Mechanisms of orofacial pain control in the central nervous system*

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Summary. Recent advances in the study of pain have revealed somatotopic- and modality-dependent processing and the integration of nociceptive signals in the brain and spinal cord. This review summarizes the uniqueness of the trigeminal sensory nucleus (TSN) in structure and function as it relates to orofacial pain control. The oral nociceptive signal is primarily processed in the rostral TSN above the obex, the nucleus principalis (Vp), and the subnuclei oralis (SpVo) and interpolaris (SpVi), while secondarily processed in the subnucleus caudalis (SpVc). In contrast, the facial nociceptive signal is primarily processed in the SpVc. The neurons projecting to the thalamus are localized mostly in the Vp, moderately in the SpVi, and modestly in the ventrolateral SpVo and the SpVc. Orofacial sensory inputs are modulated in many different ways: by interneurons in the TSN proper, through reciprocal connection between the TSN and rostral ventromedial medulla, and by the cerebral cortex. A wide variety of neuroactive substances, including

substance P, γ -aminobutyric acid, serotonin and nitric oxide (NO) could be involved in the modulatory functions of these circuits. The earliest expression of NO synthase (NOS) in the developing rat brain is observed in a discrete neuronal population in the SpVo at embryonic day 15. NOS expression in the SpVc is late at postnatal day 10. The neurons receiving intraoral signals are intimately related with the sensorimotor reflexive function through the SpVo. In summary, a better understanding of the trigeminal sensory system — which differs from the spinal system — will help to find potential therapeutic targets and lend to developing new analgesics for orofacial-specific pain with high efficacy and fewer side effects.

Introduction

Orofacial structures consist of a variety of tissues including facial skin, teeth, tongue, masticatory muscles, the temporomandibular joint, and salivary glands. Conscious appreciation of external stimuli as suitable sensory modality should be achieved on the basis of the integrity of the brain in order for the corresponding tissues to properly react to the stimulation. Orofacial structures receive frequent stimulation during food ingestion. This sensation is necessary to achieve ingestive behavior, including mastication, salivation, and swallowing, all of this being a foremost function of the brain throughout life. This unique function of the orofacial structures appears to require the exquisite organization of both peripheral and central nervous systems. The trigeminal nerves innervating the oral structures (oral nerves) relay their sensory information in the dorsomedial fields of the trigeminal sensory nuclei (TSN) (Åström, 1953; Torvik, 1956; Panneton and Burton, 1981; Jacquin *et al.*, 1983;

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Marfurt and Turner, 1984; Shigenaga *et al.*, 1986 a,b; Takemura *et al.*, 1987, 1991, 1993). Neurons in the TSN also receive direct cortical projection primarily in the dorsomedial fields (Torvik, 1956; Gojyo *et al.*, 2002). Similarly, corticospinal projection is found mostly in the dorsomedial fields in the spinal dorsal horn, where primary afferents from the body extremities terminate (Koerber and Brown, 1982; Molander and Grant, 1985; Casale *et al.*, 1988). The very sensitive exploratory function of the paws may be similarly specified as the oral structures are. The TSN neurons, which receive primary afferent inputs from the oral nerves, might be highly developed to perform processing and integration of the ascending and descending somatosensory signals generated during food ingestion.

Although the precise mechanisms of pain control in the trigeminal system are largely unknown, the trigeminal system appears to differ considerably from the spinal system. The formalin test is a commonly used experimental animal model of persistent inflammatory pain in the spinal system. The injection of formalin into the hind paw evokes licking, biting, or grooming of the injected paw, which are called pain-related behavior (PRB). PRB is a biphasic response, characterized by an immediate and short response (phase 1), followed by a quiescent period and the long sustained late responses (Phase 2; Dubuisson and Dennis, 1977; Kaneko and Hammond, 1997a, b). A biphasic PRB can be also seen in the trigeminal system. However, the PRB after formalin injection into trigeminal receptive fields is smaller than that induced by formalin injection into the hindpaw (Gilbert *et al.*, 2001; Sugiyo *et al.*, 2004). Moreover, the PRB following intraoral formalin injection is particularly small when compared with that following an extra-oral trigeminal injection (Sugiyo *et al.*, 2004).

Abnormal PRB manifests itself following experimental peripheral nerve injuries, and is interpreted as a sign of neuropathic pain such as hyperalgesia, allodynia, and phantom pain. In the spinal system, transection of a peripheral nerve or dorsal root causes a hypersensitivity of deafferented dorsal horn neurons to noxious stimulation of a nearby intact (undenervated) skin area within the same dermatome. Such a hypersensitivity of deafferented second order neurons may underlie the hyperalgesia at the edge of the denervated skin area (Sugimoto *et al.*, 1993, 1994 a,b). On the other hand, transection of the inferior alveolar nerve (IAN) induces hypersensitivity of TSN neurons outside the deafferented IAN's territory (Nomura *et al.*, 2002; Shimoda *et al.*, 2004; Tsuboi *et al.*, 2004). IAN transection causes hyperalgesia of the upper lip skin, which is outside the denervated mandibular branchial arch (Nomura *et al.*, 2002; Tsuboi *et al.*, 2004).

Orofacial pain is a very common healthcare complaint and a major concern of national public health. There are many patients who complain of intractable orofacial pain, a pain that is resistant to treatment. For developing therapeutic strategies for orofacial pain disorders, exact knowledge of the trigeminal mechanism of pain and nociception is indispensable. This review summarizes recent advances in the study of neural mechanisms specific to the processing of orofacial nociceptive signals. The first chapter introduces the morphological substrate for the transmittal of primary nociceptive signals to the second order neurons in the TSN. The morphology, content of neurochemical markers and central projection of nociceptive and non-nociceptive trigeminal primary neurons will be reviewed in the chapter. The second chapter deals with the γ -aminobutyric acid (GABA)ergic modulation of nociceptive signal transmission in the TSN. Involvement of the two major types of receptors, namely GABA_A and GABA_B, will be discussed.

Abbreviations in the text. cGMP: cyclic guanosine monophosphate, CGRP: calcitonin gene related peptide, dhI/II: laminae I and II of Spdh, dhIII/IV: laminae III and IV of Spdh, ENaC: epithelial Na⁺ channel, GABA: γ -aminobutyric acid, HRP: horse radish peroxidase, IAN: inferior alveolar nerve, NADPH-d: nicotinamide adenine dinucleotide phosphate- diaphorase, NK-1: neurokinin-1 receptor, NMDA: N-methyl-D-aspartate, nNOS: neuronal NO synthase, NO: nitric oxide, ODN: oligodeoxynucleotide, PAG: periaqueductal gray matter, PRB: pain-related behavior, Sal: saline, Sn: solitary tract nucleus, SP: substance P, Spdh: spinal dorsal horn, SP-Sap: SP conjugated to saporin, SpVc: trigeminal subnucleus caudalis, SpVi: trigeminal subnucleus interpolaris, SpVo: trigeminal subnucleus oralis, TG: trigeminal ganglion, TSN: trigeminal sensory nuclei, Vcl/II: superficial layers of SpVc, Vodm: dorsomedial subdivision of SpVo, Vor: rostradorsomedial subdivision of SpVo, Vp: trigeminal nucleus principalis.

1. Morphological substratum of the trigeminal nociceptor

1.1. Trigeminal sensory neurons

The trigeminal sensory nerve consists of primary neuronal somata located in the trigeminal ganglion (TG), peripheral axons innervating corresponding fields of the orofacial structure, and central axons whose terminals make synapses with the secondary neurons in the TSN. The soma sizes of trigeminal primary sensory neurons are

Fig. 1. Schematic representation of the central terminal fields of the trigeminal nerves in the transverse section of TSN (Takemura *et al.*, 1991). From the rostral, **A**: trigeminal nucleus principalis (Vp), **B**: trigeminal subnucleus oralis (SpVo), **C**: subnucleus interpolaris (SpVi), **D**: subnucleus caudalis (SpVc), **E**: spinal dorsal horn (Spdh). The ophthalmic division, Cor: cornea, FN: frontal nerve. The maxillary division, PN: palatine nerve, ZfN: zygomaticofacial nerve. The mandibular division, AtN: auriculotemporal nerve, BN: buccal nerve, IP: incisor tooth pulp, LN: lingual nerve, MhN: mylohyoid nerve, MN: mental nerve, MP: molar tooth pulp, R: trigeminal root, T: spinal tract of the trigeminal nerve, Vidm: dorsomedial subdivision of SpVi, Vodm: dorsomedial subdivision of SpVo. Other abbreviations as in the text.

diverse and have been implicated in different functions. There is a positive correlation between cell size and axonal diameter and peripheral conduction velocity in the rat dorsal root ganglion (Harper and Lawson, 1985). The trigeminal sensory nerve contains a smaller proportion of unmyelinated axons than do the spinal nerves. In the study using electron microscopy, unmyelinated axons in the spinal dorsal roots totaled 70–82% (Nunnemacher

and Sutherland, 1969; Steer, 1971), while those in the trigeminal sensory root comprised only 40% of the total (Young and King, 1973). The trigeminal sensory branches of the rat mandibular nerve, IAN, buccal nerve and lingual nerve, which primarily innervate the oral structures, contain a considerable proportion of large neurons in soma sizes, which would send nerve fibers to the A β range. In contrast, the auriculotemporal nerve, mylohyoid

Fig. 2. Percentage distribution of the central terminal density of the trigeminal nerve innervating the oral and facial structures in each nucleus. Oral neurons have central terminal fields more in the rostral nuclei above the obex, especially in the SpVo. Unlike the facial neurons, oral neurons have central terminal fields in the Sn, supratrigeminal nucleus (supV), and paratrigeminal nucleus (paraV). In contrast, facial neurons have central terminal fields mostly in the SpVc. Abbreviations as in Figure 1. (data from Takemura *et al.*, 1987)

nerve, and mental nerve, which primarily innervate the facial skin, contain a large proportion of small neurons (Sugimoto *et al.*, 1986). Muscle afferent neurons in the TG contain a sizable proportion of large-sized somata, which would send nerve fibers to the A β range (Ambalavanar *et al.*, 2003). The modes of percentage distribution of the somal area were 100–200 μm^2 (34.4%), 500–600 μm^2 (17.4%) and 300–400 μm^2 (35.1%) for the cutaneous branch of the mylohyoid nerve, tooth pulp and cornea, respectively (Sugimoto *et al.*, 1988). This means that the facial skin, cornea, and tooth pulp receive a comparatively dense innervation of C-fibers, A δ -fibers and A β -fibers, respectively. Surprisingly, the tooth pulp is innervated by a considerable number of large neurons in soma sizes, in the range of A β -fibers (Sugimoto *et al.*,

1989; Sugimoto and Takemura, 1993). In so far as the principal sensation of the tooth is pain, this finding is in conflict with the dogma that nociceptive information is conducted by small-sized axons, unmyelinated C-fibers, and A δ -fibers (Fields and Basbaum, 1978; Craig, 2003).

The tooth pulp is innervated by a subclass of finely myelinated and unmyelinated primary afferent neurons containing neuropeptides such as substance P (SP) (Wakisaka *et al.*, 1985; Fried *et al.*, 1989), and calcitonin gene related peptide (CGRP) (Silverman and Kruger, 1987; Fried *et al.*, 1989). Lines of evidence show that the tooth pulp is also innervated by large neurons positive for markers of mechanoreceptors and/or proprioceptors, carbonic anhydrase (Sugimoto *et al.*, 1990 a,b; Sugimoto and Takemura, 1993), parvarbumin, calretinin, and

Fig. 3. Photomicrographs showing the retrogradely HRP-labeled neurons in the transverse sections of the TSN following the injection of HRP into the thalamus. From the rostral, **A:** Vp, **B:** rostral part of SpVo containing Vor, **C:** caudal part of SpVo containing Vodm, **D:** SpVi, **E:** SpVc. Possible projection neurons to the thalamus are distributed mostly in the Vp, moderately in the SpVi, sparsely in the ventolateral division of the SpVo and seldom in the SpVc. Cun: cuneate nucleus.

calbindin-D28k (Ichikawa *et al.*, 1995, 1996). Recently a member of degenerin, epithelial Na⁺ channel (ENaC), which is a candidate for mechanoreceptive transducer, was found in nerves of the rat molar tooth pulp (Ichikawa *et al.*, 2005). Considerable numbers of myelinated nerves in the root pulp are positive for γ subunits of ENaC and

the vanilloid receptor-1-like receptor, which are mostly medium- to large-sized soma (Ichikawa and Sugimoto, 2000). They are respectively responsible for the sharp pain, induced by a dynamic liquid movement in the dentinal tubules (Narhi, 1990), and by high temperatures with a threshold $> 52^{\circ}\text{C}$ (Caterina *et al.*, 1999). In

Fig. 4. Rostro-caudal distribution of trigemino-thalamic neurons. Possible projection neurons to the thalamus are distributed mostly in the Vp, moderately in the SpVi at around the obex, and seldom in the SpVo and SpVc.

contrast, the capsaicin (vanilloid) receptor VR1, which is localized to small and medium-sized neurons, is almost absent in the tooth pulp (Ichikawa and Sugimoto, 2001). Thus the tooth pulp is supplied by a large proportion of high threshold mechanoreceptors and noxious heat receptors involved in tooth pain, unlike the skin of the spinal system. The proportional differences in sizes of neuronal somata between the oral and facial nerves can reflect the difference in the sensory modality of pain in the corresponding area.

The relatively small PRB at phase 1 and phase 2 induced by formalin injection into the orofacial area compared to the hind foot pad could stem from the relatively small proportion of small-sized neurons in the primary afferent neurons (Sugiyo *et al.*, 2004). The phase 1 PRB is considered to be a direct response of formalin injection and the phase 2 PRB is believed to be an inflammatory response. The C-fiber input into the trigeminal subnucleus caudalis (SpVc; medullary dorsal horn) is necessary for the manifestation of the phase 2 PRB (Pajot *et al.*, 2000). The relatively small PRB at phase 2 in rats formalin injected into the tongue compared with the those into the face (Sugiyo *et al.*, 2004) corroborates with the activation of C-fibers resulting in the PRB at phase 2. This is consistent with neurons innervating the oral structures having fewer small-sized neurons compared with neurons innervating the facial skin (Sugimoto *et al.*, 1986, 1988). In so far as the oral

cavity is the entrance to the digestive system, sensory modality could be sensitive to the volume, texture, and temperature of the food. It is reasonable that neurons innervating the oral structures contain a high percentage of large fibers, in the range of A-fibers, which can mediate mechanoreceptive and high threshold thermal information, to judge whether the bolus is suitable for swallowing. Prolonged information mediated by C-fibers might be less essential for eating and drinking in the oral area compared with that in the facial skin.

1.2. Central terminal fields of trigeminal nociceptor

Every branch of the trigeminal nerve has somatotopically arranged specific terminal fields in the TSN, the nucleus principalis (Vp), the spinal trigeminal nucleus, subnucleus oralis (SpVo), subnucleus interpolaris (SpVi), and SpVc (Fig. 1) (Åström, 1953; Torvik, 1956; Panneton and Burton, 1981; Jacquin *et al.*, 1983; Marfurt and Turner, 1984; Shigenaga *et al.*, 1986a, b; Takemura *et al.*, 1987, 1991, 1993). It has been established that the mandibular division is located in the dorsal or dorsomedial parts of the TSN, the ophthalmic division is located in the ventral or ventrolateral, and the maxillary division is located in the intermediate (Fig. 1). Oral and facial neurons differ in somal sizes, and the structural differences still reflect on the differences in the central terminal fields. Oral neurons have their central terminal fields primarily in the rostral

nuclei above the obex, Vp, SpVo, and SpVi, while facial neurons have them primarily in the SpVc (Fig. 2). Unlike the facial neurons, oral neurons have their central terminal fields in the supratrigeminal nucleus, solitary tract nucleus (Sn), and paratrigeminal nucleus (Fig. 1, 2). These structural characteristics are consistent with findings that all the TSN contain nociresponsive neurons (Eisenman *et al.*, 1963; Azerad *et al.*, 1982), and a tractotomy at the level of the obex eliminates almost all responses to noxious stimulation in the facial area while preserving responses in the oral structures (Young *et al.*, 1981; Dallel *et al.*, 1989). Neurons innervating the tooth pulp send their central terminals to all the TSN in the rat (Marfurt and Turner, 1984; Turner and Marfurt, 1988; Takemura *et al.*, 1991), cat (Westrum *et al.*, 1980, 1981; Arvidsson and Gobel, 1981; Shigenaga *et al.*, 1986b, 1989; Henry and Westrum, 1990), dog (Ishidori *et al.*, 1986) and monkey (Takemura *et al.*, 1993). Monkey neurons innervating the tooth pulp send their central terminals mostly to the rostral TSN above the obex but seldom to the SpVc (Takemura *et al.*, 1993). A higher animal may utilize the rostral TSN for oral nociception more than the SpVc. Putative projectional neurons to the thalamus were mostly distributed in the Vp, moderately in the SpVi, sparsely in the ventral SpVo, and seldom in the SpVc, as determined by retrograde labeling by an injection of horseradish peroxidase (HRP) into the thalamus, (Fig. 3, 4). High threshold mechanoreceptive information in the oral structures could ascend to the thalamus mostly through the Vp, while prolonged information mediated by C-fibers in the facial skin could ascend mostly through the SpVc.

Although a dichotomy exists in nociceptive processing between rostral TSN and SpVc, an intimate internuclear connection has been reported between the SpVc and the SpVo (Gobel and Purvis, 1972; Khayyat *et al.*, 1975). The microinjection of morphine into the SpVc inhibits nociceptive activities of SpVo neurons in a naloxone-reversible manner (Dallel *et al.*, 1998) — which could mediate prolonged polymodal information from

Fig. 5. Schematic drawings showing the spatial distribution of NADPH-d neurons (filled circle) and transganglionically HRP-labeled central terminal fields (fine granules) of the lingual nerve except for the chorda tympani nerve component. From the rostral, **A:** rostral part of SpVo containing Vor, **B:** caudal part of SpVo, containing Vodm, **C:** transitional zone of SpVi and SpVc, **D:** SpVc. VIII, vestibular root of the vestibulocochlear nerve. Numbers to the right of the figure represent the rostral distance in millimeters from the obex. Abbreviations as in Figure 1. (data from Takemura *et al.*, 1998)

Fig. 6. Photomicrographs of parasagittal sections showing the NADPH-d label in SpVo (large arrowhead) and the rostral Sn (small arrowhead). **A:** SpVo on E15, **B:** Enlargement of A, **C:** SpVo and rostral Sn on E17, **D:** Enlargement of C, **E:** SpVo and rostral Sn on E20, **F:** Enlargement of E. The right is rostral and the upward is dorsal. 5: trigeminal motor nucleus, 5n: trigeminal nerve, 7: facial nucleus, Aq: aqueduct, cb: cerebellum, cx: cerebral cortex, Di: diencephalon, Hi: hippocampus, Hypt: hypothalamus, IC: inferior colliculus, LV: lateral ventricle, Md: medulla, Mes: mesencephalon, Mf: mesencephalic flexure, P: pons, Pf: pontine flexure, SC: superior colliculus, Tel: telencephalon, other abbreviations as in Figure 1 and in the text. Scale bars = 500 μ m (A), 100 μ m (B), 1 mm (C, D), 200 μ m (E, F). (data from Takemura *et al.*, 1996)

thin primary afferent neurons and be involved in the sensorimotor reflexive modulation of mastication. These results imply that both facial and oral structures are intimately related in the sensorimotor reflexive function. The sensorimotor function is processed and integrated through various neuro-active substances, glutamate, GABA, serotonin, and nitric oxide (NO). Among these, NO producing neurons are localized in a discrete neuronal population in the SpVo and SpVc.

NO was originally identified physiologically as an endothelium-derived relaxing factor (Palmer *et al.*, 1987). Recently, NO has been shown to function as a retrograde neurotransmitter and a signal transducer in the brain and spinal cord (Bredt *et al.*, 1991). NO increases cyclic guanosine monophosphate (cGMP) levels and augments the sensitivity of related receptors. Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity seems to be localized in the same neurons positive for neuronal NO synthase (nNOS; Bredt *et al.*, 1991). In the spinal dorsal horn (Spdh), neuron enzymes histochemically stained for NADPH-d are localized in superficial layers of the Spdh (dhl/II: laminae I and II of Spdh). NADPH-d and nNOS are colocalized with GABA and glycine, but not with parvalbumin in laminae I-III neurons of the Spdh, and have been implicated in nociception in a model of neuropathic pain caused by *N*-methyl-D-aspartate (NMDA) receptor activation (Laing *et al.*, 1994). The number of NADPH-d neurons increased 3 days after a subcutaneous injection of complete Freund's adjuvant into the hind paws, but not 1 h or 1 day after injection (Yonehara *et al.*, 1997). The intravenous administration of *N*^ω-nitro-L-arginine methyl ester (50 mg/kg), an inhibitor of NOS, significantly blocked the adjuvant-induced thermal hyperalgesia at day 3 of inflammation, but not at day 1. NO in the Spdh could be implicated in the maintenance of chronic pain but not acute pain.

A specific neuronal population in the rostradorsomedial subdivision of SpVo (Vor) and dorsomedial subdivision of SpVo (Vodm) and the dorsomedial contiguous region of SpVo, the rostro-lateral Sn (SpVo/Sn), are constitutively positive for NADPH-d (filled circle in Fig. 5A, B; Takemura *et al.*, 1994, 1998). Similar to the Spdh, neurons in superficial layers of the SpVc (Vcl/II: laminae I and II of SpVc) are also positive for NADPH-d (filled circle in Fig. 5C, D; Takemura *et al.*, 1998). NADPH-d neurons in the SpVo/Sn are likely to receive primary afferent inputs from the intraoral structures because the spatial distribution of NADPH-d neurons and HRP-labeled central terminal fields of the lingual nerve — but not mental nerve — are identical (Fig. 5; Takemura *et al.*, 1998). NADPH-d colocalized with the calcium binding

proteins, calbindin D-28k (40%), calretinin (20%) but not with parvalbumin in neurons of the SpVo/Sn (Munekawa *et al.*, 2002). NADPH-d neurons in the SpVo/Sn do not project to the thalamus but likely project to the trigeminal motor nucleus and thus are possibly involved in the modulation of the orofacial sensorimotor reflexive activity.

The very early appearance of NADPH-d staining was observed in the SpVo/Sn, which precedes the appearance of NADPH-d elsewhere in the brain (Fig. 6, Table; Takemura *et al.*, 1996). There is general agreement that parallelism exists between the chronology of the onset of NADPH-d staining in every nucleus and the neurogenetic and/or synaptogenetic patterns observed in the striatum (Bayers, 1984), superior colliculus, and ventral lateral geniculate nucleus (González-Hernández *et al.*, 1993). The human fetus responds first to stimuli applied to nasoroal regions by the end of the 7th week. This sensitivity soon spreads from the trigeminal area to the entire body surface (Reinis and Goldman, 1980). Stimulation of the brain stem of the human fetus can induce body movements while the cerebral cortex is still inactive and does not control movements until birth (Reinis and Goldman, 1980). NADPH-d activity in the cerebral cortex appears at the last period of the perinatal stage (Table; Iwase *et al.*, 1998). The number of NADPH-d neurons peaks during embryonic development, with the somal size and fiber networks developing extensively in the postnatal days (Fig. 8; Takemura *et al.*, 1996). The NADPH-d neuronal circuitries in the SpVo/Sn develop during embryogenesis to provide orofacial sensorimotor reflexive functions necessary for suckling after birth (Fig. 6, Table; Takemura *et al.*, 1996). In contrast, NADPH-d activity in the SpVc appeared late at postnatal day 10 (Fig. 7, Table). This finding is consistent with the report that NO is implicated in nociceptive processing in the Spdh and the NO/cGMP system linked to NMDA receptor activation that develops or functions after birth (Morris *et al.*, 1994).

1.3. *c-Fos* expression in the trigeminal sensory nuclei

c-Fos is a DNA binding protein acting at the activating protein-1 consensus sequence to regulate transcription in the promoter region of several inhibitory neurotransmitter genes, enkephalin, dynorphin, and cholecystokinin (Dubner and Ruda, 1992; Uhl and Takemura, 1992). Ninety percent of dhl/II neurons expressing dynorphin also show colocalization of the *c-Fos* protein (Noguchi *et al.*, 1991). Dynorphin not only has an antinociceptive function through the κ -opioid receptor (Millan, 1986)

Table 1. Distribution of NADPH-d neurons in developing rat brain

	E15	E17-P0	P4-10	P14<
<i>Telencephalon</i>				
Cerebral cortex	—	—	+	++
Caudate putamen	—	++	+++	+++
Hippocampus	—	—	+	++
<i>Diencephalon</i>				
Thalamus	—	+	+	++
<i>Midbrain</i>				
Periaqueductal central gray	—	++	+++	+++
Superior colliculus	—	—	+	+++
Inferior colliculus	+	—	—	++
<i>Cerebellum</i>				
Molecular cell layer	—	—	—	+++
<i>Pons and Medulla</i>				
Solitary tract nucleus	++	++++	++++	++++
Trigeminal oral nucleus (SpVo)	+++	++++	+++	+++
Trigeminal caudal nucleus (SpVc)	—	—	++	+++
Reticular formation	—	++	++	++
Trigeminal ganglion	++	+		
Geniculate ganglion	+++			

Relative abundance of NADPH-d expression was estimated by visual comparison of NADPH-d neurons in number and staining intensity (— not detected, + modest, ++ moderate, +++ intense, ++++ very intense). E15, embryonic day 15; E17-P0, embryonic day 17 to postnatal day 0; P4-10, postnatal day 4 to 10; P<14, older than postnatal day 14. (data from Takemura *et al.*, 1996, and Iwase *et al.*, 1998)

but also a pronociceptive function through NMDA receptors (Caudle and Isaac, 1988; Hylden *et al.*, 1991; Shukla and LeMaire, 1994). There is accumulating evidence that dynorphin release following inflammation and nerve injury is involved in the maintenance of hyperalgesia and mechanical allodynia (Hylden *et al.*, 1991). c-Fos-positive neurons induced by noxious stimulation have been shown to colocalize with inhibitory neurotransmitters such as GABA and glycine in presumed interneurons or propriospinal neurons (i.e., nonprojection

neurons; Todd *et al.* 1994). The c-Fos-positive neurons may also contribute to nociception in different ways. One population may facilitate the activity of neurons that mediate noxious information to supraspinal centers, while another population may inhibit transmission in these pathways. There are conflicting results that intrathecally administered c-fos antisense oligodeoxynucleotide (ODN) enhances (Hunter *et al.*, 1995) and reduces (Hou *et al.*, 1997; Sugiyo *et al.*, 2001) nociceptive responses. The increased hyperalgesic behavior reported by Hunter *et*

Fig. 7. Photomicrographs of transverse sections showing the NADPH-d label in the dh (SpVc and Spdh). **A:** Spdh on E20, **B:** SpVc on P4, **C:** SpVc on P10. The right is medial and the upward is dorsal. dh: Spdh or SpVc, vh: ventral horn. Scale bars = 200 μ m (data from Takemura *et al.*, 1996)

al. (1995) following the preadministration of antisense ODN in young rats could be a consequence of the greater prevalence of κ -opioid receptors in young rats (Barr *et al.*, 1986; see also Hou *et al.*, 1997). On this basis, the analgesic effect of dynorphin (through κ -opioid receptors) is predicted to exceed the hyperalgesic effect possibly through NMDA receptors (Massardier and Hunt, 1989; Vanderah *et al.*, 1996) in young rats.

The c-Fos has been commonly used as an activity-dependent cell marker along the nociceptive pathway. It is a nuclear localized protein that is visualized immunohistochemically (see Fig. 10 for example), and its spatial and temporal expression pattern is stimulation intensity- and modality-dependent (Walther *et al.*, 1993; Chapman *et al.*, 1995; Takemura *et al.*, 2000b). For example in the spinal system, Chapman *et al.* (1995) reported that the preadministration of the NMDA receptor blocker inhibited an early nociceptive response to an intra-plantar injection of carrageenan and resulted in a significant reduction of c-Fos expression in the deep laminae, and laminae III and IV of the Spdh (dhIII/IV). Prolonged NMDA receptor blockade for 2 h reduced c-Fos expression in both dhI/II and dhIII/IV. Thus c-Fos expression in dhIII/IV likely represents the nociceptive activity of acute pain signaled by the NMDA receptor. On the other hand, c-Fos expression in the dhI/II represents the nociceptive activity of a slow and prolonged phase of pain mediated by C-fibers (Chapman and Besson, 1997).

Electrical stimulation of the TG at C-fiber activating ranges (high-intensity; 1.0 mA, 5 ms, 5 Hz) induced massive c-Fos expression primarily in the Vcl/II and

in cells of the rostral nuclei above the obex, Vp, SpVo, and SpVi (Takemura *et al.*, 2000b). This finding was in contrast to stimulation at the A-fiber activating range (low-intensity; 0.1 mA, 5ms, 5 Hz), which resulted in modest c-Fos expression in the Vcl/II and almost no c-Fos expression in the rostral nuclei. Despite the full range of expression in the Vcl/II (the ophthalmic, maxillary, and mandibular divisions), the expression in the rostral nuclei was restricted only in the dorsal and dorsomedial subdivision, which primarily receive oral sensory inputs. Unlike the ventrolateral subdivision receiving facial sensory inputs, the dorsomedial subdivision in the rostral nuclei is characterized by a substantial innervation of unmyelinated fine nociceptive neurons which contain SP, and CGRP (Sugimoto *et al.*, 1997a, b), but not TRPV1 (Bae *et al.*, 2004). Currently, c-Fos expression in the TSN most often has been reported to appear in the SpVc and SpVi/SpVc transition (Strassman and Vos, 1993; Sugimoto *et al.*, 1994 a,b; Carstens *et al.*, 1995; Hathaway *et al.*, 1995; Allen *et al.*, 1996; Bereiter and Bereiter, 1996), where c-Fos-positive neurons are induced by noxious mechanical stimuli (Strassman and Vos, 1993; Sugimoto *et al.*, 1994) and chemical irritation with capsaicin (Carstens *et al.*, 1995; Allen *et al.*, 1996) or mustard oil (Strassman and Vos, 1993; Hathaway *et al.* 1995; Bereiter and Bereiter, 1996). In contrast, c-Fos-positive neurons in the SpVo have been reported to appear only in the dorsal division, where they are induced by noxious stimulation of the tooth pulp or oral structures (Allen *et al.*, 1996; Sugimoto *et al.*, 1998a, b; He *et al.*, 2000), but not by electrical stimulation of the

Fig. 8. Camera lucida drawings of NADPH-d-labeled neurons in a representative parasagittal section for each embryonic and postnatal stage. Orientation arrows = 170 μm (data from Takemura *et al.*, 1996)

infraorbital nerve, the maxillary division (Strassman and Vos, 1993). Electrical stimulation applied to the tooth pulp always activates neurons in SpVo and SpVc, while neurons in SpVc are also consistently activated by thermal stimulation of the tooth pulp (Hu and Sessle, 1984). The overwhelming number of c-Fos-positive neurons in SpVc over SpVo may imply that SpVc receives a higher density of projections from thin fibers, which are effectively activated during inflammation and facial nociceptive condition, than do the other nuclei. The Vdm has been reported to contain nociceptive neurons that receive inputs mainly from the oral regions (Eisenman *et al.*, 1963; Azerad *et al.*, 1982; Dallel *et al.*, 1989). However, the dorsal division has neurons responsible for sensorimotor reflexes rather than sensory discriminative characters (Fukushima and Kerr 1979; Li *et al.*, 1995). Neurons in the Vdm project to the trigeminal motor nucleus (Li *et al.*, 1995), parabrachial nucleus (Allen *et al.*, 1996), and cerebellum (Falls *et al.*, 1985), but seldom project to the nucleus ventralis posteromedialis of the thalamus (Figs. 3, 4; Fukushima and Kerr, 1979; Yasui *et al.*, 1983). c-Fos-positive neurons in the Vdm not only receive nociceptive inputs but also are responsible for modulating sensorimotor reflexes involved in mastication.

2. GABAergic modulation in the trigeminal nociceptive processing

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system, though it is also present in the TG and dorsal root ganglion neurons (Szabat *et al.*, 1992; Stoyanova, 2004) and depolarizes unmyelinated primary afferent neurons (Bowery and Brown, 1974; Carlton *et al.*, 1999). GABA acts through major subtypes of receptors, A (GABA_A) and B (GABA_B), which are respectively involved in ligand gated Cl⁻ conductance and cGMP coupled regulation of Ca²⁺ and K⁺ conductance (Hammond, 1997). Substantial evidence indicates that GABA is intimately involved in nociception through actions at spinal and supraspinal/supratrigeminal (supraspinal) levels (Sawynok, 1987; Hammond, 1997). GABA has been well-documented in the TSN (Basbaum *et al.*, 1986; DiFiglia and Aronin, 1990; Ginestral and Matute, 1993; Iliakis *et al.*, 1996; Bae *et al.*, 2002) as well as Spdh (Malcangio and Bowery, 1994, 1996; Todd *et al.*, 1996; Lao and Marvizon, 2005). There is both anatomical (DiFiglia and Aronin, 1990) and pharmacological evidence (Kaneko and Hammond, 1997a, b) to suggest that the activation of nociceptive afferents is associated with a release of GABA in the medullary and/or spinal

cords. Systemic or topical administration of the GABA agonist and antagonist modulates neuronal responses in the TSN induced by noxious and non-noxious stimulation of the orofacial area (Chiang *et al.*, 1999; Takemura *et al.*, 2000a, 2001; Mitsikostas and Sanchez-del-Rio, 2001; Takeda *et al.*, 2004).

2.1. Involvement of GABA_A receptors on trigeminal nociception

Systemic administration of the GABA_A antagonist, bicuculline, or picrotoxin increases non-noxious stimulation-induced neuronal responses in the TSN (Takemura *et al.*, 2000a) and Spdh (Koyama *et al.*, 1998). In stark contrast to the non-noxious stimulation, systemic administration of bicuculline or picrotoxin decreases noxious stimulation (C-fiber activating range)-induced neuronal responses in the TSN (Takemura *et al.*, 2000a: see Fig. 10D for example) and Spdh (Koyama *et al.*, 1998). The above described stimulation intensity- or modality-dependent bidirectional effects of GABAergic ligands could be explained by the different actions of GABA on the nociception in the segmental and supraspinal sites. Segmentally administered bicuculline or picrotoxin enhanced the response to low threshold mechanoreceptors (Hwang and Yaksh, 1997; Chiang *et al.*, 1999; Mitsikostas and Sanchez-del-Rio, 2001) or to electrically defined A β afferents (Sivilotti and Woolf, 1994). However, the microinjection of bicuculline into the supraspinal site, the nucleus raphe magnus, or the periaqueductal central gray (PAG) selectively inhibits the responses of Spdh neurons to noxious heat stimuli without affecting their response to non-noxious mechanical stimuli (Sandkühler *et al.*, 1989). Under conditions of low intensity stimulation, the segmental actions appear to predominate. However, we suggest that under conditions of high intensity stimulation and nociception, the descending GABAergic disinhibitory pathways are strongly activated so that the inhibitory effects of the antagonist are more apparent. Thus the segmental GABAergic inhibitory effects could be masked by the supraspinal disinhibitory effects on nociception. Interestingly, inhibitory effects of systemic bicuculline on noxious stimulation-induced neuronal responses are strong in the ventrolateral VcI/II (receiving facial inputs) but weak in the dorsomedial VcI/II (receiving oral inputs) (Takemura *et al.*, 2000a). The somatotopically different effects of systemic bicuculline may underlie the somatotopically different distribution of neuroactive substances between dorsomedial and ventrolateral VcI/II, such as GABA (Ginestral and Matute, 1993) and 5-hydroxytryptamine receptors type 2A (Fay and Kubin, 2000).

The noxious stimulation-dependent modulation through GABA_A receptors is reversed when neurokinin-1 (NK-1)-bearing neurons in the SpVc are ablated by topical preadministration with a neurotoxin, saporin conjugated to SP (SP-Sap: Mantyh *et al.*, 1997) (Fig. 9, 10: Abe *et al.*, 2004, 2005), or C-fibers are destroyed by the neonatal administration of capsaicin (Chiang *et al.*, 1999). The NK-1, on which SP acts, is expressed by most of the lamina I neurons in the Spdh (Todd *et al.*, 2002) and SpVc (Li *et al.*, 1996). Most of the NK-1-bearing neurons project to the thalamus and/or parabrachial nucleus and are crucial for regulating pain behavior (Suzuki *et al.*, 2002; Mantyh and Hunt, 2004). The VcI/II is a primary relay nucleus receiving most C and A δ fibers of the orofacial areas, which transmit nociceptive information (Ruda and Gobel, 1980; Chiang *et al.*, 1994).

The NK-1-positive neurons in laminae I and III of the Spdh are a component of the centrally projecting circuitry (Marshall *et al.*, 1996; Todd *et al.*, 2000, 2002) that underlies descending inhibitory and facilitatory modulation to the Spdh (Olave and Maxwell, 2003). NK-1-bearing neurons are responsible for the activity of nearby excitatory short circuitry (Ren and Dubner, 1999) and ascending and descending facilitatory pathways through the nucleus reticularis gigantocellularis or disinhibitory pathways through the nucleus raphe magnus (Urban and Gebhart, 1999; Ren and Dubner, 1999, 2002; Sugiyo *et al.*, 2005). Lamina I neurons project to the PAG (Keay *et al.*, 1997). The GABAergic neurons in the PAG project to the rostroventral medulla, which contains serotonergic neurons that are responsible for inhibiting nociceptive neuronal excitability in the Spdh (Fields *et al.*, 1991). Consequently, GABAergic neurons in the supraspinal sites play a disinhibitory role against the nociceptive neurons in the Spdh/SpVc (Lin *et al.*, 1994, 1996).

Interestingly, noxious stimulation-induced internalization of the NK-1 — a marker of tachykinin signaling at the segmental level — in the spinal cord was blocked by a spinal administration of the GABA_B receptor agonist,

Fig. 9. Photomicrographs of transverse sections of the SpVc immunohistochemically stained for the NK-1 receptor in a rat pretreated with saline (Sal), non-conjugated (blank) saporin (BI-Sap), or substance P-conjugated saporin (SP-Sap) 2–4 weeks earlier. Sal- and BI-Sap-pretreated sections showed dense labeled neurons in lamina I, and sparse labeled neurons in lamina III of the SpVc. The SP-Sap-pretreated section shows almost no labeled neurons (data from Abe *et al.*, 2005)

Fig. 10. Schematic drawings and photomicrographs of a transverse section (about 1.2 mm caudal to the obex) of c-Fos-positive neurons in VcI/II induced by 10 min electrical stimulation of the TG at high-intensity (1.0 mA) with the systemic administration of saline (A–C) or bicuculline (D–F) 10 min before the stimulation in rats pretreated with Sal (A, D), Bl-Sap (B, E), or SP-Sap (C, F) 2–4 weeks previously. The c-Fos-positive neurons induced at high intensity stimulation decrease in the VcI/II of SP-Sap-treated rats compared with the Sal (A)- and Bl-Sap (B)-treated rats. The high intensity stimulation-induced c-Fos-positive neurons decrease in VcI/II with the administration of bicuculline in Sal (D) and Bl-Sap (E), but increase in SP-Sap (F)-treated rats. The fine dotted line indicates the area shown as the photomicrograph (data from Abe *et al.*, 2005)

R (+)-baclofen, but not the GABA_A receptor agonist muscimol or isoguvacine (Riley *et al.*, 2001). This suggests that presynaptic terminals to NK-1-bearing neurons express GABA_B receptors but not GABA_A receptors. Thus the segmental regulation of GABA_A receptor-mediated neuronal responses might be unaltered by SP-Sap treatment.

2.2. Involvement of GABA_B receptors on trigeminal nociception

In contrast to the effects on the GABA_A receptors, a systemically administered a GABA_B receptor agonist, baclofen, decreased noxious stimulation-induced neuronal responses in the TSN (Fromm *et al.*, 1984; Takemura *et al.*, 2001) and spinal cord (Buritova *et al.*, 1996; Castro *et al.*, 1999). The systemic administration of a

GABA_B receptor antagonist, phaclofen, increases noxious stimulation-induced neuronal responses (Takemura *et al.*, 2001). GABA_B receptors are localized in the A δ - and C-fibers (Price *et al.*, 1984). Baclofen decreases excitatory transmission from primary afferents by reducing Ca²⁺ influx into neurons and/or by potentiation of voltage-dependent K(+) currents (Takeda *et al.*, 2004). It also reduces spinal release of excitatory amino acids (Kangra *et al.*, 1991) or SP (Malcangio and Bowery, 1994). Ionophoretic administration of GABA depresses excitatory transmission in the SpVo and facilitates segmental inhibition (Fromm *et al.*, 1992). These actions are blocked by the GABA_B receptor antagonists, CGP 35348 and 2-hydroxy-saclofen (Fromm *et al.*, 1992). Chiang *et al.* (1999) reported that the intrathecal administration of a GABA_B receptor antagonist, 2-hydroxysaclofen, did not produce any significant actions

on nociceptive and non-nociceptive neurons in the SpVc. It is possible that the transient activation of SpVc neurons by pinching or tactile stimulation does not release sufficient amounts of GABA to activate the GABA_A receptor, and therefore the effects of the antagonist would not be apparent. Further detailed experiments examining somatotopically heterogeneous GABAergic modulation and stimulation intensity- and/or modality-dependent pain control would have therapeutic implications.

Conclusion

The TSN receive somatotopically specific primary afferent sensory inputs. The orofacial endogenous pain control systems in the TSN are quite different from other body areas in structural and pharmacological features. Following peripheral nerve injury, which often induces neuropathic pain, phenotypes switch between nociceptors and mechanoreceptors (Barbut *et al.*, 1981; Noguchi *et al.*, 1995; Hökfelt *et al.*, 1997). A thorough understanding of the specific mechanisms of orofacial nociception different from the mechanisms of those other body area in the central nervous system will help to develop affected new, site-specific therapeutic treatments.

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