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 supplemental 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 in 2 mol/L AgCl) was placed on the dura over the ulnar nerve. These stimuli were delivered at 2000-ms intervals. The facilitation indices of paired-pulse stimuli were calculated by fEP1/fEP2, where fEP1 denotes the peak-to-peak amplitude of the first evoked responses and fEP2 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/L AgCl 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 (calculated force: 0.166 grams). Changes in the RF size were quantified by q/p, where p and q denote the number of points on the forepaw skin that responded before and after the infarction.

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
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 (hyperedcitability and RF expansion) in the peri-infarct cortex. These postischemic 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 hyperexcitability 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 hyperexcitability may facilitate such processes as the secretion of some neurotrophins.

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**Figure 1.** Left, Average evoked potentials for the interstimulus intervals of 100 ms before (bold line) and 2 hours after the infarction (thin line). The waveform comprised a complex of positive (P1), negative (N1), and positive (P2) components. The amplitude of P1 to N1 was used as the peak-to-peak amplitude. Right, Increased facilitation indices for the interstimulus intervals of 100 ms, which was statistically significant as early as 1 hour after the infarction. h indicates hour; c, controls; error bars, SD.

**Figure 2.** One-dimensional current source density analysis of evoked potentials for the control (left) and infarcted condition (right). The analysis was computed by the following formula:

\[-I_m = \phi(x + \Delta x) - 2\phi(x) + \phi(x - \Delta x)/((\Delta x)^2).

\( I_m \) indicates source density; \( \phi \), recorded potential; \( x \), laminar depth; \( \Delta x \), recording interval in depth (=300 μm); \( \delta \), conductivity (=1). Current sinks are shown in red.
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
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