly subjects (Bagnato et al. 2006; Morgante et al. 2006; Ueki et al. 2006). The reasons for this discrepancy are not entirely clear. Differences in the PAS protocols with respect to the frequency, number and inter-stimulus interval of the two associative stimuli may contribute, but further experiments are needed to clarify this.

Several mechanisms may account for the age-related decline of PAS-induced motor cortical plasticity. It is well known that both morphological and biochemical substrates deteriorate with age (Agnati et al. 1990; Hedden and Gabrieli 2004). Neurotransmitters, which play an important role in supporting synaptic plasticity, in particular acetylcholine, dopamine and norepinephrine, show declining concentrations in the brain in the course of normal aging. Specifically, dopamine, which constitutes one of the major heterosynaptic modulators of synaptic plasticity (Jay 2003), has been shown to critically influence encoding of a new motor memory (Floel et al. 2005). In subjects older than ~50 years of age, substantially diminished motor memory encoding was found, but upregulation of dopaminergic activity restored the encoding ability in elderly subjects to levels similar to those seen in healthy young subjects (Floel et al. 2005). Likewise, acetylcholine and norepinephrine facilitate practice-dependent plasticity in the human motor cortex (Butefisch et al. 2002; Sawaki et al. 2002; Meintzschel and Ziemann 2006). As the mechanisms of practice-dependent synaptic plasticity and PAS-induced motor cortical plasticity interact closely (Ziemann et al. 2004; Stefan et al. 2006), it is well conceivable that degeneration of cholinergic, adrenergic and/or dopaminergic neuronal circuits play an important role in the age-related decline of PAS-induced motor cortical plasticity. Furthermore, neurotrophic factors involved in synaptic plasticity such as BDNF and its receptor tyrosine kinase B, which decrease with age in the human cortex (Romanczyk et al. 2002; Webster et al. 2006), may contribute to the age-related decline in PAS-induced plasticity. Finally, synaptic connectivity decreases as a function of age in the human M1 (Adams 1987), and the present and recent work suggests that the magnitude of motor cortical plasticity depends on the number and modifiability of synapses in the motor cortex (Rosenkranz et al. 2007).

Normal aging is associated with a *relative* decrease in the excitability of inhibitory circuits within M1 (Peinemann et al. 2001). Short-interval intracortical inhibition in M1 correlated negatively with age, whereas intracortical facilitation was preserved in the elderly (Peinemann et al. 2001). At the spinal level, an age-related reduction in both inhibitory and facilitatory control mechanisms of motor neuron pool recruitment has been suggested in the elderly (Morita et al. 1995; Earles et al. 2001; Kido et al. 2004). Moreover,

in elderly subjects, descending corticospinal volleys have a reduced capacity to recruit or synchronize spinal motor neurons appropriately (Pitcher et al. 2003; Oliviero et al. 2006). The site of action of PAS has been located to a cortical level (Stefan et al. 2002; Wolters et al. 2003), but spinal mechanisms may contribute as well to PAS-induced changes in corticomotor excitability (Meunier et al. 2007). These cortical and spinal age-related functional adaptations in motor control may also significantly contribute to the reduced PAS-induced plasticity in elderly subjects.

The age-related decline in PAS_{N20+2}-induced motor cortical plasticity closely compares to the age-related decline in use-dependent plasticity induced by repetitive thumb movement training (Sawaki et al. 2003). Thus, the question arises as to the relationship between stimulation- and practice-induced plasticity. Both plasticity protocols are thought to rely on mechanisms of synaptic plasticity on a cellular level (Butefisch et al. 2000; Stefan et al. 2002), but recently it has been reported that they may target different sets of circuits in the motor cortex (Rosenkranz and Rothwell 2006). Our results show that age and measures of motor cortical excitability (RMT, MEP_{1 mV}) contribute to predicting the magnitude and direction of PAS_{N20+2}-induced changes in corticomotor excitability in individual subjects. Yet, how the capacity of the human motor cortex to undergo PAS-induced plastic changes links to use-dependent plasticity remains to be established.

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