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Modification of cortical excitability in neuropathic rats: A voltage-sensitive dye study

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ABSTRACT

Recent advances in optical imaging techniques have made it possible to monitor neural activity and provided powerful tools to reveal the spatiotemporal patterns of neural activity. We used optical imaging to determine whether nerve injury affects excitability of the sensory cortex. Male Sprague–Dawley rats were subjected to neuropathic surgery consisting of a tight ligation and transection of the left tibial and sural nerves while under pentobarbital anesthesia. The rats were reanesthetized with urethane two weeks post-operatively, and the exposed cortex surfaces were stained with a voltage-sensitive dye (di-2-ANEPEQ). After electrical stimulation of the receptive field, optical signals from the cerebral cortex were recorded using an optical imaging system. Increased optical intensity and an enlarged area of activation were observed in the cerebral cortex of neuropathic rats during electrical stimulation compared to normal or sham-operated rats. Higher electric stimulation resulted in more intensity and a larger area of activation in neuropathic rats. These results suggest that cortical excitability, resulting from peripheral stimulation, may be affected by nerve injury, which indicates a degree of neural plasticity.

Experimental models suggest that chronic neuropathic pain in animals [12,19] and humans [4,6] results from neuroplastic changes such as disease or injury occurring in either the peripheral or central nervous systems. These plastic changes may be reflected in the activity of the central nervous system. The characteristic symptoms of neuropathic pain are hyperalgesia and allodynia.

Anatomical and physiological studies in animals as well as functional imaging studies in humans have demonstrated that multiple cortical areas, such as the primary and secondary somatosensory cortices, parietal operculum, insula, anterior cingulate cortex, and prefrontal cortex, are activated by painful stimuli [1,5,17,21,22]. These areas probably process different aspects of pain. Since positron emission tomography (PET) scans have been used to study functional activation by acute heat pain in the human brain [8,21], the cortical representation of pain has become one of the most active areas in pain research. For example, Peyron et al. [16] assessed the relationship between analgesic administration and hemodynamic changes in the brains of patients with neuropathic pain using PET scanning. In a functional magnetic resonance imaging (fMRI) study by Peyron et al. [17], allodynia was associated with more activated ipsilateral responses in both the secondary somatosensory cortex (SII) and the insular cortex (IC). Schweinhardt et al. [18] used fMRI to show that the perceived intensity of allodynia is correlated with increased activity in the caudal anterior insular cortex.

Optical imaging of the brain makes it possible to simultaneously observe neuronal activity at many points. Studies have shown that measurements taken using optical coherence tomography and voltage-sensitive dyes can provide simultaneous mapping of activity from multiple regions and unprecedented information regarding the spatiotemporal activity patterns within the neuronal network in rat brains [13,14,20]. Ooi et al. [14] observed neural plasticity in nerve-injured mouse cortices using optical coherence tomography. They reported significant increases in scattering intensity, which suggests many possibilities for understanding the mechanisms of the formation and progression of neural plasticity after nerve injury [14]. However, the images obtained by optical coherence tomography are often backscattered from target tissue, making scattered images unclear and sometimes indistinguishable.

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Optical imaging with voltage-sensitive dyes has been successfully used to visualize stimulus-dependent activity of the somatosensory cortex in rats [13,15,20]. However, pain studies using optical imaging with voltage-sensitive dyes are few. Therefore, we conducted this study using voltage-sensitive dye imaging to determine whether cortical excitability is changed by nerve injury.

Thirty-six adult male Sprague–Dawley rats (220–250 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and divided into three groups: naïve (no surgery), sham operation (only clearly separated sciatic nerve and branches) and neuropathic surgery. In the neuropathic group, a segment of the sciatic nerve was exposed between the mid-thigh and the popliteal fossa. The three major divisions of the sciatic nerve were clearly separated based on individual perineuria. The tibial and sural nerves were tightly ligated and then transected, while the common peroneal nerve was left intact [10]. Complete hemostasis was confirmed and the wound was closed with muscle and skin sutures. All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University Health System.

Behavioral tests were performed on the first, fourth, seventh, and 14th post-operative days. Two components of neuropathic pain (mechanical allodynia and cold allodynia) were assessed in all rats [10]. To measure mechanical allodynia, rats were placed on a metal mesh floor under a custom-made transparent plastic dome (8 cm × 8 cm × 18 cm). Innocuous mechanical stimuli were applied to the sensitive area of each hind paw with a von Frey filament every 3–4 s (8 mN bending force, 10 repetitions). The frequency of foot withdrawal out of 10 trials was expressed as a percentage (response rate (%) = no. of foot withdrawals/no. of trials × 100). To quantify cold sensitivity, we observed the withdrawal rate in response to acetone applied to each paw every 5 min (five repetitions). The frequency of foot withdrawal out of five trials of acetone application was also expressed as a percentage. After the behavioral test (14 days p.o.), animals were subjected to optical imaging.

For optical imaging, rats were reanesthetized with urethane (1.25 g/kg, i.p.) and the trachea was cannulated to allow artificial respiration. Heart rate was monitored using electrocardiography (ECG), and body temperature was maintained at 37 °C with a heating pad (Homeothermic Blanket Control Unit, Harvard Apparatus, Holliston, MA, USA). After the reflexes completely disappeared, each animal was prepared for surgery. In order to exclude the influence of respiratory movements on optical measurements, vecuronium bromide (0.2 mg/kg, Huons Co., Hwaseong, Korea) was injected, and the animal was artificially ventilated using an animal respirator (Model 683, Rodent Ventilator, Harvard Apparatus). Each animal was positioned in a stereotactic frame (Narishige Scientific Instrument Laboratory, Tokyo, Japan), and a craniotomy (10 mm × 5 mm) was performed over the right somatosensory cortex. A well of dental acrylic was built around the exposed cortex and the dura was removed. The surface of the cortex was exposed to voltage-sensitive dye (di-2-ANEPEQ, Molecular Probes, Eugene, OR, USA) for 1 h. After staining, the cortex was rinsed twice with saline and kept moist.

In order to apply electrical stimulation, a pair of stainless steel needle electrodes were placed in the peripheral receptive field where the von Frey filament or acetone had been applied during the behavioral tests. The receptive field was stimulated with a square pulse (duration: 0.1 ms, interstimulus interval: 3 s, intensity: 0, 0.6, 1.2 and 1.8 mA) using a stimulus isolation unit (World Precision Instruments, Sarasota, FL, USA). During each trial, the change in fluorescence intensity was measured for about 1880 ms. The recording surface was placed under an optical microscope (Leica Microsystems Ltd., Heerbrugg, Switzerland) equipped with a 1× objective and a 0.63× projection lens. Cortical activity was recorded using an optical imaging system (MiCAM02, Brainvision Inc., Tokyo, Japan) through a 510–550 nm excitation filter, a dichroic mirror, and a 590 nm absorption filter. A tungsten halogen lamp (150 W) was used as a light source.

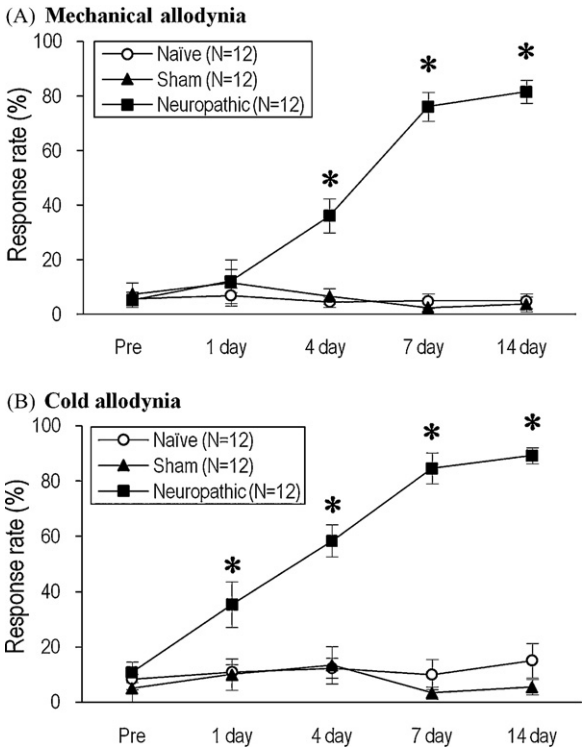

Fig. 1. Development of mechanical and cold allodynia in different groups. (A) Mechanical allodynia tested using a von Frey filament. (B) Cold allodynia induced using acetone. Each value represents the mean ± S.E.M. ((○) naïve; (▲) sham-operated; (■) neuropathic rats).

Fluorescence images were acquired at a rate of 3.7 ms/frame and were averaged 10 times using an optical imaging recording system. Image acquisition was triggered by electrocardiogram using a stimulus/non-stimulus subtraction method [13,20]. From captured images, fractional changes in optical signals (optical intensity) and areas of activation were quantified. The changes in intensity of the optical signals in the cortical area were measured by the ratio of change in the intensity of fluorescence (ΔF) to the initial intensity of fluorescence (F) expressed as percent fractional change ($\Delta F/F \times 100$). In order to analyze the area of activation, each group of 10 color images was converted to a single color image. The converted area was calculated in each captured image and expressed as a percent of the area of activation compared to the entire captured area (activated area/whole captured area × 100). The optical intensity and the activated area were analyzed using BV Analyze (Brainvision Inc., Tokyo, Japan) and MetaMorph (Universal Imaging Co., Downingtown, PA, USA) software programs.

Data are presented as the mean ± standard error of the mean (SEM). Differences in intensities of optical signals and areas of activation were analyzed using one-way ANOVA followed by Dunnett's post hoc pairwise comparisons. P values less than 0.05 were considered significant.

The frequency of foot withdrawals to repeated mechanical or thermal stimulation was plotted against time for each group (Fig. 1). It should be noted that there was no significant difference among groups (naïve, sham surgery and neuropathy group) in withdrawal behavior before surgery. After neuropathic surgery, however, rats began to withdraw the affected foot when von Frey or acetone was applied to the receptive field on the injured side. Beginning