Modulation of Associative Human Motor Cortical Plasticity by Attention

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Stefan, Katja, Matthias Wycislo, and Joseph Classen. Modulation of associative human motor cortical plasticity by attention. J. Neurophysiol. 92: 66–72, 2004; 10.1152/jn.00383.2003. The role of attention in generating motor memories remains controversial principally because it is difficult to separate the effects of attention from changes in kinematics of motor performance. We attempted to disentangle attention from performance effects by varying attention while plasticity was induced in human primary motor cortex by external stimulation in the absence of voluntary movement. A paired associative stimulation (PAS) protocol was employed consisting of repetitive application of single afferent electric stimuli, delivered to the right median nerve, paired with single-pulse transcranial magnetic stimulation (TMS) over the optimal site for activation of the right abductor pollicis brevis muscle (APB) to generate near-synchronous events in the left primary motor cortex. In experiment 1, the spatial location of attention was varied. PAS failed to induce plasticity when the subject’s attention was directed to their left hand, away from the right target hand the cortical representation of which was being stimulated by PAS. In experiment 2, the grade of attention to the target hand was manipulated. PAS-induced plasticity was maximal when the subject viewed their target hand, and its magnitude was slightly reduced when the subject could only feel their hand. Conversely, plasticity was completely blocked when the subject’s attention was diverted from the target hand by a competing cognitive task. A similar modulation by attention was observed for PAS-induced changes in the duration of the silent period evoked by TMS in voluntarily contracted muscle. Associative plasticity in the human motor cortex depends decisively on attention.

INTRODUCTION

Attention is believed by many to modulate the building of perceptual memories (Crist et al. 2001; Gilbert et al. 2000). In the motor system, the learning of sequences of motor actions is dependent on the availability of attentional resources (Hallett et al. 1996). Facilitation of motor memory formation in M1 with enhanced attention may be exclusively due to superior (kinematic) quality of training movements and be entirely independent of attention. Further, as neuronal plasticity can take place in low-level sensory cortical regions in the absence of attention (Godde et al. 2000, 2002; Watanabe et al. 2001), it is conceivable that plasticity in low-level cortical output areas such as M1 may similarly be induced in the absence of attention. To address these questions, we studied the effect of varying attention in a paradigm generating motor cortical plasticity by external stimulation, in the absence of voluntary activation, thereby avoiding contamination of varying attention by variations of performance.

Low-frequency repetitive median nerve stimulation if paired with TMS over the contralateral motor cortex leads to cortical excitability changes in human primary motor cortex (Stefan et al. 2000). This paradigm was shaped after models of associative long-term potentiation (LTP) in experimental animals. It takes advantage of the fact that transcranial magnetic stimulation (TMS) leads to transsynaptic excitation of cortical output neurons, probably via stimulation of fibers traveling horizontally with respect to the cortical surface (Rothwell 1997), and some of the somatosensory afferent information evoked by median nerve stimulation reaches the primary motor cortex in a highly somatotopically organized fashion (Classen et al. 2000; Rosén and Asanuma 1972), probably via vertically oriented cortico-cortical pathways. Paired associative stimulation (PAS)-induced excitability changes were shown to be generated rapidly, long-lasting, reversible, and topographically specific (Stefan et al. 2000). Of note, the direction of changes was demonstrated to be determined by the exact interval between the afferent and the magnetic pulse during the intervention (Wolters et al. 2003). Furthermore, PAS-induced increases or decreases of cortical excitability depended on N-methyl-D-aspartate (NMDA)-receptor activation (Stefan et al. 2002; Wolters et al. 2003). Considering these properties, we have proposed that the PAS-induced plasticity may be related to associative LTP of cortical synapses in the human cortex (Stefan et al. 2000; Wolters et al. 2003).

METHODS

The study was approved by the ethics committees of the Universities of Wuerzburg and Rostock, and all participants gave their written informed consent. Experiments were performed on 27 healthy volunteers.

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teers (16 women) aged 19–33 yr [24.6 ± 3.8 (SD) yr] in accordance with the Declaration of Helsinki. All volunteers, except three, were right handed.

**Recording**

Electromyographic (EMG) activity was recorded from the right abductor pollicis brevis muscle (APB) using surface electrodes in a belly-tendon montage. Raw signals were amplified employing a Toennies amplifier (Toennies, Freiburg, Germany) or a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) and were band-pass filtered between 20 and 2,000 Hz. EMG signals were digitized at 5 kHz by an A/D converter (model 1401 plus, Cambridge Electronics Design) and stored in a laboratory computer for display and later off-line analysis.

**Stimulation**

Focal TMS of the resting APB muscle was performed using a flat figure-eight-shaped magnetic coil (Magstim 200, The Magstim Company; wing diameter: 70 mm). The coil was held tangentially to the skull with the handle pointing backward and laterally at a 45° angle to the sagittal plane. Electrical mixed nerve stimulation was performed with a Cantata electromyograph (Dantec Medical, Skovlunde, Denmark) or a Digitimer 7AH (Digitimer, Welwyn Garden City, Hertfordshire, UK) using a standard stimulation block (cathode proximal) at a stimulation width of 200 μs.

**Experimental procedures**

Subjects were seated comfortably in a reclining chair. At first the optimal position of the magnetic coil for eliciting motor-evoked potentials (MEP) in the resting right APB was assessed over the left motor cortex at a moderately suprathreshold stimulation intensity (usually ~60% of the maximal stimulator output) and marked directly on the scalp with a soft-tip pen. At the optimal site, the resting motor threshold (RMT) was determined as the stimulus intensity needed to produce a response of ≥50 μV in the relaxed APB in ≥5 of 10 consecutive trials (Rossini et al. 1994). Thereafter, the stimulus intensity (SI) sufficient to evoke a peak-to-peak amplitude of 1 mV of the MEPs in the relaxed APB was determined (experiment 2, see following text, SI_{1 mV}).

After TMS at suprathreshold intensities, a period of electrical silence can be recorded in the contralateral contracted target muscle (Hallett 1995). Although the neurophysiological mechanisms underlying this silent period are not completely understood, evidence suggests that it represents an intracortical phenomenon, at least in its later phase. Each session carried a different grade of attention directed to the specified hand, but attention was not monitored by probing electric stimulation to the thumb (see following text). Thirteen of the 17 screened subjects fulfilled the inclusion criterion that specified that PAS must induce an enhancement of the amplitude of the MEPs recorded from right APB muscle. In the two target experiments, subjects were asked to fixate on a marker centered in front of them and to pay attention to either their right (AttIpsi) or left (AttContra) hand. Vision of the hand was occluded in both conditions. AttIpsi and AttContra sessions were separated by ≥72 h to ensure comparable conditions for probing cortical excitability. Previous studies (Stefan et al. 2000), have shown that PAS-induced changes of excitability return to baseline well before 24 h. Attention was controlled for by testing the ability of the subjects to detect and recall the number of weak electrical stimuli randomly applied to the right (AttIpsi) or left (AttContra) thumb during PAS (see following text). The results of one subject, who fell asleep during the experiment, were excluded from the analysis.

**Intervention**

PAS was performed as described previously (Stefan et al. 2000; Wolters et al. 2003). In brief, the intervention consisted of repetitive applications of single electrical stimuli delivered to the right median nerve at the level of the wrist at 300% of the perceptual threshold and followed each time after 25 ms by TMS over the motor cortex. In experiment 1, 132 pairs [median nerve stimulation plus TMS at 130% of the RMT (SI_{1.3RMT})] were delivered at 0.2 Hz over 11 min. In experiment 2, 90 pairs (median nerve stimulation plus TMS at SI_{1 mV}) were delivered at 0.05 Hz over 30 min. Unpublished results have shown that protocols of experiments 1 and 2 produce qualitatively similar effects on cortical excitability.

**Probing cortical excitability**

Complete muscle relaxation was continuously monitored by visual and auditory feedback. For amplitudes of MEPs of the testing target muscle, 20 trials were collected both before and immediately after PAS using a stimulus intensity of SI_{1 mV} (experiment 1) or SI_{1 mV} (experiment 2) and a stimulation rate of 0.1 Hz. Identical stimulus intensities were used before and after intervention. In experiments investigating the duration of the silent period and the magnitude of MEPs elicited in the active target muscle, 20 additional trials were collected in experiment 2 during voluntary contraction of the APB at 15% of maximal force immediately after testing the resting amplitudes, before and after interventional stimulation. During these probing stimulations, the subjects were instructed to attend to their right hand while fixating on a marker centered on the blank computer screen in front of them.

**Manipulation of grade of attention allocated to the target hand**

**EXPERIMENT 1.** Seventeen subjects were screened in an inclusion experiment. For this experiment, the same stimulation parameters were used for intervention as in the final experiments (0.2 Hz; 11 min; 1.3 times RMT). Subjects were asked to pay attention to the stimulated hand, but attention was not monitored by probing electric stimulation to the thumb (see following text). Thirteen of the 17 screened subjects fulfilled the inclusion criterion that specified that PAS must induce an enhancement of the amplitude of the MEPs recorded from right APB muscle. In the two target experiments, subjects were asked to fixate on a marker centered in front of them and to pay attention to either their right (AttIpsi) or left (AttContra) hand. Vision of the hand was occluded in both conditions. AttIpsi and AttContra sessions were separated by ≥72 h to ensure comparable conditions for probing cortical excitability. Previous studies (Stefan et al. 2000), have shown that PAS-induced changes of excitability return to baseline well before 24 h. Attention was controlled for by testing the ability of the subjects to detect and recall the number of weak electrical stimuli randomly applied to the right (AttIpsi) or left (AttContra) thumb during PAS (see following text). The results of one subject, who fell asleep during the experiment, were excluded from the analysis.

**EXPERIMENT 2.** Ten subjects participated in three sessions each. To avoid carry-over effects, the different sessions were separated by ≥48 h. Each session carried a different grade of attention directed to the right hand during PAS. Subjects were recruited from a large sample of volunteers in whom PAS had been demonstrated previously (Stefan et al. 2000, 2002; Wolters et al. 2003) on multiple occasions to induce enhancement of cortical excitability. In the attention-diverted condition (Att−), subjects were asked to solve continuously concatenated arithmetic tasks presented (program Superlab, Progamma, NL) on a computer screen in front of them. In the second condition, they were instructed to fixate on a marker centered on the blanked computer screen, while paying attention to their right hand (Att+). The principal design of this experiment was similar to the AttIpsi condition in experiment 1. Vision of the hand was occluded both in the first and the second condition. In the third condition (Att++), subjects were to look at their hand throughout PAS and to focus attention on their hand. Sequential arithmetic tasks (condition Att−) required the subject to report intermediate results verbally at eight times during PAS. To maximize the subjects’ attention to solving the arithmetic task, they were financially rewarded according to their performance after the completion of the experiment.
During PAS, a total of seven (experiment 1) or four (experiment 2) weak (2 times perceptual threshold) electric pulses (200 µs, D7AH; or 100 µs, D180, respectively; Digitimer) were randomly delivered to the proximal phalanx of the left (AttContra) or right (AttIpsi, Att+) thumb via ring electrodes. The number of electrical pulses to the thumb was deliberately kept low to minimize the demand on the working memory and to minimize any potential influence of these stimuli on cortical excitability. Care was taken to deliver the electric pulses to the thumb asynchronously with the PAS. After PAS, subjects were asked to report the count of the stimuli they had identified. Subjects were not provided any feedback as to the accuracy of the number reported.

The order of the experimental conditions (experiment 1: AttIpsi, AttContra; experiment 2: Att+, Att+, Att+) was counterbalanced between subjects. There was no overlap between subjects participating in experiments 1 and 2. In experiments 1 and 2, all subjects participated in all experimental conditions (either AttIpsi and AttContra, or Att+ and Att++). Recruiting the same subjects for all sessions of a particular experiment allowed us to employ a repeated-measures ANOVA design for statistical analysis. Attentional performance was defined as the absolute difference between the number of electric pulses directed to the thumb detected by the subject, and the correct number (7 in experiment 1 and 4 in experiment 2).

Data analysis

MEP-amplitudes evoked at rest (“resting amplitudes”) or at voluntary contraction (“active amplitudes”) were measured peak-to-peak in each individual trial. The duration of the silent period was determined as the time from stimulus onset to the time of voluntary EMG-activity reoccurrence (Triggs et al. 1992). For each subject, resting amplitudes, active amplitudes, and silent-period durations were averaged according to the different conditions described in the preceding text and entered into the final statistical analyses. Repeated-measures ANOVAs were employed for statistical analyses. Paired two-tailed t-test were applied for post hoc analyses. All data are given as means ± SE. Effects were considered significant, if $P < 0.05$.

RESULTS

In experiment 1, the spatial location of attention was varied (Fig. 1A). Baseline resting amplitudes, and perceptual thresholds of median nerve stimulation and of electrical stimuli delivered to the thumb were similar in both conditions (Table 1). The mean number of errors made in detecting the electrical stimuli applied to the proximal phalanx of the thumb during PAS was similar in both conditions (AttIpsi: 0.5 ± 0.7; AttContra: 0.7 ± 1.2; n.s.), suggesting a comparable grade of attention directed to the left or right hand, respectively. A repeated-measures ANOVA of the amplitudes of TMS-evoked MEPs revealed a significant interaction between period (“pre intervention,” “post intervention”) and attention-focus (“ipsilateral,” “contralateral”; $F = 6.10; P < 0.05$) while factors period and attention-focus were not significant. Resting amplitudes increased statistically significantly in AttIpsi ($P < 0.02$) but not in AttContra (Fig. 1, B and C). Increase of resting amplitudes was larger with AttIpsi compared with AttContra ($P < 0.05$).

In experiment 2, subjects participated in three sessions, each designed to manipulate the grade of attention allocated to the right target hand during PAS (Fig. 2A). Baseline resting amplitudes, and perceptual thresholds of median nerve stimulation and of electrical stimuli applied to the thumb, were similar across conditions (Table 1). The mean number of errors made in detecting four weakly suprathreshold electrical stimuli applied to the proximal phalanx of the thumb during PAS differed across conditions ($F = 5.05; P < 0.05$, ANOVA). This number was lowest in Att++ (attention and gaze focused on visible right hand) and highest in Att− (attention focused on arithmetic task) (Fig. 2A). Error rate in Att++ was significantly different from Att+ ($P < 0.05$) or Att− ($P < 0.01$); the difference between Att+ and Att− ($P = 0.069$) did not reach significance in the paired two-tailed t-test employed. A repeated-measures ANOVA of amplitudes of MEPs elicited by TMS in the resting APB muscle revealed significant effects of period (pre intervention, post intervention; $F = 13.80; P < 0.01$), attention-grade (−, +, +++; $F = 13.80; P < 0.001$) and of the interaction between period and attention-grade ($F = 23.90; P < 0.001$). After PAS, resting amplitudes increased in Att+ ($P < 0.001$) and Att++ ($P < 0.001$), whereas no increase was present in Att− (n.s.; Figs. 2B and 3B). Pairwise comparisons revealed a significantly larger increase of resting amplitudes with Att++ compared with Att+ ($P < 0.01$) or Att− ($P < 0.001$), and with Att+ compared with Att− ($P < 0.01$). We considered the possibility that the number of errors reflected the attention allocated by the subject to their hand even more comprehensively than the task because the number
of errors presumably was influenced by factors relevant for attention but outside of experimental control (e.g., individual differences in the capability to maintain the level of attention and random fluctuations). All experimental sessions (across all 3 conditions) considered, the number of errors correlated negatively with the normalized amplitude of TMS-evoked MEPs after PAS (Pearson’s correlation coefficient r = -0.478; y = -13.445x + 145.22; P < 0.01; Fig. 3C).

After PAS, active amplitudes measured at voluntary contraction remained unchanged for all experimental sessions (Att−: pre, 7.0 ± 3.6 mV; post, 7.2 ± 3.2 mV; Att+: pre, 8.0 ± 3.2 mV; post, 7.9 ± 2.8 mV; Att++: pre, 7.5 ± 4.3 mV; post, 7.0 ± 3.8 mV; repeated-measures ANOVA, n.s.; data not illustrated).

A repeated-measures ANOVA of the silent-period duration revealed significant effects of period (F = 27.6; P < 0.001); attention-grade (F = 3.6; P < 0.05) and of the interaction between period and attention-grade (F = 24.9; P < 0.01). Silent-period durations increased statistically significantly in Att+ (13 ± 3 ms; P < 0.01) and Att++ (19 ± 3 ms, P < 0.01), whereas no increase was present in Att− (2 ± 2 ms, n.s.; Figs. 2C and 3D). Pairwise comparisons revealed a significantly larger increase of silent-period duration in Att++ compared with Att− (P < 0.001), and in Att+ compared with Att− (P < 0.02); the difference between Att++ and Att+ (P = 0.072) did not quite reach significance in the two-tailed t-test employed. All three conditions considered, the number of errors correlated negatively with the duration increase after PAS (Pearson’s correlation coefficient r = -0.475; y = -3.3377x + 16.518; P < 0.01; data not illustrated).

**DISCUSSION**

The present results have shown that plasticity induced in the human motor cortex by an external stimulation protocol is strongly modulated by attention. The modulation of plastic changes ranged from enhancement to complete blockade identifying attention as a major determinant of plasticity.

We have demonstrated previously (Stefan et al. 2000; Wolters et al. 2003) that, at a constant level of attention comparable to level AttIpsi or Att+ in the present study, amplitudes of TMS-evoked MEPs are only enhanced (and the
durations of the silent periods prolonged) by paired associative stimulation when a narrow timing interval (~20-35 ms) between the two components of the associative stimulation is employed. In reference to the present study, this property of PAS-induced plasticity rules out that plastic changes of cortical excitability may have occurred as a result of attention alone rather than as the result of an interaction between attention and PAS-induced effects.

Classically, a larger influence of attention has been ascribed to higher-order cortical processing stages such as those located in prefrontal or posterior parietal areas. However, there is now strong evidence that neural activity can be affected by changes of attention even at the earliest stages of processing of somatosensory signals (Castro-Alamancos 2002; Garcia-Larrea et al. 1991; Steinmetz et al. 2000) or at a low-level cortical output stage in the primary motor cortex (Baker et al. 1999; Rosler et al. 1999; Buchner and co-workers (Buchner et al. 1999; Rougeul et al. 1979). Buchner and co-workers (Buchner et al. 1999) have shown that attention-related physiological events may influence cortical plasticity changes in primary somatosensory cortex. Our results extend these observations to the motor cortex. Together, these observations suggest that attention-dependent physiological changes even in primary brain regions may be functionally highly relevant as they influence profoundly the dynamic organization of the human brain.

Although we cannot rule out that an increase may have occurred with longer periods of exposure to PAS, perhaps the most surprising finding in the present study is the complete absence of any PAS-induced increase of MEP size in the tasks AttContra and Att-. This observation may be explained by neuroimaging (Corbetta et al. 1990; Rowe et al. 2002) as well as neurophysiological studies (e.g., refs. Classen et al. 1998a; Motter 1993), showing that cortical regions not involved in the processing of an attended task may be deactivated. Deactivation, in turn, may have interfered with neurophysiological processes underlying PAS-induced plasticity. The extra-enhancement in Att++ (attention and vision focused on the target hand), compared with Att+ (attention, but not vision, focused on the target hand), is consistent with cross-modal modulation both of processing of somatosensory signals (Taylor-Clarke et al. 2002) and of motor cortical activity (Baker et al. 1999) by visual information. This finding also shows that attention-dependent modulation of plasticity is not an all-or-none phenomenon but occurs on a continuum.

PAS-induced plasticity obeys a strict temporally asymmetric induction rule (Wolters et al. 2003), similar to spike-timing-dependent synaptic plasticity, and depends on NMDA-receptor activation (Stefan et al. 2002; Wolters et al. 2003). It is modulated by prior motor-skill learning (Wycislo and Classen 2003), similar to LTP in rat motor cortex (Rioult-Pedotti et al. 2000). Based on these and other physiological properties (Stefan et al. 2000, 2002), we have suggested that the plasticity after paired interventional stimulation may represent a human model of LTP (Stefan et al. 2000, 2002; Wolters et al. 2003), the foremost cellular candidate mechanism of memory.

As in previous studies using this (Stefan et al. 2000) or other (Ridding et al. 2000) external stimulation protocols of induction of neuronal plasticity, active amplitudes remained unaltered after intervention. Some authors (Siebner and Rothwell 2003; Touge et al. 2001) have argued that this observation would favor alterations of membrane excitability, rather than synaptic phenomena, as a physiological mechanism underlying the modulation of resting MEP amplitudes. According to these authors, interventional stimulation could have raised the membrane potential subliminally at rest, whereas levels of membrane potential would be normalized with voluntary contraction. However, the absence of a change in resting motor threshold (Stefan et al. 2000; Wolters et al. 2003), and the observation that the duration of the silent period induced in voluntary contracted muscle was modulated in a similar way as the size of the resting MEP amplitude, may provide evidence against this possibility. An alternative explanation for the lack of modulation of active amplitudes by PAS would be that, with voluntary activation, (presynaptic) inhibitory intracortical cir-

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** A: number of errors in detecting electric stimuli delivered to the thumb during PAS. B: change of normalized resting amplitude of TMS-evoked MEPs after PAS. C: correlation between number of errors as surrogate measure of attention to the target hand and magnitude of MEPs. D: change of silent-period duration after PAS. Asterisks indicate statistically significant differences. Data show means ± SE of results from 10 subjects.
cuits will be recruited. Possibly, this could mask changes in synaptic transmission from some excitatory interneurons onto the pyramidal output cells and stabilize motor cortical output. However, in the absence of invasive recordings, any hypothesis about the physiological nature of PAS-induced plasticity must remain speculative.

The influence of attention on memory formation has been examined on a behavioral level of analysis (Gilbert et al. 2000; Nissen and Bullemer 1987), and putative physiological implementations of attention have been shown to influence the formation of LTP [e.g., selective modulation of neuronal firing rate (Ngezahayo et al. 2000), modulation of oscillatory neuronal firing (Fries et al. 2001), and activation of modulatory neurotransmitters such as acetylcholine (Bear and Singer 1986)]. Our study links behavior with cortical physiology and thus appears to provide circumstantial evidence that the capacity of the cortex to express LTP may be modulated by attention.

A recent study (Conner et al. 2003) showed that disruption of basal forebrain cholinergic function impaired motor learning and inhibited cortical map reorganization. Because the basal forebrain cholinergic system is strongly implicated in attention (McGaughy et al. 2002), this finding provides indirect evidence that motor learning and its underlying cortical physiological correlates may be modulated by attention. Our results appear to support this notion. If the mechanisms studied here are involved in motor learning induced by the performance of actual movements, as suggested by experiments in rats (Rioul-Pedotti et al. 2000) and in humans (Wycislo and Classen 2003), the magnitude of movement-induced plasticity in the motor system is likely to be substantially influenced by attentional variation rather than merely by the intensity or the kinematic quality of motor performance.

The present findings offer an explanation for the clinical observation that stroke patients fare worse in the presence of unilateral spatial neglect (Denes et al. 1982). The inability to attend to the impaired body side may diminish the capacity of the brain to generate lasting memory traces of newly trained (motor) skills because a fundamental physiological mechanism underlying the formation of memories is not operational.

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