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Induction of plasticity in the human motor cortex by paired associative stimulation

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Summary

Current models of motor cortical plasticity, developed in studies on experimental animals, emphasize the importance of the conjoint activity of somatosensory afferents and intrinsic motor cortical circuits. The hypothesis that an enduring change in excitability in the cortical output circuitry can be induced in the human motor cortex by a paired-stimulation protocol was tested. Low-frequency median nerve stimulation was paired with transcranial magnetic stimulation (TMS) over the optimal cranial site for stimulating the abductor pollicis brevis muscle (APB). This protocol induced an increase in the amplitudes of the motor evoked potentials (MEPs) in the resting APB as well as a prolongation of the silent period measured in the precontracted APB following TMS; amplitudes of MEPs measured in voluntary contraction remained unchanged. Experiments testing the excitability of spinal motoneurons using F-wave studies and electrical stimulation of the brainstem suggested that the site of the plastic changes was within the motor cortex. The increases in resting amplitudes and silent period duration were conditionally dependent on the timing between the afferent and the magnetic stimulation in that they were present when events elicited by afferent and magnetic stimulation were synchronous at the level of the motor cortex. Plasticity induced by paired stimulation evolved rapidly (within 30 min), was persistent (minimum duration 30–60 min) yet reversible, and was topographically specific. This combination of features and the similarity to properties of induced enduring changes in synaptic efficacy, as elucidated in animal studies, leads us to propose that the induced plasticity may represent a signature of associative long-term potentiation of cortical synapses or closely related neuronal mechanisms in the human cortex.

Keywords: plasticity; motor cortex; transcranial magnetic stimulation

Abbreviations: ADM = abductor digiti minimi; APB = abductor pollicis brevis; APBi = abductor pollicis brevis ipsilateral to cortical interventional stimulation; BB = biceps brachii; ISI = interstimulus interval; LTP = long-term potentiation; MEP = motor evoked potential; RA = resting amplitude; RMT = resting motor threshold; SI = stimulation intensity; TA = tibialis anterior; TMS = transcranial magnetic stimulation

Introduction

The adult mammalian sensorimotor cortex is capable of reorganizing in response to various injuries or environmental changes, e. g. peripheral nerve stimulation, nerve transection, limb amputation, changes in limb position and focal lesions of the sensorimotor cortex (Donoghue *et al*., 1990; Merzenich *et al*., 1990; Sanes *et al*., 1992; Brasil-Neto *et al*., 1993; Nudo and Milliken, 1996). Furthermore, the motor cortex is reorganized in association with skill acquisition (Pascual-Leone et *al*., 1994; Karni *et al*., 1995; Pascual-Leone *et al*., 1995; Nudo *et al*., 1996) and by repetition of simple movements (Classen *et al*., 1998). Understanding the mechanisms of motor cortex plasticity is of fundamental neurobiological importance and is probably a necessary requirement for the development of strategies promoting recovery following brain damage in humans.

Of several candidate mechanisms for cortical plasticity, persistent changes in synaptic efficacy, as proposed by Hebb on theoretical grounds in 1949 (Hebb, 1949), have been favoured by many as underlying learning and memory as well as some of the cortical plasticity related to the acquisition and recovery of sensorimotor function. Ever since it was discovered in the hippocampus, long-term potentiation (LTP) has been generally regarded as the prototypical example of a mechanism involving a change in synaptic efficacy (Bliss

and Lomo, 1973). LTP is called associative, or 'Hebbian', if it occurs at an input to a postsynaptic cell conditional on (i) concomitant and synchronous activation of another input to the same cell, or (ii) concomitant and synchronous postsynaptic depolarization (Buonomano and Merzenich, 1998). This form of LTP has attracted considerable attention because it provides a model of how converging inputs from various sources, including local intracortical fibres, corticocortical and thalamocortical afferents, could interact to reshape local representational cortical patterns (e.g. Donoghue *et al*., 1996; Asanuma and Pavlides, 1997). In addition to the hippocampal cortex (Kelso and Brown, 1986; Sastry *et al*., 1986), associative LTP has been induced experimentally in a variety of different neocortical areas, including the auditory cortex (Cruikshank and Weinberger, 1996), the somatosensory cortex (Bindman *et al*., 1988; Crair and Malenka, 1995) and the visual cortex (Hirsch and Gilbert, 1993; Fregnac *et al*., 1994; Kirkwood and Bear, 1994). Of particular interest is the associative LTP that has been produced in the primary motor cortex both *in vitro* and *in vivo* using various pairing protocols (Baranyi and Feher, 1981; Baranyi and Szente, 1987; Iriki *et al*., 1989; Baranyi *et al*., 1991; Iriki *et al*., 1991; Hess and Donoghue, 1994; Hess *et al*., 1996).

In the present paper the hypothesis was tested that an enduring change in the excitability of cortical output circuits can be induced in the human motor cortex by a protocol using a design principle similar to those leading to associative LTP in previous studies on experimental animals and cortical slices. Low-frequency peripheral stimulation of somatosensory afferents was synchronously paired with transcranial magnetic stimulation (TMS) over the motor cortex, which is believed to preferentially activate intracortical fibres travelling horizontally in the cortex with respect to its surface (Rothwell, 1997). Our results, demonstrating reliable induction of motor cortical plasticity by paired associative stimulation, may be of relevance for the understanding and therapeutic manipulation of human motor cortical plasticity.

Methods

Subjects

Experiments were performed on 22 healthy volunteers (16 men, six women), aged 19–37 years (mean 27 ± 4 years) with normal results on neurological examination. The protocol was approved by the ethics committee of the University of Rostock. All subjects gave their written informed consent. All subjects were right-handed, except two who were left-handed, according to the Oldfield handedness inventory (Oldfield, 1971).

Recording

Surface EMG activity was recorded from the right abductor pollicis brevis muscle (APB) using disposable surface electrodes (silver–silver chloride; model 9013L0202, Dantec Medical, Skovlunde, Denmark) with the active electrode mounted on the muscle belly and the inactive electrode placed over the base of the metacarpophalangeal joint of the thumb. Raw signals were amplified using a Toennies amplifier (Toennies, Freiburg, Germany) and bandpass-filtered between 20 and 2000 Hz. EMG signals were sampled at 5000 Hz, digitized using an analogue–digital converter (model 1401 plus, Cambridge Electronics Design, Cambridge, UK) and stored in a laboratory computer for display and later off-line analysis.

Stimulation

Focal TMS was performed using a figure-of-eight shaped magnetic coil (diameter of each wing 9.5 cm) connected to a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. Electric mixed nerve (except F-wave studies) and digital nerve stimulation were performed using a standard stimulation block (cathode proximal, stimulus width 200 μs) connected to a Cantata electromyograph (Dantec Medical). Electric stimulation for F-wave studies and brainstem stimulation was performed using a Digitimer D 180 (maximal output 1 A, 750 V; Digitimer, Welwyn Garden City, UK) at a stimulus width of 100 μs.

Experimental procedures

Subjects were seated in a comfortable reclining chair. The optimal position of the magnetic coil for eliciting motor evoked potentials (MEP) in the right APB was assessed over the left motor cortex at a moderately suprathreshold stimulation intensity, and marked directly on the scalp with a soft-tip pen. At the optimal site, the resting motor threshold (RMT) was determined as the stimulator intensity needed to produce a response of at least 50 μV in the relaxed APB in at least five of 10 consecutive trials at a resolution of 1% of the maximal stimulator output (Rossini *et al*., 1994). Thereafter, the stimulator intensity sufficient to evoke a peak-to-peak amplitude of 1 mV in the relaxed APB was determined ($SI_{1\ mV}$). $SI_{1\ mV}$ was 1.2 ± 0.1 times RMT or 8 ± 4% of the maximal stimulator output higher than RMT. Taking all experiments into consideration, $SI_{1\ mV}$ was 44.4 ± 6.2% of the maximal stimulator output. Throughout the experiment, complete muscle relaxation was monitored by audiovisual feedback where appropriate.

In some experiments, $SI_{160\ ms}$ was assessed as the stimulator intensity sufficient to evoke a silent period of duration ~160 ms in surface EMG recordings of the APB isometrically contracted at 15% of maximum force. Force was measured using a force transducer (range 0–100 N, non-linearity <1%, contact surface area 0.7 × 1.8 cm) fed for feedback into an oscilloscope. The individual 15% force level was marked directly on the oscilloscope screen in front of the subject.

In all experiments, an interventional stimulation protocol was employed. In a typical experiment, the intervention consisted of single electrical stimuli delivered to the right median nerve at the level of the wrist at 300% of the perceptual threshold (6.0 ± 2.1 mA) and followed by TMS with the target muscle at complete rest, as monitored by audiovisual feedback. TMS was applied at an intensity of $SI_{1\ mV}$ (see above), as determined prior to the intervention. Ninety pairs of stimuli were delivered at 0.05 Hz over 30 min, with an interstimulus interval (ISI) of 25 ms.

For the measurement of resting amplitudes, 20 stimuli were delivered both before and immediately after intervention, using a stimulus intensity of $SI_{1\ mV}$ and a stimulation rate of 0.1 Hz. Identical stimulus intensities were used before and after intervention. In experiments investigating active amplitudes and the duration of the silent period, 20 additional stimuli were delivered during voluntary contraction of the APB at 15% of maximal force immediately after testing the resting amplitudes before and after interventional stimulation. The principal experimental design used in all experiments is illustrated in Fig. 1.

Variations of the standard experimental procedure are described below.

Timing of the TMS pulse in relation to median nerve stimulation during intervention

In seven subjects, the timing of the TMS pulse with reference to the median nerve stimulation was varied. ISIs of 25, 100, 525 and 5000 ms were tested in separate experimental sessions. In three subjects, an ISI of 35 ms was also tested. The order of the experimental sessions in which a specific ISI was employed was balanced between subjects. At least 2 days elapsed between any two sessions. In this experimental series, active amplitudes and silent periods were also assessed using stimulation intensities of $SI_{160\ ms}$ in addition to testing resting amplitudes at $SI_{1\ mV}$.

Duration of effects

In 11 subjects, modulation of resting amplitudes induced by prior interventional paired stimulation (ISI 25 ms) was monitored over 30 min following the intervention. The initial 20 stimuli were delivered at 0.1 Hz, and the subsequent probing magnetic stimuli were delivered at a rate of 0.05 Hz. In two volunteers, the monitoring time was extended by another 30 min, giving a total of 60 min, and one of them was retested after 24 h.

Digital nerve stimulation

In eight subjects, the interventional paired stimulation (ISI 25 ms) was performed with digital nerve stimulation instead of median nerve stimulation. Ring electrodes were attached to the proximal phalanx of the thumb, with the cathode proximal and the anode distal. Electrical stimuli were delivered at 300% of the perceptual threshold.

Somatotopy

In seven volunteers, the somatotopy of modulating the resting amplitude by interventional paired stimulation was studied in detail. The interventional paired stimulation was performed as described above over the optimal stimulation site of the APB and using a stimulator intensity eliciting a response amplitude of ~1 mV in the right unconditioned APB ($SI_{1\ mV}$). The effects of interventional paired stimulation on the resting amplitudes of the right APB were compared with those on the resting amplitudes of the right abductor digiti minimi muscle (ADM) and the right biceps brachii muscle (BB), as well as with resting amplitudes of the left ABP, ipsilateral to the cortical interventional stimulation (APBi). In one subject, resting amplitudes were also obtained from the tibialis anterior (TA) muscle. For the BB, TA and APBi, the optimal stimulation sites were determined and then stimulus intensities were identified producing a peak-to-peak amplitude of 0.4 mV (BB and TA) or 1 mV (APBi). In one subject, MEPs from BB could not be elicited reliably. To assess the excitability of the ADM, the same stimulation site and stimulus intensity ($SI_{1\ mV}$) were used as for APB. Using this stimulation site, resting amplitudes recorded from the ADM (0.4 ± 0.3 mV) were always smaller than those of the APB.

Excitability was probed for each muscle by collecting 20 trials before and after interventional paired stimulation.

Studies of resting motor thresholds

In three subjects, RMTs were assessed for the APB muscle as described above, before and after interventional paired stimulation with an ISI of 25 ms. Additionally, resting amplitudes (20 trials before and 20 trials after intervention) were also determined in the same experiments.

F-wave studies and electrical brainstem stimulation

In seven subjects, changes in the resting amplitudes of TMS-evoked MEPs following interventional paired stimulation were compared with changes in the size of F waves evoked in the relaxed APB by supramaximal electrical stimulation of the median nerve at the wrist before and after interventional paired stimulation with a 25 ms ISI. Ten to 20 F waves were recorded before and after interventional paired stimulation. Experiments were taken into consideration if the M waves elicited by peripheral nerve stimulation after the intervention were within 95–105% of the preinterventional value, suggesting stability of the excitability of the peripheral nerve.

In two subjects, electrical brainstem stimulation was performed using the method described by Ugawa and co-workers (Ugawa *et al.,* 1991). The anode (right) and cathode

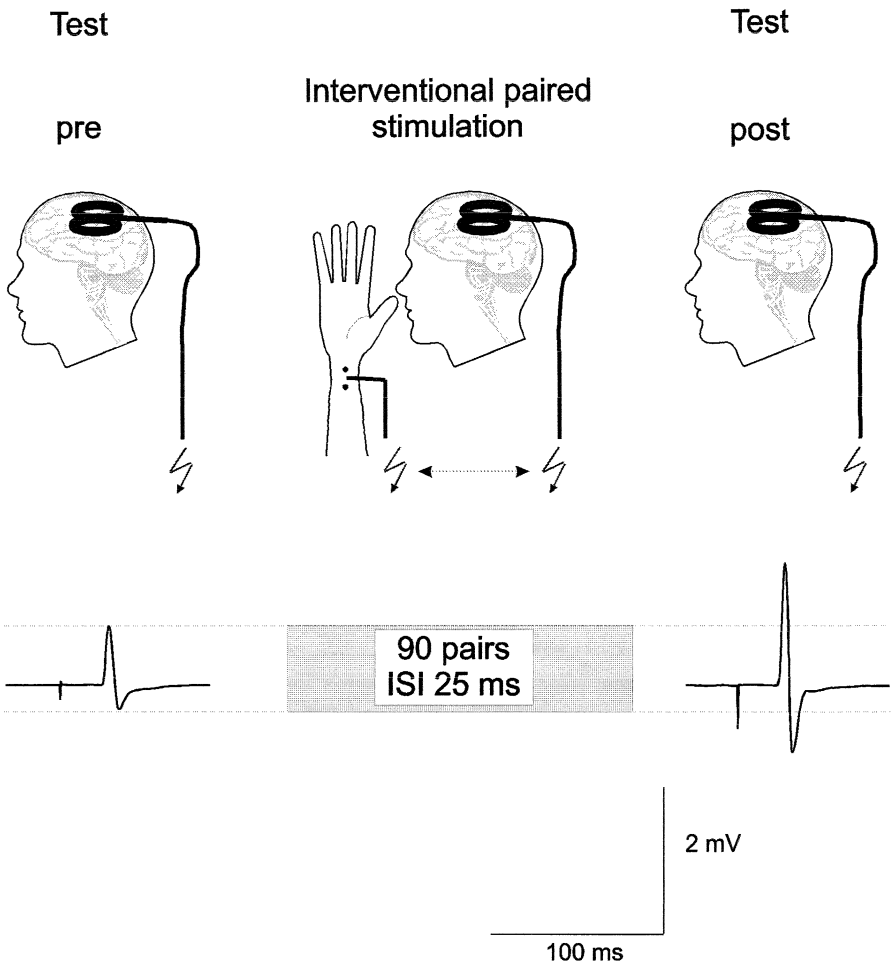

Fig. 1 Experimental design. (*Top*) Test amplitudes were elicited by single-pulse TMS before and after the intervention. During interventional stimulation, 90 pairs of stimuli, consisting of electrical stimuli delivered to the right median nerve followed by TMS over the left hemisphere at the optimal site for activating the APB muscle, were applied using a constant interstimulus interval and an interpair interval of 20 s (0.05 Hz). (*Bottom*) Effect of interventional paired stimulation with an ISI of 25 ms on the MEP of the right resting APB. Example of one subject. Each record shows the average of 20 trials.

(left) were attached to the skin overlying the mastoids. Stimulus intensity was set to produce an MEP amplitude of at least 0.5–1.0 mV in the resting APB. For both subjects, stimulus intensities were 50% of the maximal electrical stimulator output using a stimulus width of 100 μs. Magnetic stimulation was performed at $SI_{1\ mV}$. Twenty TMS stimuli and eight electrical brainstem stimuli were delivered before and after an interventional paired stimulation at an ISI of 25 ms. The TMS was randomly intermixed with brainstem stimulation to ensure that the subjects were not able to predict the modality of the stimulus about to be delivered.

Data analysis

MEP amplitudes evoked at rest (resting amplitudes) or 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 reoccurrence of voluntary EMG activity. Results were confirmed by a second investigator who was blind to the condition tested (preintervention versus postintervention). The results of both investigators were highly ($r = 0.98$) and significantly ($P < 0.001$) correlated. For each subject, resting amplitudes, active amplitudes and silent period durations were averaged separately for the time periods before and after intervention, and entered into the final statistical analyses.

For statistical analysis, repeated measures ANOVA (analysis of variance) was employed. The factors tested are explained in more detail in the Results section. If not stated otherwise, two-tailed paired *t* tests were used for *post hoc* analysis. In the experiments testing digital nerve stimulation, single-tailed *t* tests were used because an a priori hypothesis could be made about the direction of the change (increase

in resting amplitudes). Effects were considered significant if $P < 0.05$.

If not stated otherwise, all group data are given as mean ± standard deviation.

Results

In the principal experiment, the question addressed was whether repetitive stimulation of the motor cortex via two independent routes arriving synchronously at the cortical output elements is capable of inducing a lasting change in cortical excitability. One route of stimulation was electrical stimulation of the median nerve at the wrist; the other was TMS applied directly over the contralateral motor cortex. Because it takes ~20 ms for an afferent signal arising from the median nerve at the level of the wrist to travel to the somatosensory cortex and ~3 ms for it to travel from the somatosensory cortex to the motor cortex, we assumed that, in this setup, effects from both sources would be approximately synchronous at the motor cortex if median nerve stimulation at the wrist was followed by TMS at an interval of 25 ms. When all experimental sessions were considered (a total of 40 experimental sessions on 22 volunteers), paired stimulation at 25 ms ISI led to an increase in resting amplitude from a mean of 1.1 ± 0.3 mV to 1.7 ± 0.8 mV ($P < 0.001$), or, on average, by 55%. Resting amplitude increased in all but two experimental sessions (95%). The percentage increase varied between subjects and between sessions, and ranged from +5 to +185% of the baseline value. In about two-thirds (63%) of all experimental sessions the increase was at least 30%. An example of this effect is illustrated in Fig. 1.

Influence of ISI of interventional stimulation

To examine whether the increase in excitability was related to the relative timing of the two modes of stimulation, or, in other words, depended on their synchronous arrival at the motor cortex, the interval between the interventional stimulation was varied systematically from 25 to 5000 ms in seven subjects. In addition to resting amplitudes, active amplitudes and the duration of the silent period were obtained. In this experiment, $SI_{1\ mV}$ was 42.2 ± 5.3% of the maximal stimulator output and $SI_{160\ ms}$ was 49.1 ± 6.5% of the maximal stimulator output. Results are displayed in Fig. 2. For each of the three parameters (resting amplitude, active amplitude, silent period), a repeated measures ANOVA [period(pre, post) × ISI(25, 100, 525, 5000] was performed. We found a significant period × ISI interaction for resting amplitude ($F = 9.5$, $P < 0.001$) and silent period ($F = 3.3$, $P < 0.05$), suggesting significant effects of period depending on the ISI. No significant main or interaction effects were found for active amplitude. A *post hoc* analysis of resting amplitude was then performed for each ISI separately. At 25 ms ISI, the resting amplitude increased from a baseline of 1.1 ± 0.3 to 2.0 ± 0.9 mV after intervention ($P < 0.02$), corresponding to a mean increase of 77% when compared with the baseline value. Comparison between baseline and postintervention resting amplitudes did not yield any significant results for any of the other ISIs tested. Similarly to resting amplitude, *post hoc* testing yielded significant differences between the duration of the silent period at baseline and that measured after intervention exclusively at 25 ms ISI (preintervention, 165 ± 6 ms; postintervention, 183 ± 14 ms; $P < 0.01$). Thus, the effects on resting amplitude and silent period were both specific for the intervention using the shortest ISI resulting in synchronous arrival of afferent and direct stimulation at the motor cortex. In three subjects, the effect of a 35 ms ISI was tested outside the above series in which the ISI was varied systematically. Resting amplitudes increased by 48 ± 60% and silent period durations increased by 11 ± 5 ms.

Duration

In 11 subjects, the duration of the increase in the resting amplitude was examined by delivering probing TMS pulses for a period of at least 30 min following the intervention. During this time, the resting amplitudes remained elevated in all subjects (Fig. 3A). Resting amplitudes following intervention were binned in epochs of duration 5 min. Including the preinterventional epoch, consisting of 20 consecutive trials, this resulted in seven epochs (one before and six after intervention). A repeated measures ANOVA was performed on the binned data and revealed a significant effect for epoch (0–6) ($F = 5.1$, $P < 0.001$). Explorative *post hoc t*-testing, performed between all pairs of epochs, showed significant differences when the preinterventional epoch was tested against any of the postinterventional epochs. Differences between any pair of postinterventional epochs, however, were insignificant (Fig. 3A).

In two of the above subjects, resting amplitudes were obtained for a period of 60 min following intervention, and, in one of them, again after 24 h. The resting amplitudes continued to be increased for the entire period of 60 min in both subjects, and returned to baseline levels in the one subject retested at 24 h (Fig. 3B). One could argue that the duration of the effect of paired interventional stimulation could be prolonged by sustained (probing) TMS. This question was addressed in one subject in whom the first probing TMS shocks were delivered no earlier than 15 min after completion of the intervention. In this subject mean resting amplitudes increased by 19% of the baseline (not illustrated). This 19% increase fell between the 25th and 50th percentile, as determined in 11 subjects at the 15–20 min postintervention period when measured continuously and immediately after the intervention. This finding does not support the view that probing TMS had any substantial effect of its own even after interventional paired stimulation.

Mixed nerve versus digital nerve stimulation

Mixed nerve stimulation excites afferents from muscle spindles in addition to afferents from mechanoreceptors,

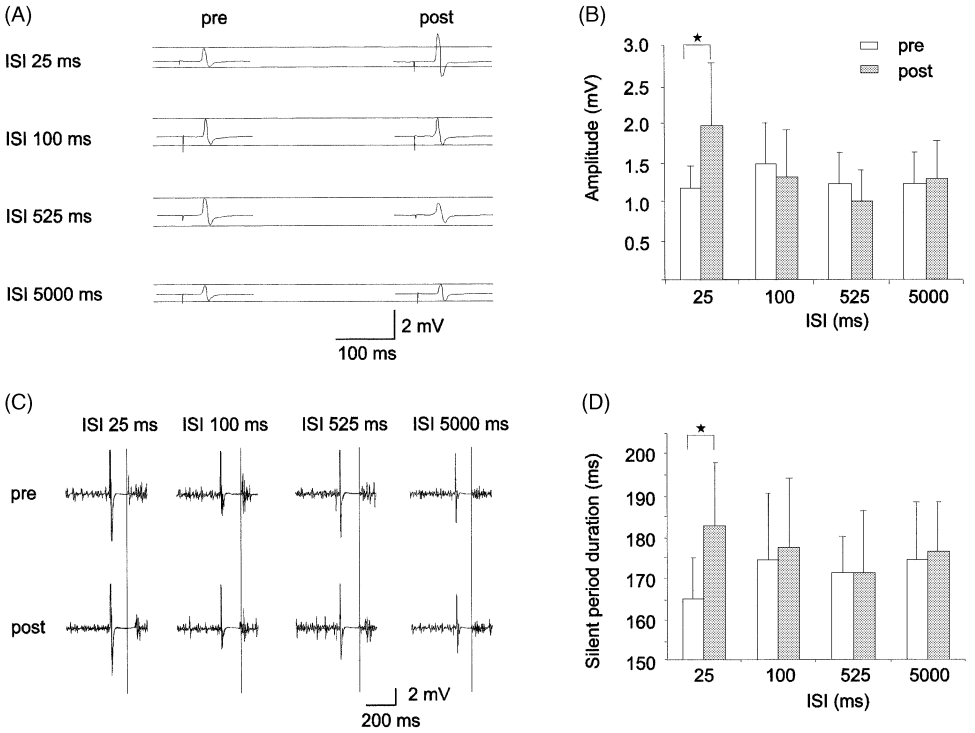

Fig. 2 Effect of different intervals between afferent conditioning and magnetic stimulation over the motor cortex during interventional paired stimulation on the magnitude of resting amplitudes (**A** and **B**) and duration of silent periods (**C** and **D**) recorded from the APB muscle. Recordings were obtained before (pre) and after (post) interventional paired stimulation. (**A**) Representative example of one subject. Recordings show averages of 20 trials. (**B**) Results for seven subjects. Resting amplitudes before (open columns) and after (shaded columns) interventional paired stimulation. Note significant increase in resting amplitudes when using an ISI of 25 ms between the afferent conditioning stimulus and TMS (asterisk; $P < 0.05$). Data show mean ± standard deviation. (**C**) Duration of silent period and size of active amplitudes before and after interventional stimulation. Representative example of one subject. Superimposition of five representative trials. Vertical lines indicate the end of silent period as determined in the baseline trials. (**D**) Results for seven subjects. Symbols and conventions as in **B**.

whereas afferents from the digital nerves are predominantly cutaneous. Therefore, data obtained from intervention using electrical stimulation of the digital nerves of the index finger were compared with data from median nerve stimulation in the same subjects in order to learn more about the role of different types of afferents in producing plasticity through interventional paired stimulation. Following interventional paired stimulation performed with digital nerve stimulation, resting amplitudes increased somewhat less (preintervention, 1.2 ± 0.1 mV; postintervention, 1.7 ± 0.6 mV; $P < 0.05$; single-tailed paired t test) than with mixed median nerve stimulation (for the same subjects, preintervention, 1. 1 ± 0.3 mV; postintervention, 1.9 ± 0.9 mV). Similarly, using digital nerve stimulation, the increase in silent period duration (preintervention, 170 ± 14 ms; postintervention, 181 ± 18 ms; $P < 0.05$; single-tailed paired t test) was somewhat smaller when compared with that of mixed nerve stimulation in the same subjects (preintervention, 164 ± 7 ms; postintervention, 181 ± 15 ms). However, there was no significant difference between the increase with digital nerve stimulation and mixed nerve stimulation for either resting amplitudes or silent period durations (Fig. 4).

Somatotopic gradient

The topographic specificity of induced plasticity was examined by comparing the effects of an interventional paired stimulation on representations of different target muscles. In addition to the right APB muscle (contralateral to the interventional cortex stimulation; $n = 7$ subjects), resting amplitudes were determined in the right ADM ($n = 6$) and BB ($n = 6$) and in the left APB ($n = 7$) in the same experimental session. The amplitudes of the different target muscles were normalized to baseline. A repeated measures ANOVA [muscle(APB, ADM, BB, APBi)] revealed a significant effect of muscle ($F = 6.5$, $P < 0.01$). *Post hoc* contrasts were calculated between APB and each of the other three target muscles using two-tailed paired t tests. There was no significant difference between the increase in resting amplitudes in the APB (+75%, compared with baseline) and ADM (+44%). Resting amplitudes increased more in the APB than in the BB (+15%) ($P < 0.05$). Resting amplitudes of the TA increased by 12% in the single subject in whom the TA was measured (in this subject, changes in resting amplitude in the other muscles were as follows: APB, +91%; ADM, not done; BB, –46%; APBi, –26%). The increase for

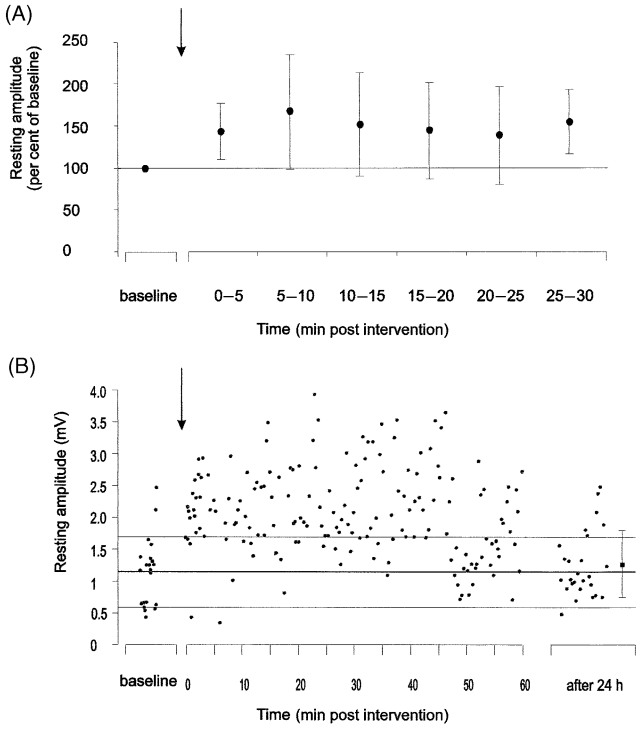

Fig. 3 Duration of increase in MEP size induced by interventional paired stimulation. (**A**) Following intervention, data were binned in epochs of 5 min duration. Mean ± standard deviation for 11 subjects. Postintervention resting amplitudes were significantly different from baseline for each of the six epochs (0–30 min). (**B**) Example of one subject. Note persistence of increased excitability for 60 min and return to baseline 24 h after intervention. The thick horizontal line designates the mean resting amplitude of trials recorded before intervention (baseline). The thin lines above and below the thick line show one standard deviation with respect to baseline data. The filled square and the error bars show mean ± standard deviation of resting amplitudes obtained after 24 h.

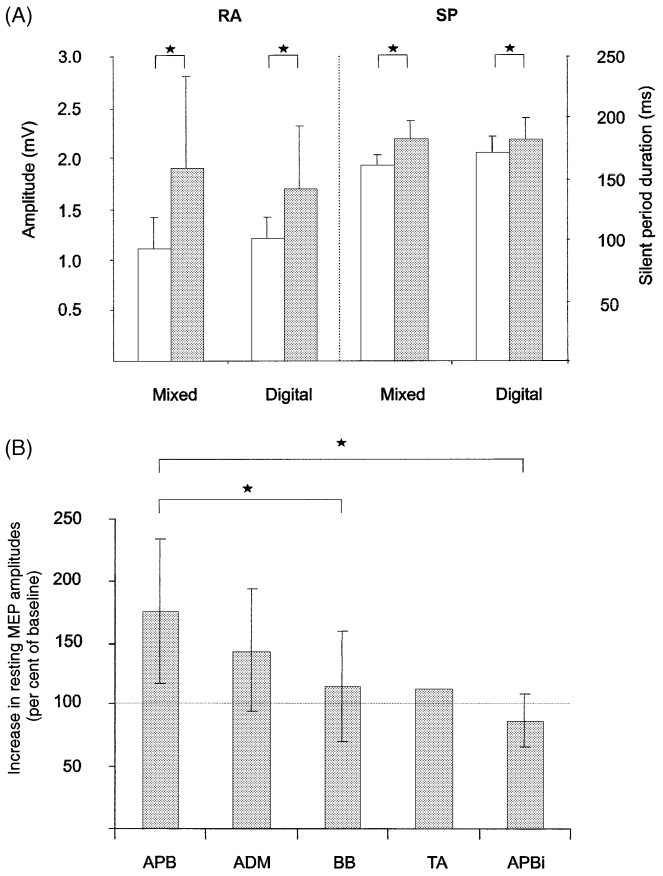

Fig. 4 (**A**) Comparison of digital nerve stimulation and (mixed) median nerve stimulation at the wrist plus TMS in the same subjects ($n = 8$) on increase in resting amplitudes (**A**) and silent period duration (**B**). Both resting amplitudes and silent period duration also increased after digital nerve stimulation ($P < 0.05$, single-tailed t test). Open columns, before intervention; shaded columns, after intervention. Asterisks indicate significant differences between preintervention and postintervention using single-tailed (digital nerve stimulation) and two-tailed (mixed nerve stimulation) paired t tests ($P < 0.05$). (**B**) Somatotopical specificity of increase in resting MEP amplitudes. The increase in resting amplitudes was maximal in the APB contralateral to cortex stimulation ($n = 7$), less pronounced in the ADM ($n = 6$), and minimal in the BB ($n = 6$) and in the TA ($n = 1$). The APB ipsilateral ($n = 7$) to cortex stimulation showed a slight decrease. Asterisks indicate significant differences from the resting amplitude increase in the APB (paired t test; $P < 0.05$).

APB was significantly larger than that for APBi ($P < 0.01$), which showed a slight decrease (–12%). Results are summarized in Fig. 5.

Resting motor thresholds

In the three subjects, resting amplitudes increased by 52 ± 36% after intervention. RMTs assessed before and after intervention remained constant (preintervention, 29 ± 1% of maximal stimulator output; postintervention, 29 ± 1% of maximal stimulator output; not illustrated).

Laminar site within the central nervous system

Two approaches were used to test the hypothesis that the increase in resting amplitudes was due to supraspinal, and indeed cortical, changes.

In one series of experiments, the effect of an interventional stimulation on TMS-evoked MEP amplitudes was compared with the effect on F-wave amplitudes. The resting amplitudes of MEPs elicited by TMS showed a significant increase following intervention (preintervention, 1.1 ± 0.5 mV; postintervention, 1.4 ± 0.7 mV; $P < 0.05$). By contrast, F waves elicited by median nerve stimulation remained unchanged (preintervention, 184 ± 50 μV; postintervention, 200 ± 100 μV; not significant) (Fig. 5A and B). Stability in the efficacy of peripheral nerve stimulation was maintained, as suggested by the fact that the magnitude of M-wave responses (preintervention, 9.4 ± 3.5 mV; postintervention, 9.4 ± 3.4 mV; not significant) (Fig. 5B) remained unchanged after intervention. Because F waves reflect the excitability of only a subportion of the spinal motor neuron pool, we additionally employed electrical brainstem stimulation. Using brainstem stimulation, the descending corticospinal tract is

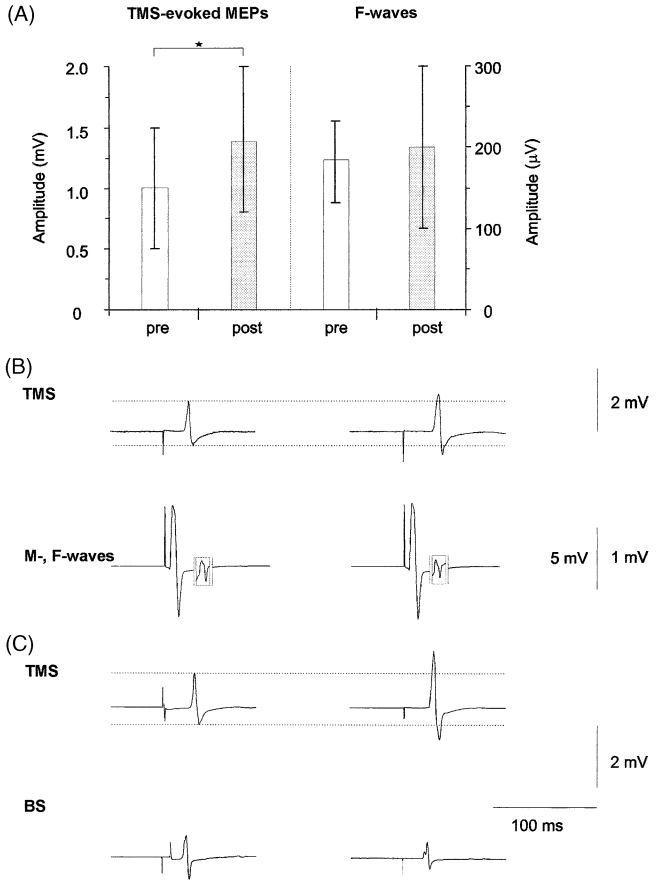

Fig. 5 Laminar site within the central nervous system. (**A**) Following interventional paired stimulation, resting amplitudes increased in the APB (left panel) while the size of F waves elicited in the APB remained constant (right panel). Data from seven subjects. Symbols and conventions as in Fig. 2B. (**B**) Comparison of TMS-evoked resting amplitudes, and of M waves and F waves elicited by peripheral nerve stimulation in a representative subject. Recordings show averages of 20 trials (TMS) or 10 trials (F waves). Vertical broken lines indicate timing of the stimulation, as shown by the stimulation artefact. Following interventional paired stimulation TMS-evoked resting amplitudes increased (upper traces) whereas F waves (insets, lower traces) did not. M-wave responses (lower traces) remained constant, suggesting stability of the efficacy of peripheral nerve stimulation. The number on the left of the lower calibration bar refers to M waves and that on the right refers to the F waves, as displayed in the inset. (**C**) Comparison of TMS-evoked and brainstem stimulation (BS)-evoked resting amplitudes. Recordings show averages of 20 trials (TMS) or eight trials (brainstem stimulation). Vertical broken lines indicate timing of the stimulation, as shown by the stimulation artefact. (Additional artefact at 10 ms in the lower left panel arose from discharge of an unrelated remote electrical circuit.) Following interventional paired stimulation, TMS-evoked resting amplitudes increased (upper traces), whereas brainstem stimulation-evoked resting amplitudes (lower traces) did not.

excited directly at the level of the foramen magnum distal to the influence of intracortical interneurons (Ugawa *et al*., 1991). In one subject, there was no significant effect on resting amplitudes elicited by either TMS or brainstem stimulation, possibly due to stress, which is known to interfere with processes mediating plasticity (Foy *et al*., 1987; Shors *et al*., 1990). In the other subject, interventional paired stimulation led to a pronounced increase in the TMS-evoked resting amplitude (preintervention, 1.7 ± 1.0 mV; postintervention, 3.0 ± 0.9 mV; $P < 0.05$). By contrast, resting amplitudes of MEPs evoked by brainstem stimulation remained essentially unchanged (preintervention, 1.3 ± 0.8 mV; postintervention, 0.9 ± 0.5 mV; not significant) (Fig. 5C).

Discussion

The present results have shown that low-frequency TMS over the primary motor cortex, if paired with peripheral stimulation of somatosensory afferents, may induce plastic changes in the human motor system.

Cortical site

The focus of attention in investigations of plasticity in the nervous system has been the cortex. However, it is well recognized that the nervous system can undergo plastic changes at multiple levels, and the importance of subcortical levels contributing to neuronal plasticity has been re-emphasized recently (e.g. Florence and Kaas, 1995; Jones and Pons, 1998; Nicolelis *et al*., 1998). In our paradigm, three lines of experimental evidence support the view that the observed plastic changes are located in the cortex. (i) The magnitude of F waves elicited by median nerve stimulation in the APB muscle tests the excitability of a portion of α-motor neurons of the median nerve. At least for this portion, excitability remained unchanged after intervention in the presence of significantly increased TMS-evoked MEPs. (ii) A similar dichotomy was observed when comparing the effect of an intervention on brainstem stimulation-evoked MEPs with that on TMS-evoked MEPs. In one subject, resting amplitudes increased after TMS but remained unchanged following brainstem stimulation. The available evidence suggests that TMS excites the cortical output elements trans-synaptically (Rothwell, 1997), whereas brainstem stimulation tests the excitability of the corticomuscular system downstream of the cortex (Ugawa *et al*., 1991). (iii) The TMS-induced silent period is thought to be generated cortically, at least in its later part (Hallett, 1995). Hence, a cortical site of any plastic changes (but not necessarily those underlying the increase in resting amplitude) can be directly derived from the observation that paired stimulation induced prolongation of the TMS-evoked silent period.

General properties—implications for a candidate mechanism

The stimulation conditions tested in the present report were chosen to match the principles of experimental design in animal studies in which motor cortical plasticity was induced by paired stimulation. TMS probably activates intracortical

fibres travelling horizontally with respect to the surface of the cortex (Rothwell, 1997) and eventually leads to the activation of postsynaptic pyramidal output cells that are, either directly (Porter, 1996) or after a synapse in cortical layer II/III (Kaneko *et al*., 1994), also the target of afferent fibres which originate in subcortical and cortical regions. Although the exact route is still under debate, somatosensory information such as that induced by median nerve stimulation probably reaches the motor cortex via corticocortical fibres at short latencies from the somatosensory cortex after a relay in the ventrolateral thalamus, or via thalamocortical fibres from the thalamus (Porter and Lemon, 1995), although the significance of the latter projection is not universally accepted (Jones, 1986). Stimulation of afferent fibres from the somatosensory cortex has been shown to produce both excitation and inhibition of the motor cortical output cells (Porter *et al*., 1990). LTP has been produced in cortical slices, as well as in intact anaesthetized experimental animals, by pairing stimulation of cortical afferents with depolarization (Baranyi and Szente, 1987) or stimulation-induced firing of the postsynaptic cell (Baranyi and Feher, 1981), and by pairing stimulation of 'vertical' (thalamocortical as well as corticocortical fibres) with stimulation of 'horizontal' intracortical fibres in cortical layers II/III (Hess and Donoghue, 1994; Hess *et al*., 1996). This type of LTP has also been demonstrated in the awake cat (Baranyi *et al*., 1991) and was termed 'associative' because of the requirement for conjoint stimulation of different pathways or targets.

As will be outlined below in more detail, the plasticity induced by paired stimulation in our study displayed a number of remarkable properties: it evolved rapidly, was persistent, yet reversible, depended on the exact timing of each stimulation modality with reference to the other modality, and was topographically specific. This combination of features renders a general change in excitability due to conditions not experimentally controlled highly unlikely. It is this combination of features and their similarity with properties of induced enduring changes of synaptic efficacy, as elucidated in the animal studies cited above, that leads us to propose that they may represent a signature of associative LTP or closely related neuronal mechanisms in the human cortex.

Rapid evolution, persistence, reversibility

The plasticity induced by paired stimulation lasted for a minimum of 30–60 min. This rather long persistence excludes short-term potentiation-like mechanisms, defined as changes in synaptic efficacy of duration <30 min, and post-tetanic potentiation, which lasts only seconds to a few minutes. Remarkably few stimulus pairs were necessary to induce this persistent change. In our paradigm, the plastic effect was already present after 30 min of intervention or after only 90 stimulus pairs. The duration of the changes, as well as the high efficacy of the interventional stimulation, is consistent with an LTP-like phenomenon. LTP, by definition, lasts >30 min. Furthermore, under some conditions induction of LTP is known to require only a few stimuli, to the extent that even as little as a single conditioning stimulus may suffice (e.g. Maren *et al*., 1994). The effect was reversible after 24 h. Although some of the structural changes associated with plasticity are known to develop rapidly (Chang *et al*., 1993; Engert and Bonhoeffer, 1999), their remarkably fast evolution, together with their reversibility within hours, makes it highly unlikely that structural changes, e.g. synaptogenesis and the sprouting of intracortical fibres, are the responsible mechanisms underlying the present plasticity.

Dependence on synchronicity

The change in resting amplitudes induced by TMS was conditionally dependent upon temporal contingency with electrical stimulation of peripheral afferents. At ISIs of duration $\geqslant$100 ms, no enhancement of excitability followed the interventional paired stimulation. However, when TMS was timed to be delivered to the motor cortex approximately synchronously with the arrival of the afferent signals at the motor cortex, resting amplitudes increased markedly. Apparently, exact synchronicity of events was not necessary, because plasticity was also induced by pairing stimuli at 35 instead of 25 ms ISI. This finding is in agreement with the observations of Baranyi and Feher. They noted that, in order for paired stimulation to be effective in producing LTP, the interval between the preceding conditioning stimulus and the following test stimulus (which caused the postsynaptic neuron to fire an action potential) must not exceed 40–60 ms (Baranyi and Feher, 1981). Recently, Markram and colleagues provided experimental evidence for a model of how synchronous neural events distributed vertically across cortical layers could modulate synaptic efficacy (Markram *et al*., 1997). These authors studied the size of the excitatory postsynaptic potentials (EPSPs) that were evoked in a postsynaptic neuron located in cortical layer V, the output layer of the motor cortex, by inducing an action potential in a presynaptic neuron. A lasting enhancement of the size of EPSPs was noted if, in addition to eliciting the postsynaptic EPSPs, the postsynaptic neuron was sufficiently depolarized by current injection to fire an action potential backpropagating into its dendritic tree. Importantly, this effect depended on the timing between the two events. It was present if the action potential in the postsynaptic neuron followed the occurrence of the EPSPs within 10 ms, whereas it was absent at an interval of 100 ms.

Topographical specificity

As outlined above, peripheral electrical stimulation provides a short-latency input to the primary motor cortex via afferents from the somatosensory cortex. This pathway comprises signals of all somatosensory qualities (Porter and Lemon, 1995). A part of the projection from the somatosensory cortex to the primary motor cortex is organized so that it exhibits high topographical specificity by connecting

homologous somatosensory and motor areas (Rosén and Asanuma, 1972; Caria *et al*., 1997). Therefore, TMS over the optimal site for stimulating the APB was also directed at the cortical region that presumably received the maximal peripheral afferent input. Hence, it may not be surprising that the maximal effect was noted for excitability of the APB. However, it is important to note that there was no evidence that the effect carried over to cortical regions not receiving dual and synchronous input by TMS and afferents. Resting amplitudes of muscles cortically represented at a distance from the target representation of the interventional stimulation did not increase (TA, APBi). Resting amplitudes in BB increased to a much lesser degree than in APB, and a small gradient was even found between increases in excitability in the APB and ADM. This finding suggests that strict topographical rules govern the induction of plasticity generated by interventional paired stimulation. This conclusion is important with respect to the mechanisms underlying the present effect, because in the motor cortex (Hess *et al*., 1996), as well as in the visual cortex (Kirkwood *et al*., 1993), LTP develops in conditioned but not in nearby pathways, whereas this specificity is not a necessary property of LTP induced in the hippocampus (Bonhoeffer *et al*., 1989; Schuman and Madison, 1994).

As mentioned above, signals originating from the somatosensory cortex and arriving at the primary motor cortex at short latencies carry information about all somatosensory qualities (Porter and Lemon, 1995). Because muscle spindles are of fundamental importance for motor control and, probably, in particular for motor plasticity (Hulliger, 1993), one may hypothesize that plasticity induced by paired stimulation would depend strongly on the stimulation of muscle spindle afferents. However, resting amplitudes or silent period durations increased not only with mixed nerve stimulation but also with digital nerve stimulation, which excites predominantly afferents from mechanoreceptors but not from muscle spindles. This result could indicate that cutaneous afferents were the principal afferent route mediating paired stimulation-induced plasticity in the absence of voluntary contraction. The results obtained by Kunesch and co-workers suggest a weak afferent signal from muscle spindles at rest. These authors failed to elicit somatosensory evoked potentials at rest when stimulating muscle spindle afferents by intraneural microstimulation, whereas stimulation of the afferents of mechanoreceptors evoked large somatosensory potentials (Kunesch *et al*., 1995). Because transmission of muscle spindle afferents to the cortex is known to be facilitated by pyramidal tract activity (Tsumoto *et al*., 1975), it may well be that the relative importances of the different qualities of afferents for motor cortical plasticity change with the state of motor activity.

Although suggestive, the evidence for associative LTP as a key mechanism in our paradigm is circumstantial. Furthermore, even if associative LTP were operative, it may not be the only mechanism. Motor cortex plasticity may also be induced by changes in membrane excitability, as has been demonstrated in experiments studying conditional learning (Woody and Engel, 1972; Aou *et al*., 1992). Because some of this plasticity may be highly specific, it is important to consider the possibility that it could underlie the present phenomena. In TMS studies, RMT has been proposed to represent a marker of membrane excitability in the pyramidal output cells, because it is relatively insensitive to pharmacological manipulations involving neurotransmission but not to those changing ionic membrane conductance (Mavroudakis *et al*., 1994; Ziemann *et al*., 1996). Although non-trivial additional assumptions are necessary in order to reconcile this concept with the presumed trans-synaptic mode of activation of pyramidal output cells by TMS, it has been found to be of heuristic and practical value in subsequent studies (Ziemann *et al*., 1998*a*; Werhahn *et al*., 1999). In our paradigm, RMTs remained unchanged following the intervention, suggesting that changes in membrane excitability, at least of those pyramidal cells activated by TMS at threshold intensity, did not substantially contribute to the increase in resting amplitudes.

Relationship to previous interventional stimulation studies

Our results may be compared with those of previous studies demonstrating stimulation-induced enhancement of excitability of the corticomuscular system (Hamdy *et al*., 1997; Ziemann *et al*., 1998*a*). These studies both suggested that changes occur at a cortical level, and in both studies the duration of excitability changes was sufficiently long to suggest involvement of LTP-like phenomena. Conditional on experimental ischaemia of the forearm, TMS applied at 0.1 Hz over the optimal site for activating the BB muscle was capable of inducing a persistent enhancement of MEPs recorded from the BB and a decrease in intracortical inhibition within the BB representation (Ziemann *et al*., 1998*a*). The authors speculated that removal of afferent input from the forearm would lead to subliminal depolarization of motor neurons controlling proximal arm muscles. 'Horizontal' input, activated by TMS and targeting the depolarized cells, would then undergo synaptic modifications, leading to sustained facilitation of the input to pyramidal output neurons controlling BB. An *N*-methyl-D-aspartate (NMDA) receptor antagonist blocked the long-lasting change in intracortical inhibition in the BB and was therefore likely mediated by LTP-like mechanisms (Ziemann *et al*., 1998*b*).

Apparently at odds with the requirement for synchronous afferent and TMS-mediated activation of the motor cortex in the present report, Hamdy and co-workers have shown that repetitive afferent stimulation may also produce a sustained increase in excitability in the homologous motor cortex area when applied on its own (Hamdy *et al*., 1998). High-frequency (10 Hz) pharyngeal stimulation over a period of 10 min induced a lasting (30–60 min) increase in excitability in the cortical representation of the pharyngeal muscles, as

tested by TMS, and was accompanied by a decrease in oesophageal response amplitudes. The mechanism(s) by which this form of plasticity is induced are unknown, but it is conceivable that LTP-like processes were active. Most notably, in this paradigm, the facilitation was seen in the (horizontal) pathways probed by TMS, which presumably were not explicitly activated by afferent stimulation. Therefore, the principle of input specificity, a characteristic of LTP in the motor cortex (see above; and Hess *et al*., 1996), would be violated, unless concurrent activation of horizontal fibres was postulated. Indeed, in the paradigm of Hamdy and co-workers (Hamdy *et al.,* 1998), activation of horizontal pathways within the central representation of pharyngeal muscles is likely to have been present for the following reasons: (i) because high-frequency pharyngeal stimulation is unpleasant, subjects will probably have activated muscles adjacent to the stimulation site; and (ii) a highly synchronized afferent volley may arrive at the motor cortex at a time when homologous cortical output neurons are jointly depolarized or firing as part of a reflex activity (Hamdy *et al*., 1997) initiated by one of the preceding stimuli.

Whatever the exact mechanism, together with the findings presented in the current paper, the above studies strongly suggest that human motor cortex excitability is modifiable by repetitive exogenous stimulation using different routes.

Significance for motor behaviour and motor learning

The paired stimulation protocol did not involve voluntary activity in the target muscle, and even required the absence of EMG activity. Yet the effects of interventional paired stimulation that were produced at rest also had an impact on the neuronal circuitry that is activated with voluntary contraction. This is demonstrated by the fact that the duration of the silent period, which, by definition, is recorded under active conditions, was found to be prolonged following intervention. This finding may indicate that TMS activates intracortical pathways that are also active with voluntary activity. This conclusion is fully in line with evidence which showed that training in repetitive thumb movements leaves a kinematic memory trace in movements evoked by TMS in the resting thumb following training (Classen *et al*., 1998). Recent data support the view that the silent period reflects an inhibitory postsynaptic potential (IPSP) mediated by $GABA_B$ receptor activation (Werhahn *et al*., 1999). Therefore, a further implication of the prolongation of silent period duration is that interventional paired stimulation facilitates the action of inhibitory interneurons generating the IPSP. Although LTP is also known to exist for synapses of inhibitory to pyramidal cells (Buonomano and Merzenich, 1998), our finding would be just as compatible with facilitated excitatory input to the inhibitory interneuron.

The current findings lend further support to the models of plasticity that have been proposed to underlie motor learning (Donoghue *et al*., 1996; Asanuma and Pavlides, 1997). Ablation studies conducted in animals suggest that afferents from the somatosensory cortex play an important role in the acquisition of new motor skills. Monkeys trained in a highly demanding motor task could retain the newly acquired skill even after removal of the somatosensory cortex. However, when the somatosensory cortex was removed prior to training, learning was substantially impaired (Pavlides *et al*., 1993). Drawing on these behavioural experiments and many examples of LTP generated in the motor cortex, Asanuma and Pavlides have proposed that one neuronal substrate of motor learning would be long-term enhancement of synaptic efficacy in the primary motor cortex, generated by repetitive activation of somatosensory afferents (Asanuma and Pavlides, 1997). Donoghue and colleagues suggested that the contacts made by intracortical horizontal fibres onto cortical neurons may be an ideal candidate for the site of such plastic synaptic changes within the motor cortex (Donoghue *et al*., 1996). This hypothesis was recently substantiated by experiments demonstrating the strengthening of horizontal connections occurring with behavioural evidence for the acquisition of a motor skill (Rioult-Pedotti *et al*., 1998). Our findings underline both the importance of repetitive activation of somatosensory afferents for motor cortical plasticity and a prominent role for horizontal fibres in mediating such plastic changes.

In summary, we have shown that a paradigm similar to that eliciting associative LTP in animal models can result in long-lasting, LTP-like plasticity in the human motor cortex. This finding may have significance for understanding motor learning and for rehabilitation after brain injury.

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