Somatosensory Evoked Potentials in Rat Cerebral Cortex Before and After Middle Cerebral Artery Occlusion

Kaoru Sakatani, MD, Hideaki Iizuka, MD, and Wise Young, PhD, MD

We recorded somatosensory evoked potentials in pentobarbital-anesthetized rats before and after middle cerebral artery occlusion. Trigeminal (vibrissae), median (forelimb), and sciatic (hind limb) nerve stimuli produced consistent, robust, and sharply localized responses in the trigeminal, forelimb, and hind limb regions of the somatosensory cortex of 18 rats. These regions are situated at sequentially greater distances from the center of infarcts produced by middle cerebral artery occlusion. In eight rats, occlusion 1-2 mm below the rhinal fissure abolished somatosensory evoked potentials in all three cortical region within minutes. Positive wavelets preceding the primary cortical response were also diminished by the occlusion, suggesting that ischemia affected the thalamocortical white matter. Four of these eight rats did not show histologically apparent ischemic involvement of the hind limb cortical region at 3 hours after occlusion; sciatic nerve evoked potentials recovered substantially in all four rats, and the amplitudes exceeded baseline (129±30% at 1 hour, 173±33% at 3 hours) in three of the four rats. Three of the eight rats did not have gross ischemic involvement of the forelimb cortical region; median nerve evoked potentials recovered fully in all eight rats, but the amplitudes did not exceed baseline. All eight rats had evidence of ischemic damage in the trigeminal cortex; no rat showed full recovery in this region, and all but one had trigeminal evoked potentials that were <20% of baseline amplitudes by 3 hours after occlusion. Two rats had large infarcts involving the hind limb and forelimb cortical regions and were studied for 24 hours; these rats did not recover evoked potentials in the three regions at 24 hours, although one rat transiently recovered hind limb evoked potentials to 83% of baseline amplitude at 3 hours. Amplitude of the hind limb somatosensory evoked potentials recorded in the contralateral hemisphere doubled after occlusion. Thus, middle cerebral artery occlusion causes widespread loss of somatosensory evoked potentials in the hind limb, forelimb, and trigeminal cortical regions of the ischemic hemisphere of rats. These regions show distinctly different temporal patterns of recovery. Our findings suggest that cortical evoked potentials may be useful for monitoring ischemic damage in these cortical regions after middle cerebral artery occlusion (Stroke 1990;21:124-132)

Middle cerebral artery occlusion (MCAO) in rats has become a widely used model of focal ischemia for studying mechanisms and treatments of cerebral infarction.1-4 Unlike global ischemia models,5-7 MCAO produces focal cerebral ischemia,8,9 MCAO 1-2 mm below the rhinal fissure typically causes an infarct in the frontopolar cortex, with variable extension into the frontoparietal cortex and the temporal cortex,10 and large regional changes of brain water, Na⁺, K⁺, and Ca²⁺ concentrations.2,3 This MCAO model spares the lenticulostriate branches of the middle cerebral artery (MCA)8,9 and causes infarcts largely localized to the cerebral cortex.

The rat primary somatosensory cortex has three anatomically and physiologically distinct regions: the hind limb area (HL), the forelimb area (FL), and the parietal 1 area (Par1).11,12 Par1 is situated most laterally and receives projections mainly from the cutaneous mechanoreceptors of the mystacial vibrissae, the head, and the neck. HL is located most medially, and FL lies between HL and Par1. Peripheral nerve stimulation produces localized field potentials in the somatosensory cortex. The typical ischemic infarct produced by MCAO encroaches on these regions. We
mapped cortical somatosensory evoked potential (SEP) responses to sciatic, median, and trigeminal nerve stimulation in rats before and after MCAO.

Materials and Methods

We anesthetized 18 adult Long-Evans rats weighing 320–450 g with 40 mg/kg i.p. pentobarbital. After tracheotomies, the rats were paralyzed with 1 mg/kg i.v. gallamine triethiodide and ventilated with a rodent respirator (Harvard Instruments, South Natick, Massachusetts). Catheters were inserted into the right femoral vein and the left femoral artery. Blood pressures were maintained at 80–120 mm Hg. Each rat was mounted in a stereotactic frame. For the mapping studies, the cortex was exposed with a right-sided square craniotomy extending from 3 mm anterior to 4 mm posterior to the bregma and from 1 mm to 8 mm lateral of the sagittal suture. Cerebrospinal fluid was drained via the cisterna magna to reduce cortical swelling. The dura was not opened. The bone edges were marked at 1-mm intervals to designate the location of the recording sites. A mineral oil pool was created above the craniotomy border of the sciatic nerve ACR overlapped the posteromedial border of the median nerve ACR. The electrodes were fixed to the skull with dental cement. After baseline SEPs were recorded, the right MCA was exposed through a craniotomy anterior to the foramen of the mandibular nerve and occluded 1–2 mm below the rhinal fissure by radiofrequency coagulation with fine forceps. The coagulated MCA was then divided to ensure complete occlusion. Sequential SEPs from the three cortical areas were recorded from 30 seconds to 3 hours after MCAO. The peak-to-peak amplitude of the primary cortical response was measured. The amplitude of the wavelets preceding the primary cortical response were measured from the baseline immediately preceding the wavelet to the peak of the wavelet.

The eight rats with MCAO were examined histologically after the end of SEP recording. All eight rats were anesthetized with pentobarbital and perfused with 10% buffered formalin; six rats were killed 3 hours after MCAO and the other two were killed 24 hours after MCAO. The brains were removed, and 10-μm-thick paraffin sections were cut from the infarct zone and the recording sites. Alternate sections were stained with hematoxylin and eosin, the Klüver-Barrera method, or the Fink-Heimer silver impregnation method.

Results

The spatial distribution of the cortical responses to sciatic median, and trigeminal (vibrissae) nerve stimulation were mapped in 10 rats (Figure 1). Stimulation characteristically produced large primary cortical responses with positive-negative-positive waveforms. The first large positivity of the primary cortical response is called the initial positivity (IP). At least two positive wavelets preceded the IP. Median and trigeminal nerve SEPs had more prominent wavelets than sciatic nerve SEP. The positive wavelet immediately preceding the IP had a wider spatial distribution than the IP and has been attributed to ascending activity in the thalamocortical white matter.

The cortical responses were sharply localized on the surface of the cortex. We defined an area of maximal response (AMR) as a cortical region in which SEP peak-to-peak amplitudes were 80–100% of the largest recorded response; AMRs seldom exceeded 1.0–1.5 mm in diameter. A cortical region in which SEP peak-to-peak amplitude was 40–100% of the largest recorded response is designated an area of cortical response (ACR); ACRs had diameters as large as 4–5 mm.

In all 10 rats in the mapping study, the SEP generator sites were consistently situated within the areas shown in Figure 2. Sciatic nerve SEPs were greatest 1–2 mm posterior to the bregma and 2–3 mm lateral of the midline. Median nerve SEPs were greatest 0–1 mm anterior to the bregma and 4–5 mm lateral of the midline. These sciatic and median nerve ACRs corresponded closely to HL and FL, respectively, of the parietal cortex.
FIGURE 1. Maps of somatosensory evoked potentials recorded from rat cortex and activated by contralateral sciatic (A), median (B), and trigeminal (C) nerve stimulation. Right dorsolateral view of rat brain indicates recording sites, coordinates in millimeters from bregma and from midline. Sizes of filled squares indicate relative amplitudes of primary cortical responses recorded at each site. Traces at right are averages of 20 responses at each site. Positivity is up. Vertical calibration bar indicates 400 μV; horizontal bar, 10 msec.
AMRs, however, were distinct. The location of the trigeminal nerve SEP generator site depended on the vibrissae stimulated. For example, stimulating the anterior middle vibrissae evoked responses that were greatest 2–3 mm posterior to the bregma and 7–8 mm lateral of the midline. Stimulating the posterior middle vibrissae shifted the AMR posteromedially to 3–4 mm posterior to the bregma and 5–6 mm lateral of the midline. The trigeminal nerve ACR corresponded to Par11,12 of the parietal cortex. The medial border of the trigeminal nerve ACR approached but did not overlap the lateral border of the median nerve ACR.

Peak latencies of the SEPs varied by recording site in the 10 rats. Moving the recording electrode from the AMR increased the latencies of the IP (Figure 1). At the AMR, IP latencies of the sciatic and median nerve SEPs were 11.5–13 and 7.0–8.5 msec, respectively. The trigeminal nerve SEP had the shortest IP latency, 6.5–8.0 msec, at the AMR. In four experiments, we cooled the rats to room temperature and observed a doubling of the SEP peak latencies. This

**FIGURE 2.** Summary diagram of three cortical somatosensory evoked potential (SEP) generator sites in rats. Response amplitudes are represented by shading at each site as percent of maximal response. Coordinates are millimeters from bregma and from midline. Typical middle cerebral artery (MCA) branching pattern illustrates relation between generator sites and artery. F, frontal branch; P, parietal branch.

**FIGURE 3.** Effect of middle cerebral artery occlusion (MCAo) on median nerve somatosensory evoked responses in rats. Top: Responses before MCAo; arrows point to two early positive wavelets. Middle: Within 30 seconds after MCAo, primary response was greatly diminished, whereas second positive wavelet (dashed arrow) showed relatively small decrease. Bottom: By 3 minutes after MCAo, both primary cortical response and adjacent positive wavelet were abolished, and only first positive wavelet (solid arrow) remained. Positivity is up.
FIGURE 4. Effect of middle cerebral artery occlusion (MCAo) on somatosensory evoked potentials (SEPs) in rats from sciatic, median, and trigeminal nerve stimulation. MCAo abolished responses from all three cortical areas within 5 minutes. Sciatic and median nerve SEPs recovered. Sciatic nerve SEP amplitude exceeded baseline by 3 hours after MCAo. Trigeminal nerve SEP showed partial transient recovery at 1 hour after MCAo. Positivity is up.
TABLE 1. SEP Amplitude and Ischemic Damage in Eight Rats After Middle Cerebral Artery Occlusion

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<tr>
<th>Rat</th>
<th>SEP amplitude (%)</th>
<th>Ischemic damage</th>
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<tr>
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<td>HL 1 hr</td>
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<tr>
<td>1</td>
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SEP, somatosensory evoked potential; HL, hind limb area; FL, fore limb area; + +, many cortical neurons damaged in cortex; +, some damaged neurons scattered through cortex; –, no damaged neurons observed histologically; ••, infarct involving entire cortical region; •, partial involvement by infarct.

Three rats (4, 5, and 6) had no gross ischemic involvement of FL 3 hours after MCAO; median nerve SEP recovered fully in all three, but mean amplitude did not exceed baseline. Ischemic damage was observed in Parl of all six rats examined 3 hours after MCAO; none of these rats showed substantial recovery of the cortical response in this region, and only one rat had trigeminal SEP amplitudes of as much as 20% of baseline by 3 hours after MCAO. In the two rats (7 and 8) examined 24 hours after MCAO, the infarct could be easily distinguished from surrounding regions and completely involved FL and Parl as well as part of HL; these rats had no SEPs in the three regions 24 hours after MCAO, although one rat (7) transiently recovered sciatic nerve SEPs to 83% of baseline amplitude 3 hours after MCAO.

Because of the widespread losses of both cortical and subcortical SEP components, we wondered whether MCAO transiently reduced general brain excitability. SEPs recorded from the nonischemic contralateral hemisphere in four rats indicated that MCAO abolished SEPs only in the ipsilateral hemisphere. MCAO consistently increased sciatic nerve SEP amplitudes in the contralateral hemisphere (Figure 5). By 3 hours after MCAO, SEP amplitudes were 190% of baseline. Such increased SEP amplitudes in the contralateral hemisphere have been reported previously.3

Discussion

Our results indicate that sciatic, median, and trigeminal nerve stimulation produce robust and sharply localized responses in specific cortical regions. Recorded from the surface of the cortex, the responses often exceeded 1 mV in amplitude and required minimal averaging. AMRs were very consistent from rat to rat and were sharply delineated. For example, SEPs usually could not be detected 3–4 mm from the AMR. The spatial distribution of the cortical responses corresponded to the somatotopic distribu-
tion of cortical unit activity elicited by mechanical stimulation of the body surface. The three somatosensory cortical regions activated by trigeminal, median, and sciatic nerve stimulation are conveniently situated at sequentially greater distances from the center of the infarct caused by MCAO. The trigeminal SEP is generated in Par1, usually situated within the parietal border of the infarct. We recently found that treatment with 21-aminosteroids significantly reduces shifts of ions and water in this region. Since MCAO reduced trigeminal nerve SEP amplitudes by >80% in seven of eight rats, even partial recovery in a few rats after treatment would be highly significant. The infarct produced by MCAO generally encroaches upon FL in our experience. The time course of SEP loss and recovery in this region may be useful for assessing the effects of ischemia in this part of the cortex. In contrast, ischemic changes were seen in HL of only 50% of the rats. Prolonged losses of sciatic nerve SEP in more rats would indicate unusually extensive ischemic involvement.

MCAO rapidly abolished the primary cortical responses in all three cortical regions for at least 30 minutes, even though ischemic damage was not always seen in HL and FL. Such widespread loss of SEPs may reflect transient ischemia in the entire MCA territory before the brain readjusts collateral blood flow to compensate for the occlusion. Artery-to-artery anastomoses connect the MCA with the anterior and posterior cerebral arteries. We have observed retrograde blood flow in the distal ends of transected frontal and parietal MCA branches. This retrograde blood flow does not take place immediately after occlusion and sometimes requires several minutes to start.

Several alternative explanations for the widespread depression of SEPs should be considered. First, diffusion of K+ from ischemic areas to the surrounding cortex may be responsible for the widespread SEP depression. Second, K+ may also diffuse into the white matter underlying the cortex and block axons conducting HL SEP. This possibility is supported by the loss of positive wavelets preceding the IP in all three regions, suggesting blockade of thalamocortical white matter conduction. However, many of the rats showed rapid loss of the primary cortical responses and delayed loss of the subcortical responses. Third, widespread suppression of cortical SEPs may be synaptically mediated. Neurons in ischemic cortex may become excited as they depolarize, releasing neurotransmitters into other areas of the cortex and transiently inhibiting neuronal excitability. However, MCAO did not depress SEP in the contralateral cortex, which should receive significant innervation from the ischemic cortex.

In five of eight rats, SEP responses recovered in HL, and SEP amplitudes in three of the five rats were dramatically greater than baseline. In Figure 4, for example, the sciatic SEP amplitude increased to nearly twice baseline. The mechanism of the augmented SEP response is unclear. Cellular swelling may have reduced the extracellular volume in HL and increased the electrical impedance of extracellular diffusion paths. Since field potentials generated by neurons result from extracellular currents, an increase in tissue impedance would increase the amplitude of field potentials. Extracellular K+ concentrations may also be elevated in this region, perhaps due to spreading depression, depolarizing the cells and bringing them closer to threshold. Finally, changes in the somatosensory injury to the cortex may be responsible for the increased amplitude of the responses. The nucleus gracilis and the nucleus cuneatus both receive strong inhibitory cortical input. Thus, disinhibition may have increased the input to the cortex. This is supported by our observation of greater SEP responses in the contralateral cortex.

Several investigators have used neurophysiologic tests to assess cortical excitability after regional and global ischemia. To our knowledge, SEP changes in the rat MCAO model have not been previously described. While the presence of evoked potentials may not necessarily indicate normal cortical function, their loss from three distinct cortical regions represents strong evidence for widespread transient cortical dysfunction after MCAO. The different patterns of recovery in the three regions suggest that SEPs will be useful for the serial monitoring of cortical function in rats after MCAO.

Acknowledgments

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