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Remote changes in cortical excitability after stroke

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Summary

Changes in the cerebral metabolism and the excitability of brain areas remote from an ischaemic brain lesion have been reported in animals and humans and implicated as a mechanism relevant to functional recovery. The aim of the present study was to determine whether changes in the inhibitory and excitatory activity in motor cortex of the non-affected hemisphere are present in stroke patients, and whether these changes are related to the extent of the patients' recovery of function. Transcranial magnetic stimulation (TMS) was used to study the first dorsal interosseus muscle (FDI) of the non-affected hand in 13 patients with good recovery of hand function after stroke, and was compared with left hemispheric stimulation in 13 healthy age-matched volunteers. In the first experiment, paired-pulse TMS with the conditioning stimulus (CS) set at 80% of the subject's motor threshold (MT) and interstimulus intervals (ISIs) of 2, 3, 10 and 15 ms was used. In the second experiment, different intensities of CS were used to study its inhibitory effect on a succeeding suprathreshold test stimulus at an ISI that was kept constant at 2 ms. In a third experiment, the rise in motor evoked potential (MEP) amplitudes with increasing stimulus intensities was measured. In two additional control experiments, the effect of left versus right hemispheric stimulation in normal volunteers and good versus poor recovery of hand function in patients after stroke on the excitability of inhibitory and excitatory activity was studied. MT, mean test MEP and recruitment curves were similar in patients and healthy volunteers. In those patients with good recovery, paired-pulse excitability was increased at ISIs of 2 and 3 ms, similar to healthy volunteers at ISIs of 10 and 15 ms. When tested with different CS intensities at an ISI of 2 ms, inhibitory activity was similar in patients and healthy subjects at small CS intensities, but faded rapidly at higher CS intensities in patients. In contrast, in patients with poor recovery, this increase in cortical excitability at higher CS intensities was not seen. The similarity of MT, mean test MEP and recruitment curves in patients and healthy volunteers indicates that the overall cortico-motoneuronal excitability has not changed in patients. The similarity of the inhibitory effect at low CS intensities in the patients with good recovery and healthy subjects, and the steeper increase of conditioned MEP amplitude at higher CS intensities in the recovering patients suggest that in the patients' contralesional motor cortex the balance of excitatory and inhibitory activity was shifted towards an increase of excitatory activity in the neuronal circuits tested at ISIs of 2 and 3 ms. This shares similarities to mechanisms implicated as relevant for reorganizational processes after experimental brain injury and may be relevant for functional recovery after stroke. The absence of changes in cortical excitability in patients with poor recovery supports the relevance of our findings for recovery.

Keywords: brain infarction; diaschisis; GABA-related inhibition; human motor cortex; transcranial magnetic stimulation

Abbreviations: CS = conditioning stimulus; FDI = first dorsal interosseus muscle; ICI = intracortical inhibition; ISI = interstimulus interval; M1 = primary motor cortex; MEP = motor evoked potential; MT = motor threshold; TMS = transcranial magnetic stimulation

Introduction

During the past two decades, neuroanatomical and neurophysiological studies in animals, and neurophysiological and neuroimaging studies in humans have demonstrated that the adult brain is capable of extensive functional recovery. In the motor cortex, representation can reorganize rapidly in response to different stimuli, such as peripheral nerve lesions (Sanes *et al*., 1988; Donoghue *et al*., 1990), ischaemic nerve block (Brasil-Neto *et al*., 1993) or motor performance (Nudo

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Table 1 *Patient characteristics*

Age	Gender	Handedness	Motricity Index*	Site of lesion
Patients with good recovery of hand function (Experiments 1–3)				
50	M	R	100	Left MCA infarction (cortical)
37	F	R	76	Dissection of left ICA with left MCA infarction (cortical involvement)
41	F	R	92	Left MCA infarction due to embolus (cortical and subcortical involvement)
64	M	R	76	Right MCA and ACA infarction (cortical and subcortical involvement)
62	M	L	72	Right MCA infarction (cortical and subcortical involvement)
62	M	R	76	Left MCA infarction (cortical and subcortical involvement) due to CEA of left ICA
76	F	R	61	Right MCA infarction (subcortical involvement)
65	M	R	76	Right MCA infarction (cortical involvement)
66	F	R	72	Left MCA infarction (cortical and subcortical involvement)
56	F	L	72	Right MCA infarction (subcortical involvement)
64	M	R	76	Left MCA infarction (subcortical involvment, capsula interna)
75	F	R	76	Left MCA infarction (subcortical involvement)
55	M	R	76	Right MCA infarction (subcortical involvement, capsula interna)
Patients with poor recovery of their hand function (Experiment 5)				
55	F	R	0	Right MCA infarction (cortical and subcortical involvement)
52	M	R	28	Left MCA infarction (cortical and subcortical involvement) due to dissection of left ICA
37	F	R	28	Left MCA infarction (cortical and subcortical involvement)
46	M	R	51	Left MCA and PCA infarction due to occlusion of left ICA and left VA (cortical and subcortical involvement)
52	M	R	28	Right MCA infarction due to occlusion of right ICA

*Demeurisse *et al*. (1980). ACA = anterior cerebral artery; CEA = cortical endarterectomy; ICA = internal carotid artery; MCA = middle cerebral artery; PCA = posterior cerebral artery; VA = vertebral artery.

et al., 1996; Classen *et al*., 1998; Bütefisch *et al*., 2000). The underlying mechanisms involve the unmasking of existing, but latent, horizontal connections (for a review see Sanes and Donoghue, 2000) or modulation of synaptic efficacy such as long-term potentiation (LTP) or long-term depression (LTD) (for a review see Hess *et al*., 1996). The neurotransmitter systems involved in mediating these effects include the inhibitory GABAergic system (Hess and Donoghue, 1994; Hess *et al*., 1996) as well as the excitatory glutamatergic system with activation of *N*-methyl-D-aspartate (NMDA) receptors (Hess *et al*., 1996).

Changes in the cerebral metabolism and in the excitability of brain areas remote from a lesion have been reported in animals and humans and implicated as mechanisms relevant for functional recovery (termed diaschisis; for reviews see Feeney and Baron, 1986; Andrews, 1991; Seitz *et al*., 1999). In rats, following an ischaemic lesion in the primary motor cortex (M1), long-term changes in the inhibitory and excitatory neurotransmitter systems of the homotopic cortex of the non-affected hemisphere have been described and implicated as processes relevant for functional recovery after stroke (for a review see Witte, 1998). More specifically, following a lesion to the M1, downregulation of $GABA_A$ receptor function has been described in the non-affected contralateral motor cortex (Buchkremer-Ratzmann *et al*., 1996; Schiene *et al*., 1996; Buchkremer-Ratzmann and Witte, 1997; Qü *et al*., 1998; Neumann-Haefelin and Witte, 2000). This was accompanied by upregulation of NMDA receptors (Mittmann *et al*., 1998) and facilitation of LTP induction in the perilesional area (Hagemann *et al*., 1998), suggesting that the regulation of excitatory and inhibitory neurotransmitter systems in the cerebral cortex remote from a lesion may play a role in the recovery after stroke (Nudo, 1999).

Transcranial magnetic stimulation (TMS) using the paired-pulse technique at different interstimulus intervals (ISIs) (Kujirai *et al*., 1993) allows the study of the excitatory and $GABA_A$-mediated inhibitory system of human motor cortex non-invasively (Ziemann *et al*., 1996*a*, *b*). In this paradigm, a suprathreshold test pulse is preceded by a subthreshold conditioning stimulus (CS) at different ISIs. Because inhibitory and excitatory circuits are stimulated simultaneously, varying the intensity of the CS allows the study of the

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threshold and excitability of inhibitory and excitatory activity in more detail (Schäfer *et al*., 1997; Chen *et al*., 1998; Fischer *et al*., 2002).

Here we report the results of a study designed to test the hypothesis that remote changes in intracortical excitatory and inhibitory activity such as those described in animal experiments are present in the non-affected hemisphere of patients recovering from ischaemic cerebral infarction.

Methods

The experiments were approved by the ethics committee of the Heinrich-Heine-University Düsseldorf and conducted according to the Declaration of Helsinki. All subjects studied gave their written informed consent.

Experimental design

There were three principal experiments (Experiments 1–3). In these experiments, 13 patients with good recovery of their hand function (see patient characteristics, Table 1) and 13 healthy age-matched volunteers participated. These three experiments were performed on each subject in a single session with the electrodes and coil in the same position throughout the experiments. The motor cortex of the non-affected hemisphere was studied in patients, and the left hemisphere in healthy volunteers. The motor threshold (MT) was determined at the beginning of the experiments, and the intensity of the conditioning and test pulses adjusted accordingly (see below). In the first experiment, cortical inhibition and excitation were studied using paired-pulse TMS at different ISIs and a constant conditioning pulse intensity (Kujirai *et al*., 1993). In the second experiment, a paradigm was used that is analogous to the recruitment curve (Ridding and Rothwell, 1997) where the size of an evoked response (motor evoked potential; MEP) is plotted against the intensity of the stimulus, and the threshold and the slope of this curve provide information about the excitability of the motor cortex. By varying the intensity of the CS in the paired-pulse paradigm, the threshold and excitability of inhibitory and excitatory activity can be studied (Schäfer *et al*., 1997; Chen *et al*., 1998; Fischer *et al*., 2002). In the third experiment, the increase in MEP amplitudes with increasing stimulus intensities (recruitment curve) (Ridding and Rothwell, 1997) was measured as an estimate of corticomotoneuronal excitability (Mavroudakis *et al*., 1994; Ziemann *et al*., 1996*a*). In addition to these three principal experiments, two control experiments were conducted (Experiments 4 and 5). In Experiment 4, the effect of left versus right hemispheric stimulation on the threshold and excitability of inhibitory and excitatory activity was studied in 10 healthy volunteers. In Experiment 5, changes in cortical excitability of the non-affected motor cortex was compared in patients with good and poor recovery of hand function.

Subjects

All stroke patients referred to our hospital were assessed for inclusion in the study. Thirteen patients (eight female, age 59 ± 3 years; for patient characteristics, see Table 1) fulfilled the following inclusion criteria and were included in the study: first cerebral infarction within the last month, single lesion as defined by MRI of the brain affecting the primary motor output system of the hand either at a cortical (M1) or subcortical level (internal capsule), dense paresis of the hand for >3 days after their cerebral infarction, good functional recovery of hand function as defined by the ability to perform selective movements of the fingers within the first month following the event (at the time of study), no other neurological disorder, no contraindication for TMS, no intake of CNS-active drugs, and the ability to give informed consent. Hand function was assessed by the Motricity Index, Arm section, where function is estimated on a scale from 0 to 100, with 0 being no function and 100 full function (Demeurisse *et al*., 1980). The left hemisphere was studied in six and the right hemisphere in seven patients. This was compared with the results of the left hemisphere of 13 healthy right handed age-matched volunteers (six female, age 51 ± 3.7 years). As a control for possible side to side hemispheric asymmetry, both hemispheres of 10 healthy right-handed healthy volunteers (four female, age 50 ± 5 years) were studied in a separate experiment (Experiment 4). Six of the 13 subjects reported in the principal experiments (Experiments 1–3) participated in this study. Another five patients (two female, age 48.4 ± 3.2 years; for patient characteristics, see Table 1) fulfilled all the above-mentioned inclusion criteria with the exception of the recovery of their hand function. These five patients were not able to move their hand voluntarily within the first month following the event (at the time of study). They were studied in a separate experiment (Experiment 5).The left hemisphere was studied in three and the right in two patients.

Transcranial magnetic stimulation

Surface electromyographic (EMG) (bandpass 1 Hz–1 kHz) activity was recorded from the first dorsal interosseus (FDI) muscle, using surface electrodes (11 mm diameter) in a belly-tendon montage and data acquisition set (LabVIEW ™, National Instruments Corp., USA). The raw EMG was digitized at a rate of 5 kHz and stored on a PC for off-line analysis. TMS was applied through a figure of eight-shaped coil (7 cm wing diameter) using two Magstim 200 stimulators connected via a Bistim module (Magstim Company, UK). The coil was positioned on the scalp over the motor cortex at the optimal site for stimulating the FDI muscle. The position was marked with a soft tip pen directly on the scalp. The MT of the FDI muscle was determined to the nearest 1% of the maximum stimulator output and defined as the minimum stimulus intensity to evoke an MEP of >50 μV in at least five of 10 trials (Rossini *et al*., 1994).

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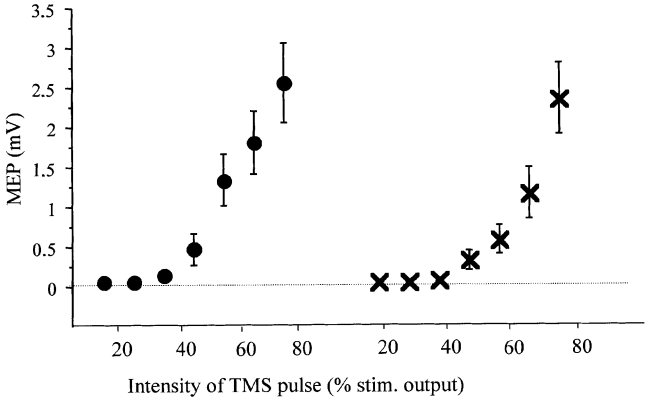

Fig. 1 Recruitment curves in normal volunteers (filled circles) and patients (crosses). Experiment 3. The intensity of the TMS pulse is expressed as a percentage of maximal stimulator output. For each stimulus intensity, the mean and SE of the corresponding MEP amplitudes is plotted. Responses were recorded in the relaxed FDI muscle.

Experiment 1

Paired stimuli were delivered at ISIs of 2, 3, 10 and 15 ms (10 pairs of stimuli for each ISI) to the motor cortex of the non-affected hemisphere in patients and to the left hemisphere in healthy volunteers. The intensity of the CS was 80% of resting MT; the intensity of the test stimulus was 110% of the resting MT. Paired pulses were intermixed with single test and conditioning pulses. The sequence of timing of stimuli was controlled by customized software. EMG was monitored at a gain of 50 μV per division to ensure complete muscle relaxation.

Experiment 2

Single test pulses, conditioning pulses (five of each) and paired pulses (five pairs) at an ISI of 2 ms were delivered to the motor cortex of the non-affected hemisphere in patients and to the left hemisphere in healthy volunteers. The ISI of 2 ms was chosen because in healthy volunteers it consistently led to intracortical inhibition (ICI) (Kujirai *et al*., 1993; Chen *et al*., 1998; Sanger *et al*., 2001) and largely avoided the I wave facilitation at higher CS intensities (Ziemann *et al*., 1998; Chen and Garg, 2000) which may obscure ICI (Awiszus *et al*., 1999). The intensity of the CS was set at 20–100% of the subject's resting MT and administered randomly at 10% increments. The intensity of the test pulse was 110% of the resting MT. Paired pulses were intermixed with single test and conditioning pulses. The sequence of timing of stimuli was controlled by customized software. EMG was monitored at a gain of 50 μV per division to ensure complete muscle relaxation.

Experiment 3

MEP recruitment was studied at stimulus intensities of 20–100% of maximal stimulator output and administered randomly at 10% increments. At each intensity, five single pulses were delivered to the appropriate scalp position.

Experiment 4

The right and left motor cortex of 10 healthy right-handed volunteers were studied in a randomized counterbalanced design. As in Experiment 2, the intensity of the CS was set at 20–100% of the subject's resting MT and administered randomly at 10% increments while the intensity of the test pulse was kept constant at 110% of the resting MT. Paired pulses were intermixed with single test and conditioning pulses.

Experiment 5

Cortical excitability was studied in the non-affected hemisphere of five patients with poor recovery of hand function. Similarly to Experiment 2, the intensity of the CS was set at 30–100% of the subject's resting MT and administered randomly at 10% increments while the intensity of the test pulse was kept constant at 110% of the resting MT. Paired pulses were intermixed with single test and conditioning pulses.

Data analysis and statistical methods

MEP amplitudes were measured off-line. Recordings with EMG background activity were excluded from further analysis.

For the paired-pulse paradigm, MEP amplitudes at different ISIs were calculated as a percentage of the mean amplitude of the test pulse alone. Data of the 2 and 3 ms ISIs and 10 and 15 ms ISIs were pooled for statistical analysis. CS intensities were expressed as a percentage of the subject's resting MT and as a percentage of the stimulator output. Because the CS intensities were applied in 10% increments of the subject's MT and the MT differed considerably among the different subjects, the CS intensities when expressed as a percentage of stimulator output were grouped in bins of 5% (Experiment 2) and 10% (Experiment 5) of maximal stimulator output. Because of these differences in MT, CS intensity bins contained a different number of subjects. Only bins with $n > 5$ were analysed.

Effect of age, test MEP amplitude and MT in the principal experiments (Experiments 1–3)

Separate one-way factorial ANOVAs (analyses of variance) were used to assess the effect of subject type (patient versus healthy subject) on age, the MT and mean test amplitude (derived from the second experiment).

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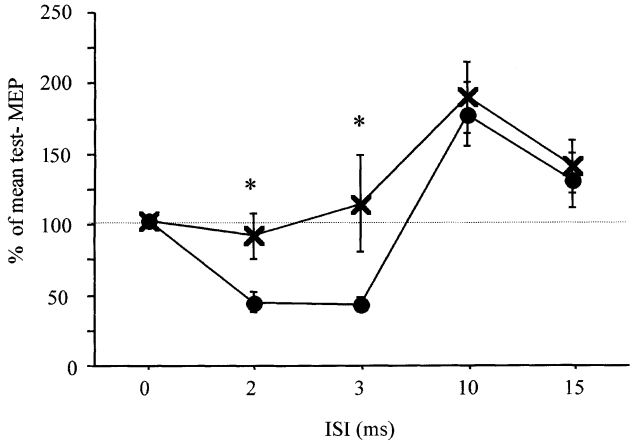

Fig. 2 Inhibition and facilitation produced by paired-pulse TMS at different ISIs in normal volunteers (filled circles) and patients (crosses), Experiment 1. The conditioning pulse at 80% of the subject's MT preceded the test pulse by 2, 3, 10 and 15 ms. Responses were recorded in the relaxed FDI muscle. The MEP amplitudes at different ISIs were calculated as a percentage of the mean amplitude of the test pulse alone. Mean ± 1 SE. Asterisks indicate significant differences of the MEP amplitude between patients and healthy volunteers.

Effect of stimulus intensity and subject type on the MEP amplitude (recruitment curve, Experiment 3)

For each subject, the mean MEP amplitude at each stimulus intensity was calculated. A two-way factorial ANOVA was used to assess the effect of subject type and intensity on the MEP amplitude.

Effects of a different ISI and CS on inhibition and facilitation

The effects of ISI, CS (expressed as %MT) and subject type on the conditioned MEP amplitudes were examined by two separate repeated-measures ANOVAs, with ISI and CS as the within-group factor (repeated measure) and subject as the between-group factor. The effect of different CS and ISI on the conditioned amplitude was analysed for patients and healthy subjects separately with paired *t* tests.

In all the statistical tests, an alpha level of 0.05 was used. Bonferroni correction for multiple comparisons was done when multiple *t* tests were performed. All data are expressed as mean ± SE.

Results

Principal experiments (Experiments 1–3)

Subjects

For Experiments 1–3, the age of patients (59.4 ± 3.2 years) and healthy subjects (51.3 ± 3.7 years) was similar and showed no statistically significant difference. The Motricity Index of the patients was 77 ± 0.2/100. All subjects participated in the three experiments.

MT, MEP amplitudes evoked by single test stimulus and recruitment curves in the patients with good recovery and normal subjects (Experiments 1–3)

Subject type had no statistically significant effect on MT (mean MT expressed as a percentage of maximal stimulator output in normals 49.2 ± 3.3 versus 52.1 ± 2.5 in patients), mean MEP test amplitude of FDI muscle (in normals 0.650 ± 0.152 mV versus 0.61 ± 0.087 mV in patients) and rise in MEP amplitudes with increasing stimulus intensity (Fig. 1) (ANOVA, intensity: $F = 95.65$, $P < 0.0001$; subject type, NS; intensity × subject type, NS).

Effect of different ISIs on ICI and intracortical facilitation (Experiment 1)

Group data are shown in Fig. 2. ANOVA showed that the effects of the different ISIs ($F = 32.64$, $P < 0.0001$) and type of subject ($F = 6.48$, $P = 0.014$) were significant, while the interaction between ISI and subject was not significant. *Post hoc* comparison with *t* test showed that inhibition at ISIs of 2 and 3 ms was significantly reduced in patients when compared with healthy volunteers ($P = 0.006$) while facilitation was similar at ISIs of 10 and 15 ms (Fig. 2).

When tested for each group separately, the conditioned MEP amplitude was significantly inhibited at ISIs of 2 and 3 ms ($P < 0.0001$) and significantly facilitated at ISIs of 10 and 15 ms ($P = 0.006$) in healthy subjects. In patients, no significant inhibition of the conditioned MEP amplitude at the 2 and 3 ms interval was seen. Similarly to the healthy subjects, there was significant facilitation of the conditioned MEP amplitude at 10 and 15 ms intervals ($P = 0.0003$).

Threshold of inhibitory and excitatory activity in patients and normal volunteers (Experiment 2)

The intensity of the conditioning pulse had a statistically significant effect on the conditioned MEP amplitude ($F = 17.77$, $P < 0.0001$), while the factor subject type had no significant effect. The interaction of stimulus intensity and subject type was significant ($F = 3.65$, $P = 0.05$), indicating that the effects of changes in CS intensity are different in patients and healthy subjects: at CS intensities <20% of maximal stimulator output (Fig. 3A) corresponding to < 30% of MT (Fig. 3B), no inhibition of conditioned MEP was seen in patients or normals. At CS intensities of 25 and 30% of maximal

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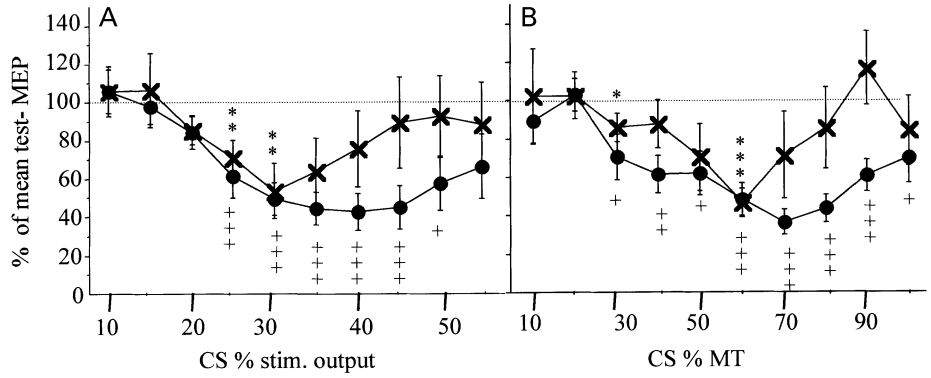

Fig. 3 Inhibition and facilitation produced by paired-pulse TMS at a constant ISI of 2 ms with the conditioning pulse (CS) at different intensities of the stimulator output in normal volunteers (NV, filled circles) and patients (PT, crosses), Experiment 2. CS preceded the test pulse by 2 ms. Responses were recorded in the relaxed FDI muscle of the non-affected hand. The MEP amplitudes at different CS intensities were calculated as a percentage of the mean amplitude of the test pulse alone. (**A**) CS intensity is expressed as a percentage of stimulator output. (**B**) CS intensity is expressed as a percentage of the subject's MT. Mean ± 1 SE. Significant inhibition of the MEP amplitude is indicated by * in patients (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$) and by + in healthy volunteers (+$P < 0.05$, ++$P < 0.01$, +++$P < 0.005$).

stimulator output (Fig. 3A, left part of the U-shaped curve) corresponding to 30–60% of MT (Fig. 3B, left part of the U-shaped curve), significant inhibition of the conditioned test MEP amplitude was seen in both patients and normals. In contrast, at higher stimulus intensities (>30% of maximal maximal stimulator output, >60% MT), patients and normal volunteers showed a different pattern; as indicated by the increase of conditioned MEP at a steeper rate [>30% maximal stimulator output (Fig. 3A, right part of the U-shaped curve), >60% MT (Fig. 3B, right part of the U-shaped curve)], inhibition fades at lower CS intensities in patients when compared with normal volunteers and even exceeds the size of the MEP amplitude evoked by the single test pulse in some patients. Figure 4 illustrates this facilitatory effect in two representative patients with cortical and subcortical lesion of either hemisphere. This facilitatory effect was absent in normal subjects. Consequently, maximal inhibition occurred at higher intensities in normals (40% of maximal stimulator output or 70% of MT) compared with patients (30% of maximal stimulator output or 60% of MT).

MT and intensity of CS that produces maximal inhibition in patients and normal volunteers

In Fig. 5, each subject's CS intensity (expressed as a percentage of maximal stimulator output) that produced the greatest inhibition is plotted against the subject's MT. In normal volunteers, a linear relationship between these two parameters is seen ($P < 0.0001$), indicating that at a higher MT a higher CS intensity is required to evoke the greatest inhibition. In contrast, the relationship of these two parameters was not linear in the patients.

Control experiments (Experiments 4 and 5)

Threshold of inhibitory and excitatory activity: effect of left versus right hemispheric stimulation in healthy volunteers (Experiment 4)

Motor threshold was 51.0 ± 3.7% in the right FDI and 51.4 ± 3.1% in the left FDI. Mean test MEP evoked by a single test pulse was 0.566 ± 0.04 mV in the right FDI and 0.490 ± 0.03 mV in the left FDI. There was no significant effect of the site of stimulation (between-group factor) and conditioned MEP amplitude. The intensity of the conditioning pulse was significant (within-group factor, $P < 0.0001$), while the interaction between site of stimulation and intensity of the conditioning pulse was not significant.

Threshold of inhibitory and excitatory activity in patients with good versus poor recovery (Experiment 5)

Arm function as assessed by the Motricity Index was severely impaired in the patients with poor recovery (27 ± 0.31/100) when compared with those with good recovery (77 ± 0.20/100). In the non-affected hemisphere, the MT of FDI was similar in patients with poor recovery (52.2 ± 5.7% of maximal stimulator output) and those with good recovery (52.1 ± 2.5%). Similarly, the mean test MEP evoked by a single test pulse was comparable between the two groups (0.655 ± 0.40 mV in patients with poor recovery versus 0.612 ± 0.08 mV in patients with good recovery). Figure 6 shows that inhibition of the conditioned MEP started at higher CS intensities in patients with poor recovery (50% MT) when compared with the healthy volunteers (30% MT) and patients with good recovery (30% MT). When CS was expressed as a percentage of maximal stimulator output, this difference is

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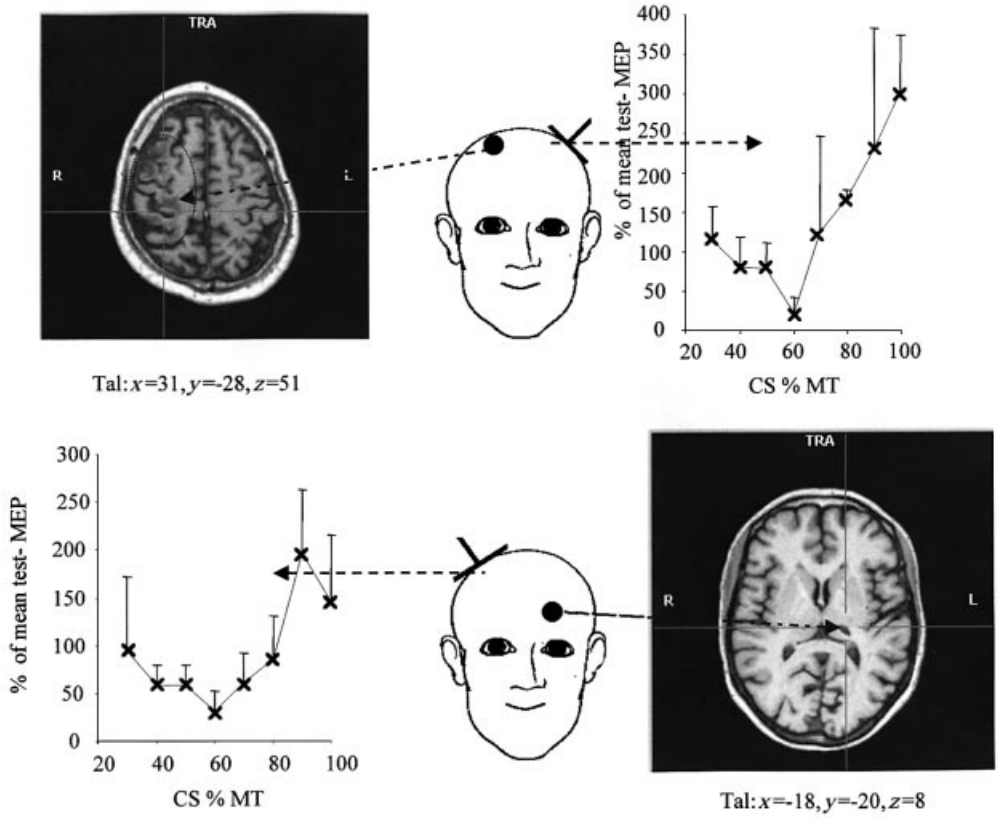

Fig. 4 Increase in cortical excitability in patients with subcortical and cortical lesions of either hemisphere: inhibition and facilitation produced by paired-pulse TMS at a constant ISI of 2 ms with the conditioning pulse (CS) at different intensities of the stimulator output in two representative patients (Experiment 2). CS preceded the test pulse by 2 ms. Responses were recorded in the relaxed FDI muscle of the non-affected hand. The MEP amplitudes at different CS intensities were calculated as a percentage of the mean amplitude of the test pulse alone. The MRT of the subject's brain was transformed into Talaraich space using Brain Voyager™ (BrainInnovation BV, The Netherlands, and Max Planck Society eV, Germany). The Talaraich coordinates are given for the location of the lesion as indicated by the intersection of the vertical and horizontal line. Upper panel: a patient with extensive infarction involving the M1 hand area (note that the hand knob of the non-affected hemisphere is clearly visible). Stimulation of the motor cortex of the non-affected hemisphere shows facilitation with higher CS intensities. Lower panel: a similar facilitatory effect of the CS at higher intensities is seen in a patient with infarction of the left internal capsule.

less clear: 30–35% in patients with poor recovery versus 25% in patients with good recovery and healthy volunteers. With higher CS intensities, the facilitatory effect described for the patients with good recovery (Experiments 1 and 2) is not seen. Instead, maximal inhibition is seen in patients with poor recovery.

Discussion

The main finding of this study is that in patients recovering from ischaemic infarction, remote changes in the excitability in the homotopic motor cortex of the non-affected hemisphere occur and that these changes are due to a shift in the balance between excitatory and inhibitory activity towards an increase of excitatory activity. Because measures of corticomotorneuronal excitability such as MT and threshold of intracortical inhibitory and excitatory activity were similar in the right and left hemisphere of normal subjects, these changes are not due to side to side asymmetry in patients and normal subjects. The similarity of MT, mean test MEP and recruitment curves in both groups indicates that the overall corticomotoneuronal excitability has not changed in patients. Therefore, the ischaemic infarction of the primary motor output system of the hand did not elicit gross changes in corticomotoneuronal excitability of the non-affected homotopic motor cortex of our patients, a result consistent with two previous reports (Liepert *et al*., 2000; Shimizu *et al*., 2002). However, a decreased MT of ~5% was reported for a the non-

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affected motor cortex of 18 stroke patients (Kimiskidis *et al*., 2002), indicating that further studies are needed to analyse this issue in more detail.

Because the increase in paired-pulse excitability was not accompanied by an increase in cortical motor neuron or spinal motor neuron excitability, the cortical site of our finding is supported (Kujirai *et al*., 1993; Ziemann *et al*., 1996*c*; Nakamura *et al*., 1997; Di Lazzaro *et al*., 1998). There is evidence that the inhibitory effect of the conditioning pulse at short ISIs arises from GABAergic interneurons (Ziemann *et al*., 1996*a*, *b*) of small areas close to the tested corticospinal neurons (Ashby *et al*., 1999). Therefore, the inhibitory effect of CS at low intensities demonstrated in our study in healthy subjects and patients probably occurs at a cortical site and may be mediated by GABA. When inhibition is studied with the conventional paired-pulse TMS paradigm at ISIs of 2 and 3 ms and with the intensity of CS set at 80% of MT, the conditioned MEP amplitude was significantly larger in patients when compared with the healthy subjects. Moreover, in patients, a facilitatory effect was seen at an ISI of 3 ms. A larger conditioned MEP amplitude at a short ISI was reported previously for the unaffected hemisphere in patients with cortical and subcortical strokes in the subacute phase (13.8 ± 4.6 days of their recovery; Liepert *et al*., 2000) and for patients with a cortical site of their stroke up to 2.9 months (Shimizu *et al*., 2002). Generally, it is thought that the smaller effect of the conditioning pulse on the MEP amplitude evoked by the test pulse is due to reduced inhibitory activity of neuronal circuits tested at short ISIs. However, as already mentioned by Ridding *et al*. (1995), it is conceivable that this abnormality is due to increased excitability of the neuronal circuits responsible for excitatory effects at ISIs of 2 and 3 ms. This hypothesis is supported by our findings of the second experiment (see below). Furthermore, as indicated by our finding of similar facilitatory effects of the conditioning pulse on the MEP amplitude evoked by the test pulse at ISIs of 10 and 15 ms in patients and normal volunteers (Experiment 1), the excitability of the neuronal circuits responsible for the excitatory effects at these ISIs is normal. This was reported previously (Liepert *et al*., 2000) and would indicate that intracortical facilitation at ISIs of 2 and 3 ms and ISIs of 10 and 15 ms is mediated by different neuronal circuits.

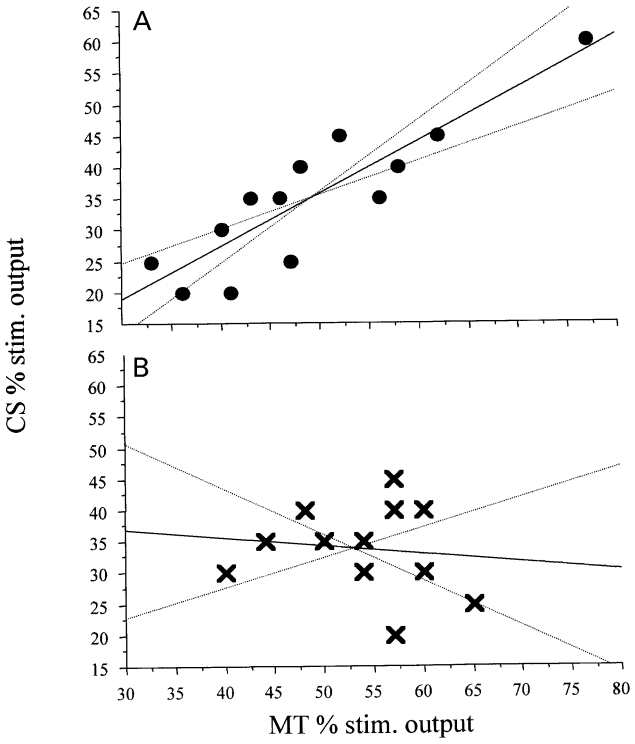

Fig. 5 Relationship between the CS intensity that is necessary for eliciting maximal inhibition and MT in normal volunteers (filled circles) and patients (crosses). For each subject, the intensity of the CS that produced the greatest reduction in MEP amplitude was plotted against the subject's MT. Regression was calculated. (**A**) In normal volunteers, a linear relationship between CS and MT was demonstrated: CS = –6.352 + 0.841 × MT; r^2 = 0782, $P < 0.0001$. (**B**) In patients, no such relationship was seen: CS = 37.83 – 007 × MT; r^2 = 001, $P = 0.77$.

In the second experiment, the effect of a wide range of CS intensities on the conditioned MEP at an ISI of 2 ms was tested. In normal volunteers, the intensity of the conditioning pulse had a significant influence on the magnitude of inhibition of the conditioned MEP of FDI muscle. Analogously to the interpretation of the stimulus response curve for the MEP amplitude evoked by different stimulus intensities (Ridding and Rothwell, 1997), we would suggest for our paradigm that increasing inhibition seen with increasing CS intensity (left half of the U-shaped curve) indicates either that as intensity of CS increases successively more inhibitory interneurons were recruited or that the activity of inhibitory interneurons increased with increasing CS intensity. Significant inhibition was present over a wide range of CS intensities (up to 50% of maximum stimulator output) in normal subjects. This is similar to findings reported for other intrinsic hand muscles (Schäfer *et al*., 1997; Chen *et al*., 1998), and supports the view that in motor cortex the threshold for activation of inhibitory interneurons is lower than for excitatory interneurons (Schäfer *et al*., 1997; Chen *et al*., 1998).

In patients, the intensity of the conditioning pulse had a significant influence on the magnitude of inhibition of conditioned MEP of FDI muscles. Further, the similarity of the slope of the left half of the U-shaped curve in patients and healthy volunteers, and the similarity of the CS intensity producing significant inhibition (Fig. 3A) suggest that the threshold for the activation and activity of inhibitory interneurons is similar in healthy subjects and patients. In contrast to normals, however, inhibition fades rapidly with increasing CS intensities in patients (right half of the U-shaped curve; inhibition was significant at CS of 25–35% of maximal stimulator output) and suggests that in patients, the balance of excitatory and inhibitory activity was shifted towards an increase of excitatory activity or lowered thresh-

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old for the activation of excitatory interneurons. The finding could also be explained by subthreshold activation of the corticospinal neuron by the CS at higher intensities. However, this would require that the threshold for activating the corticospinal neurons is lowered in patients when compared with healthy volunteers. In view of a similar MT in both

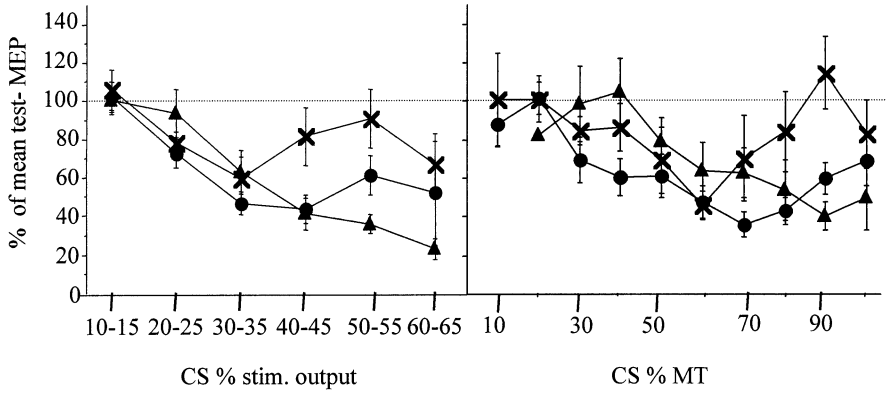

Fig. 6 Differences in cortical excitability in patients with good and poor recovery. Inhibition and facilitation produced by paired-pulse TMS at a constant ISI of 2 ms with the conditioning pulse (CS) at different intensities of the stimulator output in normal volunteers (filled circles), patients with good recovery (crosses) and patients with poor recovery of hand function (triangles) (Experiments 2 and 5). CS preceded the test pulse by 2 ms. Responses were recorded in the relaxed FDI muscle of the non-affected hand. The MEP amplitudes at different CS intensities were calculated as a percentage of the mean amplitude of the test pulse alone. Left graph: CS intensity is expressed as a percentage of stimulator output. Right graph: CS intensity is expressed as a percentage of the subject's MT. Mean ± 1 SE.

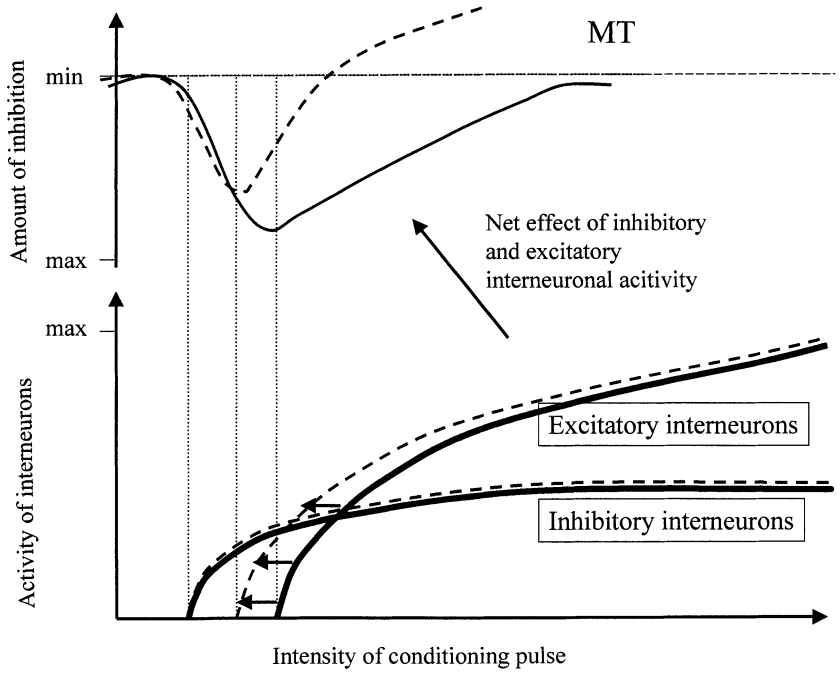

Fig. 7 Schematic of excitatory and inhibitory activity. In the lower part of the diagram, the intracortical excitatory and inhibitory interneuronal activity of normal subjects (solid line) and patients (dotted line) is plotted against the intensity of the conditioning pulse. With increasing CS intensity, both intracortical inhibitory and excitatory activity increases. In either group, the inhibitory interneurons are recruited earlier (at lower CS intensities) than the excitatory interneurons. In patients, however, the threshold for the excitatory interneurons is shifted towards lower intensities, as indicated by the shift of the dotted line to the left (arrows). In the upper diagram, the net effect of inhibitory and excitatory activity as measured by the inhibitory effect of the CS on the conditioned MEP amplitude (amount of inhibition) is plotted against the intensity of CS. Because of the earlier recruitment of excitatory interneurons in patients, the net effect of inhibitory and excitatory activity is shifted towards less inhibition that is of shorter duration.

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groups, this is less likely. Accordingly, the larger conditioned MEP amplitudes seen at short ISIs with CS set at 80% of MT (Fig. 2) or 95% of active threshold (Liepert *et al*., 2000) in patients are due to an increase in excitatory activity of the neuronal circuits responsible for excitatory effects at short ISIs supervening inhibitory activity at these intensities (Fig. 7; modified after Schäfer *et al*. 1997).

Because intracortical inhibition can be modulated intracortically (Kujirai *et al*., 1993), transcallosally (Ferbert *et al*., 1992) or by cortical and subcortical areas anatomically projecting to the MI (Classen *et al*., 1997), further experiments are needed to determine the underlying mechanism of the increase in excitatory activity demonstrated in the non-affected hemisphere of the patients in this study. However, two findings of our study argue against the possibility that the increased cortical excitability is due to loss of trancallosally mediated inhibition of the homotopic M1 hand area (Shimizu *et al*., 2002). First, although all patients with poor recovery of the hand function had extensive cortical lesions involving M1, none of them showed a similar increase in excitatory activity. Secondly, in the patient group with good recovery of hand function, patients with subcortical lesions showed similar changes when compared with patients with cortical lesions (see Fig. 4).

The relative independence of absolute CS intensities necessary to elicit inhibition and MT supports the concept that ICI is due to different mechanisms from those for MT (Ziemann *et al*., 1996*b*; Chen *et al*., 1998) and was reported previously for distal and proximal lower and upper limb muscles of healthy subjects, displaying a wide variety of MTs but similar CS intensity required to elicit inhibition. Another possible explanation is that due to the fact that we can only look at a net effect of excitatory and inhibitory activity, the increase in excitatory activity of the neuronal circuits responsible for excitatory effects at short ISIs supervenes inhibitory activity at higher CS.

Facilitatory I (indirect) wave interaction in motor cortex can lead to a facilitatory effect at short ISIs (Ziemann *et al*., 1998; Chen and Garg, 2000). Although in their studies of healthy volunteers at ISIs of 2 and 3 ms a trough was seen (Ziemann *et al*., 1998; Chen and Garg, 2000), it is conceivable that this could be the underlying mechanism of the facilitatory effect in our patients. Because I wave interaction is under GABAergic control, the downregulation of $GABA_A$ receptors as described in the non-affected motor cortex of animals following a lesion to the motor cortex (Buchkremer-Ratzmann *et al*., 1996; Schiene *et al*., 1996; Buchkremer-Ratzmann and Witte, 1997; Qü *et al*., 1998; Neumann-Haefelin and Witte, 2000) could possibly result in an increased facilitatory effect seen in our patients.

It is conceivable that these changes reflect differential regulation in the excitatory and inhibitory neurotransmitter systems and may play a role in the functional recovery of patients (Witte, 1998; Nudo, 1999). Indeed, in patients with poor recovery, no increase in cortical excitability at higher CS intensities was seen when compared with the patients with good recovery (Fig. 6). The absence of changes in cortical excitability in patients with poor recovery certainly supports the relevance of our findings for recovery. However, we do not know whether a strong causal relationship between lack of increased cortical excitability and poor recovery exists. Recovery is most probably a complex process in which a number of different factors come into play (for reviews see Nudo *et al*., 1999; Seitz *et al*., 2002).

In conclusion, our findings document an increase of intracortical excitatory activity remote from the lesion in humans recovering from infarction of their brain, that is consistent with previous animal studies (Buchkremer-Ratzmann *et al*., 1996; Schiene *et al*., 1996; Buchkremer-Ratzmann and Witte, 1997; Neumann-Haefelin and Witte, 2000), that may play a role in the functional recovery of patients (Witte, 1998; Nudo, 1999).

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