Diffuse-reflectance imaging of peri-infarct depolarization in a rat middle cerebral artery occlusion model

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1. Introduction
In focal cerebral ischemia, spreading depolarization is one of the key events that determine the brain tissue survival. In the infarct core, impairment of energy metabolism causes anoxic depolarization (AD), which considerably increases energy consumption, accelerating irreversible neuronal damage. In the peri-infarct penumbra region, where tissue is still reversible in spite of the limited blood flow, peri-infarct depolarization (PID) occurs, exacerbating energy deficit and hence expanding the infarct area. Thus, a method for noninvasive, real-time monitoring of AD and PIDs is crucially important to diagnose brain tissue viability. Spreading depolarization is generally accompanied by massive ion movements across the cellular membrane. This changes cellular/subcellular morphological characteristics in the tissue, causing change in light scattering. Since in the near-infrared (NIR) spectral region, the reduced scattering coefficient of the gray matter is two orders of magnitude larger than the absorption coefficient, the use of light scattering signal enables sensitive monitoring of the events associated with spreading depolarization.

We previously observed AD by diffuse reflectance measurement in a rat hypoxic brain model [1,2]. In this study, we performed transcranial NIR diffuse reflectance imaging of the rat brain during middle cerebral artery (MCA) occlusion, which caused PIDs, by using a charge-coupled device (CCD) and examined spatiotemporal characteristics of PIDs.

2. Materials and Methods
Sprague-Dawley male rats weighing 200-280 g were anesthetized with pentobarbital sodium (50 mg/kg animal weight) and placed in a stereotactic frame. After shaving the head, the scalp was incised at the midline and the parietal bone was exposed. For transcranial reflectance imaging of the rat brain, we used illumination with NIR light obtained from a white light lamp with a band-pass filter (800 ± 70 nm). The light was incident onto the entire cortex through the intact skull at an oblique angle to avoid specular reflection. The diffusely reflected light from the brain was imaged with a 8-bit CCD. To clearly visualize spatiotemporal change in the diffuse light reflectance, we created difference images at each time point by using an image acquisition and calculating software. A focal cerebral ischemia was induced by distal MCA occlusion. The left temporals muscle was separated from the temporal bone and removed. A window (3 mm x 4 mm) was drilled in the temporal bone and left MCA was occluded using bipolar electric coagulation.

3. Results and Discussion
Figure 1 shows difference NIR diffuse-reflectance images from the rat brain after MCA occlusion. About 1 min after occlusion, diffuse reflectance decreased focally in the left outermost region, and the dark region spread over the entire cortex with the elapse of time. The decreased reflectance indicated decrease in the light scattering, which is attributable to cell swelling accompanied by the PID. The number of times of the depolarization wave ranged from four to ten within 1 hour after MCA occlusion and mean velocity of the waves was about 2.0 mm/min, which were consistent with those recorded by electrodes [3]. These results showed the usefulness of the NIR diffuse reflectance to imaging to visualize spatiotemporal characteristics of PIDs in a rat MCA occlusion model.

Figure 1 (a) Photograph and (b-f) difference NIR diffuse-reflectance images for a rat brain after MCA occlusion.

4. Conclusion
Diffuse-reflectance imaging is useful for understanding pathophysiology of PIDs and for developing new diagnostic and therapeutic methods for ischemic stroke.

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References