

Orofacial proprioceptive thalamus of the rat

Atsushi Yoshida¹ · Takashi Fujio¹ · Fumihiko Sato¹ · Md Sams Sazzad Ali¹ · Tahsinul Haque^{1,4} · Haruka Ohara¹ · Masayuki Moritani² · Takafumi Kato¹ · Jonathan O. Dostrovsky³ · Yoshihisa Tachibana¹

Received: 27 July 2016 / Accepted: 26 December 2016 / Published online: 5 April 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract The ascending pathway mediating proprioception from the orofacial region is still not fully known. The present study elucidated the relay of jaw-closing muscle spindle (JCMS) inputs from brainstem to thalamus in rats. We injected an anterograde tracer into the electrophysiologically identified supratrigeminal nucleus (Su5), known to receive JCMS input. Many thalamic axon terminals were labeled and were found mainly contralaterally in a small, unpredicted area of the caudo-ventromedial edge (VPMcvm) of ventral posteromedial thalamic nucleus (VPM). Electrical stimulation of the masseter nerve and passive jaw movements induced large responses in the VPMcvm. The VPMcvm is far from the rostradorsal part of ventral posterolateral thalamic nucleus (VPL) where proprioceptive inputs from the body are represented. After injection of a retrograde tracer into the electrophysiologically identified VPMcvm, many neurons were labeled almost exclusively in the contralateral Su5, whereas no labeled neurons were found in the principal sensory trigeminal nucleus (Pr5) and spinal trigeminal nucleus (Sp5). In contrast, after injection of a retrograde tracer into the core of VPM, many neurons

were labeled contralaterally in the Pr5 and Sp5, but none in the Su5. We conclude that JCMS input excites trigeminothalamic projection neurons in the Su5 which project primarily to the VPMcvm in marked contrast to other proprioceptors and sensory receptors in the orofacial region which project to the core VPM. These findings suggest that lesions or deep brain stimulation in the human equivalent of VPMcvm may be useful for treatment of movement disorders (e.g., orofacial tremor) without affecting other sensations.

Keywords Orofacial deep sensation · VPM · Trigeminal · Supratrigeminal nucleus · Thalamotomy · DBS

Abbreviations

3V	Third ventricle
7n	Facial nerve
10	Dorsal motor nucleus of vagus
12	Hypoglossal nucleus
ABC	Avidin-biotin-peroxidase complex
APT	Anterior pretectal nucleus
AVM	Area ventral to the Mo5 and medial to the Pr5
BDA	Biotinylated dextranamine
CL	Centrolateral thalamic nucleus
CM	Central medial thalamic nucleus
contra	Contralateral
Cu	Cuneate nucleus
DAB	Diaminobenzidine
DBS	Deep brain stimulation
FG	Fluorogold
fr	Fasciculus retroflexus
Gr	Gracile nucleus
HRP	Horseradish peroxidase
I5	Intertrigeminal region

✉ Atsushi Yoshida
yoshida@dent.osaka-u.ac.jp

¹ Department of Oral Anatomy and Neurobiology, Graduate School of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan

² Department of Physical Therapy, Faculty of Health Science, Morinomiya University of Medical Sciences, Osaka 559-8611, Japan

³ Department of Physiology and Faculty of Dentistry, University of Toronto, Toronto, ON M5S 1A8, Canada

⁴ Present Address: Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, King Saud University, Riyadh 11545, Saudi Arabia

IMD	Intermediodorsal thalamic nucleus
ipsi	Ipsilateral
JCMS	Jaw-closing muscle spindle
KF	Kölliker–Fuse nucleus
LHb	Lateral habenular nucleus
LPB	Lateral parabrachial nucleus
m5	Trigeminal motor nerve
Me5	Mesencephalic trigeminal nucleus
me5	Mesencephalic trigeminal tract
MD	Mediodorsal thalamic nucleus
MHb	Medial habenular nucleus
ml	Medial lemniscus
Mo5	Trigeminal motor nucleus
MPB	Medial parabrachial nucleus
PB	Parabrachial nucleus
PC	Paracentral thalamic nucleus
PF	Parafascicular thalamic nucleus
PhB	Phosphate buffer
PhBS	Phosphate buffered saline
Po	Posterior thalamic nucleus
Pr5	Principal sensory trigeminal nucleus
PV	Paraventricular thalamic nucleus
scp	Superior cerebellar peduncle
SO	Superior olive
Sol	Nucleus of the solitary tract
Sp5	Spinal trigeminal nucleus
sp5	Spinal trigeminal tract
Sp5C	Sp5, caudal part
Sp5I	Sp5, interpoler part
Sp5O	Sp5, oral part
Su5	Supratrigeminal nucleus
TMB	Tetramethyl benzidine
Vim	Ventral intermediate thalamic nucleus
VM	Ventromedial thalamic nucleus
VPL	Ventral posterolateral thalamic nucleus
VPLo	Oral part of the VPL
VPM	Ventral posteromedial thalamic nucleus
VPMcvm	Caudo-ventromedial edge of the VPM
VPPC	Parvicellular part of ventral posterior thalamic nucleus
WGA-HRP	Wheat-germ agglutinin conjugated horseradish peroxidase

Introduction

Proprioceptive sensation from the body is conveyed to the rostr dorsolateral shell or rostral (oral) region (or VPLo) of the ventral posterolateral thalamic nucleus (VPL) (monkey, Friedman and Jones 1981; Maendly et al. 1981; Jones and Friedman 1982; Jones et al. 1982; cat: Andersson et al. 1966; rat; Francis et al. 2008). However, little is known about the thalamic relay of proprioceptive inputs from the

orofacial area. Thus, the major aim of this study is to obtain detailed information on the rat thalamic relay of orofacial proprioceptive information. Jones and Friedman (1982) predicted that the relay site for orofacial proprioception is located in the rostr dorsolateral part of the ventral posteromedial thalamic nucleus (VPM) (medially adjacent to the VPLo), but direct evidence is still lacking. Although all cutaneous, deep and proprioceptive sensations from the body are conveyed by the primary afferents arising from the dorsal root ganglion neurons, the orofacial sensations are conveyed by the primary afferents arising not only from the trigeminal ganglion neurons (comparable to the dorsal root ganglion neurons) but also from the mesencephalic trigeminal nucleus (Me5) neurons (for review, see Dubner et al. 1978; Taylor 1990). Luo and Dessem (1995) and Luo et al. (1995) reported the existence of neurons in the rat supratrigeminal nucleus (Su5) projecting to the contralateral VPM, but they did not determine the detailed projection termination sites in the VPM. In addition, earlier studies including those of Luo and colleagues cited above, were conducted without defining the exact location of the Su5. Furthermore the definition of Su5 in previous rat studies are inconsistent and even contradictory (e.g., rat brain atlas by Swanson, 1992, and Paxinos and Watson 1998, 2007, 2014). Therefore, we (Fujio et al. 2016) have recently determined the exact location of the rat Su5 according to the original definition of the Su5 by Lorente de Nó (1992, 1993): the Su5 is a premotoneuron pool in the trigeminal reflex arc. We cytoarchitectonically identified a restricted area in the supratrigeminal region which receives strong projections from Me5 neurons conveying inputs from the jaw-closing muscle spindles (JCMSs), and, thus, we defined this area as the “Su5”. This study also revealed that the location of our “Su5” does not correspond to the location of Su5 shown in the rat atlases by Paxinos and Watson (1998, 2007, 2014), but overlaps to some extent with the “trigeminal transition zone” which was newly introduced in the supratrigeminal region by Paxinos and Watson (2007, 2014). Therefore in the present study, we applied our own definition of the Su5 (Fujio et al. 2016) to identify the thalamic “orofacial proprioception-center” receiving JCMS inputs. To this end, we examined the thalamic projections from the Su5 by using neuronal tract tracing and electrophysiological recording methods in rats.

Earlier electrophysiological studies demonstrated that cutaneous and proprioceptive sensations from the body are separately encoded in different regions of VPL (in the core (middle) region and rostr dorsolateral shell or rostral (oral) region of VPL, respectively) (human: Ohye et al. 1989; Lenz et al. 1990, 1994; monkey: Friedman and Jones 1981; Maendly et al. 1981; Jones and Friedman 1982; Jones et al. 1982; cat: Andersson et al. 1966; rat: Francis et al. 2008). These studies also demonstrated that all kinds of

proprioceptive inputs from the muscle spindle, fascia, tendon and joint capsule converge in the rostradorsal shell or rostral (oral) region of VPL. In contrast, Luo and Dessem (1995) suggested that the VPM integrates the information from the Su5 receiving JCMS inputs via the Me5 neurons and the information from the principal sensory trigeminal nucleus (Pr5) and spinal trigeminal nucleus (Sp5) receiving the orofacial sensations (including non-Me5 deep sensory inputs) via the trigeminal ganglion neurons. Thus, the present study was designed to re-examine whether the JCMS proprioceptive sensations mediated via the Su5 neurons and the other orofacial sensations mediated via the Pr5 and Sp5 neurons are encoded convergently or separately in the VPM, by comparing thalamic terminal distributions of distinct populations of trigeminal neurons.

Determining the exact thalamic relay of orofacial proprioception may provide useful information for the neurosurgical treatment of some orofacial tremor and movement disorders in patients. Indeed, electrical lesions (thalamotomy) or deep brain stimulation (DBS) in the ventral intermediate thalamic nucleus (Vim), which corresponds to the VPLo in non-human primates and receives proprioceptive inputs from the body, is applied to improve motor symptoms in various movement-disorder patients (e.g. parkinsonian patients) (Narabayashi and Ohye 1980; Ohye et al. 1989; Lenz et al. 1990, 1994; Ohye 2000; Hamani et al. 2006). There exist several orofacial movement disorders such as oromandibular dystonia, essential tremor and parkinsonian resting tremor in the jaw (Clark et al. 1993; Erer and Jankovic 2007; Wolraich et al. 2010; Karp and Alter 2016). However, surgical targets for treating these disorders remain unclear. The results of the present study showing the exact thalamic relay of orofacial proprioception in rats may provide useful insights for the treatment of some of these disorders.

Materials and methods

Animals

A total of 30 male Wistar rats ranging in weight from 230 to 330 g was used. 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. We made efforts to minimize the number of animals used.

Experimental procedures

In all animals used, sodium pentobarbital (55 mg/kg) with supplementary doses of sodium pentobarbital (10 mg/ml) was intraperitoneally injected for anesthesia

at such a level that neither corneal reflex nor spontaneous eye movements were apparent. In the first, second and third experiments, an incision was made in the buccal skin on the right side, in order to expose the masseter nerve (which innervates the JCMSs), and it was dissected free from the masseter muscle. In order to expose the lingual nerve on the right side, in the second and fourth experiments, after an incision was made in the submandibular skin, the mylohyoid muscle was torn, and the lingual nerve was then dissected free. To expose the infraorbital nerve distal to the infraorbital foramen, an incision was made in the infraorbital skin, and the nerve was dissected free. All incisions of the skin were performed under local anesthesia with lidocaine hydrochloride. For the electrical stimulation of these nerves (single pulse, 200 μ s duration, 1 Hz), silver hook bipolar stimulation electrodes were attached to the individual nerves. Subsequently, the animal's head was mounted in a stereotaxic apparatus. The rectal temperature was maintained between 36 and 38 °C with a heating pad, and ECG was continuously monitored. The atlases of the rat brain by Paxinos and Watson (1998, 2014) were chiefly used for the delineation of brain structures and determination of coordinates for stereotaxic micropipette insertion.

In the first experiment, we injected an anterograde tracer, biotinylated dextranamine (BDA, 10,000 MW, Molecular Probes, Eugene, OR, USA), into the Su5. First, the right parietal bone was partly removed with the aid of a dental drill. Then, a small part of the dura was cut to insert a glass micropipette filled with 4% BDA dissolved in saline, obliquely with an 18° rostral-to-caudal inclination into the rostralateral pons. According to the criteria reported in our previous study (Fujio et al. 2016), the Su5 was identified by recording of the field potentials in response to electrical stimulation of the masseter nerve and by unit recordings of responses to passive, sustained jaw-opening movements. For jaw-opening movements (muscle stretch), the mandible was manually depressed with a hand-held plastic probe inserted into the mouth. Signals recorded from the microelectrode were amplified, filtered (300–3 kHz), and stored in a computer at the sampling rate of 20 kHz (field potentials) or 10 kHz (unit activity). Subsequently, BDA was injected iontophoretically with 2.0 μ A positive pulses (300 ms, 2 Hz) for 7–10 min into the identified Su5. After the tracer injection, stimulation electrodes were detached from the masseter nerve, and all wounds were sutured. Then, 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 the 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.

In the second experiment, we used local field potential and unit recordings to find the regions of VPM and surrounding thalamus which received sensory inputs from the JCMSs elicited by electrical stimulation of the masseter nerve and passive, sustained jaw-opening movements, and from the tongue elicited by electrical stimulation of the lingual nerve. Before the animals were fixed to a stereotaxic apparatus, an incision was made in the anterior neck skin, the sternothyroid muscle was torn, and the trachea was cannulated. Then, a small bur hole was made on the parietal part of the skull on the left side to expose the dura. A small part of the dura was cut to vertically insert a glass microelectrode, filled with 2.0 M potassium citrate, into the brain. The evoked field potentials were mapped in and around the caudo-ventromedial part in the VPM (4.16 mm caudal to bregma, 2.10 mm lateral, 3.30 mm dorsal to the interaural line) where the densest BDA-labeled terminals were found in the first experiment as described below in the “Results” section. For the mapping of field potentials, the microelectrode was driven down in 25 μm steps and the distance between successive recording tracks was 100 μm . During the electrophysiological mapping, the animals were immobilized with a neuromuscular junction blocker, 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. After the mapping, the microelectrode was changed to the one filled with 3% horseradish peroxidase (HRP, Toyobo, Osaka, Japan) dissolved in saline for the confirmation of recording sites (but not for the tracing study). The HRP injection was iontophoretically performed by delivering 2.0 μA positive pulses (300 ms, 2 Hz) for 20 s.

In the third and fourth experiments, a retrograde tracer, wheat-germ agglutinin conjugated horseradish peroxidase (WGA-HRP, Sigma Chemical Co., St. Louis, MO) or Fluorogold (FG, Fluorochrome, Englewood, CO, USA) was injected into two distinct regions of the VPM. In these experiments, the cannulation of the trachea and artificial ventilation were not performed. A glass microelectrode filled with 3% WGA-HRP dissolved in 0.05 M Tris buffer or with 1% FG in 0.1 M sodium acetate buffer was inserted into the VPM. In the third experiment, WGA-HRP or FG was iontophoretically injected by delivering 2.0 μA positive pulses (300 ms, 2 Hz) for 7–12 min for WGA-HRP or for 10–15 min for FG into the region of VPM where the largest field potentials were recorded during the electrical stimulation of the masseter nerve and during passive, sustained jaw-opening movements. In the fourth experiment, WGA-HRP or FG was injected into the VPM where the largest field potentials were recorded during the electrical stimulation of the lingual nerve or infraorbital nerve. After the tracer injections in the third and fourth experiments, the animals were treated in the same way as the

above-mentioned animals receiving the BDA injection into the Su5.

Histology

After a postinjection survival of 6–8 days in BDA or FG injection cases, or 2–3 days in WGA-HRP injection cases, or soon after HRP injection cases, the animals were re-anesthetized deeply with an i.p. injection of sodium pentobarbital (100 mg/kg) and perfused through the ascending aorta with 100 ml of 0.02 M phosphate-buffered saline (PhBS, pH 7.4) followed by 300 ml of a fixative. A fixative containing 1.0% paraformaldehyde and 1.0% glutaraldehyde in 0.1 M phosphate buffer (PhB) (pH 7.4) was used for WGA-HRP injection cases, and one containing 4% paraformaldehyde in 0.1 M PhB (pH 7.4) was used for the other cases. Then, the entire brain was removed and placed in 25% sucrose in 0.02 M PhB at 4 °C for a few days. It was cut coronally (60- μm thickness) on a freezing microtome. The serial sections were alternately divided into three sets.

For the detection of BDA, all sets of alternate serial sections were washed in 0.02 M PhBS (pH 7.4) and preincubated in 0.02 M PhBS containing 0.01% H_2O_2 and 0.75% Triton X-100, as described previously (Sato et al. 2013; Akhter et al. 2014). The sections were then incubated in 0.02 M PhBS containing avidin–biotin–peroxidase complex (ABC) diluted at 1:100. The sections were reacted in a diaminobenzidine (DAB) solution [0.1 M PhB (pH 7.4) containing 0.04% DAB, 0.006% H_2O_2 and 0.08% nickel ammonium sulfate]. For the detection of HRP injection sites, all sets of alternate serial sections were reacted in a DAB solution. For the detection of WGA-HRP, two sets of alternate serial sections were processed according to the tetramethyl benzidine (TMB) method (Mesulam 1978). The remaining set of sections was reacted in a DAB solution. In FG injection cases, one set of alternate serial sections was not reacted. For the detection of FG, the remaining two sets of the sections were incubated in 0.02 M PhBS containing 3% normal goat serum, 0.2% Triton X-100 and polyclonal rabbit anti-FG primary antibody (Chemicon, CA, USA: product No. AB153) diluted at 1:10,000. The sections were then incubated in 0.02 M PhBS containing biotinylated goat anti-rabbit immunoglobulin G (Vector) diluted at 1:400, and in 0.02 M PhBS containing ABC diluted at 1:100. Subsequently, the sections were incubated in a DAB solution. In the control experiments where the FG injection was not made or the anti-FG primary antibody was omitted, no labeling was detected.

Finally, all sets of sections in all experiments were mounted on gelatin-coated slides, and dried. One set of sections in BDA injection cases, two sets of sections in HRP injection cases, one set of DAB-reacted sections in WGA-HRP injection cases, and one set of above-reacted sections

in FG injection cases were counterstained with Thionin or Neutral Red. Then, all of the sections in all experiments, except for sections reacted with TMB, were dehydrated in graded alcohols, cleared in xylene, and coverslipped. Sections reacted with TMB were cleared directly with xylene without dehydration in alcohol.

Data analysis

The field potentials recorded in all of the above experiments were stored on a personal computer, and offline analysis was performed with computer assistance (PowerLab 8/30, ADInstruments, Sydney, Australia). Responses to seven to ten successive peripheral stimuli were averaged at each recording site.

We drew the brain structures, tracer injection sites, retrogradely labeled neuronal cell bodies, anterogradely labeled axonal fibers and terminals, and electrode penetration tracks by use of a camera lucida attached to a light microscope. The extent of the FG injection site was judged by observing the sections under an epifluorescence microscope with filters providing ultraviolet excitation light, as described previously (Yoshida et al. 1991, 2009). The extent of the WGA-HRP injection site was judged by observing the sections reacted with DAB, as described previously (Yoshida et al. 1991, 1992). The number of retrogradely labeled neurons was counted in every third section. Photomicrographs

of selected sections were taken by a digital camera (Pixera Pro 150ES, CA, USA) connected to the light microscope. Photomicrographs of retrogradely WGA-HRP-labeled neuronal cell bodies were taken under the dark field illumination with polarizing filters. All photomicrographs were processed using Photoshop CS2 (Adobe Systems, CA, USA) with a slight adjustment of the image's contrast.

Results

Anterograde labeling of thalamic projections from Su5 neurons

In the first experiment, we examined the thalamic projection patterns of Su5 neurons that receive proprioceptive inputs from JCMS receptors. The electrophysiological identification of the Su5 was described elsewhere (Fujio et al. 2016). Briefly, we targeted the brainstem region (assumed to be Su5) where we could record a small negative field potential (with around 1 msec latency) in response to electrical stimulation of the masseter nerve (innervating JCMS receptors; e.g., Fig. 1A) and/or neurons that increased their firing during passive, sustained jaw-opening movements (e.g., Fig. 1B). Subsequently, we injected the anterograde tracer, BDA, into this electrophysiologically identified site in Su5. The locations of the Su5 and tracer injection

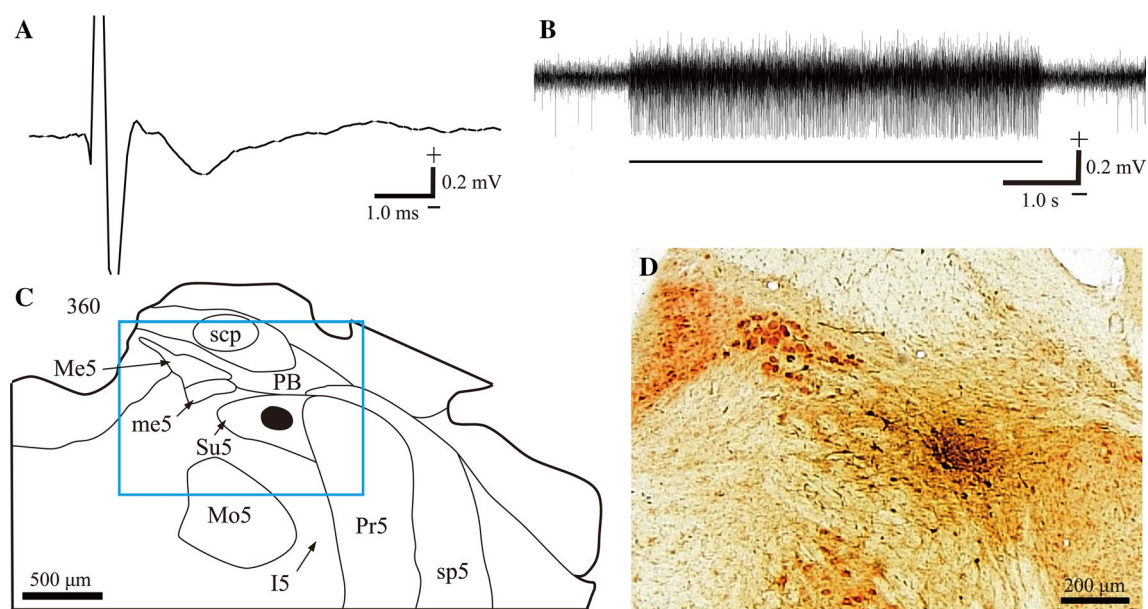


Fig. 1 Field potential recorded in the supratrigeminal nucleus (Su5) (A, B) and location of a tracer injection site into the recording site (C, D) in case R810. A Field potential in response to electrical stimulation of the masseter nerve. B Extracellular multi-unit discharges recorded during a sustained jaw-opening movement (for about 5 s indicated by the horizontal bar). C, D Reconstruction showing loca-

tion of injection site (a filled area) of the anterograde tracer, biotinylated dextranamine (BDA) in the Su5 360 μ m rostral to the caudal end of the trigeminal motor nucleus (Mo5). The blue boxed area in C indicates the area shown in D. The section D is counterstained with Neutral red, and the injection site is revealed by the dark brown patch. For abbreviations see list

sites were histologically confirmed; the Su5 was an area in the supratrigeminal region at the level of the caudal two-thirds of the trigeminal motor nucleus (Mo5) and the level slightly caudal to the Mo5, and was positioned medially adjacent to the dorsomedial part of Pr5, as described previously (Fujio et al. 2016; see also “Introduction” section). All of the injection sites covered some portion of the Su5 in 11 rats. Among these cases, the BDA injection sites were strictly confined to the Su5 in three rats. In a representative case, R810, the injection site was located in the center of the Su5 (Fig. 1C, D). We examined the distribution of anterogradely BDA-labeled axon fibers and terminals in and around the VPM (e.g., Fig. 2). In this case, the VPM was located between 2.30 and 4.52 mm caudal to bregma on the side contralateral to the BDA injection site. In the neighboring region, the parvicellular part of ventral posterior thalamic nucleus (VPPC), the gustatory thalamus, was cytoarchitecturally identified on the basis of well-known criteria described by Yasui et al. (1989) and by the Paxinos and Watson (1998) stereotaxic atlas. Many anterogradely BDA-labeled axon fibers and terminals were observed in the caudo-ventromedial edge of the VPM (termed VPMcvm) bilaterally with a contralateral predominance to the BDA injection site; the densest labeled terminals were seen at the level 4.16 mm caudal to bregma (Fig. 2C, E, F). Note that the VPMcvm could not be exactly distinguished from the remaining core part of VPM (termed core VPM: that is, the VPM excluding the VPMcvm) only on the basis of its cytoarchitecture (e.g., Figs. 2E, 3C). A small number of labeled fibers and terminals were seen in the VPPC bilaterally with a contralateral predominance. Labeled fibers and terminals were also observed scattered in the parafascicular nucleus and paracentral nucleus predominantly on the ipsilateral side. Importantly, no labeled terminals were found in the core VPM, posterior thalamic nucleus, submedial thalamic nucleus, or VPL.

Electrophysiological mapping of thalamic proprioceptive neurons

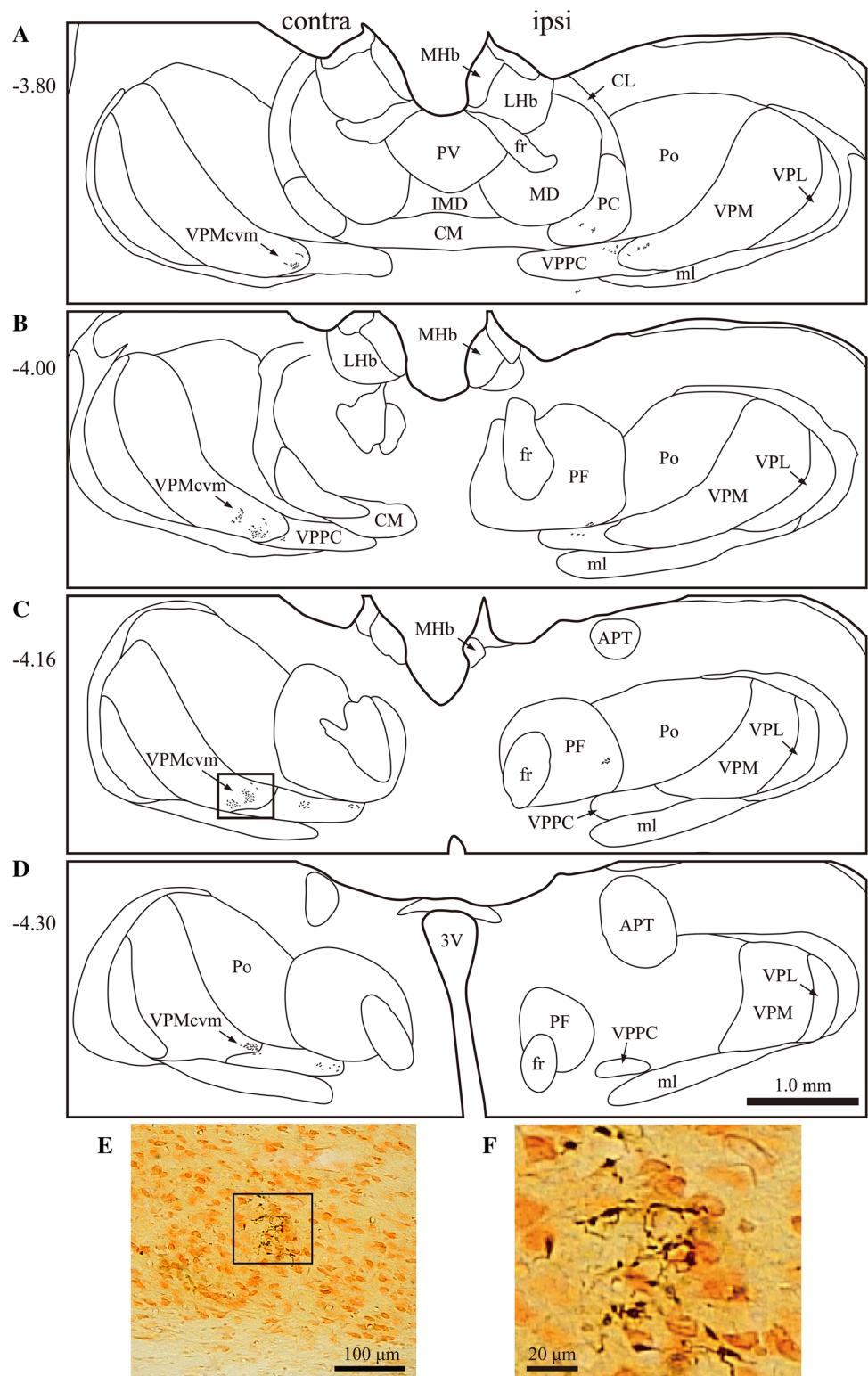
In the second experiment, we performed electrophysiological mapping of VPMcvm by recording the field potentials evoked by electrical stimulation of the masseter nerve or lingual nerve, and neuronal discharges to jaw-opening (e.g., Fig. 3) in three rats. During the mapping, these rats were immobilized with a neuromuscular junction blocker, and artificially ventilated, in order to minimize the influence of jaw-muscle contraction induced by electrical stimulation of each nerve. In a representative case, R909, large negative field potentials could be recorded only in the VPMcvm after masseter nerve stimulation; the exact locations of all recording sites were later confirmed histologically (Fig. 3A–C). At the hot spot of VPMcvm (circled number

6 in Fig. 3B, C), the largest negative potentials with 4.0 msec latency were evoked by masseter nerve stimulation, and importantly, no responses were evoked by lingual nerve stimulation (circled number 6 in Fig. 3D). Multi-unit discharges that increased during passive, sustained jaw-opening movements were also recorded at this site (Fig. 3E). In the ventromedial part of the core VPM (circled number 3 in Fig. 3A, about 0.84 mm rostral to the site indicated by circled number 6), large negative potentials with 4.2 ms latency were evoked by lingual nerve stimulation, whereas no responses were evoked by masseter nerve stimulation (circled number 3 in Fig. 3D).

Retrograde labeling of neurons projecting to VPMcvm

In the first experiment, it was demonstrated that there is a projection from the Su5 to the VPMcvm, but not to the core VPM. However, it remains unclear whether the VPMcvm receives projections from the Pr5 and Sp5. In the third experiment, aimed at addressing this issue, a retrograde tracer, WGA-HRP or FG, was injected into the VPMcvm in ten rats. In each rat, the VPMcvm was identified on the basis of electrophysiological recordings as in the second experiment. Figure 4 shows the field potentials recorded in the VPMcvm in case R613, after the electrical stimulation of the masseter nerve (Fig. 4A), and multi-unit responses during passive, sustained jaw-opening movements (Fig. 4B). WGA-HRP was injected in six of the ten rats, and FG in the other four rats. In all of the ten cases, the injection sites were histologically confirmed to cover at least a portion of the VPMcvm (e.g., Fig. 4C, D, F), and some neurons were retrogradely labeled in the Su5. The resultant distribution of the retrogradely labeled neurons is representatively shown in Table 1. In two of the ten cases (case R613 with a WGA-HRP injection and case R706 with an FG injection) in which injection sites were confined to the VPMcvm (e.g., Fig. 4C, D), the retrogradely labeled neurons were seen only on the side contralateral to the injection site (e.g., Fig. 5). In case R613, for example, a large number of labeled neurons ($n = 159$) were found throughout the Su5, especially in its caudal part (Fig. 5B–D, G). A considerable number of labeled neurons ($n = 87$) were also found in the dorsomedial edge of the Pr5 laterally adjacent to the Su5 (Fig. 5B–D, G). Only very few neurons were labeled in the intertrigeminal region ($n = 5$) (Fig. 5B–D, G), and in the area ventral to the Mo5 and medial to the Pr5 ($n = 7$) (Fig. 5C). On the other hand, we found no labeled neurons in any of the following structures: the parabrachial nucleus (Fig. 5A, B, F), remaining areas of the Pr5 (Fig. 5A–E, G) and Sp5 (Fig. 5E), the Mo5 (Fig. 5A–D, G) and the parvicellular reticular formation (Fig. 5A–E, G). In two

Fig. 2 Distribution of anterogradely labeled axon terminals (small dots) in the dorsal thalamus after a BDA injection in the Su5 in case R810. The injection site is presented in Fig. 1C, D. Coronal sections are arranged rostrocaudally from A to D. Numbers denote distance in millimeters caudal (–) to bregma on the contralateral (contra) side to the injection site. Note that the largest number of BDA-labeled terminals was seen, at the level 4.16 mm caudal to bregma, in the caudo-ventromedial edge (VPMcvm) of the ventral posteromedial thalamic nucleus (VPM) contralateral to the BDA injection site. In this case, the VPM was located at the levels from 2.30 to 4.52 mm caudal to bregma on the contralateral side. The boxed area in C indicates the area shown in E. E, F Photomicrographs showing BDA-labeled axon fibers and terminals in the contralateral VPMcvm. Area boxed in E corresponds to the area in F. Sections E, F are counterstained with Neutral red. For abbreviations see list



other cases of the ten cases (R204, R211), the injection sites covered the VPMcvm, but extended into the VPPC medial to the VPMcvm (Fig. 4E). A small number of retrogradely labeled cells were also found bilaterally in the parabrachial nucleus dorsal or rostradorsal to the Su5

(Table 1). In the remaining six cases of the ten cases, the injection sites covered the VPMcvm but extended into the core VPM lateral or rostradorsal to the VPMcvm. The retrogradely labeled cells were also seen in the complex of the Pr5 and Sp5 (oral, interpolar and caudal

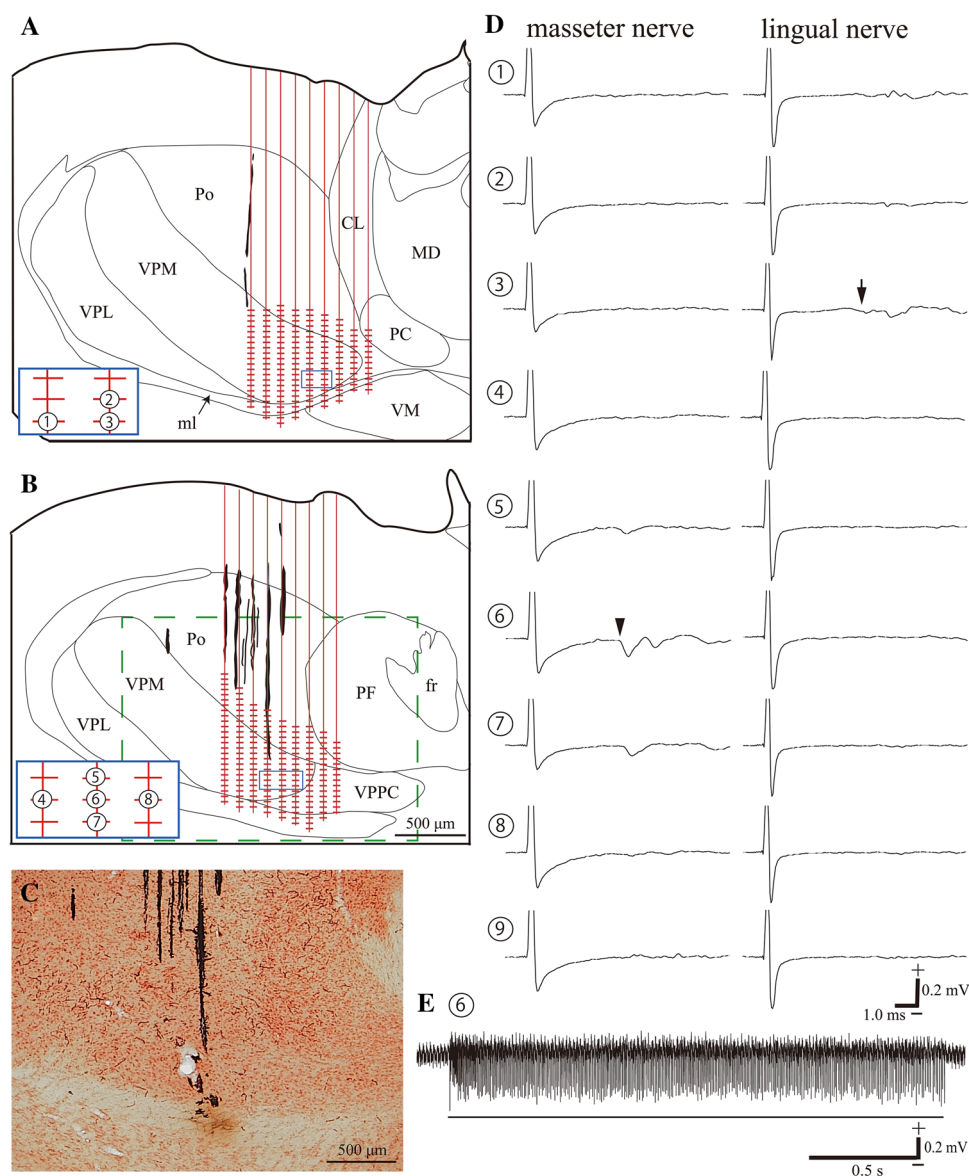


Fig. 3 Drawings (**A**, **B**) and a photomicrograph (**C**) showing recording sites, and field potentials recorded in and around the VPMcvm (**D**, **E**) in case R909. **A**, **B** Sections showing the contralateral side to the stimulated nerve are arranged rostrocaudally. **A–C** Thick, black vertical lines indicate bleeding caused by microelectrode penetration. **A**, **B** Each electrode penetration track is indicated by a red vertical line and is reconstructed on the basis of microdrive readings and visible electrode tracks observed in the sections. Short red, transverse bars indicate recording sites. Each small, blue boxed area, which is presented in the core VPM (**A**) or VPMcvm (**B**), indicates an area shown in the large, blue boxed inset at lower left. **B**, **C** The area bracketed with dashed green lines in **B** indicates the area shown in **C**.

parts): the injection site and labeled neuron numbers in a representative case R100 are indicated in Fig. 4E, F and Table 1, respectively.

D Field potentials evoked by masseter nerve stimulation and lingual nerve stimulation are presented in the left and right columns, respectively. In this case, the animal was paralyzed with a neuromuscular junction blocker. Field potentials indicated by circled numbers were recorded at the site indicated by the same circled numbers shown in the insets of **A** or **B**. Field potentials indicated by circled number 9 were recorded at the site just caudal to the site indicated by circled number 6. An arrow and an arrowhead in **D** indicate 4.2 and 4.0 ms latency, respectively. **E** Extracellular multi-unit discharges recorded at the site indicated by circled number 6 during a sustained jaw-opening movement (for about 2.3 s indicated by a horizontal line). For abbreviations see list

Retrograde labeling of neurons projecting to core VPM

In the fourth experiment, we sought to compare the distribution patterns of neurons (including the Su5 neurons) projecting to the VPMcvm with those of Pr5/Sp5 neurons

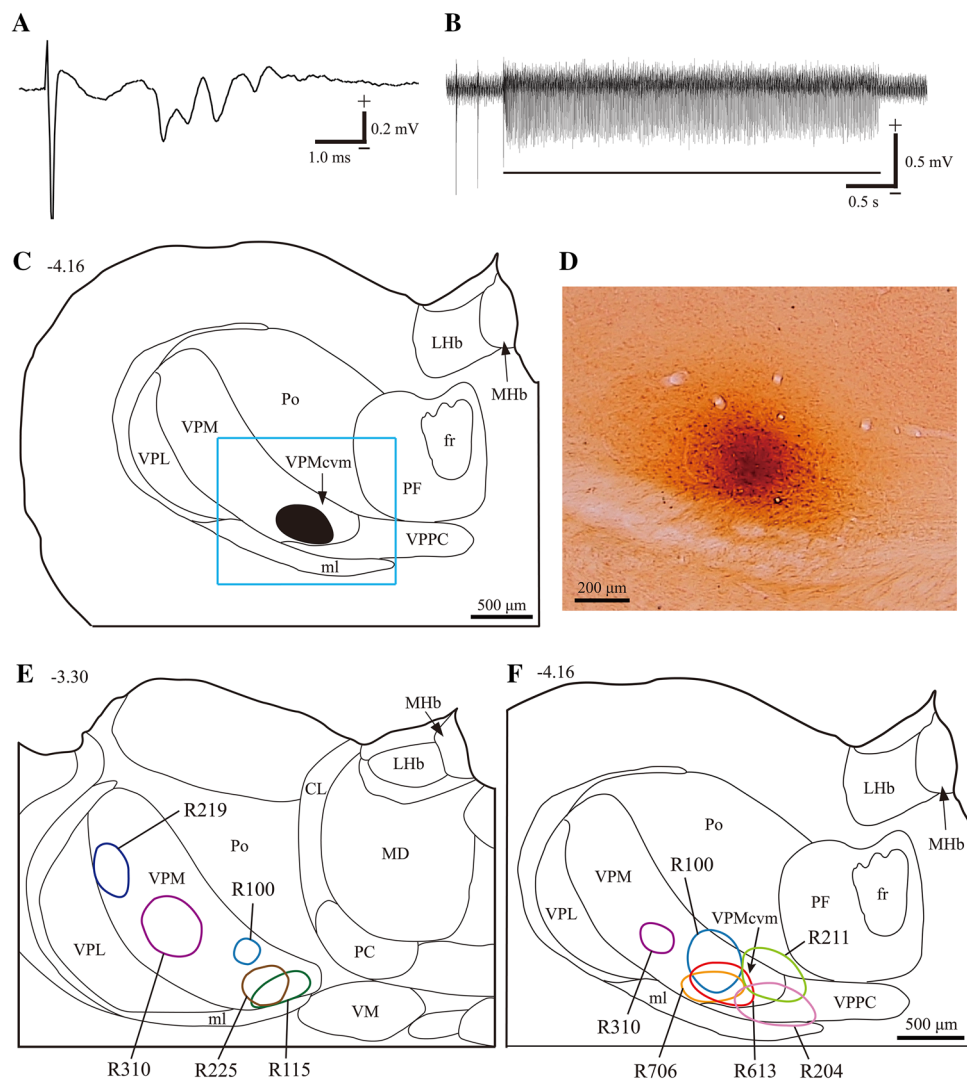


Fig. 4 Field potential recorded in the VPMcvm (**A**, **B**) and locations of tracer injection sites (**C**–**F**). The examples shown in **A**–**D** were obtained in case R613. **A** Field potential evoked by electrical stimulation of the masseter nerve contralateral to the recording site. Note that, in this case, the animal was not paralyzed with a neuromuscular junction blocker. **B** Extracellular multi-unit discharges recorded during a sustained jaw-opening movement (for about 3.7 s indicated by a horizontal line). **C**, **D** A drawing and a photomicrograph showing the injection site of wheat-germ agglutinin conjugated horseradish peroxidase (WGA-HRP) (a filled area in **C**) made at the recording site. The injection center was found in the VPMcvm contralateral to the stimulated masseter nerve in a coronal section 4.16 mm caudal (–) to

bregma. The blue boxed area in **C** indicates the region shown in **D**. The section **D** was reacted with diaminobenzidine (DAB) and weakly counterstained with *Neutral red*. **E**, **F** Semi-schematic drawings of the location of injection sites in the coronal section 3.30 and 4.16 mm caudal (–) to bregma in nine cases (including case R613 shown in **C**). The injection site in case R115 is also shown in Fig. 6B. Injection sites in cases R310 and R100 are present in both of the sections since they extended rostrocaudally to both levels. Note that WGA-HRP and FG were injected in cases R613, R204, R211, R115 and R310 and cases R706, R100, R225 and R219, respectively (also see Table 1). For abbreviations see list

projecting to the core VPM. Thus, we first injected a retrograde tracer in the core VPM of four rats at the site where the largest field potentials evoked by electrical stimulation of the lingual nerve were recorded (Fig. 6A). In two cases (R115 with WGA-HRP injection and R225 with FG injection) out of the four cases, the injection site covered the ventromedial part of VPM rostral to the VPMcvm, but did not appear to extend into the VPMcvm (Figs. 4E, 6B, C).

The distribution patterns of retrogradely labeled neurons were different between the VPMcvm injection and the core VPM injection (Table 1). In case R115, for example, many labeled neurons ($n=236$) were found in the Pr5 (except for its dorsomedial border) at the rostral level of Pr5 on the side contralateral to the injection site, whereas no neurons in the Su5 (Fig. 6D, E). Very few neurons ($n=2$) were labeled in the intertrigeminal region, and no neurons were labeled in

Table 1 Distribution of retrogradely labeled neurons after tracer injections in and around VPM

Main injection site	Case No.	Tracer	Contralateral							Ipsilateral	
			Su5	Dorsomedial edge of Pr5	Pr5	Sp5	I5	AVM	PB	PB	
3rd experiment											
VPMcvm	R613	WGA-HRP	159	87	0	0	5	7	0	0	
VPMcvm	R706	FG	76	60	0	9	3	0	0	0	
VPMcvm + VPPC	R204	WGA-HRP	41	53	0	0	3	0	21	18	
VPMcvm + VPPC	R211	WGA-HRP	30	60	0	0	6	14	18	27	
VPMcvm + core VPM	R100	FG	38	95	161	42	11	14	0	0	
4th experiment											
Core (lingual) VPM	R115	WGA-HRP	0	20	236	34	2	0	0	0	
Core (lingual) VPM	R225	FG	0	10	118	19	1	0	0	0	
Core (infraorbital) VPM	R219	FG	0	0	259	97	0	4	0	0	
Core (infraorbital) VPM	R310	WGA-HRP	0	0	145	55	0	15	0	0	

Numbers of retrogradely labeled neurons were counted in every third section. For abbreviations see list

the area ventral to the Mo5 and medial to the Pr5, and the parabrachial nucleus. More caudally, a considerable number of labeled neurons ($n=34$) were also seen in the Sp5, especially in the rostradorsal region of the caudal part of Sp5 (Fig. 6F).

In the second part of the fourth experiment, we injected a retrograde tracer in the core VPM where the largest field potentials were recorded after the electrical stimulation of the infraorbital nerve in two rats (case R219 with FG injection and case R310 with WGA-HRP injection). These injection sites were confined to the core VPM far from the VPMcvm (Fig. 4E, F). No neurons were labeled in the Su5 and dorsomedial edge of the Pr5, but many retrogradely FG-labeled neurons were widely distributed in the dorsoventrally middle two-thirds of Pr5 and the Sp5, and especially in the caudal part of Sp5 (Table 1).

Discussion

The present study has for the first time revealed the detailed thalamic relay of JCMS sensory inputs; the relay site was confined to a small region in the VPM (i.e., VPMcvm) which had not been predicted in previous studies. This finding provides an important contribution to our understanding of the organization of ascending sensory proprioceptive pathways in the rat and could lead to new therapeutic approaches for the neurosurgical treatment of patients suffering from orofacial movement disorders.

Characteristics of experimental procedures

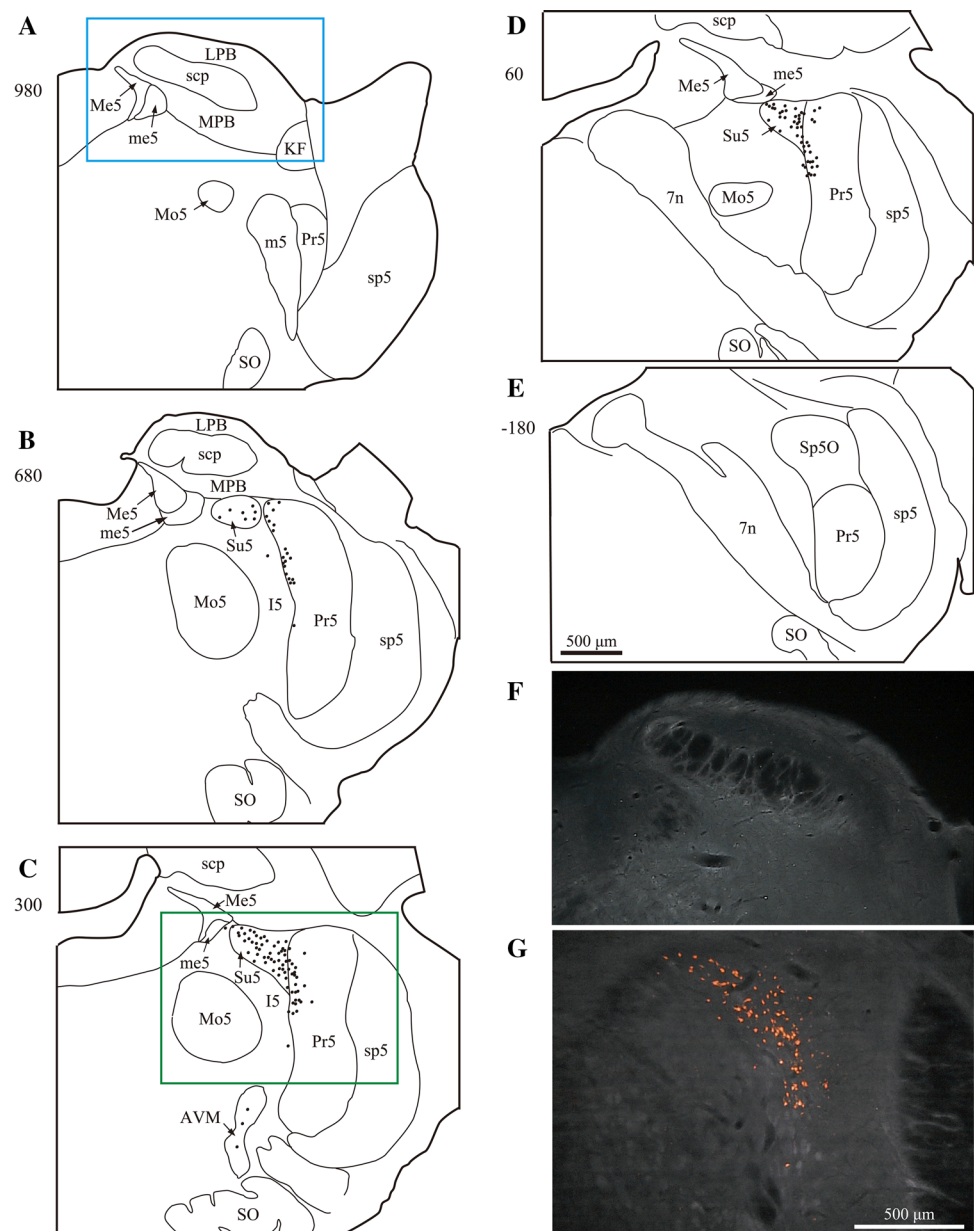
It has been generally recognized that forelimb and hindlimb proprioceptive information is conveyed to the rostradorsal

shell or rostral (oral) region (or VPLo) of VPL, and that these signals are relayed by neurons in the external cuneate nucleus and nucleus Z at least in rats, respectively (Low et al. 1986; Leong and Tan 1987; Bolton and Tracey 1992). Jones and Friedman (1982) predicted that the thalamic relay site of orofacial proprioception would be located in the rostradorsal aspect of the VPM. However, their prediction is not consistent with our result: the thalamic relay site of orofacial proprioception was not in the “rostral” VPM, but in the “caudo-ventromedial” edge (VPMcvm) of VPM. The VPMcvm is a very small part of the VPM and thus would be difficult to delineate only by using electrophysiological mapping of the very large VPM without the present morphological data which identify its position and extent. We should mention that our present results regarding the Su5-VPMcvm projection have been established based on our previous findings (Fujio et al. 2016), which precisely defined the location of rat Su5 and demonstrated that the Su5 neurons almost exclusively receive the JCMS inputs.

Characteristics of Su5 neurons projecting to VPMcvm

One of the main goals of our study was to examine the distribution patterns of neurons projecting to the electrophysiologically identified VPMcvm. The projection neurons to the VPMcvm were found not only in the Su5 but also in the dorsomedial edge of Pr5 laterally adjacent to the Su5 (Fig. 5B–D, G). Interestingly, our previous study (Fujio et al. 2016) showed that both the Su5 and the dorsomedial edge of Pr5 laterally adjacent to the Su5 receive projections from the Me5 primary afferents innervating the JCMSs. These findings suggest that the neurons in the dorsomedial edge of Pr5 are functionally in the same class as the Su5 neurons, but not the same as the core Pr5 neurons.

Fig. 5 Distribution of retrogradely labeled neurons (*dots*) in the pons after WGA-HRP injection in the VPMcvm in case R613. The injection site is presented in Fig. 4C, D. Coronal sections contralateral to the injection site are arranged rostrocaudally from **A** to **E**. *Numbers* denote distance in micrometers rostral and caudal (–) to the caudal end of Mo5. In this case, the rostral end of Su5 was located 720 μm rostral to the caudal end of Mo5. The *blue boxed area* in **A** and *green boxed area* in **C** indicate areas shown in **F** and **G**, respectively. **F, G** Dark field photomicrographs of non-counterstained sections



It is known that the Me5 neurons conveying JCMS inputs centrally project not only to the Su5 but also to the dorsomedial region of the oral part of Sp5 and the parvicellular reticular formation, all of which contain premotoneurons projecting to the Mo5 (Luo et al. 2001). However, the present study has shown that the projection neurons to the VPMcvm are located only in the Su5, and not in the dorsomedial region of the oral part of Sp5 and the parvicellular reticular formation. This difference strongly suggests that the Su5 neurons play a specially important role in the processing of proprioceptive signals and their relay to the thalamus, and, then, possibly to the cerebral cortex.

Parallel pathways to VPM

The first and second experiments of the present study have clearly shown that the JCMS inputs are conveyed by the Su5 neurons mainly to a very small area (VPMcvm) in the VPM, but not to the core VPM (see summary diagram in Fig. 7). In the third experiment, in the cases where retrograde tracer injections were confined to the VPMcvm, labeled neurons were mainly found only in the Su5, and none in the Pr5, Sp5 and parabrachial nucleus. In the cases where the injections extended into the core VPM, the retrogradely labeled neurons were found not only in the Su5 but also in the Pr5 and Sp5. Collectively, these findings strongly suggest that the VPMcvm

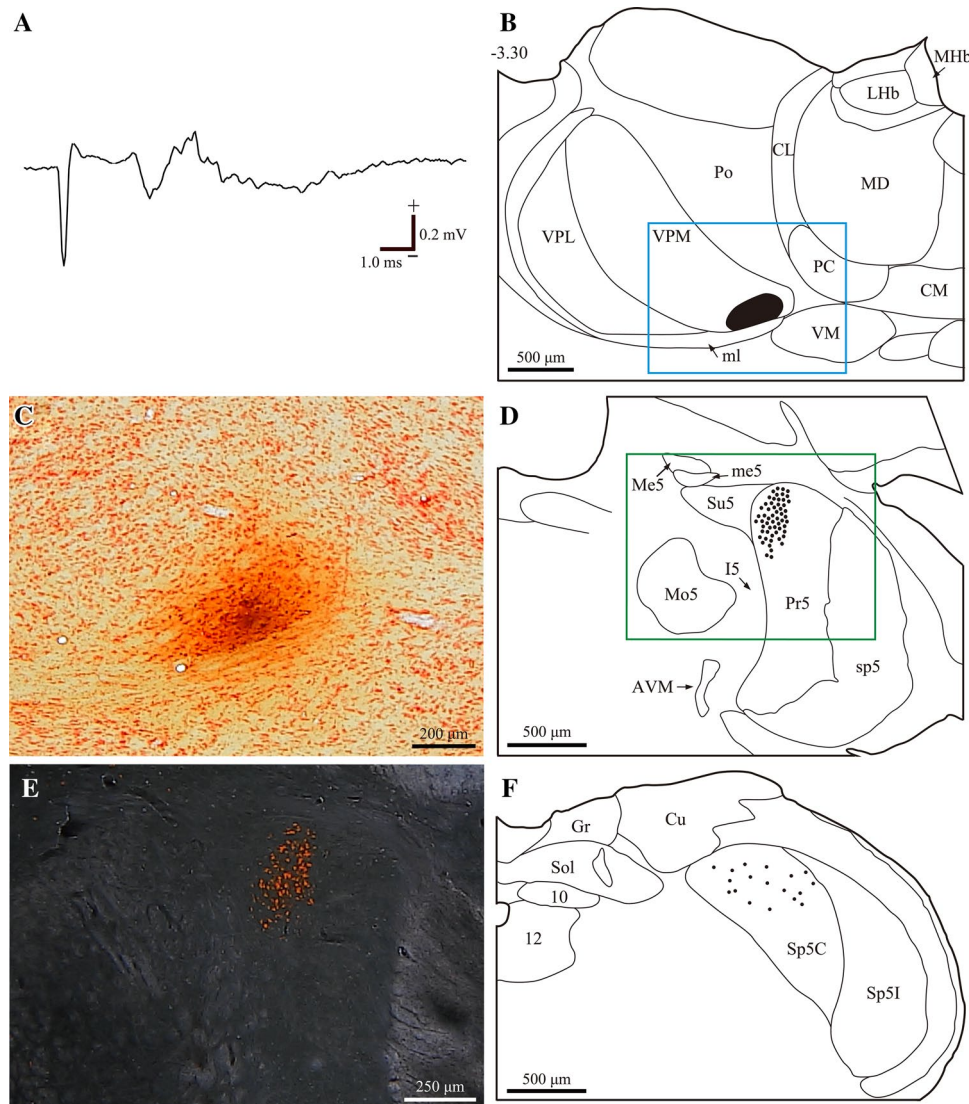


Fig. 6 Field potential evoked in the core VPM (A) and WGA-HRP injection site made at the recording site (B, C), and the resultant distribution of retrogradely WGA-HRP labeled neurons (D–F) in case R115. **A** The average field potential evoked by electrical stimulation of the lingual nerve, which was recorded in the core part of VPM contralateral to the lingual nerve. **B** The recording site in the core VPM just rostral to the VPMcvm (at the level 3.30 mm caudal to bregma), where WGA-HRP was subsequently injected (its injection site is denoted by a filled area). The injection site is also shown in Fig. 4E. The blue boxed area in B indicates the area shown in C. The

section in C was reacted with DAB and counterstained with *Neutral red*. **D** Retrogradely labeled neurons (dots) in the dorsomedial part of the principal sensory trigeminal nucleus (Pr5) at the level 450 μm rostral to the caudal end of Mo5 on the side contralateral to the injection site. The green boxed area in D indicates the area shown in E (dark field photomicrograph of non-counterstained section). **F** Retrogradely labeled neurons (dots) in the rostral region of the caudal part of spinal trigeminal nucleus (630 μm rostral to the obex) on the side contralateral to the injection site. For abbreviations see list

only receives the JCMS inputs via the Su5 neurons, and does not receive the orofacial inputs via the Pr5, Sp5 and parabrachial nucleus. In the fourth experiment, after retrograde tracers were injected into the lingual nerve and infraorbital nerve area of core VPM, labeled neurons were found mainly in the Pr5 and Sp5, and none in the Su5. These findings confirm the existence of projections from the Pr5 and Sp5 to the core VPM, as shown elsewhere (Fukushima and Kerr 1979; Shigenaga et al. 1983;

Bruce et al. 1987; Kemplay and Webster 1989). Collectively, these findings have indicated the existence of two distinct trigeminothalamic pathways: the Su5-VPMcvm pathway and the Pr5/Sp5-core VPM pathway. Since the neurons in the Pr5 and Sp5 are known to receive primary afferent projections from trigeminal ganglion neurons (Marfurt 1981; Takemura et al. 1991), the present findings have further demonstrated that the Su5-VPMcvm pathway conveys only the JCMS inputs, while the Pr5/

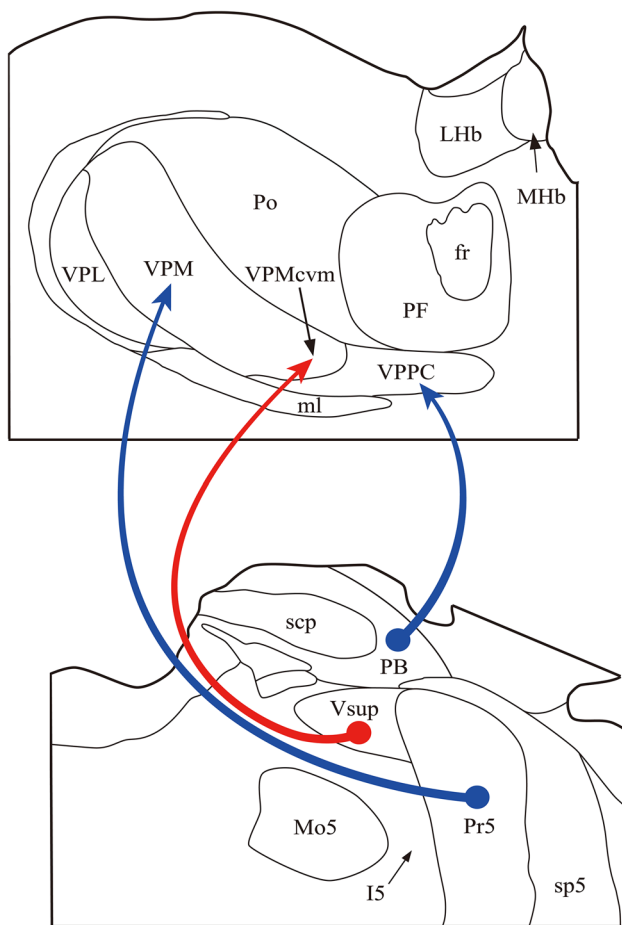


Fig. 7 Summary diagram showing the ascending thalamic projection (*red*) from the Su5 to the VPMcvm in comparison with those (*blue*) from the Pr5 (and spinal trigeminal nucleus) and parabrachial nucleus (PB), which have previously been described (for references, see text). Note that the diagram shows only the contralateral thalamic projections. However, in fact, the PB projection to the parvocellular part of ventral posterior thalamic nucleus (VPPC) is bilateral. For abbreviations see list

Sp5-core VPM pathway conveys all of the other orofacial sensations including the other proprioceptive sensations.

Additionally, in the third experiment where the injection site covering the VPMcvm extended medially into the VPPC, retrogradely labeled neurons were found in the parabrachial nucleus as well as in the Su5. The VPPC (often called the gustatory thalamus) is known to receive direct projections from the parabrachial nucleus (Norgren and Leonard 1973; Cechetto and Saper 1987; Krout and Loewy 2000) (Fig. 7).

The proprioceptive processing by the Pr5/Sp5-core VPM pathway was established by the following previous findings. The primary afferents innervating the tendons of masseter and temporal muscles arise from the trigeminal ganglion neurons in the cat (Cody et al. 1972; Lund et al. 1978). The nociceptive primary afferents innervating the

fascia of masseter muscle also arise from the trigeminal ganglion neurons and project to the Pr5 and Sp5 in the rat (Dessem et al. 2007). The primary afferents innervating the capsule of the temporomandibular joint terminate in the Pr5 and Sp5 in cats (Romfh et al. 1979; Capra 1987). The core VPM of the rat receives the projections from the Sp5 secondary neurons that respond to electrical stimulation of the capsule of the temporomandibular joint and the fascia of the masseter muscle (Ohya 1992; Ohya et al. 1993). Collectively, these earlier findings support that the proprioceptive sensations arising from the JCMSs and from the other orofacial deep tissues (e.g., tendon, fascia and joint capsule) are transmitted by separate pathways to separate portions of the VPM (VPMcvm and core VPM).

Characteristics of Su5-VPMcvm pathway

The present study shows that the Su5 neurons do not project to the posterior thalamic nucleus and submedial thalamic nucleus, although the neurons in the Pr5 and Sp5 are known to project to these thalamic nuclei in addition to the core VPM (Dado and Giesler 1990; Chiaia et al. 1991a, b; Fabri and Burton 1991; Yoshida et al. 1991; Diamond et al. 1992a, b; Veinante and Deschênes 1999; Pierret et al. 2000). It has been demonstrated that the posterior thalamic nucleus receives not only general somatic inputs but also auditory, visual and vestibular inputs and plays a role as the interface between sensory and motor functions (Groenewegen and Witter 2004), and that the submedial thalamic nucleus receives nociceptive inputs and projects to the pre-frontal cortical area involved in autonomic and limbic functions (Dado and Giesler 1990; Yoshida et al. 1991, 1992). Therefore, the thalamic processing of JCMS inputs relayed through the Su5-VPMcvm pathway may have a specific but as yet unknown role, compared to that of the other orofacial sensory inputs (including the other proprioceptive inputs) conveyed through the Pr5 and Sp5.

Clinical application

The Vim in humans is considered to be homologous to the oral part (VPLo) of VPL in non-human primates. The Vim neurons respond to the passive and active movements of body parts and receive proprioceptive inputs (group Ia muscle spindle inputs) from the body. Lesions (thalamotomy) or DBS in the Vim have been effectively applied to parkinsonian and essential tremor patients to treat their tremor (Narabayashi and Ohye 1980; Ohye et al. 1989; Lenz et al. 1990, 1994; Ohye 2000; Hamani et al. 2006). On the other hand, in the present study, the “orofacial proprioceptive thalamus” VPMcvm which receives only JCMS inputs has for the first time been identified in rats. This finding suggests the existence of a similar region in other

animals including humans. Thalamotomy or DBS in such a specific orofacial proprioceptive thalamic region corresponding to the rat VPMcvm might be an effective target for patients suffering from orofacial movement disorders, especially essential orofacial tremor (Clark et al. 1993; Erer and Jankovic 2007; Wolraich et al. 2010; Karp and Alter 2016). Since the present study has demonstrated that the VPMcvm only receives JCMS inputs, targeting the “orofacial proprioceptive thalamus” in human surgery might exert a curative effect on the patients without affecting other orofacial sensations.

Acknowledgements We are indebted to Dr. Akiko Tomita, Dr. Yume Uemura, Ms. Etsuko Ikenoue and Ms. Yumi Tsutsumi for their technical help. This work was supported by Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant 26293391 and 16K15775 to A.Y. and Grant 15H06387 to O.H.)

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- Akhter F, Haque T, Sato F, Kato T, Ohara H, Fujio T, Tsutsumi K, Uchino K, Sessle BJ, Yoshida A (2014) Projections from the dorsal peduncular cortex to the trigeminal subnucleus caudalis (medullary dorsal horn) and other lower brainstem areas in rats. *Neuroscience* 266:23–37
- Andersson SA, Landgren S, Wolsk D (1966) The thalamic relay and cortical projection of group I muscle afferents from the forelimb of the cat. *J Physiol (Lond)* 183:576–591
- Bolton PS, Tracey DJ (1992) Neurons in the dorsal column nuclei of the rat respond to stimulation of neck mechanoreceptors and project to the thalamus. *Brain Res* 595:175–179
- Bruce LL, McHaffie JG, Stein BE (1987) The organization of trigeminothalamic and trigeminothalamic neurons in rodents: a double-labeling study with fluorescent dyes. *J Comp Neurol* 262:315–330
- Capra NF (1987) Localization and central projections of primary afferent neurons that innervate the temporomandibular joint in cats. *Somatosens Res* 4:201–213
- Cechetto DF, Saper CB (1987) Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *J Comp Neurol* 262:27–45
- Chiaia NL, Rhoades RW, Bennett-Clarke CA, Fish SE, Killackey HP (1991a) Thalamic processing of vibrissal information in the rat. I. Afferent input to the medial ventral posterior and posterior nuclei. *J Comp Neurol* 314:201–216
- Chiaia NL, Rhoades RW, Fish SE, Killackey HP (1991b) Thalamic processing of vibrissal information in the rat: II. Morphological and functional properties of medial ventral posterior nucleus and posterior nucleus neurons. *J Comp Neurol* 314:217–236
- Clark GT, Koyano K, Browne PA (1993) Oral motor disorders in humans. *J Calif Dent Assoc* 21:19–30
- Cody FW, Lee RW, Taylor A (1972) A functional analysis of the components of the mesencephalic nucleus of the fifth nerve in the cat. *J Physiol (Lond)* 226:249–261
- Dado RJ, Giesler GJ Jr (1990) Afferent input to nucleus submedialis in rats: retrograde labeling of neurons in the spinal cord and caudal medulla. *J Neurosci* 10:2672–2686
- Dessem D, Moritani M, Ambalavanar R (2007) Nociceptive craniofacial muscle primary afferent neurons synapse in both the rostral and caudal brain stem. *J Neurophysiol* 98:214–223
- Diamond ME, Armstrong-James M, Budway MJ, Ebner FF (1992a) Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex. *J Comp Neurol* 319:66–84
- Diamond ME, Armstrong-James M, Ebner FF (1992b) Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus. *J Comp Neurol* 318:462–476
- Dubner R, Sessle BJ, Storey AT (1978) The neural basis of oral and facial function. Plenum Press, New York
- Erer S, Jankovic J (2007) Hereditary chin tremor in Parkinson’s disease. *Clin Neurol Neurosurg* 109:784–785
- Fabri M, Burton H (1991) Topography of connections between primary somatosensory cortex and posterior complex in rat: a multiple fluorescent tracer study. *Brain Res* 538:351–357
- Francis JT, Xu S, Chapin JK (2008) Proprioceptive and cutaneous representations in the rat ventral posterolateral thalamus. *J Neurophysiol* 99:2291–2304
- Friedman DP, Jones EG (1981) Thalamic input to areas 3a and 2 in monkeys. *J Neurophysiol* 45:59–85
- Fujio T, Sato F, Tachibana Y, Kato T, Tomita A, Higashiyama K, Ono T, Maeda Y, Yoshida A (2016) Revisiting the supratrigeminal nucleus in the rat. *Neuroscience* 324:307–320
- Fukushima T, Kerr FW (1979) Organization of trigeminothalamic tracts and other thalamic afferent systems of the brainstem in the rat: presence of gelatinosa neurons with thalamic connections. *J Comp Neurol* 183:169–184
- Groenewegen HJ, Witter MP (2004) Thalamus. In: Paxinos G (ed) *The rat nervous system*. Elsevier Academic Press, Amsterdam, pp 407–453
- Hamani C, Dostrovsky JO, Lozano AM (2006) The motor thalamus in neurosurgery. *Neurosurgery* 58:146–158
- Jones EG, Friedman DP (1982) Projection pattern of functional components of thalamic ventrobasal complex on monkey somatosensory cortex. *J Neurophysiol* 48:521–544
- Jones EG, Friedman DP, Hendry SH (1982) Thalamic basis of place- and modality-specific columns in monkey somatosensory cortex: a correlative anatomical and physiological study. *J Neurophysiol* 48:545–568
- Karp BI, Alter K (2016) Botulinum toxin treatment of blepharospasm, orofacial/oromandibular dystonia, and hemifacial spasm. *Semin Neurol* 36:84–91
- Kemplay S, Webster KE (1989) A quantitative study of the projections of the gracile, cuneate and trigeminal nuclei and of the medullary reticular formation to the thalamus in the rat. *Neuroscience* 32:153–167
- Krout KE, Loewy AD (2000) Parabrachial nucleus projections to midline and intralaminar thalamic nuclei of the rat. *J Comp Neurol* 428:475–494
- Lenz FA, Kwan HC, Dostrovsky JO, Tasker RR, Murphy JT, Lenz YE (1990) Single unit analysis of the human ventral thalamic nuclear group. Activity correlated with movement. *Brain* 113:1795–1821
- Lenz FA, Kwan HC, Martin R, Tasker R, Richardson RT, Dostrovsky JO (1994) Characteristics of somatotopic organization and spontaneous neuronal activity in the region of the thalamic principal sensory nucleus in patients with spinal cord transection. *J Neurophysiol* 72:1570–1587

- Leong SK, Tan CK (1987) Central projection of rat sciatic nerve fibres as revealed by Ricinus communis agglutinin and horseradish peroxidase tracers. *J Anat* 154:15–26
- Lorente de Nó R (1922) Contribución al conocimiento del nervio trigémino. Libro en honor de Dn. S. Ramón y Cajal. Moya, Madrid, 2:13
- Lorente de Nó R (1933) Vestibulo-ocular reflex arc. *Arch Neurol Psychiatr* 30:245–291
- Low JS, Mantle-St John LA, Tracey DJ (1986) Nucleus z in the rat: spinal afferents from collaterals of dorsal spinocerebellar tract neurons. *J Comp Neurol* 243:510–526
- Lund JP, Richmond FJ, Touloumis C, Patry Y, Lamarre Y (1978) The distribution of Golgi tendon organs and muscle spindles in masseter and temporalis muscles of the cat. *Neuroscience* 3:259–270
- Luo P, Dessem D (1995) Inputs from identified jaw-muscle spindle afferents to trigeminothalamic neurons in the rat: a double-labeling study using retrograde HRP and intracellular biotinamide. *J Comp Neurol* 353:50–66
- Luo P, Wong R, Dessem D (1995) Ultrastructural basis for synaptic transmission between jaw-muscle spindle afferents and trigeminothalamic neurons in the rostral trigeminal sensory nuclei of the rat. *J Comp Neurol* 363:109–128
- Luo P, Moritani M, Dessem D (2001) Jaw-muscle spindle afferent pathways to the trigeminal motor nucleus in the rat. *J Comp Neurol* 435:341–353
- Maendly R, Rüegg DG, Wiesendanger M, Wiesendanger R, Lagowska J, Hess B (1981) Thalamic relay for group I muscle afferents of forelimb nerves in the monkey. *J Neurophysiol* 46:901–917
- Marfurt CF (1981) The central projections of trigeminal primary afferent neurons in the cat as determined by the transganglionic transport of horseradish peroxidase. *J Comp Neurol* 203:785–798
- Mesulam MM (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. *J Histochem Cytochem* 126:106–117
- Narabayashi H, Ohye C (1980) Importance of microstereotomectomy for tremor alleviation. *Appl Neurophysiol* 43:222–227
- Norgren R, Leonard CM (1973) Ascending central gustatory pathways. *J Comp Neurol* 150:217–237
- Ohya A (1992) Responses of trigeminal subnucleus interpolaris neurons to afferent inputs from deep oral structures. *Brain Res Bull* 29:773–781
- Ohya A, Tsuruoka M, Imai E, Fukunaga H, Shinya A, Furuya R, Kawawa T, Matsui Y (1993) Thalamic- and cerebellar-projecting interpolaris neuron responses to afferent inputs. *Brain Res Bull* 32:615–621
- Ohye C (2000) Use of selective thalamotomy for various kinds of movement disorder, based on basic studies. *Stereotact Funct Neurosurg* 75:54–65
- Ohye C, Shibasaki T, Hirai T, Wada H, Hirato M, Kawashima Y (1989) Further physiological observations on the ventralis intermedius neurons in the human thalamus. *J Neurophysiol* 61:488–500
- Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edn. Academic Press, Sydney
- Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates, 6th edn. Academic Press, Sydney
- Paxinos G, Watson C (2014) The rat brain in stereotaxic coordinates, 7th edn. Academic Press, Sydney
- Pierret T, Lavallée P, Deschênes M (2000) Parallel streams for the relay of vibrissal information through thalamic barreloids. *J Neurosci* 20:7455–7462
- Romfh JH, Capra NF, Gatipon GB (1979) Trigeminal nerve and temporomandibular joint of the cat: a horseradish peroxidase study. *Exp Neurol* 65:99–106
- Sato F, Akhter F, Haque T, Kato T, Takeda R, Nagase Y, Sessle BJ, Yoshida A (2013) Projections from the insular cortex to pain-receptive trigeminal caudal subnucleus (medullary dorsal horn) and other lower brainstem areas in rats. *Neuroscience* 233:9–27
- Shigenaga Y, Nakatani Z, Nishimori T, Suemune S, Kuroda R, Matano S (1983) The cells of origin of cat trigeminothalamic projections: especially in the caudal medulla. *Brain Res* 277:201–222
- Takemura M, Sugimoto T, Shigenaga Y (1991) Difference in central projection of primary afferents innervating facial and intraoral structures in the rat. *Exp Neurol* 111:324–331
- Taylor A (1990) Neurophysiology of the jaws and teeth. Macmillan Press, London
- Veinante P, Deschênes M (1999) Single- and multi-whisker channels in the ascending projections from the principal trigeminal nucleus in the rat. *J Neurosci* 19:5085–5095
- Wolraich D, Vasile Marchis-Crisan C, Redding N, Khella SL, Mirza N (2010) Laryngeal tremor: co-occurrence with other movement disorders. *ORL J Otorhinolaryngol Relat Spec* 72:291–294
- Yasui Y, Saper CB, Cechetti DF (1989) Calcitonin gene-related peptide immunoreactivity in the visceral sensory cortex, thalamus, and related pathways in the rat. *J Comp Neurol* 290:487–501
- Yoshida A, Dostrovsky JO, Sessle BJ, Chiang CY (1991) Trigeminal projections to the nucleus submedius of the thalamus in the rat. *J Comp Neurol* 307:609–625
- Yoshida A, Dostrovsky JO, Chiang CY (1992) The afferent and efferent connections of the nucleus submedius in the rat. *J Comp Neurol* 324:115–133
- Yoshida A, Taki I, Chang Z, Iida C, Haque T, Tomita A, Seki S, Yamamoto S, Masuda Y, Moritani M, Shigenaga Y (2009) Corticofugal projections to trigeminal motoneurons innervating antagonistic jaw muscles in rats as demonstrated by anterograde and retrograde tract tracing. *J Comp Neurol* 514:368–386