Hyperexcitability-Associated Rapid Plasticity After a Focal Cerebral Ischemia

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Background and Purpose—This article addresses how neuroplastic changes are initiated after an ischemic stroke.

Methods—A focal cerebral ischemia was photochemically induced on the primary somatosensory cortex of rats, and in vivo electrophysiological recordings were performed on the peri-infarct cortex before and from 1 to 6 hours after the infarction.

Results—Paired-pulse analysis of evoked field potentials to peripheral electrical stimuli showed statistically significant neuronal hyperexcitability that was associated with rapid expansion of receptive fields (146.1% at 1 hour and 553.6% at 6 hours) as early as 1 hour after the infarction (P<0.05). Current source density analysis revealed increased current sinks in cortical layer II/III.

Conclusions—Our electrophysiological results showed, for the first time to our knowledge, rapid plastic changes in the peri-infarct cortex during the hyperacute stage of an ischemic stroke. Manipulation of this rapid plasticity may affect subsequent plastic changes. (Stroke. 2004;35:e346-e348.)

Key Words: cerebral ischemia  □  neuronal plasticity  □  somatosensory evoked potentials  □  rats  □  electrophysiology

Although recent studies on animals1 and humans2 have strongly implicated peri-infarct cortical plasticity in functional recovery, it remains unknown how such changes are initiated. We addressed this issue, focusing on the hyperacute stage of stroke.

Materials and Methods

Animal Preparation and Focal Cerebral Ischemia

Male Sprague-Dawley rats (400 to 500 grams; Clea Japan, Inc) were anesthetized with a mixture of ketamine hydrochloride (40 mg/kg, intramuscularly) and xylazine (4 mg/kg, intramuscularly) and maintained with supplementary doses. A focal cerebral ischemia was photochemically induced on the primary somatosensory cortex (4.0 mm lateral and 3.5 mm posterior to the bregma). In brief, after the administration of a solution of Rose Bengal (1.3 mg/100 grams intramuscularly) and xylazine (4 mg/kg, intramuscularly) and main-

Electrophysiological Recordings

Cortical surface recordings (band-pass filter: 15 Hz to 3 kHz) were performed before and at 1, 2, 4, and 6 hours after the infarction (n=9). The Ag/AgCl ball-shaped recording electrode (diameter of 100 μm; 5 kHz at 1000 Hz) was placed on the dura over the ulnar area of the right forepaw barrel subfield, and a reference electrode was placed on the posterior cortex. The recording site (forepaw barrel subfield) was 3.8 to 4.0 mm lateral and 0.6 to 0.9 mm anterior (>4.0 mm anterior to the infarction core) to the bregma.

The evoked field potentials were recorded to single and paired electrical stimuli (interstimulus intervals of 10 to 100 ms) given from a hook electrode placed directly on the left ulnar nerve. These stimuli were delivered at 2000-ms intervals. The facilitation indices of paired-pulse stimuli were calculated by \( fEP_{1}/fEP_{2} \), where \( fEP_{1} \) denotes the peak-to-peak amplitude of the first evoked responses and \( fEP_{2} \) denotes that of the second.3 Stimulus intensities were made constant so that digits 4 and 5 twitched (9 to 10 V).

One-dimensional current source density analysis was performed on the data for >6 hours after the infarction. After the dura was opened, a glass electrode (2 mol/LNa at 1 kHz) was inserted in the right forepaw barrel subfield (depth: 0 to 2100 μm), and the evoked potentials were sampled at 300-μm intervals.

Multi-unit activities were recorded to examine receptive fields (RFs) on the forepaw skin with a von Frey hair-type probe (calcu-

Statistical Analysis

Statistical differences of the evoked potentials and of the facilitation indices between the control and the infarcted groups were assessed by 1-way ANOVA and Dunnett post-hoc tests. \( P<0.05 \) was considered significant.

Results

Stained sections revealed a clearly demarcated region of necrotic tissue damage with a radius of 1.5±0.2 mm on the

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cortical surface, without affecting the underlying white matter.

After the infarction, the amplitude of the evoked potentials to single stimuli increased as a function of time (controls [mean ± SD: 1.7 ± 0.46 mV], 1 [2.58 ± 0.78 mV], 2 [2.99 ± 1.52 mV], and 4 hours [3.4 ± 1.9 mV]), but it took 6 hours (4.09 ± 2.13 mV) for it to reach statistical significance (Dunnett post-hoc tests, P < 0.05). However, the facilitation indices of paired-pulse stimuli clearly indicated a statistical significance for the interstimulus intervals of 100 ms as early as 1 (1.05 ± 0.26), 2 (1.02 ± 0.24), 4 (1.06 ± 0.23), and 6 hours (1.02 ± 0.23) after the infarction, as compared with those of the controls (0.63 ± 0.33) (Dunnett post-hoc tests, P < 0.05; Figure 1).

To locate laminar excitability changes, current source density analysis was performed. In the controls, as in other primary sensory cortices, an initial current sink in layer IV was followed by sinks in the superficial and deep layers. The infarcted group (n = 4) consistently indicated, while showing current flows similar to those of the controls (n = 4), a significant increase of the sink currents in layer II/III (300 and 600 μm; Figure 2).

Because this layer has been shown to be particularly susceptible to plastic changes during cortical reorganization, we recorded multi-unit activities from layer II/III (450 to 600 μm; n = 8) to examine the RF size before and after the infarct. The results clearly showed the rapid expansion of RFs (Figure 3). The RF size of the forepaw began increasing within 1 hour of the infarct (146.1%), coinciding with excitability changes in paired-pulse protocols, and kept increasing to 211.7%, 281.9%, 358.3%, 545.3%, and 553.6% of the controls at subsequent hourly recordings (2 to 6 hours). The direction of the expansion was relatively concentric, implying that the phenomenon was induced not as a function of distance from the infarct but as that of neuronal connectivity.

**Discussion**

This in vivo study established the first evidence on the immediate onset of ischemia-induced plastic changes (hypereexcitability and RF expansion) in the peri-infarct cortex. These posts ischemic changes were observed much earlier than those reported in previous studies on central lesions in vivo or using an in vitro preparation examining a different cortical location. Both ischemia-induced hypereexcitability and RF expansion may be induced by the reduction of GABAergic activities, which were indeed suggested by the increased facilitation indices (Figure 1). RF expansion may contribute to functional recovery through an increased impact of peripheral stimuli on peri-infarct neurons, whereas hypereexcitability may facilitate such processes as the secretion of some neurotrophins.
in an activity-dependent manner. If this is the case, then the electrophysiological changes we observed may play a role in inducing subsequent plastic changes such as long-term potentiation. In this regard, further studies are required.

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References


Figure 3. An example for the expansion of RFs before (left) and 2 hours after the infarction (right). RFs are shown as shaded areas.
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