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THE ROLE OF TRIGEMINAL INTERPOLARIS-CAUDALIS TRANSITION ZONE IN PERSISTENT OROFACIAL PAIN

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The sensory information from dental and craniofacial regions is first relayed in the spinal trigeminal nuclear complex (spinal trigeminal nucleus), which is further divided rostrocaudally into the subnuclei oralis (Vo), interpolaris (Vi) and caudalis (Vc) (Olszewski, 1950). It is widely accepted that nociceptive input from the cranial orofacial region is initially processed in the Vc (Sessle 2000) that exhibits considerable similarity with spinal dorsal horn and thus termed the medullary dorsal horn (MDH) (Gobel 1981). Despite apparent homology in nociceptive processing, recent observations have identified features of trigeminal pain processing in the MDH that are distinctly different from that of the spinal dorsal horn (Bereiter *et al*., 2000). Further, studies have pointed out increased excitability and sensitization of trigeminal pain pathways in non-laminar regions of the spinal trigeminal nuclear complex, particularly the trigeminal Vi/Vc transition zone, following injury and noxious stimulation of the dental and craniofacial region. Multiple lines of evidence suggest that the trigeminal Vi/Vc transition zone plays an important role in deep tissue pain processing, integrating nociceptive orofacial input, and the development of persistent orofacial pain.

I. Vi/Vc Neuronal Activation after Orofacial Injury

At the obex level, the ventral portion of the laminated Vc merges with the caudal Vi. Thus, rostral Vc with imperfectly laminated structures appears dorsally with Vi (ventral)atthe same coronalplane to form the trigeminal Vi/Vc transition zone (Fig. 1A). The dorsal portion of the Vi/Vc transition zone in the rat mainly involves the rostral end of the laminated Vc and the ventral Vi/Vc mainly includes the caudal Vi. Immunostaining with the anti-calcitonin gene-related peptide (CGRP) antibodies, the superficial laminae of the Vc can be clearly seen in the dorsal lateral portion of the Vi/Vc (Fig. 1A). The ventral pole of the Vi/Vc, the CGRP staining in the Vi appears less well organized, whereas the CGRP-like immunoreactivity (LI) is distributed across the full length of the superficial MDH (Fig. 1B). Somatotopy exists in the trigeminal transition zone. For example, the mandibular structures masseter muscle and temporomandibular joint (TMJ) are represented at the dorsomedial Vi/Vc (Klineberg, 1971; Nishimori *et al*., 1986; Capra, 1987; Takemura *et al*., 1987; Pfaller and Arvidsson, 1988; Shigenaga *et al*., 1988; Arvidsson and Raappana 1989); and corneal afferents terminate in the ventral Vi/Vc (Pozo and Cervero 1993; Hirata *et al*., 1999).

An interesting finding in recent years is that neuronal activation, as indicated by Fos protein expression, is consistently observed in the trigeminal Vi/Vc transition zone after a variety of noxious stimuli applied to the dental and craniofacial regions, including dural blood vessel and facial stimulation (Strassman and Vos, 1993, Strassman *et al*., 1994), corneal stimulation (Bereiter *et al*., 1994) and intravitreal capsaicin (Chang *et al*., 2010), noxious stimulation of the tooth (Coimbra and Coimbra, 1994), oral mucous membrane (Sugimoto *et al*., 1994) and the pulp (Chattipakorn *et al*., 2002, 2005; Oakden and Boissonade, 1998), chemical stimulation of the rat's tongue (Carstens *et al*., 1995), mustard oil and adjuvant stimulation of the TMJ (Hathaway *et al*., 1995; Zhou *et al*. 1999), and masseter muscle

inflammation (Imbe *et al*., 1999; Ro *et al*., 2003). These studies reveal an interesting pattern of trigeminal nociceptive neuronal activation (Fig. 2). First, stimulation-induced trigeminal Fos protein expression exhibits a bi-modal distribution, with one peak at the caudal Vc/C1,2 and a rostral peak at the Vi/Vc (Strassman and Vos, 1993). Second, while stimulus-induced Fos-LI in the Vc/C1,2, particularly in superficial laminae, is predominantly ipsilateral to the side of stimulation, Fos-LI in the Vi/Vc transition zone is often expressed bilaterally in the ventral Vi/Vc despite a unilateral injury.

The dual representation of orofacial nociceptive input in the spinal trigeminal complex is consistent with anatomical findings. It is known that sensory inputs from the orofacial region project to two regions of the spinal trigeminal complex, the Vc and caudal Vi (Arvidsson *et al*., 1992; Capra, and Dessem, 1992). The location of the terminal field in the caudal Vi is constant but the terminal field in the Vc shows somatopic differences. In general, primary afferent fibers from the perioral and perinasal regions, or the most anterior face (tongue, upper lip and snout), terminate most rostrally in the Vc, and fibers from progressively more posterior facial regions (lower lip, cornea, supraorbital vibrassae) terminate at successively lower levels (Shigenaga *et al*., 1986). Accordingly, primary afferent fibers that convey noxious stimuli from more caudal and lateral regions of the face have progressively more caudal terminations in reference to the obex. The fibers that innervate the circumoral zone terminate near the obex, whereas intermediate and peripheral zones terminate more caudally in the Vc. The anatomical relationship between the orofacial input and central termination explains the Fos expression pattern after noxious stimulation. For example, the terminal field for the lower lip is clearly caudal and separated from the Vi/Vc zone; so are the two distinct Fos peaks (Fig. 2). The primary terminal field for the upper lip is immediately caudal or slightly overlapped with the Vi/Vc zone (Arvidsson *et al*., 1992). Consistently, the caudal peak of Fos labeling is apparently missing after stimulation of the upper lip, as the Vi/Vc and caudal Fos peaks merge at the obex-Vi/Vc level (Fig. 2) (Strassman and Vos 1993).

What is the significance of neuronal activation at the Vi/Vc level? It has been shown that urethane anesthesia itself can induce Fos at the Vi/Vc, but not at the caudal Vc (Strassman and Vos, 1993). Methohexital (Brevital) anesthesia also induces Fos in the Vi/Vc (Zhou *et al*., 1999). However, the masseter inflammation-induced Fos in Vi/Vc cannot be explained by the effect of Brevital anesthesia alone (Imbe *et al*., 1999). Specific neuronal activation at the Vi/Vc transition zone after noxious stimulation, which correlates with behavioral hyperalgesia, suggests a previously unrecognized role of this region in trigeminal pain processing.

II. Functional Input and Output of the Vi/Vc Transition Zone

A. Peripheral Input

By comparing the masseteric inflammation induced Fos-LI to that induced by anesthesia and skin-cut over the masseter muscle, it was found that the bilateral periobex peak of Fos-LI was primarily a response to masseteric inflammation, while the skin-cut mainly induced the caudal ipsilateral peak of Fos-LI (Imbe *et al*., 1999; Ikeda *et al*., 2003). These findings suggest that the Vi/Vc transition zone plays an important role in the responses to deep tissue injury. This hypothesis is supported by recent studies discussed below.

Utilizing a double tracing protocol (Capra and Wax, 1989), we studied the central termination of masseter muscle afferents in the trigeminal transition zone, compared with that of the cutaneous afferents (Wang *et al*. 2006). Different neuronal tracers were injected either centrally (FluoroGold; ventral Vi/Vc or MDH) or peripherally (wheat germ agglutinin-conjugated horseradish peroxidase or cholera toxin B; masseter or overlying

skin), in the same rat and the convergence of the tracers in the trigeminal ganglion was examined. A population of small- to medium-sized neurons was double-labeled after injections of the tracers into the masseter and Vi/Vc, masseter and MDH, or the skin and MDH. However, only a few double-labeled neurons were occasionally observed after injections of the tracers into the skin and Vi/Vc. These results indicate that while both masseter and cutaneous inputs project to the MDH, masseter afferents provide an additional input to the Vi/Vc. The ventral Vi/Vc also receives direct corneal input (Pozo and Cervero, 1993; Tashiro *et al*., 2010).

Behavioral pharmacology studies show that injection of an N-methyl-D-aspartate receptor antagonist, AP-5, into the Vi/Vc and MDH attenuated masseter inflammatory hyperalgesia. In contrast, hyperalgesia after inflammation of the skin overlying the masseter was attenuated by injection of AP-5 into the MDH but not Vi/Vc. Similar results were obtained by injecting glial inhibitors and IL-10, an anti-inflammatory cytokine, into the Vi/Vc or MDH (Shimizu *et al*. 2009b). These anatomical and behavioral observations support the view that there is differential involvement of trigeminal transition zone, and laminated subnucleus caudalis in orofacial deep and cutaneous hyperalgesia.

B. Visceral Input

The afferent projections to the ipsilateral dorsal Vi/Vc have been identified following HRP injection into the cervical vagus nerve (Gwyn *et al*., 1985). Electrical stimulation of the vagus nerve induces expression of Fos-LI in the Vi/Vc transition zone (Gieroba and Blessing, 1994; Yousfi-Malki and Puizillout, 1994). The vagotomy produces a decrease in the masseteric inflammation-induced Fos-LI in the Vi/Vc transition zone (Imbe *et al*., 1999). Taken together, a portion of the orofacial injury-induced Vi/Vc neuronal activation is likely a result of somatic-visceral integration that is mediated by vagal afferents to the brain.

C. Caudalis Input

The multiple lines of evidence indicate that neuronal activation in the transition zone, particularly the ventral pole, depends on input from the caudal Vc. Neurons in the caudal Vc project to rostral subnuclei of the spinal trigeminal nucleus (Ikeda *et al*., 1982; Lovick and Wolstencroft, 1983; Jacquin *et al*., 1990). The ascending pathway from caudal Vc modulates the activity of neurons in the more rostral subnuclei of the spinal trigeminal nucleus (Greenwood and Sessle, 1976; Scibetta and King 1969). Inhibition of caudal Vc affects Vi/Vc neuronal activity and evoked transmitter release (Bereiter *et al*., 2002; Hirata *et al*., 2003). Topical application of glutamate and morphine to the Vc/C1 region either facilitated or inhibited evoked activity of ~30% of corneal units tested in the Vi/Vc (Meng *et al*., 1998). Following injection of the retrograde tracer Fluorogold into the Vi/Vc transition zone, retrogradely labeled cells were observed in the Vc/C1,2 region (Sato *et al*., 2005). After lesions of the bilateral Vc, there is a selective reduction of inflammation-induced Fos-expressing neurons in the ventral Vi/Vc, but not in dorsal or intermediate Vi/Vc, or nucleus tractus solitarius (Sugiyo *et al*., 2005). These findings support that trigeminal transition zone activity, particularly the ventral Vi/Vc, is modulated by the activation of the caudal laminated Vc zone.

D. Rostral Projections

Neurons in the spinal trigeminal complex project to a variety of rostral brain structures related to somatosensory, as well as somatoautonomic and somatovisceral processing. It has been shown that neurons in the ventral portion of the Vi/Vc transition zone have a major projection to the nucleus submedius of the thalamus (Sm) (Yoshida *et al*., 1991). This Vi/Vc-Sm pathway is activated after orofacial injury. Through a combined Fluorogold retrograde tracing and Fos protein immunocytochemistry double labeling approach, rostrally

projecting neurons that are activated after masseter inflammation can be identified (Ikeda *et al*., 2003). In the ventral portion of the Vi/Vc transition zone, about 40 percent of neurons exhibiting inflammation-induced Fos-LI project to the Sm. However, very few Fos-labeled neurons in the dorsal Vi/Vc project to the Sm. Anesthesia alone also induces Fos expression in ventral Vi/Vc neurons but these neurons do not project to Sm (Ikeda *et al*., 2003). About 20 percent of Fos-positive neurons in dorsal and ventral Vi/Vc project to the parabrachial nucleus and about 5 percent of Fos-positive neurons project to either the lateral hypothalamus or medial ventroposterior thalamic nucleus. The Sm plays a role in nociceptive processing, particularly related to aversive and emotional responses (Craig and Burton 1981). The parabrachial nucleus is involved in processing trigeminal nociceptive input and integrating emotional and autonomic responses (see Feil and Herbert 1995). The major projections from Vi/Vc are to the Sm and parabrachial nucleus, supporting the conclusion that this region plays a role in autonomic and hormonal functions and is emotionality related to persistent pain (Bereiter *et al*., 1996; Ikeda *et al*., 2003) (Fig. 3).

E. Reciprocal Interactions with the Brainstem Descending Circuitry

The rostral ventromedial medulla (RVM) is a key structure in descending pain modulation, which includes the midline nucleus raphe magnus and the adjacent reticular formation ventral to the gigantocellular reticular nucleus (Fields *et al*., 2006). In addition to inhibitory descending input, RVM facilitates neuropathic pain, secondary hyperalgesia and persistent pain (Porreca *et al*., 2002; Vanegas and Schaible, 2004; Ren and Dubner, 2008). The Vi/Vc transition zone has access to the RVM. Following the injection of Fluorogold into the RVM 7 days before injection of an inflammatory agent, Complete Freund's Adjuvant (CFA), into the masseter muscle and perfusion of the rat at 2-hour post-inflammation, a population of neurons in the ventral Vi/Vc exhibited Fluorogold/Fos double staining, suggesting the connection between Vi/Vc and RVM and the activation of the Vi/Vc-RVM pathway after inflammation. This Vi/Vc-RVM projection pathway appears selective for the ventral portion of the Vi/Vc since no double-labeled neurons were found in the dorsal Vi/Vc or laminae I–IV of Vc (Sugiyo *et al*., 2005). Injection of an anterograde tracer, phaseolus vulgaris leucoagglutinin, into the RVM, resulted in labeling profiles overlapped with the region that showed Fluorogold/Fos double labeling in the Vi/Vc, suggesting that the connections between RVM and Vi/Vc are reciprocal. Excitotoxic lesions of the RVM or Vi/ Vc with ibotenic acid led to the elimination or attenuation of masseter hyperalgesia/allodynia developed after masseter inflammation (Sugiyo *et al*., 2005). Thus, there are reciprocal connections between the ventral Vi/Vc transition zone and RVM. The Vi/Vc-RVM pathway is activated after orofacial deep tissue injury and involved in descending facilitation of orofacial hyperalgesia.

III. Cellular and Chemical Mediators: Role of Neuron-Glia-Cytokine Interactions

Nerve signals arising from sites of tissue or nerve injury lead to long-term increases in excitability and plasticity in the central nervous system, often referred to as central sensitization. Central sensitization is brought about by a series of cellular events including neuronal depolarization, removal of the voltage-dependent magnesium block of the N-methyl-D-aspartate receptor (NMDAR); phosphorylation of NMDAR, alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and neurokinin (NK) 1 receptors and an increase in synaptic strength (Dubner and Ren, 2004). Ample evidence indicates that central sensitization underlies mechanisms of persistent pain (Woolf and Salter, 2000; Dubner and Ren, 2004).

There has been an increasing awareness of neuroimmune interactions and their role in the etiology of diseases including chronic pain (Ren and Dubner, 2010). The neuroimmune interactions are reciprocal, or bi-directional, and involve central glial cells, cytokines, and neurotransmitters and their receptors. The emerging literature strongly implicates a role for glia and proinflammatory cytokines in the genesis and maintenance of persistent or chronic pain (Watkins and Maier, 2005).

The Vi/Vc transition zone contributes to central sensitization after orofacial injury. Sensitization of Vi/Vc neurons occurs after orofacial injury, which involves a cascade of cellular events including the activation of neurotransmitter receptors and neuron-glia-cytokine interactions. In response to orofacial tissue injury, glia in the Vi/Vc transition zone exhibit hyperactivity and inflammatory cytokines released, contributing to activity-dependent plasticity and hyperalgesia (Guo *et al*., 2007).

A. Vi/Vc Glial Response to Injury

Following peripheral injury, microglia and astrocytes show increased levels of activity or a hyperactive state, and are often referred to as “activated”. The specific expression of cellular markers is used to assess levels of glial activity. Among the most commonly used, glial fibrillary acidic protein (GFAP), an astrocytic intermediate cytoskeleton filament, is a marker of astrocytes and cluster of differentiation molecule 11b (CD11b), also known as Mac-1 or CR3, is the α-integrin marker of microglia. The monoclonal antibody OX-42 is commonly used to detect CD11b in brain microglia. Two calcium-binding peptides have been used as a functional marker of glia: S100B for astrocytes and Iba1 (ionized calcium-binding adapter molecule) for microglia.

Masseter inflammation induces glial hyperactivity in the Vi/Vc transition zone (Guo *et al*., 2007). Following injection of the inflammatory agent CFA into the masseter muscle, reactive astrocytes were clearly seen in the Vi/Vc transition zone, indicated by GFAP immunostaining. The activated astrocytes typically exhibited hypertrophy with thicker processes and larger and densely stained cell bodies. The increase in GFAP levels is seen as early as at 0.5-hours and lasts for about one week after inflammation. The activation of astrocytes after inflammation is associated with upregulation of connexin 43 (Cx43), an astrocytic gap junction protein. Double immunohistochemistry shows that the Cx43-LI co-localizes with GFAP, but not with CD11b, a marker of microglia, NeuN, a neuronal marker, or Cx36, a neuronal gap junction protein. The levels of CD11b are also upregulated by masseter inflammation. The glial hyperactivity induced by masseter inflammation in the Vi/Vc transition zone correlates with the development of hyperalgesia (Sugiyo *et al*., 2005; Watanabe *et al*., 2005).

B. Role of Inflammatory Cytokine IL-1beta

The hyperactivity of glia induced by masseter inflammation is accompanied by an increase in the inflammatory cytokine levels. Compared to the naive rats, the immunostaining for interleukin-1beta (IL-1beta a prototypic proinflammatory cytokine, is increased significantly in the Vi/Vc transition zone (Guo *et al*., 2007). Western blot shows the increase of IL-1beta in the Vi/Vc transition zone with a time course similar to that of astrocyte hypertrophy after masseter inflammation. Interestingly, IL-1beta is selectively induced in astrocytes, but not in microglia or neurons, in the ventral Vi/Vc transiton zone. The selective localization of IL-1beta in astrocytes has also been reported in other animal models, including bone cancer pain (Zhang *et al*., 2005), tissue and nerve injury (Kawasaki *et al*., 2008; Wei *et al*., 2008; Weyerbacher *et al*., 2010), and intracerebral hemorrhage (Wasserman *et al*., 2007). These findings suggest that astrocytes provide an alternative source of IL-1beta, in addition to the known release of IL-1beta from microglia after injury (Clark *et al*., 2006; Kawasaki *et al*.,

2008). The CFA-induced increases in IL-1beta levels are reduced after the treatment with propentofylline, a glial inhibitor (Guo *et al*., 2007), suggesting that glial activation is upstream to cytokine induction in the Vi/Vc after inflammation.

The IL-1beta in the Vi/Vc plays a role in the development of orofacial hyper-algesia. The mechanical allodynia and hyperalgesia in the orofacial region can be assessed by applying a series of von Frey microfilaments to the skin. The response frequencies to a range of von Frey filament forces are determined. A stimulus-response frequency relationship is established and 50 perecnt effective force values are derived. The EF_{50} is defined as the force that induces 50 percent response frequency in rats. A significant reduction of EF_{50} vs. the baseline level indicates mechanical allodynia and hyperalgesia (Guo *et al*., 2004). When an IL-1 receptor antagonist (IL-1ra) was administered intrathecally via osmotic pumps through a cannula implanted at the level of the obex prior to the induction of masseter inflammation, the behavioral allodynia and hyperalgesia were significantly attenuated (Guo *et al*., 2007). Direct injection of IL-1beta into the ventral Vi/Vc transition zone produces mechanical allodynia and hyperalgesia (Shimizu *et al*.,2009a).

C. Glia, Cytokine and NMDA Receptor Activation

The Vi/Vc glial hyperactivity and inflammatory cytokine release facilitates central sensitization through interactions with the NMDAR. NMDAR phosphorylation is widely accepted as an indication of synaptic plasticity and correlates with the time course of persistent pain (Guo *et al*., 2002, 2004; Brenner *et al*., 2004). IL-1beta facilitates NMDAR phosphorylation in an *ex vivo* medullary slice preparation (Guo *et al*., 2007). Incubation of IL-1beta in the medullary slices induced a significant and dose-dependent increase in phospho-ser896-NR1 (P-NR1) levels in the Vi/Vc transition zone. In contrast, the application of TNF-alpha, the other prototypic inflammatory cytokine, does not affect P-NR1 levels at the dose tested (Guo *et al*., 2007). Microinjection of IL-1beta into the ventral Vi/Vc transition zone in vivo also produced an increase in P-ser896-NR1 levels that is blocked by IL-1ra (Guo *et al*., 2007).

The IL-1beta-induced NR1 phosphorylation is blocked by chelerythrine, a PKC inhibitor, confirming that PKC is involved in this effect. 2APB, a membrane permeable IP3 receptor antagonist also blocks IL-1beta-induced NR1 phosphorylation. The NMDAR channel blocker MK-801 does not affect the IL-1beta-induced increase in P-ser896-NR1 (Guo *et al*., 2007). The involvement of the PKC inhibitor in IL-1beta-facilitated P-NR1 suggests that a key link between IL-1beta and subsequent activation of NMDAR involves phospholipases PLA2 or PLC, since PKC is a downstream effector of arachidonic acid and diacylglycerol. This hypothesis has been tested. The PLC inhibitor U73122 and PLA2 inhibitor AACOCF3 blocked the effect of IL-1beta on NMDAR phosphorylation in Vi/Vc. It is well known that IL-1R signaling leads to transcriptional regulation of cellular function (Martin and Wesche, 2002). These findings show that the effect of IL-1beta on NMDAR phosphorylation is attributable to the post-translational regulation through IL-1R signaling; and that the intermediate pathway involves PLC, PLA2 and subsequent PKC activation and intracellular Ca^{2+} release.

Direct administration of IL-1beta into the ventral trigeminal transition zone produced orofacial hyperalgesia that lasted for hours (Shimizu *et al*., 2009a). Pretreatment with glial inhibitors does not block IL-1beta-induced hyperalgesia even with relatively high doses that have been shown to be effective in attenuating hyperalgesia after injury (Wei *et al*., 2008; Shimizu *et al*., 2009b). Although the glial inhibitor/modulator propentofylline do not block IL-1beta-induced hyperalgesia, propentofylline was able to attenuate hyperalgesia after masseter inflammation (Shimizu *et al*., unpublished observations). Likewise, fluorocitrate is unable to block IL-1beta-induced NMDA receptor phosphorylation in the Vi/Vc transition

zone in vitro, but attenuated masseter inflammatory hyperalgesia (Guo *et al*., 2007). Thus, IL-1beta-induced hyperalgesia appears to be downstream to glial activity and involves activation of NMDAR. Direct application of IL-1beta bypasses glial cells, to produce neuronal hyperexcitability and hyperalgesia.

The NMDAR plays a major role in central sensitization and pain hypersensitivity, including orofacial hyperalgesia (Chiang *et al*., 1997; Bereiter and Bereiter, 2000; Guo *et al*., 2007; Wang *et al*., 2009). Injection of the NMDAR antagonist into the Vi/Vc transition zone attenuates masseter inflammatory hyperalgesia (Wang *et al*., 2006). The IL-1beta signaling facilitates NMDAR activity in neurons (Viviani *et al*., 2003; Yang *et al*., 2005; Guo *et al*., 2007; Zhang *et al*., 2008). IL-1beta exaggerates NMDA and glutamate-evoked hippocampal neuron death in the rat (Ma *et al*., 2002-2003). Consistently, IL-1R colocalizes with the NMDAR NR1 subunit in Vi/Vc neurons (Guo *et al*., 2007). Thus, IL-1R signaling may selectively regulate NMDAR function and increase synaptic strength via post-translational phosphorylation and contribute to inflammation-induced pain hypersensitivity. Collective evidence suggests that glial activation, inflammatory cytokine release and NMDAR activation are sequential events in the nervous system responses to injury, and that IL-1beta and its interaction with NMDA receptors plays a critical role in the central mechanisms of hyperalgesia.

IV. Functional Significance of Vi/Vc in Trigeminal Pain Processing

Noxious stimulation of the different dental and craniofacial regions induces bimodal neuronal activation in the spinal trigeminal complex. While the caudal peak of neuronal activity varies rostrocaudally and mediolaterally in the Vc according to somatotopy, the rostral peak is consistently located at the obex or the trigeminal transition zone level (Strassman and Vos, 1993). Further, anesthetic alone induces Fos protein expression and increased phosphorylation of the extracellular signal-regulated kinase in the Vi/Vc transition zone (Imbe *et al*., 1999; Shoda *et al*., 2009). These findings suggest that neuronal activation in the ventral Vi/Vc transition zone lacks somatotopy, although there are selective primary afferent inputs to the Vi/Vc transition zone (Klineberg, 1971; Shigenaga *et al*., 1988; Pozo and Cervero 1993; Zhou *et al*., 1999; Hirata *et al*., 1999; Imbe *et al*., 1999; Wang *et al*., 2006). Evidence suggests that the Vi/Vc response, particularly the ventral Vi/Vc, to injury is related to the integration of somatosensory and visceral/autonomic functions, and the engagement of descending pain modulation, but less concerned with somatotopically organized pain behavior, that is mainly mediated by laminated Vc (Fig. 3).

A. Somatovisceral Somatoautonomic Integration

It is suggested that anesthetics-induced Fos expression in the brainstem nuclei could be explained at least in part by its cardiovascular effects, since they induce a decrease in the arterial blood pressure and heart rate (Rocha and Herbert, 1997). The administration of nitroglycerin, a vasodilator, also induces hypotension and bilateral Fos-LI in the ventral portion of Vi/Vc (Tassorelli and Joseph, 1995; Bereiter *et al*. 1994) show that mustard oil stimulation of the cornea induces Fos-LI in a group of neurons located in the ventral Vi/Vc and that local anesthesia of this area greatly attenuated the mustard oil-induced adrenal and autonomic responses. Vi/Vc neuronal activation is also related to injury-produced environmental stress and visceral input. Adrenalectomy and vagotomy selectively reduced Fos expression at the Vi/Vc transition zone with little effect on the caudal Fos peak (Imbe *et al*., 1999), suggesting that Vi/Vc neuronal activation depends on the integrity of the adrenal cortex and vagus nerve. Thus, in addition to somatotopically organized nociceptive responses, orofacial injury is also coupled to somatovisceral and somatoautonomic activity that contributes to central neural activation mediated through the Vi/Vc.

B. Descending Modulation

Trigeminal neurons have connections with neurons in the nucleus raphe magnus, the major component of the RVM (Beitz *et al*., 1983; Basbaum *et al*., 1986). The spinal trigeminal nucleus receives serotonergic and enkephalinergic projections from the nucleus raphe magnus (Beitz, 1982; Beitz *et al*., 1987). Pain modulating neurons in nucleus raphe magnus also have terminals in the spinal trigeminal nucleus (Mason and Fields, 1989), which may modulate trigeminal nociceptive transmission (Chiang *et al*., 1994).

The Vi/Vc neurons also relay nociceptive input from the orofacial deep tissues to RVM neurons (Sugiyo *et al*., 2005). Inflammation-activated neurons in the Vi/Vc transition zone project to the RVM and there is RVM neuronal activation after TMJ inflammation (Zhou *et al*., 1999). An anterograde tracing study suggests that RVM neurons project to the area of the ventral trigeminal transition zone, where activated RVM projecting neurons are localized (Sugiyo *et al*., 2005). This suggests reciprocal neural pathways between these two regions. These results further emphasize the importance of Vi/Vc neurons in engaging descending pain modulation.

Behavioral studies confirm the functional significance of the RVM-Vi/Vc connections (Sugiyo *et al*., 2005; Shimizu *et al*., 2009a). Unilateral CFA-induced inflammation of the masseter muscle produces mechanical allodynia and hyper-algesia in the orofacial region overlapping the masseter muscle. Excitotoxic lesions of the RVM or Vi/Vc transition zone lead to significant attenuation of behavioral hyperalgesia after masseter inflammation, indicating that modulatory inputs from the RVM enhance the hyperalgesia/allodynia, found after masseter inflammation. These results are consistent with the view that hyper-algesia in animal models of inflammatory and neuropathic pain are closely linked to the activation of descending pathways (Porreca *et al*., 2002; Ren and Dubner, 2008; Vanegas and Schaible, 2004). Masseter inflammation-induced hyperalgesia is maintained by descending facilitatory drive that is recruited by the Vi/Vc-RVM circuitry, at least partially.

C. Deep vs. Cutaneous Injury

Deep orofacial tissue inflammation produces stronger central neuronal activation in the spinal trigeminal complex, including the Vi/Vc region than does cutaneous inflammation (Zhou *et al*., 1999; Imbe *et al*., 2001). Compared to cutaneous CFA injection, the injection of CFA into the TMJ produces a significantly stronger inflammation associated with greater Fos expression. It has been shown that mustard oil injections into the TMJ versus cutaneous tissues produces greater activation of masticatory muscles (Yu *et al*., 1993) than the cutaneous mustard oil injections and that TMJ inflammation resulted in more widespread excitation of medullary dorsal horn neurons than perioral cutaneous inflammation (Iwata *et al*., 1999).

There is also a differential involvement of the Vi/Vc transition zone in deep vs. cutaneous orofacial hyperalgesia. Injection of the NMDAR antagonist AP-5, anti-inflammatory cytokine IL-10 or glial inhibitors fluorocitrate and minocycline into the ventral Vi/Vc transition zone only attenuates hyperalgesia after masseter inflammation, without an effect on hyperalgesia associated with cutaneous injury (Wang *et al*., 2006; Shimizu *et al*., 2009b). In contrast, both masseter and cutaneous hyperalgesia are attenuated after the injection of these agents into the caudal Vc. Western blot analysis shows a selective enhancement of NMDAR phosphorylation and GFAP upregulation in the Vi/Vc transition zone after masseter, but not cutaneous inflammation (Shimizu *et al*., 2009b). These observations indicate that, in addition to Vc, deep orofacial input engages Vi/Vc neurons in developing central sensitization and hyperalgesia. Noxious cutaneous or superficial input has access to the Vi/Vc (Strassman and Vos, 1993; Zhou *et al*., 1999). The development of cutaneous

hyperalgesia depends on caudal Vc and does not require coordination by Vi/Vc neurons (Shimizu *et al*., 2009b; Chang *et al*., 2010). Thus, while both deep and cutaneous orofacial nociceptive inputs are processed in the laminated Vc, the ventral pole of Vi/Vc is involved in the coordination of sensorimotor functions of the trigeminal system associated with the response to and recuperation from deep tissue injury. This response includes roles in nociceptive hyperexcitability involving glia and NMDAR, somatovisceral and somatoautomic integration, and descending pain modulation (also see Dubner and Ren, 2006).

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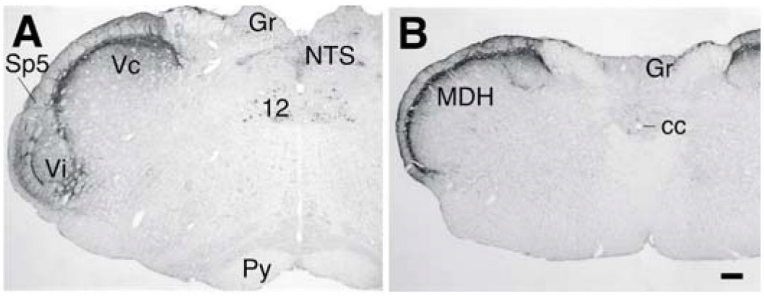

Fig. 1.

Digital photomicrograph illustrating the trigeminal Vi/Vc transition zone and laminated subnucleus caudalis (Vc). The sections in A and B were immunostained against anti-CGRP antibodies to illustrate the appearance of the Vi/Vc transition zone (A, about 0.4 mm rostral to the obex) and laminated Vc, or Medullary Dorsal Horn (MDH) (B, about 1.0 mm caudal to the obex). Note the the delineation of the Vi in the ventral Vi/Vc transition zone by calcitonin gene-related peptide staining (A). Scale bar = 0.2 mm. 12, hypoglossal nucleus; cc, central canal; Gr, gracile nucleus; NTS, Nucleus Tractus Solitarius; Py, pyramidal tract; Sp5, spinal trigeminal tract; Vi, subnucleus interpolaris of the spinal trigeminal complex. (Adapted from Wang *et al*., 2006, with permission from John Willey and Sons, License number: 2514340553127).

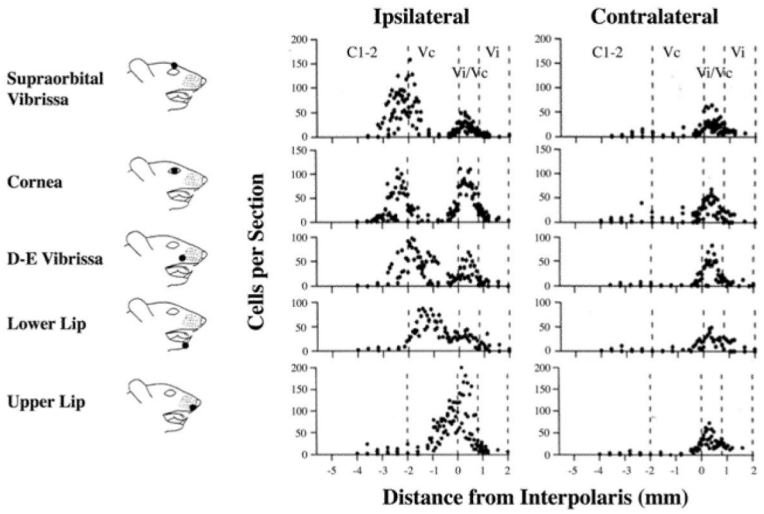

Fig. 2.

Rostrocaudal distribution of Fos-immunoreactive neurons in the spinal trigeminal complex after orofacial stimulation. Cartoons on the right show site of stimulation. The number of Fos-positive cells is plotted against the distance from the subnucleus interpolaris. 0 ≈ obex level, positive = rostral, negative = caudal. Note bi-modal distribution of Fos-positive cells along the Vi/Vc and Vc/C1,2 ipsilateral to stimulation and one peak of Fos-positive cells at the Vi/Vc level contralateral to stimulation. (Adapted from Strassman and Vos, 1993, with permission from John Willey and Sons, License number: 2514340716050).

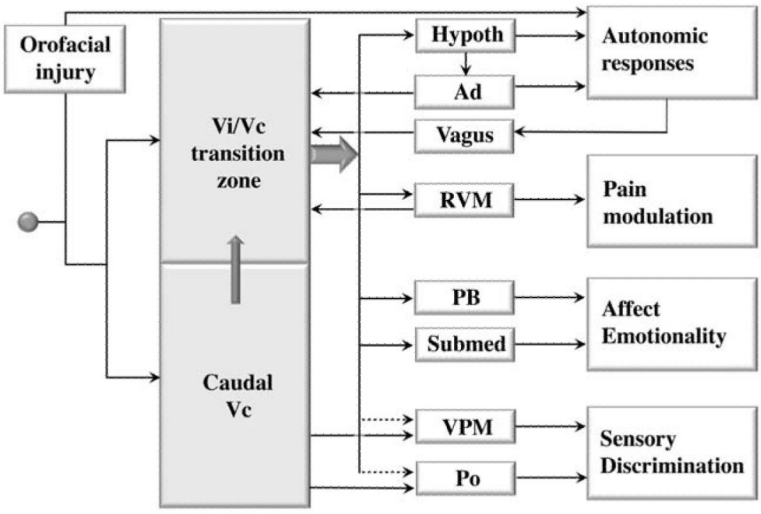

Fig. 3.

Summary of the role of the trigeminal Vi/Vc transition zone in persistent orofacial pain. Orofacial injury-related inputs not only activate caudal Vc neurons, but also reach the Vi/Vc transition zone. The Vi/Vc output accesses hypothalamus (Hypoth), rostral ventromedial medulla (RVM), parabrachial nucleus (PB), and nucleus submedius of the thalamus (Submed) to play a role in autonomic responses to injury, descending pain modulation and pain-related emotionality. The Vi/Vc transition zone also receives input from RVM and Vi/Vc neuronal activation is regulated by caudal Vc through internuclear connections, the adrenal cortex (Ad) through circulating glucocorticoids and vagal afferents. Also shown is the Vc output that is relayed through the thalamic medial ventrolateral nucleus (VPM) and posterior thalamic nucleus (PO) for discriminative pain. The Vi/Vc transition zone may play a minor role in discriminative pain (dashed arrows).