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INTRACORTICAL SIGNAL PROCESSING OF PERIODONTAL LIGAMENT SENSATIONS IN RAT

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Abstract—The somatosensory information from the orofacial region, including the periodontal ligament (PDL), is processed in a manner that differs from that used for other body somatosensory information in the related cortices. It was reported that electrical stimulation to rat PDL elicited activation of the insular oral region (IOR) and the primary (S1) and secondary (S2) somatosensory cortices. However, the physiological relationship between S1 and S2/IOR is not well understood. To address this issue, we performed *in vivo* optical imaging using a voltage-sensitive dye. Our results demonstrated that the electrical stimulation to the PDL of the mandibular incisor evoked the simultaneous activation of S1 and the S2/IOR. The stimulation to the initial response area of the S1 evoked responses in the S2/IOR, and vice versa. An injection of tetrodotoxin (TTX) to the cortical region between S1 and S2/IOR attenuated such elicited responses only in the non-stimulated cortical partner site. The cortico-cortical interaction between S1 and S2/IOR was suppressed by the application of TTX, indicating that these two cortical regions bi-directionally communicate the signal processing of PDL sensations. An injection of FluoroGold™ (FG) to the initial response area in S1 or the S2/IOR showed that FG-positive cells were scattered in the non-injected cortical counterpart. This morphological result demonstrated the presence of a bi-directional intracortical connection between the initial response areas in S1 and the S2/IOR. These findings suggest the presence of a mutual connection between S1 and the S2/IOR as an intracortical signal processing network for orofacial nociception.

Key words: rats, optical imaging, voltage-sensitive dye imaging, electric stimulation, somatosensory cortex, insular cortex.

INTRODUCTION

Patients who undergo orthodontic treatment frequently complain of acute and transient pain. One reason for this pain is that the orthodontic tooth movement elicits local inflammation (Proffit et al., 2013) accompanied by nociception in the periodontal ligament (PDL). Other types of somatosensory stimuli are also received by the PDL during orthodontic treatment, and the sensory information from the PDL is transmitted to the trigeminal sensory complex (i.e., the spinal nucleus and principal sensory nucleus of the trigeminal nerve). Information about an epicritic sensation, i.e., a tactile/pressure sensation, is transmitted to the principal sensory nucleus, whereas emotional sensations (i.e., the sense of temperature and pain) are processed in the spinal trigeminal nucleus (Saper et al., 2013). Nociceptive fibers of the medial system, including those extending from the PDL, terminate mainly in the subnucleus caudalis of the spinal nucleus, and this sensory information finally reaches the cerebral cortex via the parvocellular part of the ventral posteromedial thalamic nucleus (VPMpc), the amygdala, and the parabrachial nucleus (Møller, 2012; Basbaum and Jessell, 2013).

In general, somatosensory information is sequentially processed in the primary somatosensory cortex (S1) and then the secondary somatosensory cortex (S2) (Hendry and Hsiao, 2008). However, in their 2008 study of rats, Liao and Yen reported the morphological findings that (1) somatosensory projections from the thalamus terminate in S1 and S2 independently, and (2) there are bi-directional projections between S1 and S2. At that time, the presence of the independent thalamic projections to S1 and the S2/ insular oral region (IOR) had already been reported for several animal species by other research groups (Darian-Smith et al., 1966 [cat]; Rowe and Sessle, 1968 [cat]; Carvell and Simons, 1987 [mouse]; Krubitzer and Kaas, 1987 [squirrel]; Aldes, 1988 [rat]). However, it is not yet clear how the sensory information

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Abbreviations: BDA, biotinylated dextran amine; FG, FluoroGold; IC, insular cortex; IOR, insular oral region; MCA, middle cerebral artery; PDL, periodontal ligament; RF, rhinal fissure; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; S2/IOR, S2 and the insular oral region; TTX, tetrodotoxin; VPMpc, parvocellular part of the ventral posteromedial thalamic nucleus.

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from the PDL is transmitted to the somatosensory cortices S1 and S2.

Although somatosensory input assembles into a somatotopic map in S1 and S2 (which show a spatial localization of the different parts of the body), S1 is distinct from S2 in terms of physiological roles. S1 performs somatotopic discrimination and/or identification, whereas S2 plays a key role in the recognition of stimulus intensities and the memory formation concerning pain experience (Hendry and Hsiao, 2008). Sensory information from the oral region in rats is processed in the ventral part of this somatotopic map (Remple et al., 2003; Nakamura et al., 2015; Horinuki et al., 2015).

Nakamura et al. (2015) and Horinuki et al. (2015) reported that in the rat, the cortical areas relating to intraoral somatosensation of the dental pulp and the PDL are located in not only S1 but also the S2/IOR. In addition, Horinuki et al. (2015) demonstrated that the latency of the S2/IOR response to stimulation of the PDL is equal to or shorter than that of S1. Although much effort has focused on the elucidation of cortical neural networks that process somatosensory information, the cortical mechanism underlying the signal processing of PDL nociception has been largely unexplored. In the present study, we sought to clarify the mechanisms underlying the signal processing of PDL sensations in the rat S1 and S2/IOR. We used an *in vivo* optical imaging modality and a morphological technique to examine the mechanisms, with a focus on the intracortical relationship between somatosensory areas including the IOR.

EXPERIMENTAL PROCEDURES

Male Sprague–Dawley rats (Sankyo Labo, Tokyo) were used. All animal experiments performed in this study were approved by the Meikai University Animal Ethics Committee (approval no. A1536) and were conducted in accordance with institutional guidelines for the care and use of experimental animals described in the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of rats used. A total of 27 rats were used, and the numbers of rats used in the individual procedures are noted below.

In vivo optical imaging

Cortical responses were measured by an *in vivo* optical imaging technique using a voltage-sensitive dye (RH-1691, Optical Imaging, New York, NY). The data acquisition was performed using an imaging system composed of a stereomicroscope (Leica Microsystems, Wetzler, Germany) equipped with a CCD camera (MiCAM02, BrainVision, Tokyo), as described (Mizoguchi et al., 2011; Fujita et al., 2012; Nakamura et al., 2015; Horinuki et al., 2015).

Atropine methyl bromide (5.0 mg/kg, i.p.) was administered as a premedication to 140- to 300-g rats at postnatal week 5–6 (n = 23), and the rats were then anesthetized with urethane (Sigma–Aldrich, Tokyo) (1.5 g/kg, i.p.). Urethane was additionally applied to maintain surgical levels of anesthesia as needed. Each rat underwent a tracheotomy and intubation, and was then fixed to a custom-made stereotaxic frame which was tilted 60° laterally and maintained at approx. 37 °C by a heating pad (BWT-100, Bio Research Center, Aichi, Japan). After the anesthetized rat was fixed in place, the left temporal muscle and zygomatic arch were carefully removed, and a craniotomy was performed to expose both the somatosensory and insular cortices in the same field of view (Fig. 1A; refer to Remple et al., 2003 and Nakamura et al., 2015). Lidocaine (2% gel, AstraZeneca, Tokyo) was administered to the incisions to ensure complete analgesia as needed. The left insular cortex (IC) and surrounding cortical surface was stained with RH-1691, and fluorescent images of the voltage-sensitive dye findings were observed with the CCD camera.

The frame size acquired by the CCD camera was a 6.4×4.8 mm^2 imaging area consisting of 184×124 pixels. The cortical surface immersed with RH-1691 was intermittently illuminated at 632-nm excitation wavelength, which was generated using a tungsten–halogen lamp (CLS150XD, Leica Microsystems) through an excitation filter and a dichroic mirror. The emission fluorescence was obtained through the CCD camera with an absorption filter (λ > 650- nm-long pass, Andover, Salem, NH). Fluorescent images were acquired at a rate of 4 ms/frame over a 500-ms period.

Because the fluorescence of RH-1691 showed acute bleaching, we performed an image subtraction of values in the absence of any stimuli from each recording in order to reduce the noise. Thus, one image set was built up from paired recordings with and without stimulation, and 32–40 consecutive images in response to the stimuli were averaged.

Imaging data processing

For all optical imaging experiments, the optical signal is presented as a ratio ($\Delta F/F$), in which ΔF shows the change in the fluorescence intensity and F is the initial fluorescence intensity. The calculated ratio was processed with a spatial filter (9×9 pixels). We defined a 'significant response' as a signal exceeding three times the standard deviation (SD) of the baseline noise. The analyses and processing of all imaging data were performed with the software BrainVision Analyzer ver. 1208 (BrainVision, Tokyo). The area and amplitude of an optical signal ($\Delta F/F$) are presented as a pseudo-color map; for example, images are arranged according to the elapsed time order. Multiple images from multiple rats were aligned and superimposed at an intersectional point of the rhinal fissure (RF) and the middle cerebral artery (MCA) as a marker. These alignments and superimpositions of images were conducted using Adobe Illustrator (ver. CS6; Adobe Systems, San Jose, CA).

The response area (mm^2) was calculated as follows: The entire area of the acquired image was 6.4×5.28 mm^2, because only the vertical ratio of the imaging area was converted into 1.1. This area corresponded to 106,720 pixels. The response area was

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