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SOMATOTOPIC DIRECT PROJECTIONS FROM OROFACIAL AREAS OF PRIMARY SOMATOSENSORY CORTEX TO PONS AND MEDULLA, ESPECIALLY TO TRIGEMINAL SENSORY NUCLEAR COMPLEX, IN RATS

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Abstract—The primary somatosensory cortex (S1) projects to the thalamus and brainstem somatosensory nuclei and modulates somatosensory information ascending to the S1 itself. However, the projections from the S1 to the brainstem second-order somatosensory neuron pools have not been fully studied. To address this in rats, we first revealed the somatotopic representation of orofacial areas in the S1 by recording cortical surface potentials evoked by stimulation of the lingual, mental, infraorbital, and frontal nerves. We then examined the morphology of descending projections from the electrophysiologically defined orofacial S1 areas to the pons and medulla after injections of an anterograde tracer, biotinylated dextranamine (BDA), into the orofacial S1 areas. BDA-labeled axon terminals were seen mostly in the trigeminal sensory nuclear complex (TSNC) and had a strong contralateral predominance. They also showed a somatotopic arrangement in dorsoventral and superficial-deep directions within almost all rostrocaudal TSNC levels, and in a rostrocaudal direction within the trigeminal caudal subnucleus. In the principal nucleus (Vp) or oral subnucleus (Vo) of TSNC, the BDA-labeled axon terminals showed a somatotopic arrangement closely matched to that of the electrophysiologically defined projection sites of orofacial primary afferents; these projection sites were marked by injections of a retrograde tracer, Fluorogold (FG), into the Vp or Vo. The FG injections labeled a large number of S1 neurons, with a strong contralateral predominance, in a somatotopic manner, which corresponded to that presented in the electrophysiologically defined orofacial S1 areas. The present results suggest that the orofacial S1 projections to somatotopically matched regions of trigeminal second-order somatosensory neuron pools may allow the orofacial S1 to accurately modulate orofacial somatosensory transmission to higher brain centers including the orofacial S1 itself.

Key words: SI, topography, somatotopy, feedback, corticofugal.

General somatic sensation is somatotopically transmitted mainly to the contralateral primary somatosensory cortex (S1) through primary somatosensory afferents, second-order neurons in the lower brainstem, and then third-order neurons in the contralateral thalamus (for review, see Werner and Whitsel, 1973). It has been generally recognized that the S1 has descending projections to the subcortical relay neurons to modulate the sensory information ascending to the S1 itself (for review, see Nuñez and Malmierca, 2007). In the case of the thalamic neurons, their reciprocal connections with the S1 have been well documented (Diamond et al., 1969; Jones et al., 1979; Land et al., 1995; Deschênes et al., 1998), and similarly for the second-order somatosensory neurons, topographic descending projections from the contralateral S1 to the dorsal column nuclei have also been documented morphologically and electrophysiologically (Towe and Jabbur, 1961; Weisberg and Rustioni, 1976, 1979; Cheema et al., 1983; Martínez-Lorenzana et al., 2001). In the dorsal column nuclei, the lowest threshold for spikes elicited by stimulation of S1 is seen in neurons with a hindpaw receptive field, which corresponds to the receptive field of the stimulated S1 (Cole and Gordon, 1992; Malmierca and Nuñez, 1998, 2004). For other second-order somatosensory neuron pools, the existence of S1 projections to the trigeminal sensory nuclear complex (TSNC), which principally receives orofacial somatosensory inputs has also been dem-

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Abbreviations: Agl, lateral part of the agranular cortex; AP, area postrema; Au, auditory cortex; BDA, biotinylated dextranamine; c-inf.orb-S1, caudal part of inf.orb-S1; Cu, cuneate nucleus; ECu, external cuneate nucleus; FG, Fluorogold; front-S1, frontal nerve area of S1; front-V1 region, frontal nerve region of Vp; Gr, gracile nucleus; HRP, horseradish peroxidase; inf.orb-S1, infraorbital nerve area of S1; inf.orb-V2 region, infraorbital nerve region of Vp; Ins, insular cortex; IO, inferior olive; l-inf.orb-V2 region, lateral part of inf.orb-V2 region; ling-S1, lingual nerve area of S1; ling-V3 region, lingual nerve region of Vor; ment-S1, mental nerve area of S1; ment-V3 region, mental nerve region of Vor; m-inf.orb-V2 region, medial part of inf.orb-V2 region; PaV, paratrigeminal nucleus; Pb, parabrachial nucleus; PBS, phosphate buffered saline; PtA, parietal association cortex; py, pyramidal tract; pyx, pyramidal decussation; r-inf.orb-S1, rostral part of inf.orb-S1; scp, superior cerebellar peduncle; SO, superior olive; Sol, nucleus of the solitary tract; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; TSNC, trigeminal sensory nuclear complex; Vc, trigeminal caudal subnucleus; Vi, trigeminal interpolar subnucleus; Vidm, dorsomedial part of Vi; VIImo, facial nucleus; Vis, visual cortex; Vivl, ventrorateral part of Vi; Vmes, trigeminal mesencephalic nucleus; Vmodl, dorsolateral part of the trigeminal motor nucleus; Vmovm, ventromedial part of the trigeminal motor nucleus; Vo, trigeminal oral subnucleus; Vodm, dorsomedial part of Vo; Vor, rostrodorsomedial part of Vo; Vovl, ventrolateral part of Vo; Vp, trigeminal principal nucleus; Vsup, supratrigeminal nucleus; Vtr, trigeminal spinal tract; XII, hypoglossal nucleus; 7g, genu of 7N; 7N, facial nerve.

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onstrated in several animals (monkey, Mettler, 1935a,b; Kuypers, 1960; cat, Brodal et al., 1956; Dubner and Sessle, 1971; Wold and Brodal, 1973; Dunn and Tolbert, 1982; rat, Torvik, 1957; Wise et al., 1979; Killackey et al., 1989; mouse, Welker et al., 1988). The direct projections from the whisker barrel areas in the S1 to all the levels of TSNC have also been demonstrated by means of anterograde tract-tracing (rat, Wise and Jones, 1977; Jacquin et al., 1990c; mouse, Welker et al., 1988). In addition, electrophysiological studies have shown that cortical somatosensory neurons receiving certain facial inputs have direct descending projections to the cat's trigeminal principal nucleus (Vp) and caudal subnucleus (Vc), which may receive analogous facial inputs (Dubner and Sessle, 1971), and that stimulation of a barrel column in the S1 excites multiwhisker neurons in the rat's trigeminal interpolar subnucleus (Vi) whose receptive field includes the whiskers represented in the stimulated S1 barrel column (Woolston et al., 1983; Furuta et al., 2010).

Collectively, these earlier studies suggest that regions of the S1 project somatotopically to second-order somatosensory neurons with a receptive field corresponding to the somatotopically related S1 region. However, the detailed somatotopy in the descending projections from the various regions of the orofacial S1 to the second-order somatosensory neurons has not been revealed.

Therefore, the aims of the present study were first to delineate various orofacial afferent projection sites in the S1, and then to examine the features of descending projections from these delineated orofacial S1 areas to the second-order somatosensory neuron pools including the TSNC, and, lastly, to examine the somatotopy of the projections.

EXPERIMENTAL PROCEDURES

Animals, recordings, and tracer injections

A total of 114 male Wistar rats in the weight range 280–330 g were used in the present study. All experimental procedures were approved by the Osaka University Graduate School of Dentistry Intramural Animal Care and Use Committee in accordance with the guidelines of NIH, USA, and all efforts were made to minimize the number of animals used. Animals were anesthetized with an i.p. injection of sodium pentobarbital (55 mg/kg), with supplementary doses of sodium pentobarbital (10 mg/ml) being given if necessary to maintain the animal in a deep level of anesthesia throughout the experiment. The electrocardiogram and rectal temperature were monitored continuously and maintained within physiological limits.

In experiment 1, either the lingual nerve, mental nerve, infraorbital nerve, or frontal nerve was exposed on the right side, and bipolar silver hook electrodes were attached for electrical stimulation in 39 rats. The trachea was cannulated for artificial ventilation, and head of the animal was fixed to a stereotaxic apparatus. In order to exclude the influence of movements induced by peripheral nerve stimulation, animals were immobilized with vecuronium bromide (0.02 mg/kg, i.v.) and artificially ventilated. To keep the animals immobilized, vecuronium bromide was added (0.02 mg/kg, i.v.) several times throughout the experiment. By reference to the atlas by Paxinos and Watson (1998) and representations of orofacial area in the S1 reported by Chapin and Lin (1984) and Remple et al. (2003), a small burr hole was made on the parietal part of the skull on the left side to expose the dura overlying the S1. To identify the different orofacial S1 areas to which somatosensory information from various orofacial tissues was conveyed, field potentials evoked by stimulation of the peripheral nerves (single rectangular pulses of 0.2 ms duration, 1 Hz) were recorded through an AgCl-coated monopolar, silver, ball electrode (diameter: 0.3 mm) placed gently on the dura. The recording points on the dura were spaced 0.5 mm (or 1.0 mm) apart in rostrocaudal and dorsoventral directions. Evoked surface field potentials were recorded on magnetic tape (PC204, Sony Magnescale, Tokyo, Japan), and later replayed and analyzed with computer assistance (PawerLab 8/30, ADInstruments, Sydney, Australia). Responses to 10 successive peripheral stimuli were averaged at each site. The peak amplitudes of responses with latencies of 8–12 ms were measured. We defined four S1 receptive field areas where the responses whose peak amplitudes were larger than the half of the largest peak amplitude could be evoked by stimulation of the lingual nerve, mental nerve, infraorbital nerve, or frontal nerve: these areas are referred to as the S1 lingual nerve area (ling-S1), S1 mental nerve area (ment-S1), S1 infraorbital nerve area (inf.orb-S1), and S1 frontal nerve area (front-S1), respectively. The inf.orb-S1 was further divided into its rostral part (r-inf.orb-S1) and caudal part (c-inf.orb-S1) because it was much larger than the other S1 receptive field areas.

After finishing the recordings, to mark the center of S1 receptive field areas identified on the cortical surface, an injection of 3% horseradish peroxidase (HRP, Toyobo, Osaka, Japan) was made within the S1 at a depth of 50 μm beneath the center. Furthermore, in order to mark two reference points for the stereotaxic S1 location, HRP was also injected into two points at a depth of 50 μm in the cortex (3 mm ventral to bregma at the rostrocaudal level of bregma, and 3 mm rostral and 3 mm ventral to bregma, or 3 mm caudal and 3 mm ventral to bregma). For these injections, a glass micropipette (tip diameter 5–10 μm) filled with 3% HRP dissolved in saline was inserted into the cortex through a small hole made by cutting the dura, and HRP was iontophoretically injected (positive pulses, 2 μA, 300 ms, 2 Hz, 1 min). Subsequently, the animals were perfused with a fixative as described later in the text.

In experiment 2, one of the four peripheral nerves mentioned above was exposed on the right side, and bipolar silver hook electrodes were attached for electrical stimulation in 41 rats. The head of the animal was fixed in a stereotaxic apparatus, and a small burr hole was made on the parietal part of the skull overlying the center of one of the four orofacial S1 areas identified in experiment 1, in order to reveal the dura on the left side. A small part of the dura was cut to expose the S1 surface. The platform on which the animal was placed was rotated so that the exposed cortical surface was horizontal. A glass microelectrode (tip diameter 10–15 μm) filled with an anterograde tracer, 4% biotinylated dextranamine (BDA, 10,000 MW, Molecular Probes, Eugene, OR, USA) dissolved in saline was inserted perpendicularly into the exposed S1. By recording the intracortical field potentials evoked by stimulation of the peripheral nerve, the layer V of the orofacial S1 area identified in experiment 1 was located, and BDA was then iontophoretically injected in it (positive pulses, 2 μA, 300 ms, 2 Hz, 60 min). BDA was successfully injected into the ling-S1, ment-S1, r-inf.orb-S1, c-inf.orb-S1, and front-S1 in three rats for each of the five injection sites.

After BDA injections, the bone defect was filled with dental cement. All wounds were sutured closed, an antibiotic (cefotiam hydrochloride, 66 mg/kg) and an analgesic (flurbiprofen axetil, 3.3 mg/kg) were given i.p., and the animals were allowed to recover from anesthesia in their cages. During 7–8 days of postinjection survival, the animals were monitored on a daily basis to assess body weight, general behaviors, and any postoperative complications such as bleeding or inflammation. At the end of the survival period, all animals were re-anesthetized deeply with an i.p. injec-