Associative Plasticity in Human Motor Cortex During Voluntary Muscle Contraction

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1Center for Sensory-Motor Interaction, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark; and 2Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College of London, London, United Kingdom

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Kujirai, Kayoko, Takashi Kujirai, Thomas Sinkjaer, and John C. Rothwell. Associative plasticity in human motor cortex during voluntary muscle contraction. J Neurophysiol 96: 1337–1346, 2006. First published May 24, 2006; doi:10.1152/jn.01140.2005. TMS pulses over the hand area of motor cortex activate different subpopulations of synaptic connections if the direction of the induced current in the brain is reversed from posterior-anterior (PA) direction to anterior-posterior (AP). We tested whether this also made a difference to the after-effects of paired associative stimulation (PAS: ulnar nerve stimulation followed 25 ms later by a transcranial magnetic stimulation pulse). If 50 pairs of stimuli (0.1 Hz) were applied using conventional suprathreshold PA-PAS in resting subjects, there was no effect on motor-evoked potentials (MEPs) in the first dorsal interosseous muscle. In contrast if the same number of pulses were given while subjects made a small tonic (5% maximum) contraction, MEPS were facilitated and resting motor threshold reduced when AP but not PA pulses were used. Subsequent experiments employed subthreshold TMS (95% of the active motor threshold) during voluntary muscle contraction. MEP facilitation accompanied by reduced AP threshold occurred when PAS was given using AP pulses (AP-Sub-PAS), whereas PAS using PA pulses (PA-Sub-PAS) had no excitatory effect. There was no facilitation if the ulnar nerve stimulus was replaced by digital nerve stimulation. There was a tendency for short interval intracortical inhibition (SICI) to decrease and intracortical facilitation (ICF) to increase after AP-Sub-PAS. We propose that the increased effectiveness of AP-Sub-PAS over PA-Sub-PAS is due to the fact that AP TMS more readily activates I3 inputs to corticospinal neurons and hence that these are an important component of associative plasticity in the human motor cortex.

INTRODUCTION

Recently Stefan et al. (2000) introduced a new transcranial magnetic stimulation (TMS) technique that offered the possibility of studying Hebbian-like mechanisms of synaptic plasticity in the cortex of conscious humans. The method relies on the pairing of two different inputs to the motor cortex so that they arrive approximately at the same time. Thus peripheral afferents to the cortex are activated by an electrical stimulus to the median nerve at the wrist and then 20–25 ms later (the approximate time taken for peripheral afferents to reach the cortex), a TMS pulse is given over the hand area of the contralateral motor cortex. If these pairs are repeated 90 times with an interval of 20 s between each pairing, then a single-pulse TMS of the motor hand area evokes a larger muscle response than before pairing. If the timings are changed so that the TMS pulse is applied before input from the peripheral stimulus reaches cortex (TMS pulse 10 ms before median nerve), then the amplitude of motor-evoked potentials (MEPs) is reduced (Wolters et al. 2003). The method is known as paired associative stimulation (PAS) and the facilitation/depression of motor responses lasts $\leq$1 h.

It is thought that PAS may be related to mechanisms of associative plasticity that have been studied in animal preparations (Markram et al. 1997). In many systems, it has been found that if a weak excitatory synaptic input repeatedly arrives at a neuron shortly before the neuron has fired an action potential, then the strength of the connection is increased. If the timing of the stimuli is reversed so that the input arrives just after neural discharge, then the strength of the connection is reduced. There are also cases in which the rules are reversed such that synaptic strengthening occurs if the weak input arrives after rather than before the postsynaptic action potential. The hypothesis in human studies using PAS is that the median nerve stimulus and TMS both evoke inputs onto a shared neuron in the cortex. Repeated pairings then result in an increase in the net efficiency with which subsequent TMS pulses can activate corticospinal neurons. However, there is little information about the possible locus of interaction between pulses.

One unexplained feature of the human PAS paradigm is that the intensity of the TMS pulse has to be sufficient to evoke a MEP response in relaxed muscles to induce the facilitatory effect of PAS (Stefan et al. 2000; Wolters et al. 2003; Ziemann et al. 2004). This could be because the TMS pulse has to evoke a particular amount of synaptic activation at the shared neuron for Hebbian plasticity to occur, the TMS pulse has to evoke activity in a particular set of (high-threshold) inputs for PAS to occur, or the afferent input produced by the evoked muscle twitch has a reinforcing effect on PAS. The latter seems unlikely in view of the fact that the input would arrive much later than the original pairs of input.

The aim of the present paper was to test the hypothesis that PAS requires activation of a particular set of cortical synapses by the TMS pulse to generate a lasting effect. Single motor-unit recordings as well as direct recordings of descending activity in the corticospinal tract have shown that a TMS pulse activates a series of excitatory inputs to corticospinal neurons. A posterior-anterior (PA) directed current pulse preferentially activates a series of excitatory inputs to corticospinal neurons. This could be because the TMS pulse has to evoke activity in a particular set of (high-threshold) inputs for PAS to occur, or the afferent input produced by the evoked muscle twitch has a reinforcing effect on PAS. The latter seems unlikely in view of the fact that the input would arrive much later than the original pairs of input.

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were delivered at a rate of 0.1 Hz for 500 s during sustained voluntary muscle contraction (5% MVC). AMT, active motor threshold.

| TABLE 2. Preferential current-directions in the brain and stimulus intensities to recruit different kinds of I wave |
|------------------------|---------------------------------------------------------------|--------------------------|
| I Waves | Preferential Current-Direction in the Brain | Stimulus Intensity |
| I1 wave | Posterior-anterior (PA) | Around motor threshold |
| I3 wave | Anterior-posterior (AP) | Around motor threshold |
| Both I1 and I3 waves | Posterior-anterior (PA) | Suprathereshold |

excites the earliest (I1) inputs whereas an oppositely directed anterior-posterior (AP) pulse tends to recruit later arriving inputs which may correspond to I3 inputs (Table 1) (Sakai et al. 1997; but see Di Lazzaro et al. 2001). We have therefore tested whether PAS is equally efficient when AP pulses are used rather than the usual PA pulses. A limiting factor, however, is that the differential recruitment of AP and PA pulses is only observed reliably at very low stimulus intensities at around the active motor threshold. We first showed that PAS can be evoked during voluntary muscle contraction and that the threshold for this effect is lower than when PAS is applied at rest. Then, we applied PAS during muscle contraction using two directions of TMS pulses: PA and AP. The results show that under these conditions, PAS using subthreshold AP pulses is superior to PAS using subthreshold PA pulses. We hypothesize that this is because the synaptic inputs that cause I3 activity are particularly important in producing the long-term potentiation (LTP)-like effects of PAS.

METHODS

Subjects

Forty seven healthy volunteers (28 males and 19 females; mean age, 25.9 yr; range, 21–48 yr) who gave written informed consent according to the local ethical committee approval participated in the study. Before the study, all participants completed the adult safety screen questionnaire (Keel et al. 2000). None of the participants had any contraindications to TMS (Wassermann 1998). All of them except for three volunteers were right handed according to Edinburgh Handedness Inventory (Oldfield 1971). Most of the subjects participated in different protocols on separate days. The data from participants were sufficient to induce a long-term effect during muscle contraction, we shortened the intervention period to 500 s.

Subjects were seated in a comfortable chair in front of a computer monitor that displayed the electromyographic activity (EMG) of the FDI muscle. In the modified PAS procedure, the subject was required to maintain muscle activation at 5% MVC for 500 s with help of visual feedback in terms of rectified EMG from the dominant FDI on the display. The hand and fingers were placed with the palm down. Single ulnar nerve stimulation (1 ms in duration) at an intensity just above the motor threshold (mean M-wave in amplitude; around 200 μV) was delivered at the wrist of the dominant arm. TMS was given 25 ms after nerve stimulation over the contralateral motor cortex through a figure-eight coil (diameter of each wing, 70 mm) connected to a Magstim 200 magnetic stimulator with a monophasic current waveform (Magstim, Whitland, UK). The coil was held tangential to the scalp with the handle pointing backward and 45° away from the midline. This coil position produces PA directed induced current in the brain and preferentially recruits I1 waves (Sakai et al. 1997). The intensity of TMS was adjusted to produce MEPs of ~1 mV in peak-to-peak amplitude in the absence of the preceding nerve stimulation. Experiments in which the induced current was reversed from PA to AP were performed by rotating the coil around the intersection of the coil windings at the hot-spot identified with PA stimulation. The EMG electrodes (Ag/AgCl, 0.9 cm in diameter) were placed over the belly of the FDI with the reference electrode over the first interphalangeal joint of the index finger. EMG signals were amplified and band-pass filtered (20 Hz to 1.5 kHz), digitized at 5 kHz by an A/D converter (PCI-6040E, National Instruments, Austin), and stored in a laboratory computer for display and later off-line analysis. Data acquisition was employed by the computer program (Center for Sensory-Motor Interaction, Aalborg, Denmark).

0.1 Hz PAS with subthreshold intensity applying AP or PA pulses (AP-Sub-PAS or PA-Sub-PAS, respectively)

The ulnar nerve stimulation was paired with TMS at an intensity of 95% active motor threshold (AMT) using either AP or PA induced current in the brain during sustained voluntary contraction (5% MVC) of the FDI (Table 2). Fifty pairs of stimuli were delivered at a rate of 0.1 Hz for 500 s. The interstimulus interval between the ulnar nerve stimulation and the following TMS was fixed at 25 ms. During the intervention, EMG was monitored to check that M-waves evoked by the ulnar nerve stimulation remained constant in amplitude (~200 μV in peak-to-peak). TMS was applied at the optimal scalp site for evoking MEPs in the FDI. The coil was held tangential to the scalp with the handle pointing backward and 45° away from the midline for the application of the PA-directed pulse. For the AP pulse, the position of the coil handle was reversed around the intersection of coil windings. Then, the coil angle was adjusted to obtain MEPs with onset latency 2–3 ms later than that of MEPs induced by a PA pulse. The coil

| TABLE 2. PAS applying different kinds of TMS pulse under voluntary muscle contraction |
|------------------------|---------------------------------------------------------------|--------------------------|
| PAS Type | Current Direction in the Brain | Stimulus Intensity | Interstimulus Interval (ISI), ms |
| AP-Subthreshold-PAS | Anterior-posterior (AP) | Subthreshold (0.95 × AMT) | 25 |
| PA-Subthreshold-PAS | Posterior-anterior (PA) | Subthreshold (0.95 × AMT) | 25 |
| PA-1 mV-PAS | Posterior-anterior (PA) | Suprathereshold (to evoke MEPs around 1 mV) | 25 |
| PA-Subthreshold-PAS ISI 28 | Posterior-anterior (PA) | Subthreshold (0.95 × AMT) | 28 |

Ultrasound stimulation was paired with transcranial magnetic stimulation (TMS) using different kinds of induced-current in the brain. Fifty pairs of stimuli were delivered at a rate of 0.1 Hz for 500 s during sustained voluntary muscle contraction (5% MVC). AMT, active motor threshold.
position was marked on the scalp using colored ink in both PA and AP
directions.

0.1 Hz PA-Subthreshold-PAS using an ISI of 28 ms
(PA-Sub-PAS ISI 28)

Because MEPs to AP stimulation occur 2–3 ms later than after PA
stimulation and because PAS depends on the timing between the two
inputs, we also investigated PAS using PA pulses with an ISI = 28 ms
(PA-Sub-PAS ISI 28). In this way, the relative timing of corticospinal
discharge with respect to the sensory input should be comparable
between PA and AP stimulation. To avoid the activation of the later
axonal input by suprathreshold stimulation, it was confirmed that
TMS pulse was subthreshold during PAS by checking EMG activity.

Measurements of motor corticospinal excitability

TIME COURSE. Post-PAS cortico-spinal excitability measuring the
peak-to-peak amplitude of MEPs to PA pulses was evaluated imme-
diately (0 min), 10, 20, and 30 min after each intervention. The size
of MEPs was investigated at rest with the test stimulus intensity
unchanged before and after the intervention. The test stimulus intensi-
ity was set at 1.25 times of the resting MEP threshold (RMT) to a
single PA pulse to evoke MEPs around 1 mV in size before PAS. At
each time point, 10 MEPs were obtained with an intertrial interval of
4 s.

RESTING AND ACTIVE MEP THRESHOLDS. MEP threshold was de-
defined as the lowest stimulus intensity that produced MEP >50 µV at
rest (RMT) or MEP >100 µV during weak muscle contraction of the
FDI (AMT) in ≥5 of 10 consecutive trials (Rossini et al. 1994). Thresholds
were measured before and after the intervention using a
figure-eight coil to induce either PA or AP current in the brain in steps of
1% of the maximum stimulator output.

The intervention was carried out after confirming the reproducibil-
ity of motor thresholds and MEP amplitudes over at least two
consecutive sets of trials. For the measurement at rest, subjects were
encouraged to be fully relaxed.

RECRUITMENT CURVES. Recruitment curves were recorded using
PA and AP pulses in relaxed and in active conditions (5% MVC). Six
subjects were studied, except for AP stimulation during the active
condition when five subjects were available. Stimuli were applied at
the optimal scalp sites for evoking MEPs in the FDI. Five stimuli were
applied at an intensity 6% below the threshold. Then, the stimulus
intensity was increased by 3% and another five stimuli were applied.
This process was repeated until the amplitude of the MEPs reached
maximum. The recruitment curve during muscle contraction was
examined in the first 20 min after PAS. This was because enhance-
ment of MEPs evoked during active PAS disappeared 30 min after PAS
whereas the aftereffect continued for more than 30 min in the resting
muscle (unpublished data).

SHORT INTERVAL INTRACORTICAL INHIBITION (SICI) AND INTRACOR-
TICAL FACILITATION (ICF). To investigate the cortical excitability,
SICI and ICF (the interstimulus interval [ISI]; 3 and 10 ms, respec-
tively) were evaluated, applying the paired-pulse TMS technique in
the standard PA direction (Kujirai et al. 1993). The conditioning
stimulus had an intensity of 95% of AMT. In absolute terms, this was
about 2% of the stimulator output below AMT. SICI and ICF were
expressed as a ratio of the mean amplitudes of conditioned and
unconditioned responses. The test stimulus evoked MEPs of 0.5–1.0
mV. Seven trials for each condition were randomly delivered. After
PAS the measures were repeated using two amplitudes of test-MEP:
in the “no-adjust” condition we used the same test-stimulus intensity
as the baseline before PAS and in the “adjust” condition we changed
the intensity to obtain similar test-MEP sizes to those pre PAS,
although the conditioning TMS intensity remained unchanged.

Control studies

Three different interventions using subthreshold AP-TMS pulses
were applied—1) 0.1 Hz AP-rTMS alone; To examine the effect of
rTMS alone, we applied subthreshold rTMS (95% of AMT) with an
AP pulse during the activation of FDI at 5% MVC for 500 s. 2) 0.1 Hz
AP-subPAS-rTMS with digital nerve stimulation: 1) stimulation of
the index finger (AP-Cutaneous-2D-PAS); the effect of stimulation of
cutaneous nerve adjacent to the FDI and 2) stimulation of the ring
and the little fingers (AP-Cutaneous-4D+5D-PAS): the effect of stimula-
tion of cutaneous afferents in the ulnar nerve.

Digital stimulation (0.2 ms in duration) using a pair of ring
electrodes was applied to the index finger or to the ring and the little
fingers. The cathode was placed around the proximal interphalangeal
joint and the anode placed around distal phalangeal joint, and
the intensity was set to around three times that of the sensory perception
threshold. The ISI between the digital nerve stimulation and TMS was
set at 27 ms. PAS was applied under sustained contraction of the FDI
(5% MVC) for 500 s.

Statistical procedures

The effect of PAS on the time course of MEP sizes or on MEP
thresholds was analyzed using one-way repeated-measures ANOVA
(within-subject factors: condition, time). The effect of different conditions
during PAS (rest and active) on MEP sizes was evaluated with two-way
repeated-measures ANOVA (within-subject factors: condition, time).
The effect of different kinds of PAS on MEP sizes or on MEP
thresholds was evaluated using two-way repeated-measures ANOVA
(between group factor; PAS type, within-subject factor: time).
The effect of the different kinds of PAS on SICI or on ICF was evaluated
with four-way repeated-measures ANOVA (between group factor;
PAS type, within-subject factor; pre- and post-PAS, ISI, with and
without adjustment of test size). Recruitment curves before and after
PAS were compared by two-way repeated-measures ANOVA (within-
subject factors: pre- and post-PAS, and intensity). Effects were
considered significant when P < 0.05. Post hoc paired t-test (2-tailed)
was employed for further analysis. Latencies of MEPs before and after
PAS were compared using paired t-test. Data are expressed as
means ± SE.

RESULTS

0.1 Hz PA-1mV-PAS at rest versus during muscle activation

In preliminary experiments on four subjects, we compared
the aftereffect of 50 pairs of 0.1 Hz PA-1mV-PAS given when
subjects were relaxed with the effect of the same PAS given
during voluntary muscle contraction (Fig. 1: note that the MEP
amplitude during active PAS was adjusted to be the same as
that during relaxed PAS which meant that the TMS intensity
was smaller when the experiment was performed during muscle
contraction). Because of the small number of subjects, we
analyzed the data by averaging the time points at 0 and 10 min
(early) after PAS and at 20 and 30 min (late). There was a
significant interaction between condition (rest and active) ×
time (pre, early, late) in two-way ANOVA [F(2,12) = 4.92,
P < 0.05]. This was due to the fact that the facilitation lasted
longer when the target muscle was active compared with when
it was relaxed. This was confirmed in subsequent one-factor
ANOVA, which showed no significant effect of time on MEP
sizes after PAS at rest, whereas there was a significant change
in the MEP amplitude after PAS under voluntary muscle
contraction [F(4,12) = 4.09, P < 0.05]. Post hoc paired t-test
showed significant facilitation in the active condition at 20 and 30 min but not in the relaxed condition.

Figure 2 illustrates a more detailed analysis of the effects of active PAS in eight subjects, showing effects both on the MEP amplitude as well as on the MEP threshold to AP or PA pulses in active or relaxed muscle. As in Fig. 1, the MEPs were facilitated for at least 30 min [1-factor ANOVA, $F(4,28)=5.14$, $P<0.01$; Fig. 2A]; thresholds were unchanged apart from the resting AP threshold which was reduced [1-factor ANOVA, $F(4,28)=5.21$, $P<0.01$; Fig. 2B].

0.1 Hz-Subthreshold-PAS during muscle contraction: comparing the effects of subthreshold PA and AP TMS pulses

As noted in the Introduction, although PA and AP TMS pulses tend to activate different subpopulations of synaptic inputs to corticospinal neurons, this difference is only evident at threshold intensities and disappears at higher levels. Because it was previously thought that the aftereffect of PAS could only be obtained with suprathreshold TMS pulses, comparisons of PA and AP-PAS would not have been valid. The finding that PAS cannot only be obtained but is more effective during voluntary contraction, both in terms of the smaller number of stimulus pairings required as well as the lower intensity of TMS, allowed us to conduct the main series of experiments by applying PA-PAS and AP-PAS during muscle contraction.

In eight subjects, we compared the aftereffects of three kinds of PAS: PA-1mV-PAS and PAS using subthreshold PA and AP pulses (PA-Sub-PAS and AP-Sub-PAS, respectively). An ISI of 25 ms was used in all three conditions. As mentioned in Methods, for the subthreshold PA pulse, we also explored a longer ISI of 28 ms to compensate for the difference in latencies of MEPs evoked by PA and AP stimulation (PA-Sub-PAS ISI 28 ms). All four PASs were applied at the rate of 0.1 Hz for 500 s during contraction of the FDI. The mean intensities of the TMS pulses in these experiments were 20.4 ± 2.4, 31.2 ± 2.9, 45.4 ± 2.7, and 31.8 ± 2.9% of stimulator output, respectively.

Two-factor ANOVA revealed a significant effect of PAS type [AP-Sub-PAS, PA-Sub-PAS, PA-1mV-PAS, and PA-Sub-PAS ISI 28 ms; $F(3,27)=4.31$, $P<0.05$] and a time × PAS type interaction [$F(12,108)=2.29$, $P<0.05$]. Subse-

FIG. 1. Shown is 0.1 Hz PA-1mV-paired associative stimulation (PAS) for 500 s at rest vs. during muscle activation. PAS consisted of a pair of stimuli: single ulnar nerve stimulation at the wrist and transcranial magnetic stimulation (TMS) over the contralateral motor cortex. TMS pulse was given to induce posterior-anterior (PA) current in the brain, and the stimulus intensity was set to evoke motor-evoked potentials (MEPs) of −1 mV in the 1st dorsal interosseous muscle (FDI) either at rest (PA-1mV-PAS rest) or during sustained muscle contraction (5% of MVC) of the FDI (PA-1mV-PAS active). Fifty pairs of stimuli were delivered at 0.1 Hz for 500 s. The graph shows how the peak-to-peak amplitude of the MEP (means ± SE from 4 subjects) changed over the 30 min after PAS. When data were analyzed by averaging time points at 0 and 10 min (early) after PAS and at 20 and 30 min (late), there was a significant interaction between condition (rest and active) × time (pre, early, late) in two-way repeated measures ANOVA [$F(4,28)=4.92$, $P<0.05$]. There was a significant change in MEP amplitude in active state [$F(4, 12)=4.09$, $P<0.05$, 1-factor ANOVA]. *$P<0.05$, **$P<0.01$; significant facilitation in comparison to pre-PAS by post hoc paired t-test.

FIG. 2. Change in MEP amplitude and threshold after 0.1 Hz PA-1mV-PAS during active muscle contraction. A: time course of changes in MEP amplitude (means ± SE) before and after PAS (data from 8 subjects). MEPs were significantly increased after PAS [$F(4,28)=5.14$, $P<0.01$ by 1 factor ANOVA]. *$P<0.05$, **$P<0.01$; significant facilitation in comparison to pre-PAS by post hoc paired t-test. B: resting motor threshold and active motor threshold to either a PA or an AP TMS pulse. The resting AP threshold was decreased after PAS [1-factor ANOVA, $F(4,28)=5.21$, $P<0.01$] while resting PA threshold remained unchanged. Active motor thresholds were not changed. *$P<0.01$, **$P<0.001$ by post hoc paired t-test.
sequent one-factor ANOVAs on each type of PAS, with time as the main factor showed that both PA-1mV-PAS and AP-Sub-PAS facilitated the MEPs $[F(4,28) = 5.14, P < 0.01$ and $F(4,28) = 2.85, P < 0.05$, respectively]. PA-Sub-PAS ISI 28 ms had a significant main effect of TIME $[F(4, 24) = 6.84, P < 0.001]$. *P < 0.05, **P < 0.01; significant difference in comparison with pre-PAS by post hoc paired $t$-test.

Significant facilitation appeared to start just after PAS when AP-Sub-PAS was applied, whereas significant facilitation started about 10 min after PA-1mV-PAS and gradually increased. However, a follow-up two-factor comparison of the time courses of PA-1mV-PAS and AP-Sub-PAS failed to reveal any significant effect of PAS type or any significant interaction with time, suggesting that the effect of the two methods was equal. In contrast to subthreshold AP pulse, conditioning with a subthreshold PA pulse did not produce any significant facilitation at either ISI.

We also measured how the three forms of PAS (AP-Sub-PAS, PA-Sub-PAS and PA-1mV-PAS) affected the RMT to a single AP pulse of TMS 20 min after PAS ($n = 7$). There was a significant interaction of PAS type $\times$ time $[F(2,18) = 19.94, P < 0.0001]$. The AP threshold was reduced after AP-Sub-PAS and PA-1mV-PAS but not after PA-Sub-PAS. **P < 0.01, ***P < 0.001; post hoc paired $t$-test.

Recruitment pattern of MEPs after 0.1 Hz Subthreshold-PAS using AP pulse

In six subjects, we investigated the facilitation of MEPs by AP-Sub-PAS in more detail by constructing recruitment curves.
for MEPs evoked by single PA and AP pulses in rest and active conditions (Fig. 4). Because the recruitment of MEPs started around motor threshold, data evoked by intensities below motor threshold were excluded from the statistical analysis. Recruitment curves for MEPs evoked either by PA or AP pulses increased after AP-Sub-PAS whether the MEPs were evoked at rest or during background activity [main effect of PAS: $F(1,4) = 11.50, P < 0.05$ and $F(1,3) = 17.72, P < 0.05$, respectively for PA pulses; $F(1,3) = 13.2, P < 0.05$ and $F(1,5) = 7.36, P < 0.05$, respectively for AP pulses]. For MEPs evoked at rest, there was a significant PAS × intensity interaction for both PA and AP pulses [$F(8,32) = 3.86, P < 0.01$ and $F(4,12) = 3.44, P < 0.05$ by PA and AP pulses, respectively].

To gain more insight into the nature of the facilitation after AP-Sub-PAS, we also examined the form of the MEP responses before and after PAS. The onset latencies of the responses are given in Table 3 and show that the onset of MEPs evoked by AP stimulation was 2–3 ms later than PA stimulation, consistent with the recruitment of different sets of synaptic inputs to corticospinal neurons (Sakai et al. 1997). This difference was preserved after AP-Sub-PAS. However, inspection of individual MEPs showed that the PAS had subtly changed their form. MEPs before and after AP-Sub-PAS from three different individuals are superimposed in Fig. 5. Only data from responses during active contraction are illustrated. Data collected at rest is difficult to interpret due to the additional time taken for descending corticospinal volleys to recruit spinal motoneurons from their resting potential to firing threshold.

In subject 1, MEP facilitation after AP-Sub-PAS begins when the stimulus intensity of the test pulse is 9% above AMT (Fig. 5). At this intensity, the main part of the increase in the amplitude of MEPs recruited by a PA pulse occurs 2–3 ms after

<table>
<thead>
<tr>
<th>TMS Pulse Type</th>
<th>Onset Latency of MEPs, ms Pre-PAS</th>
<th>Onset Latency of MEPs, ms Post-PAS</th>
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<tbody>
<tr>
<td>PA pulse</td>
<td>$21.4 \pm 0.50$</td>
<td>$21.7 \pm 0.64$</td>
</tr>
<tr>
<td>AP pulse</td>
<td>$24.0 \pm 0.44$</td>
<td>$24.2 \pm 0.61$</td>
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Values are means ± SE ($n = 6$). Latencies of MEPs were examined by PA and AP TMS pulses under contraction of the FDI (5% of MVC). The onset latency of MEPs evoked by AP stimulation was 2–3 ms later than PA stimulation. AP-subthreshold-PAS did not change the onset latency of MEPs either by PA or AP pulse ($P > 0.05$, 2-tailed paired t-test).
the onset of the response. This is approximately the same latency as the onset of responses to AP stimulation. Thus facilitation preferentially occurred in the later part of the MEP response. In contrast, the early part of the MEP remained unchanged. When stimulus intensity was increased further (e.g., AMT +15%), facilitation after PAS was seen in all parts of the MEP. A similar effect was observed in the records from subject 2. In subject 3, facilitation always affected all parts of the MEP response even at low intensities of stimulation. The tentative conclusion is that in most individuals, facilitation after PAS preferentially involves increased effectiveness of transmission in synaptic inputs to corticospinal neurons that are best activated by an AP pulse.

We compared RMT to a single AP pulse after AP-Sub-PAS and AP-rTMS alone. Two-way ANOVA revealed a significant time × intervention type interaction \( F(1,14) = 31.96, P < 0.0001 \). Ten minutes after the intervention, the threshold was significantly decreased after AP-Sub-PAS while it remained unchanged after AP-rTMS alone (Fig. 6B).

**SICI and ICF after 0.1 Hz AP-Sub-PAS and 0.1 Hz PA-1mV-PAS**

In eight subjects, SICI and ICF were evaluated with a paired-pulse paradigm at interstimulus intervals of 3 and 10 ms, approximately 10 min after the end of PAS (Fig. 7). As noted in METHODS, the size of the test MEP response increased after PAS (AP-Sub-PAS—pre: 971.8 ± 100.9, post: 1189.4 ± 209.2 μV; PA-1mV-PAS—891.6 ± 114.8, post: 1342.9 ± 122.4 μV). To control for this, we made a second set of measurements after reducing the intensity of the test pulse [AP-Sub-PAS—pre: 56.3 ± 1.6, post (Adjust): 53.7 ± 1.6%; PA-1mV-PAS—pre: 53.7 ± 3.0, post (Adjust): 50.5 ± 3.4%] so that the amplitude of the test MEP remained constant. Neither type of PAS changed AMT to a PA pulse, so the intensity of the conditioning pulse was kept constant at 95% of AMT. There were no significant differences in the size of test MEPs and the intensity of TMS pulses between AP-Sub-PAS and PA-1mV-PAS in both adjust and nonadjust conditions \( (P > 0.05, \text{paired } t\text{-test}). \)
DISCUSSION

All previous paradigms in which PAS has been used to produce lasting facilitation of MEPs have required a suprathreshold (~20% above RMT) PA pulse (Stefan et al. 2000, 2002; Wolters et al. 2003; Ziemann et al. 2004). The present study shows that the number of PAS pairings and the intensity of the TMS pulses can be reduced if PAS is given while subjects make a mild voluntary contraction of the target muscle. In addition we found that during contraction, subthreshold AP-directed current pulses are more efficient than subthreshold PA pulses in producing the facilitation of MEPs. We argue in the following text that this is compatible with the idea that the cortical circuits targeted by AP stimulation play a prominent role in PAS facilitation. This may also explain why this type of facilitation is accompanied by a reduction in the threshold to single AP pulses as well as the fact that MEP facilitation seems preferentially to involve later parts of the evoked surface potential.

Activation of the target muscle accelerates induction of plasticity

The first set of experiments showed that “standard” PA-1mV-PAS failed to facilitate MEPs if only 50 pairs of stimuli were given (0.1 Hz) at rest. This is consistent with previous studies in which long-lasting effects of PA-1mV-PAS were obtained after 90–200 stimuli (Stefan et al. 2000; Ziemann et al. 2004). However, the present study showed that if the 50 pairs of stimuli were applied during tonic muscle contraction, then MEP facilitation occurred. There are two possible reasons that could explain why muscle activation accelerates the induction of lasting MEP facilitation.

We analyzed the data using an ANOVA in which “type of PAS,” “pre- and post-PAS,” “ISI,” and “Adjust and Non-adjust” were the four main factors. There were significant interactions among type of PAS, pre- and post-PAS, and adjust and non-adjust [F(1,14) = 5.84, P < 0.05], type of PAS × pre- and post-PAS [F(1,14) = 6.35, P < 0.05], and type of PAS × adjust and non-adjust [F(1,14) = 5.84, P < 0.05]. As reported previously by Stefan et al. (2002), PA-1mV-PAS has no effect on the amount of SICI using a post hoc paired t-test. Nevertheless there was a tendency for SICI to be less effective after 0.1 Hz AP-Sub-PAS when the test MEP size was not adjusted (P < 0.05, paired t-test). ICF tended to increase after 0.1 Hz AP-Sub-PAS (P < 0.01, paired t-test) when the test size was adjusted, whereas it tended to decrease after PA-1mV-PAS.
First, voluntary contraction increases the recruitment of I waves (Di Lazzaro et al. 1998) and reduces the excitability of intracortical inhibitory systems as detected with paired pulse tests of SICI (Ridding et al. 1995). As noted by Stefan et al. (2002), the removal of ongoing inhibition might favor the development of LTP-like effects. The effect is also similar to data obtained in some animal preparations: the spike-time-dependent induction of LTP in slices of neocortex can be strengthened at 0.1 Hz if the postsynaptic cells are depolarized by another input (Nelson et al. 2002).

The second possibility is that performance of the contrac-
tion, which required subjects to monitor a visual display of their muscle activation, focused their attention onto the FDI muscle. This would be compatible with the observation of Stefan et al. (2004) that visuospatial attention increases the facilitatory effects of PAS. However, they did not test whether this would also decrease the time needed to achieve facilitatory effects, so a direct comparison with the present results is not possible at the present time.

We also found that PAS was accompanied by a decrease in the threshold to AP stimulation, whereas there was no effect on the threshold to PA stimulation (see also Stefan et al. 2000). Several authors have argued that the two forms of TMS are thought to activate different sets of synaptic inputs to corticospinal neurons and that this is responsible for the 2- to 3-ms difference in the onset latency of surface EMG responses. Sakai et al. (1997) who studied EMG responses in single motor units suggested that these were equivalent to the I3 (AP stimulation) and I1 (PA stimulation) inputs, whereas Di Lazzaro et al. (2001), on the basis of direct recordings of corticospinal volleys in the cervical cord, suggested that the difference may be more complex. Whatever the exact mechanism, the conclusion is that PAS has a greater effect on transmission in cortical pathways activated by AP than PA stimulation.

Facilitation after AP-Sub-PAS

Because PAS was more effective when applied during muscle contraction and differences in recruitment of inputs by AP and PA TMS are most pronounced at the threshold, we induced PAS in the remainder of the experiments during weak voluntary contraction of the target muscle to maximize our chances of observing the aftereffects of PAS with subthreshold TMS pulses.

The data showed that PAS using subthreshold AP stimuli, but not PA stimuli, was capable of producing lasting facilitation of MEPs. This PAS produced a similar amount of facilitation as PAS evoked by a suprathreshold PA pulse (PA-1mV-PAS). It also decreased the threshold to subsequent AP test pulses but had no effect on the PA threshold. Our hypothesis is that the mechanism responsible for MEP facilitation is similar for both AP-Sub-PAS and for conventional PA-1mV-PAS. We speculate that in both cases, lasting facilitation was predominantly due to changes in the effectiveness of cortical pathways recruited by an AP pulse. It is known that PA pulses can activate these same cortical pathways but only at suprathre-
hold intensities. This may explain why PAS evoked with PA pulses requires a higher TMS intensity than with AP pulses.

The preferential effect of PAS on pathways activated by AP pulses was also evident in the form of surface EMG responses before and after PAS. In the majority of subjects, particularly around threshold intensities, facilitation of MEPs began some 2–3 ms after the onset of responses evoked by a PA pulse at a latency similar to the onset latency of AP responses. This is compatible with the idea that the cortical pathways with delayed inputs to corticospinal neurons are preferentially facilitated by this form of PAS. Their inputs to the spinal cord arrive 2–3 ms after the earliest corticospinal volleys so that the onset of EMG facilitation is delayed. Cortical pathways with faster inputs activated by PA pulses are presumably less affected so that the onset of the EMG response is very similar after PAS. However, we note that this distinction may differ from individual to individual and probably explains why in some sub-
jects, MEP facilitation occurs throughout the whole of the response evoked by PA stimulation. Further studies using other methods such as single motor-unit studies with peristimulus time histograms (PSTH) or even invasive recordings of descending corticospinal volleys from the cervical epidural space might give more direct information about changes of the recruitment pattern of I waves in individuals.

Timing considerations and contribution of possible I-wave inputs to pyramidal neurons (I1 and I3 inputs) during PAS

Timing considerations suggest that in our experiments af-
ferent input during PAS could have interacted with early (e.g., I1 input) and late inputs (e.g., I3 input) to corticospinal neu-
rons. The ISI of 25 ms was chosen according to Wolters et al. (2003) so that there was time for the afferent ulnar nerve volley to reach the sensory cortex (latency of N20 response) plus 2- to 4-ms conduction time from the sensory to motor cortex (Gold-
ing et al. 1970), before the TMS pulse was applied. Interest-
ingly, Ziemann et al. (2004) used an ISI of about 20 ms (N20 latency). In their case, the afferent input would arrive after firing of the initial input (I1 input) produced by the TMS pulse and could only interact with later arriving inputs such as those preferentially activated by AP pulses (I3 input). Thus later (e.g., I3) inputs to corticospinal neurons are likely to be an important target for PAS facilitation.

When we applied an even longer ISI = 28 ms, the facilita-
tory effect was replaced by an initial reduction in cortical excitability. Presumably at such intervals, there would be time for other neuronal elements to be recruited by the afferent input which might have complex effects on motor cortex circuits.

Effects of PAS on SICI and ICF

The paired-pulse paradigm responsible for SICI preferen-
tially suppresses late facilitatory inputs of the corticospinal neuron (the I3 wave). As reported by Stefan et al. (2002), we found that PA-1mV-PAS had no effect on SICI, whereas AP-Sub-PAS had a tendency to increase ICF. Although this was not a strong effect, it suggests that AP-Sub-PAS has a greater effect on excitatory inputs than on changes in the excitability of inhibitory circuits.

Proprionic input and cutaneous input

We also found that AP-Sub-PAS required the activation of a mixed nerve (ulnar) during paired stimulation, and that no effect was seen if stimulation of predominantly cutaneous nerves (the ring and the little fingers) were used instead. This contrasts with the original observations of Stefan et al. (2000),

\[ \text{equation} \]

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\[ \text{equation} \]

\[ \text{equation} \]
using PA-1mV-PAS at rest, who found there was no significant difference in the effects between cutaneous and mixed nerve PAS. This may reflect the specificity of our method: the lower intensity of AP stimulation may require pairing with proprioceptive inputs, whereas higher intensity of PA stimulation may activate circuits that respond to either proprioceptive and/or cutaneous inputs. Another possibility is that proprioceptive inputs might be transmitted more efficiently to the cortex than cutaneous inputs during voluntary muscle contraction (Kunesh et al. 1995; Tsutomo et al. 1975).

Possible mechanism of associative plasticity in the human motor cortex

The present data strongly suggest that synaptic inputs to corticospinal neurons that are preferentially targeted by AP pulses play an important role in the facilitatory after-effects of PAS. In a simple model of the circuits involved this may represent a difference between I1 (difficult to facilitate, recruited by PA stimulation) and I3 (easier to facilitate, recruited by AP stimulation) inputs. Such long-term effects could be due to two forms of plasticity: LTP/LTD-like plasticity in synaptic transmission and non-synaptic plasticity involving changes in the intrinsic properties of neural membranes (Daoudal and Debanne 2003; Johnston et al. 2003). The latter can lead to reduced threshold for action potential initiation as well as reduced afterhyperpolarization and enhanced backpropagation of action potentials arising at the initial segment to dendrites. There is good evidence that conventional PAS involves some degree of LTP/LTD-like synaptic plasticity and the same is likely to be true here. However, changes in membrane properties may also be involved and could contribute, for example, to the lowering of threshold to AP stimulation after 0.1 Hz AP-Sub-PAS.

Conclusion

The present data support the hypothesis that associative plasticity using time-locked ulnar nerve and TMS inputs is primarily due to a change in the excitability of inputs to corticospinal neurons activated by AP directed TMS pulses. These may be equivalent to those responsible for recruiting the I3 wave in the corticospinal tract. The PAS method using AP subthreshold stimulation at 0.1 Hz may in the future allow us to study more specific effects of spike-timing-dependent plasticity in the human cortex.

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