Interhemispheric Asymmetries of Motor Cortex Excitability in the Postacute Stroke Stage
A Paired-Pulse Transcranial Magnetic Stimulation Study
Paola Cicinelli, MD; Patrizio Pasqualetti, PhD; Marina Zaccagnini, MD; Raimondo Traversa, MD; Massimiliano Oliveri, MD; Paolo Maria Rossini, MD

Background and Purpose—Changes in the intracortical inhibition (ICI) and facilitation (ICF) of motor cortex paired-pulse transcranial magnetic stimulation were reported in the affected (AH) and unaffected (UH) hemispheres of stroke patients and reflect some of the mechanisms related to motor cortex plasticity and different degrees of functional recovery. The interhemispheric differences of the ICI/ICF slopes have been found to have a nearly identical time course in the 2 hemispheres of healthy subjects, and whether such symmetry is modified after monohemispheric stroke has not yet been examined. Our goal was to investigate the interhemispheric asymmetries of the time course of ICI/ICF between the AH and UH of stroke patients in the postacute phase of recovery.

Methods—ICI/ICF recovery curves to subthreshold-conditioning suprathreshold-test magnetic stimuli were recorded from the paretic and nonparetic hand muscles of 10 well-recovered stroke patients and compared with those of a population of 10 control subjects.

Results—In the healthy subjects, ICI/ICF showed a symmetrical time evolution between the 2 hemispheres. In stroke patients, the ICI/ICF slopes were significantly different between the UH and AH; the intracortical inhibition was reduced in the AH and normal in the UH.

Conclusions—The defective AH ICI associated with the effective UH ICI could represent a marker of poststroke cortical plasticity implicated as a mechanism relevant to functional recovery. Analysis of the interhemispheric asymmetries of the ICI/ICF recovery curves might provide a valuable neurophysiological parameter in the prognosis and follow-up of patients with monohemispheric stroke. (Stroke. 2003;34:2653-2658.)

Key Words: evoked potentials, motor ■ motor cortex ■ recovery of function ■ rehabilitation ■ stroke

Neurophysiological mechanisms underlying functional recovery or permanent sequelae from brain lesions such as stroke are still poorly understood. Some initial motor deficits improve because of the resolution of perilesional edema and/or diachisis in brain areas distant from the site of the lesion. An aspect likely responsible for motor recovery beyond the acute period after a vascular lesion is brain plasticity. Evidence of a significant causal link between injury-induced neural network reorganization and recovery of functions has been largely demonstrated in animal studies and in humans.1–4 Changes in cortical organization encompass a wide variety of phenomena and mechanisms, including unmasking of existent but functionally silent corticocortical connections or modulation of synaptic efficacy such as long-term potentiation, long-term depression, and formation of new synapses. Most studies have focused on identifying molecules and physiological processes that are permissive for plasticity, and several intracortical processes have been hypothesized to play a crucial role in this phenomenon.5–8 Experimental studies have suggested that plastic changes usually require the downregulation of local inhibitory circuits within the M1 and that local disinhibition can unmask latent intracortical connections contributing to cortical reorganization.9–11 Overall, understanding of the physiological processes that are permit functional plasticity is a central issue when looking for better ways to modulate neural reorganization for optimal behavioral gain.3,5,7 Among brain imaging techniques, transcranial magnetic stimulation (TMS) has been used in different ways to identify short- and long-term patterns of motor cortex reorganization during recovery of motor function.1–4 TMS in a paired-pulse paradigm (paired TMS) is now considered a gold standard in testing the excitability of inhibitory and excitatory intracortical circuits within the human motor cortex and in evaluating their role in the modulation of motor cortical output. Neuropharmacological TMS studies supported the hypothesis that the depression of motor responses [motor evoked potentials (MEPs)] evoked by a suprathreshold “test” magnetic stimulus by a previous

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TMS studies performed in stroke patients have reported that paired paradigm reflect the excitability of separate inhibitory and excitatory interneuronal circuits in the motor cortex and that the threshold for activation of inhibitory interneurons is lower than for excitatory interneurons. There is evidence that ICI and ICF as obtained via conditioning-test stimuli reflect the excitability of separate inhibitory and excitatory interneuronal circuits in the motor cortex and that the threshold for activation of inhibitory interneurons is lower than for excitatory interneurons. The relationship between changes in ICI/ICF recovery curves and motor cortex plasticity has been investigated in the human motor cortex in different physiological and pathological conditions. Paired TMS studies performed in stroke patients have reported that changes in the intracortical excitability of the affected (AH) and unaffected (UH) hemispheres could represent a neurophysiological marker correlated with recovery of motor functions. Analysis of the interhemispheric asymmetry of the ICI/ICF of the UH and AH has not been approached yet. Interhemispheric differences of TMS-linked brain responses are minimal in healthy subjects and are known to be less influenced by the different experimental conditions and more stable intersubjectively and intrasubjectively. Together with other TMS parameters, the timing and shape of the ICI/ICF recovery curves are also very symmetrical in the 2 hemispheres of healthy subjects; whether such symmetry is modified after monohemispheric stroke represents the aim of the present study. Here, we analyzed the interhemispheric asymmetry of the time course of ICI/ICF between the AH and UH of a group of subacute stroke patients with mild to moderate motor deficits and compared this parameter with that obtained from a population of control subjects.

### Materials and Methods

Ten first-ever stroke inpatients (8 men, 2 women; mean age, 66.6 ± 7.2 years) were recruited from a large stroke population in our rehabilitation hospital after they gave informed consent. These were patients in a postacute stage who had suffered a monohemispheric vascular lesion occurring 30.6 ± 7.4 days (range, 20 to 42 days) previously. Criteria for their inclusion were (1) CT or MRI documenting a unique monohemispheric vascular lesion, (2) age < 80 years, and (3) presence of a mild to moderate motor deficit. Exclusion criteria were concomitant neuropathies, systemic vasculopathies, dementia or severe aphasia making patients uncooperative, and absence of voluntary motor recruitment in the affected hand. Six and 4 patients suffered left (LH) and right (RH) hemispheric lesions, respectively. The stroke was ischemic in 8 patients and hemorrhagic in the other 2 patients; according to brain CT or MRI findings, 3 patients had a subcortical lesion, and the remaining 7 had a cortical lesion. Neurological status was evaluated with the Canadian Neurological Scale from which subscores for hand functionality was extrapolated. Patients presented mild to moderate hemiparesis, and all of them had partially recovered hand movements (Canadian Stroke hand item, from 1 to 15; Table 1) MEPs were recorded with surface Ag/AgCl disk electrodes from the opponens pollicis (Opp) and abductor digitii minimi (ADM) muscles in a belly-tendon montage. The amplified (100 to 500 μV/div) and bandpass-filtered (0.1 Hz to 2 kHz) electromyographic raw signal was digitized and fed into a laboratory computer. Auditory feedback of the electromyographic signal was given to ensure complete voluntary relaxation of the target muscles. ICI and ICF were studied by use of a paired conditioning-test shock paradigm. Conditioning and test stimuli were given through a focal figure-8 coil (each loop measured 70 mm in diameter) connected to 2 magnetic stimulators via a Bi-Stim module (MagStim, Whitland) discharging a maximum output of 2.2 T. The coil was placed flat on the scalp area approximately over the motor strip at the optimal site for hand MEP elicitation (“hot spot” site) and oriented in such a way that electric currents induced in the brain flowed in a posterior-to-anterior direction across the hand area of the motor cortex. The effect of the first (conditioning) stimulus on the second (test) stimulus was investigated at ISIs of 1, 3, 5, 7, 10, and 15 ms. First, the hot spot site was identified in each patient; then, the resting (RMT) and active (AMT) motor thresholds were evaluated in the UH and AH according to the recommendations of the International Federation of Clinical Neurophysiology Committee. The conditioning stimulus was set to such a low intensity (5% below AMT) that any effect on the size of the test response should be ascribed to purely intracortical mechanisms, whereas test pulse intensities were regulated 20% above the RMT. In each set of experiments, test and conditioning shocks at different intervals were randomly interleaved, with each pair of stimuli delivered at a repetition rate ranging between 0.18 and 0.25 Hz. At least 4 trials were obtained for each experimental condition. A control population of 10 healthy volunteers (6 men, 4 women; mean age, 57 ± 3.2 years) was used for comparison; normative data of the measured parameters, including interhemispheric differences, have been published elsewhere. The experimental protocol was approved by the local ethics committee.

### TABLE 1. Clinical and Neuroimaging Findings in Stroke Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Side</th>
<th>CT/MRI</th>
<th>Canadian Neurological Scale</th>
<th>Hand Score</th>
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</thead>
<tbody>
<tr>
<td>P.R.</td>
<td>55</td>
<td>M</td>
<td>Left</td>
<td>Temporo-parietal</td>
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<td>1</td>
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<tr>
<td>D.C.L.</td>
<td>70</td>
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<td>Right</td>
<td>Capsular</td>
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<td>1</td>
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<tr>
<td>M.A.</td>
<td>56</td>
<td>F</td>
<td>Left</td>
<td>Parieto-occipital</td>
<td>9</td>
<td>1</td>
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<tr>
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<td>62</td>
<td>M</td>
<td>Left</td>
<td>Fronto-parieto-temporal</td>
<td>9.5</td>
<td>1.5</td>
</tr>
<tr>
<td>P.E.</td>
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<td>M</td>
<td>Left</td>
<td>Fronto-parieto-temporal</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>M.R.</td>
<td>72</td>
<td>M</td>
<td>Right</td>
<td>Capsular</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>M.P.</td>
<td>63</td>
<td>M</td>
<td>Left</td>
<td>Parietal</td>
<td>9</td>
<td>1</td>
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<tr>
<td>C.F.</td>
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<td>M</td>
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<td>Fronto-parietal</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>F.A.</td>
<td>72</td>
<td>M</td>
<td>Right</td>
<td>Temporo-parieto-occipital</td>
<td>9.5</td>
<td>1.5</td>
</tr>
<tr>
<td>R.M.</td>
<td>73</td>
<td>M</td>
<td>Right</td>
<td>Capsular</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>
Statistical Analysis
Conditioned MEP amplitudes to paired stimulation were expressed as percentages of test MEP amplitudes to single stimulation; median values were calculated at each ISI and transformed by means of square root to obtain a better heteroscedasticity and gaussianity. Therefore, the lack of inhibition/facilitation is now represented by the reference value of 10. According to the study design, both hemispheres were evaluated in control subjects (RH and LH) and in patients (AH and UH). We performed a preliminary equivalence test of interhemispheric differences in control subjects. Assuming the null hypothesis that an RH-LH difference <5% could be considered neurophysiologically not relevant, a 1-sample t-test indicated that such a difference was never significant at each ISI, allowing us to arbitrarily align RH to AH and LH to UH. The main analysis was based on analysis of variance (ANOVA) for repeated measures with hemisphere and ISI as within-subjects factors and group as the between-subjects factor. Pillai’s trace was chosen to assess significance; 95% confidence interval (CIs) were calculated by means of Sidak’s procedure to control the α inflation.

Results
Motor Threshold
The RMT and AMT to single magnetic stimuli were significantly higher in the AH (RMT, 69.1±11.5%; AMT, 42.0±8.3%; P<0.01) than in the UH (RMT, 59.3±8.4%; AMT, 35.0±4.8%) (Table 2). ANOVA for repeated measures revealed a strong hemisphere×group interaction [Pillai’s trace=0.638; F(2,17)=14.999; P<0.001]. This overall effect was due to the interhemispheric asymmetry in stroke patients [RMT: t(9)=4.346, P=0.002; AMT: t(9)=4.269, P=0.002] opposed to the symmetry in healthy subjects [RMT: t(9)=0.294, P=0.775; AMT: t(9)=0.667, P=0.522]. Specific comparisons indicated that RMT and AMT of the patients’ UH were not significantly different from subjects’ RH or LH (consistently, P>0.20), whereas a clear hypoexcitability was found when patients’ AH was compared with subjects’ RH or LH (consistently, P<0.03).

ICI/ICF Curves
In the AH, a partial loss of ICI was observed at ISI of 1 ms (Opp, 48.3±30%; ADM, 51.9±46%) and 3 ms (Opp, 54.3±28%; ADM, 54.3±34%), whereas in the UH, the pattern of inhibition was similar to that observed in control subjects (Table 3 and Figure 1). At ISIs of 5 and 7 ms, the effect of the conditioning stimulus on the test MEP amplitude turned from inhibition to facilitation, and at ISIs of 10 and 15 ms, consistent but not significant facilitation of the conditioned MEP (ICF) was present; no differences were detected between the ICF of the AH and UH and control group subjects. The ICI/ICF recovery curves were not qualitatively different between cortical and subcortical lesion patients, but because of the small sample size in the latter group (3 subcortical lesion patients), this result should be confirmed in a statistically valid analysis in a larger population of patients. Doubly multivariate ANOVA indicated the significant triple interaction of hemisphere×ISI×group [Pillai’s trace=0.204; F(10,180)=2.04; P=0.032], which was found similarly for both muscles (ADM, P=0.027; Opp, P=0.021; Figure 2). When ANOVA was applied separately to each group, no hemisphere×ISI interaction was found in control subjects [Pillai’s trace=0.074; F(5,45)=0.718; P=0.614], whereas in patients, a clear interaction was found [Pillai’s trace=0.46; F(10,90)=2.69; P=0.006]. This indicates that in control subjects the interhemispheric differences did not change according to ISI, whereas in patients the AH-UH asymmetry showed a modulation when ISI 4.8% (Table 2). ANOVA for repeated measures revealed a strong hemisphere effect of the conditioning stimulus on the test MEP amplitude (Opp, 48.3±30%; ADM, 54.3±28%) and 3 ms (Opp, 54.3±28%; ADM, 54.3±34%), whereas in the UH, the pattern of inhibition was similar to that observed in control subjects (Table 3 and Figure 1). At ISIs of 5 and 7 ms, the effect of the conditioning stimulus on the test MEP amplitude turned from inhibition to facilitation, and at ISIs of 10 and 15 ms, consistent but not significant facilitation of the conditioned MEP (ICF) was present; no differences were detected between the ICF of the AH and UH and control group subjects. The ICI/ICF recovery curves were not qualitatively different between cortical and subcortical lesion patients, but because of the small sample size in the latter group (3 subcortical lesion patients), this result should be confirmed in a statistically valid analysis in a larger population of patients. Doubly multivariate ANOVA indicated the significant triple interaction of hemisphere×ISI×group [Pillai’s trace=0.204; F(10,180)=2.04; P=0.032], which was found similarly for both muscles (ADM, P=0.027; Opp, P=0.021; Figure 2). When ANOVA was applied separately to each group, no hemisphere×ISI interaction was found in control subjects [Pillai’s trace=0.074; F(5,45)=0.718; P=0.614], whereas in patients, a clear interaction was found [Pillai’s trace=0.46; F(10,90)=2.69; P=0.006]. This indicates that in control subjects the interhemispheric differences did not change according to ISI, whereas in patients the AH-UH asymmetry showed a modulation when ISI 4.8% (Table 2). ANOVA for repeated measures revealed a strong hemisphere effect of the conditioning stimulus on the test MEP amplitude (Opp, 48.3±30%; ADM, 54.3±28%) and 3 ms (Opp, 54.3±28%; ADM, 54.3±34%), whereas in the UH, the pattern of inhibition was similar to that observed in control subjects (Table 3 and Figure 1). At ISIs of 5 and 7 ms, the effect of the conditioning stimulus on the test MEP amplitude turned from inhibition to facilitation, and at ISIs of 10 and 15 ms, consistent but not significant facilitation of the conditioned MEP (ICF) was present; no differences were detected between the ICF of the AH and UH and control group subjects. The ICI/ICF recovery curves were not qualitatively different between cortical and subcortical lesion patients, but because of the small sample size in the latter group (3 subcortical lesion patients), this result should be confirmed in a statistically valid analysis in a larger population of patients. Doubly multivariate ANOVA indicated the significant triple interaction of hemisphere×ISI×group [Pillai’s trace=0.204; F(10,180)=2.04; P=0.032], which was found similarly for both muscles (ADM, P=0.027; Opp, P=0.021; Figure 2). When ANOVA was applied separately to each group, no hemisphere×ISI interaction was found in control subjects [Pillai’s trace=0.074; F(5,45)=0.718; P=0.614], whereas in patients, a clear interaction was found [Pillai’s trace=0.46; F(10,90)=2.69; P=0.006]. This indicates that in control subjects the interhemispheric differences did not change according to ISI, whereas in patients the AH-UH asymmetry showed a modulation when ISI

### TABLE 2. Motor Thresholds (Percent of the Stimulator Output) to Single Magnetic Stimuli at Rest (RMT) and During Active Contraction (AMT) of the Target Muscles in Healthy Subjects and Stroke Patients

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects</th>
<th>Stroke Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RMT, %</td>
<td>AMT, %</td>
</tr>
<tr>
<td>RH UH AH</td>
<td>58.8±3.7 59.0±4.3 69.1±11.5</td>
<td>31.2±6.7 30.8±5.4 35.0±4.8 42.0±8.3</td>
</tr>
</tbody>
</table>

Values are mean±SD.
changed between 1 and 15 ms. A significant inhibition asymmetry was found at ISIs of 1 and 3 ms and a slight and not significant facilitation asymmetry was observed at ISI of 15 ms (Figure 3).

Discussion
Analysis of the ICI/ICF to paired TMS of the UH and AH of a population of postacute stroke patients with a mild to moderate motor impairment showed significant interhemi-

Figure 1. ICI to paired TMS at ISIs of 1 and 3 ms in a paradigmatic stroke patient. Conditioning stimulus delivered 1 and 3 ms before the test stimulus produced only a weak inhibition of the test MEP in the AH, whereas it was able to induce a marked inhibition until the disappearance of the test MEP in the UH.

Figure 2. ICI/ICF recovery curves (with 95% CIs) of Opp and ADM in stroke patients and normal control subjects. In the AH, only a trend toward a reduction of inhibition is observed at ISIs of 1 and 3 ms.
spheric asymmetries of the early inhibitory part of the recovery curves resulting from an enhanced intracortical excitability of the AH (defective AH ICI) and a normal intracortical inhibition of the UH (effective UH ICI). The mean excitability threshold to single stimuli was significantly higher in the AH than in UH and normal control subjects. The different pattern of excitability changes in the AH is due to activation of different motor cortex excitable elements; the increased excitability to paired stimuli might reflect a reduced activity of M1 intracortical inhibitory circuits, and the decreased excitability to single stimuli is probably related to loss of excitable elements (death or totally unresponsive), together with changes in the membrane properties of the still-functioning corticospinal neurons that remained altered after stroke. Several paired TMS studies performed in stroke patients in the acute phase and in the early stage of recovery have demonstrated a significant disinhibition in the hemisphere with the vascular lesion (AH). This loss of inhibition in the AH was thought to be a compensatory mechanism, and it was speculated that a reduction in GABA activity was achieved to facilitate cortical plasticity and to promote the best possible recovery of motor functions.19–22 For what concerns the intracortical excitability of the UH, some conflicting results have been reported. In fact, although some authors found a correlation between the persistence of the UH intracortical disinhibition and poor motor recovery, others have failed to demonstrate any correlation between the return to a normal intracortical excitability in the UH and good recovery.8,20–22 In our population of subacute stroke patients with a relatively good clinical outcome, the defective AH ICI could be due either to a persistent functional defect in the intracortical inhibitory GABAergic circuits within the M1 or to an increased excitability and lowered threshold for activation of those interneurons responsible for the excitatory effects at short ISIs.8 It is conceivable that these changes in the excitability pattern might represent a reaction of the motor cortex of the AH to maintain an appropriate corticospinal output to the paretic hand as a mechanism correlated to recovery processes. Nevertheless, the motor inhibition of the AH is not measurable in stroke patients with poor outcome in whom MEPs are missing or severely reduced in amplitude, and it is not possible to affirm that these changes in AH inhibition indicate recovery processes or simply represent an epiphenomenon. In this respect, follow-up from acute to stabilized stages is needed. The time course of UH ICI was not different from that of control group subjects. A progressive increase in excitability of the UH was regarded as a bad prognostic indicator because of the loss of transcallosal (inhibitory) modulation from a severely damaged AH.25 In this respect, the effective UH ICI could be due to early reestablishment or continuous maintenance of a normal amount of transcallosal inhibitory influences coming from the stroke hemisphere; therefore, this could be interpreted as a good neurophysiological marker for clinical outcome. Analysis of the ICF recovery curves of the AH and UH and their interhemispheric asymmetries did not show any differences compared with that of control group subjects. This finding is in agreement with paired TMS studies that have failed to unveil any dysfunction in the glutamatergic system19,21 and is supported by experimental studies in which an increase in N-methyl-D-aspartate–mediated receptors was documented only transiently after a vascular lesion.11

On the basis of the present findings, we can speculate that a significant interhemispheric asymmetry of inhibition caused by a defective AH ICI associated with an effective UH ICI could be considered a good neurophysiological marker of cortical plasticity implicated as a mechanism relevant for poststroke functional recovery. Analysis of the ICI/ICF interhemispheric asymmetries might provide a valuable neuro-

Figure 3. Interhemispheric asymmetries (with 95% CIs) in control subjects and stroke patients. No changes were observed in control subjects, whereas a clear modulation according to ISIs was found in stroke patients, with significant asymmetry at ISI of 1 and 3 ms.
physiological parameter in the prognosis and follow-up of patients with monohemispheric stroke.

References


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