The Level of Cortical Afferent Inhibition in Acute Stroke Correlates With Long-Term Functional Recovery in Humans

Vincenzo Di Lazzaro, MD; Paolo Proifice, MD; Fabio Pilato, MD; Fioravante Capone, MD; Federico Ranieri, MD; Lucia Florio, MD; Cesare Colosimo, MD; Emanuele Pravatà, MD; Patrizio Pasqualetti, PhD; Michele Dileone, MD

Background and Purpose—Using transcranial magnetic stimulation, we investigated short-interval intracortical inhibition and short-latency afferent inhibition in acute ischemic stroke.

Methods—We evaluated short-interval intracortical inhibition and short-latency afferent inhibition in the affected hemisphere and unaffected hemisphere in 16 patients and correlated electrophysiological parameters with outcome at 6 months.

Results—Affected hemisphere short-latency afferent inhibition was significantly reduced in patients, and short-latency afferent inhibition level correlated with functional outcome. Reduced afferent inhibition in acute stroke correlates with long-term recovery. (Stroke. 2012;43:250-252.)

Key Words: GABA ■ transcranial magnetic stimulation

Changes in gamma-aminobutyric acid (GABA)-ergic activity in perilesional cortex after stroke have a central role in recovery.1 Inhibitory circuits of human cerebral cortex can be evaluated using paired-pulse transcranial magnetic stimulation, short-interval intracortical inhibition (SICI), or by coupling peripheral nerve stimulation with transcranial magnetic stimulation in short-latency afferent inhibition (SAI).2 Both inhibitory phenomena are mediated by inhibitory interneurons that use GABA$_A$ receptors, but different receptor subtypes are involved in SICI and SAI.2

We investigated SICI and SAI in acute stroke and evaluated the correlation between the level of cortical inhibition and functional outcome at 6 months.

Methods and Patients
Sixteen patients (mean age, 66.8±13.4 years) with first-ever stroke were recruited. Acute-phase evaluation was based on the National Institutes of Health Stroke Scale. Outcome at 6 months was assessed using modified Rankin Scale (mRS). This study was performed according to the Declaration of Helsinki and was approved by the local ethics committee. Patients gave their informed consent before participation.

Patients underwent brain magnetic resonance imaging. Seven patients had a subcortical stroke, whereas 9 patients showed cortical and subcortical involvement. To evaluate whether SAI changes were correlated with structural abnormalities of cholinergic systems, we estimated the damage extent of pathways emanating from nucleus basalis of Meynert: medial pathway, Capsular Lateral pathway, and Perisylvian Lateral pathway.3 For further details, see Supplemental Methods and Supplemental Figure I (http://stroke.ahajournals.org).

Magnetic Stimulation
We evaluated active motor threshold and resting motor threshold, amplitude of motor-evoked potentials (MEP), SICI at 2 ms interstimulus interval, and SAI at interstimulus intervals from N20 latency plus 2, 3, and 4 ms. We evaluated both affected hemispheres (AH) and unaffected hemispheres (UH).

Because it has been suggested that a change in the slope of input–output curve may influence the amount of cortical inhibition,4 we also obtained AH input–output curve using increasing stimulus intensities and evaluated whether there was a correlation between slope of input–output curves and amount of AH-SAI.

Data obtained in patients were compared with those obtained in 13 healthy subjects (mean age, 70.4±11 years).

Statistical Analysis
Comparison between AH and UH was performed by means of paired $t$-test, after checking frequency distributions and, eventually, transformed raw values, to achieve a better fit to gaussianity and a reduction of biasing effects of outliers (such as for MEP values). Comparisons of stroke patients versus healthy subjects were performed by means of $t$-test for independent samples.

Associations between electrophysiological findings and clinical outcome (mRS at 6 months) were assessed by means of nonparametric Spearman’s rho. The potential effect of the lesion site on mRS was assessed with Mann-Whitney $U$ test. Electrophysiological mea-

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From the Institute of Neurology (V.D., P.Pr., F.P., F.C., F.R., L.F., M.D.), Università Cattolica, Rome, Italy; Department of Neuroscience (V.D., P.Pa.), AFAr-Fatebenefratelli, “San Giovanni Calibita-Fatebenefratelli” Hospital, Rome, Italy; Institute of Radiology (C.C., E.P.), Università Cattolica, Rome, Italy.

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Correspondence to Vincenzo Di Lazzaro, MD, Istituto di Neurologia, Università Cattolica, L.go A. Gemelli 8, 00168 Rome, Italy. E-mail vdlazzaro@rm.unicatt.it
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sures associated with clinical outcome were further investigated through partial correlation analysis. More specifically, the correlation between AH-SAI and mRS-6-months was controlled for baseline National Institutes of Health Stroke Scale with the following formula:

\[
\text{Partial correlation} = \frac{\text{Bivariate correlation} - \text{Partial correlation}}{\sqrt{1 - \text{Bivariate correlation}^2}}
\]

Here, the left term indicates partial correlation between SAI and mRS, controlling for National Institutes of Health Stroke Scale, and the right term comprises the usual bivariate nonparametric correlation. This measure, after the appropriate transformation, follows a t-distribution with \((n-3)\) degrees of freedom.

Correlation between AH-SAI and the recruitment slope (indexed by the linear increase of MEP amplitude with respect to stimulation increase) was evaluated with Spearman’s rho. Because stroke-induced functional changes in cortical excitability may be influenced by stroke location and distribution,5 we evaluated the effect of lesion site (subcortical or cortical–subcortical) on SAI using Mann-Whitney U test.

Nonparametric Spearman’s \(\rho\) was used to correlate AH-SAI with percentages of lesional voxels in cholinergic pathways. Significance levels were adjusted according to Bonferroni procedure to control the risk of \(\alpha\)-inflation.

Results

Results are summarized in Figure. AH-SAI and AH-MEP amplitude were lower than corresponding UH and control values.

No evidence of association between electrophysiological parameters and stroke severity in the acute phase was found (consistently \(P > 0.05\)). Looking at correlations with clinical status at 6 months, the only significant associations were found with AH-SAI (Table).

When the effect of AH-SAI on mRS was adjusted for the confounding effect of baseline clinical status (National Institutes of Health Stroke Scale at T0), the nonparametric partial correlation remained significant (\(\rho = 0.66; P = 0.016\)), suggesting its relevance even equalizing for baseline clinical status.

There was no correlation between AH-SAI and either slope of the input–output curve (Spearman’s \(\rho = 0.12; P = 0.676\)) or site of the lesion (Mann-Whitney \(U, P = 0.958\)). Also, there was no correlation between site of the lesion and recovery at 6 months (Mann-Whitney \(U, P = 0.99\)).

Involvement of cholinergic pathways was limited (Supplemental Table I), and there was no correlation between AH-SAI and either percentage of lesional voxels of medial pathway (\(\rho = -0.39; P = 0.52\)), lateral cholinergic pathways.
(ρ = −0.47; P = 0.264), and lateral perforant pathway (ρ = −0.43; P = 0.376) or percentage of lesional voxels in the 3 pathways considered together (Spearman’s ρ = −0.5; P = 0.17).

Discussion
We report for the first time a suppression of afferent inhibition in acute stroke. AH-SAI level was correlated with recovery at 6 months.

SAI is produced by afferent inputs, and central cholinergic pathways are involved in SAI; thus, a lesion of these circuits might explain its reduction. However, the absence of consistent sensory deficits and/or abnormalities of N20 wave of somatosensory evoked potentials, the limited involvement of cholinergic pathways, and the absence of any correlation between involvement of cholinergic pathways and level of SAI, make this hypothesis unlikely.

We speculate that SAI suppression might be produced by functional changes in central inhibitory circuits. Because SAI is probably mediated by the α5-subunit,2 we suggest that its suppression might be related to a reduction of activity related to this subunit. Interestingly, a recent experimental study showed that pharmacological antagonization of α5-subunit activity promotes functional recovery after stroke.1

Long-term potentiation can be induced in motor cortex by stimulation of sensory cortex,6 and it has been proposed that long-term potentiation produced by sensory inputs might promote cortical reorganization after a lesion.7 Thus, it can be speculated that reduced SAI level could enhance sensory stimuli-related long-term potentiation phenomena in the motor cortex with a positive effect on relearning related recovery.

Disclosures
None.

References

Table. Bivariate Correlation Between Electrophysiological Findings and Clinical Recovery (modified Rankin Scale Score at 6 months)

<table>
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<tr>
<th></th>
<th>Spearman’s ρ</th>
<th>Corrected P Value</th>
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<td>RMT, AH</td>
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<td>MEP amplitude, UH</td>
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<td>&gt;0.90</td>
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<td>MEP amplitude, AH</td>
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<td>SICI, AH</td>
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</table>

RMT indicates resting motor threshold; UH, unaffected hemisphere; AH, affected hemisphere; AMT, active motor threshold; MEP, motor-evoked potential; SAI, short-latency afferent inhibition; SICI, short-interval intracortical inhibition.

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Supplemental Material

Supplemental Methods

Patients and Methods

Patients

Sixteen patients (mean age, 66.8±13.4 years) with first-ever stroke were recruited. Inclusion criteria were 1) ischemic stroke (both cortical and subcortical) involving the middle cerebral artery territory; 2) <10 days post-stroke; 3) hand weakness; 4) recordable MEP after TMS of the lesioned hemisphere. Exclusion criteria were: 1) seizure history; 2) hemorrhagic stroke; 3) concomitant neurological or other severe medical problems; 4) complete paralysis of the hand; 5) inability to give informed consent; 6) treatment with drugs acting on CNS; 7) contraindications for TMS and MRI studies; 8) absent N20 somatosensory evoked potentials from the lesioned hemisphere and/or pronounced sensory deficit (a score of 2 at the sensory item of the National Institutes of Health Stroke Scale), this was done because SAI is produced by the arrival of somatosensory inputs to the brain, thus, an impairment of the afferent pathways might interfere with SAI testing.

All patients underwent a standardised protocol of rehabilitation based on physical therapy for two months.

Neuroradiological and Neurophysiological methods

MRI

MRI was performed with a 1.5 T scanner (GE Signa, General Electric, Milwaukee, WI, USA), including fast-spin-echo (FSE) T2-weighted, fluid-attenuated-inversion-recovery (FLAIR), gradient-echo T2*-weighted, and FSE T1-weighted sequences. Diffusion-weighted-imaging (DWI) was obtained with echoplanar (EPI) spin-echo sequences (TR = 8600; TE = 83; slice thickness = 4 mm; gap = 0.4 mm; b-value = 1000 s/mm²). The corresponding apparent diffusion coefficient maps
(ADCs) were computed using a GE Advantage PC Workstation (GE Medical Systems; Milwaukee, WI, USA). The MRICro v1.4 software \(^1\) was used to delineate the boundary of the lesions as seen on DWI-ADC images, directly on the individual MRI images for each slice and to assess the lesions volume. In order to evaluate whether SAI changes were correlated to structural abnormalities of the central cholinergic system, we estimated the damage extent of the pathways emanating from the nucleus basalis of Meynert (nbM), since it was demonstrated that these projections are organized into discrete, ascertainable fibre bundles in the hemispheres \(^2\). These fibres provide the main cholinergic input for the neocortex and were accurately described and illustrated on anatomical templates in humans by Selden et al. \(^2\). The medial pathway (Mp), originates from nbM and courses around the corpus callosum within the cingulum to reach the orbitofrontal, subcallosal, cingulate and retrosplenial cortices; the Capsular Lateral pathway (CLp) runs across the external capsule and the uncinate fasciculus, and finally reaches the frontoparietal cortices and the temporal lobe; the Perisylvian Lateral pathway (PLp) travels within the claustrum to supply the frontoparietal cortex, the insula and the superior temporal gyrus. The entity of Mp, CLp and PLp damage from stroke was estimated by mapping the extent of a given lesion within each pathway using a procedure similar to that proposed by Babiloni et al. in a previous study \(^3\), as follows. In order to allow intersubject comparison, the Brainvoyager QX v1.10 software (Brain Innovation, Maastricht, The Netherlands) was used to transfer all patient’s DWI images into the Talairach space \(^4\) and to subsequently obtain normalized regions-of-interest (ROIs) of the lesions in the standard anatomical space.

To provide anatomical references representative of the study group, the Mp, CLp and PLp trajectories, as shown in Selden’s templates, were drawn on a Talairach-space custom atlas which was obtained by averaging the brains of 12 age-matched subjects with normal morphologic MRI findings. To this purpose, inversion-recovery-fast-spoiled-gradient-echo (IR-FSPGR) T1-weighted scans (matrix 256×256, FOV 256 mm, slice thickness 1.4 mm, in-plane voxel size 1 mm×1 mm) were acquired, and the statistical parametric mapping (SPM) v8.0 software (The Wellcome Trust Centre for Neuroimaging, London, UK) was
used to smooth with a 4mm-FWHM Gaussian filter and to subsequently average the obtained volumes; the resulting template was then transferred into Talairach space. Brainvoyager was further used to superimpose the patients normalized lesional ROIs onto the cholinergic trajectories and to finally count the intersecting voxels, an example is shown in Figure 1. The percentage of intersecting voxels of each pathway was used as a measure of the cholinergic damage and was tested for correlation with SAI findings.

**Transcranial magnetic stimulation**

**Single pulse cortical stimulation**

Magnetic stimulation was performed with a high-power Magstim 200 (MagstimCo., Whitland, Dyfed). A figure-of-eight coil with external loop diameters of 9cm was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous muscle (FDI). The induced current flowed in a postero-anterior direction. We evaluated active (AMT) and resting (RMT) motor threshold and amplitude of motor evoked potentials (MEPs). The resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 50 µV in 50% of 10 trials) at rest. The active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 200 µV in 50% of 10 trials) during isometric contraction of the tested muscle. The MEP amplitude was evaluated using a stimulus intensity of 120% RMT with the muscle at rest. Ten data sweeps were collected, and the mean peak-to-peak amplitude of the MEPs was calculated.

We evaluated the RMT, AMT and MEP amplitude of the affected (AH) and unaffected (UH) hemispheres.

**Short interval intracortical inhibition (SICI)**

We evaluated SICI to paired TMS both for the AH and the UH. SICI was studied using a paired pulse magnetic stimulation paradigm: two magnetic stimuli were given through the same stimulating coil, using a Bistim module, over the motor cortex and the effect of the first (conditioning) stimulus on the second (test) stimulus was investigated. The conditioning stimulus
was set at an intensity of 5% (of stimulator output) below AMT. The second, test, shock intensity was adjusted to evoke a MEP in relaxed FDI with an amplitude of approximately 0.5 mV peak-to-peak. The timing of the conditioning shock was altered in relation to the test shock. Interstimulus interval (ISI) of 2 ms was investigated. Ten single pulse and 10 paired pulse stimulations were performed. Subject was given audio-visual feedback at high gain to assist in maintaining complete relaxation. We calculated the amount of inhibition of the conditioned MEPs.

**Short latency afferent inhibition**

Conditioning peripheral stimuli were single pulses of electrical stimulation (200 µs duration) applied through bipolar electrodes to the median nerve at the wrist (cathode proximal). Conditioning stimulus intensity was set just above the motor threshold necessary to evoke a visible twitch of the thenar muscles. The afferent inhibition induced by the peripheral conditioning stimulus was tested at different interstimulus intervals (ISIs). ISIs were determined relative to the latency of the N20 component of the somatosensory evoked potential obtained after stimulation of the median nerve. The active electrode for recording the N20 potential evoked by median nerve stimulation was attached 3 cm posterior to C3 (10–20 system) for the right median nerve stimulation and to C4 for left median nerve stimulation, the reference was 3 cm posterior to C4 (or C3 for left median nerve stimulation). Five hundred responses were averaged to identify the latency of the N20 peak. ISIs from the latency of the N20 plus 2 milliseconds to the latency of the N20 plus 4 milliseconds were investigated in steps of 1 ms. Ten repeats were delivered for cortical stimulation alone and for conditioned stimulation at each ISI in pseudo-randomised order. Subjects were given audio-visual feedback of the EMG signal at high gain to assist in maintaining complete relaxation; trials contaminated by EMG activity were discarded. The amplitude of the conditioned responses was expressed as a percentage of the amplitude of the test response. We averaged the percentage of inhibition of the conditioned responses at the three different ISIs to obtain a grand mean of SAI.
**Supplemental Figure**

Example of MRI estimation of the damage to the cholinergic fibers emanating from the nbM in one representative patient (#6 supplemental table) with acute ischemic lesion in the left hemisphere. Lesional voxels (purple) intersecting each cholinergic trajectory (green, red and yellow, respectively) are plotted with dark gray color. (Mp = Medial pathway; nbM = nucleus basalis of Meynert; CLp = Capsular division of the Lateral pathway; PLp = Perysilvian division of the Lateral pathway). Maps were superimposed on a custom-created brain template for anatomical reference. See supplementary method section S1 for details. Talairach (Tal) Y-coordinates of the coronal sections are given.
Supplemental Table: Neuroradiological findings

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<th>MRI findings</th>
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<th>Plp</th>
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NI=not involved
Supplemental References


