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Research Report

The synaptic microcircuitry associated with primary afferent terminals in the interpolaris and caudalis of trigeminal sensory nuclear complex

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Abstract

Previous ultrastructural studies indicating a higher number of axoaxonic contacts on individual low-threshold mechanoreceptive afferents in the principalis (Vp) than in the oralis (Vo) of cat trigeminal sensory nuclear complex (TSNC) suggest that the synaptic microcircuitry associated with primary afferents manifests unique differences across the sensory nuclei of TSNC. To address this issue, we analyzed synaptic microcircuits associated with fast adapting vibrissa afferent terminals in the interpolaris (Vi) and caudalis (Vc, laminae III/IV) by using intraaxonal injections of horseradish peroxidase (HRP) in cats. Forty-two and 65 HRP-labeled boutons were analyzed in the Vi and Vc, respectively. The labeled boutons contained clear, spherical vesicles. They most frequently formed asymmetric axodendritic synapses and were commonly postsynaptic to unlabeled axon terminals containing pleomorphic vesicles (p-endings) with symmetric junctions. The examination of synaptic contacts over the entire surface of individual boutons indicated that the afferent boutons made contacts with an average of two postsynaptic targets in the Vi and Vc. In contrast, axoaxonic contacts, and labeled boutons participating in synaptic triads, where p-endings contacted both the boutons and their postsynaptic targets, were, on average, higher in the Vi than in the Vc. These results suggest that the output of sensory information conveyed through low-threshold mechanoreceptive afferents is more strongly controlled at the level of the first synapse by presynaptic and postsynaptic mechanisms in the Vi responsible for sensory discriminative functions than in the Vc for sensorimotor reflexive functions.

Theme: Sensory systems
Topic: Somatic afferents

Keywords: Intraaxonal; Horseradish peroxidase; Ultrastructure; Presynaptic; Postsynaptic

1. Introduction

Since Olszewski (1950) [20] divided the trigeminal sensory nuclear complex (TSNC) into four nuclei: the principalis (Vp), oralis (Vo), interpolaris (Vi), and caudalis (Vc), numerous investigations have explored the structure–function relationship in the TSNC (for review, see [27]).

At the light microscopic (LM) level, Shigenaga and his colleagues using transganglionic [29,30] and retrograde [28] transport of horseradish peroxidase (HRP), and intra-axonal [17,18,31,33] and intracellular [32,36,38] injections of HRP in cats have provided evidence suggesting that the morphological differentiation of the TSNC has an important role for eliciting different functions following the same sensory signal. This suggestion is supported by the

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following findings: first, although intraoral and facial structures are represented dorsoventrally in the four trigeminal sensory nuclei (Vp, Vo, Vi, and Vc), the dorsomedial subnucleus (Vpd) of Vp receives projections from the intraoral structure only but the ventrolateral subnucleus (Vpv) of Vp from the two [29,30]. Second, neurons in the Vpv, Vi, and laminae I and V of the Vc project mainly to the contralateral posteromedial ventral nucleus (VPM) of the thalamus but the Vpd to the ipsilateral VPM [29] (also see Refs. [9,11,35]): many Vo neurons innervate the trigeminal motor nucleus or motoneurons [32,36,38]. Third, the terminal morphology (e.g., the shape, number, and size) of physiologically defined, low-threshold mechanoreceptive afferents manifests unique differences across the nuclei [17,18,31,33]; also see Refs. [12–14].

In addition, on the basis of the above-described LM studies, Shigenaga and his colleagues have examined the ultrastructure of functionally identified, intraaxonally HRP-labeled, low-threshold mechanoreceptive afferent terminals from the periodontal ligaments in the Vpv and Vo [1,2], the vibrissae in the Vpv [19], and the tongue in the Vpd and Vo [39] in cats. The results of these electron microscopic (EM) studies have documented that the average number of contacts made by afferent boutons onto their second-order neurons is almost the same for primary afferents in the same nucleus (Vp or Vo). In contrast, the number of axon terminals presynaptic to afferents is rather variable not only among different classes of the afferent in the same nucleus (Vp or Vo) but also between different nuclei (Vp vs. Vo) for the same afferent class. Comparable EM studies in the Vi and Vc are not available. Comparisons of EM data on primary afferent terminals among different nuclei are essential, with due attention to the suggestion that the TSNC can be functionally divided into two major groups: the Vp, Vi, and lamina I of the Vc for sensory discrimination, and Vo and Vc (laminae III/IV) for sensorimotor reflexive functions (see above; for reviews, also see [21,27]).

In the present study, we performed an ultrastructural analysis of functionally identified, intraaxonally HRP-labeled afferent terminals in the cat, in which fast adapting (FA) vibrissa afferents were examined, as representative of low-threshold mechanoreceptive afferents, in the Vi and Vc, and compared the present data with those previously reported on the ultrastructures of primary afferent terminals in the Vp and Vo [2,19,39].

2. Materials and methods

Experiments were conducted on two adult cats (2.5–3.0 kg). The care and treatment of animals were in accordance with Osaka University and National Institutes of Health guidelines. All experiments described here were approved by the Osaka University Intramural Animal Care and Use Committee. Food and water were available ad libitum, and the cats were maintained one per cage under standard laboratory conditions: 12-h light/dark cycle at 22 ± 2 °C. All efforts were made to minimize the number of animals used and their suffering. Anesthesia was induced by ketamine (35 mg/kg, i.m.) followed by sodium pentobarbital (40 mg/kg, i.v.), with supplemental doses of sodium pentobarbital (10 mg/ml, i.v.) given to maintain the animals in a deep level of anesthesia throughout the experiments. End-tidal % CO_2 and rectal temperature were monitored continuously and maintained within physiological limits, and the depth of anesthesia was monitored frequently by checking the animal's pupil size and pulse rate.

The infraorbital foramen was exposed to insert bipolar electrodes into the infraorbital sulcus for electrical stimulation. Animals were placed in a stereotaxic frame, and part of the occipital bone was removed to expose the caudal pons and the medulla. Cisternal drainage and bilateral pneumothorax were performed to reduce pulsations of the brainstem. Animals were then immobilized with pancuronium bromide (0.07 mg/kg, i.v.) and artificially ventilated.

Intraaxonal recordings and injections of HRP (Toyobo, Japan) were achieved by using micropipettes with broken tips (0.7–1.2 μm) filled by capillary action with a solution of 3% HRP in 0.3 M KCl and 0.05 M Tris buffer at pH 7.6. Penetrations were made into the trigeminal spinal tract at the level of caudal Vo and intraaxonal recordings were identified by a sudden negative-going DC potential shift and appearance of a large amplitude of an action potential by stimulation of the infraorbital nerve (a single pulse with 0.2 ms duration at 1 Hz; e.g., Fig. 1A). After an axon was penetrated, it was characterized physiologically by noting the location at the receptive field and determining its response properties (manually delivered by deflection of vibrissa or non-vibrissa hairs). Once FA vibrissa afferent was identified (e.g., Fig. 1B), HRP was iontophoresed with 10–15 nA depolarizing continuous currents for 5–10 min. In the two animals, two and five fibers were filled, since previous studies reported that synaptic arrangements showed no significant differences between fibers in the same nucleus [2,19,39]. Fifteen or sixteen hours after the injection of HRP, the animals were deeply anesthetized and perfused through the ascending aorta with 2.5% glutaraldehyde, 1.0% paraformaldehyde, 0.3% picric acid, and 0.2 M $CaCl_2$ in 0.1 M phosphate buffer (pH 7.3). The lower brainstem including the pons and medulla was removed and stored in the same fixative overnight at 4 °C. Serial 70 μm-thick transverse sections were cut on a Vibratome. These sections were processed with 3,3′-diaminobenzidine tetrahydrochloride. The wet sections were examined, and six or seven sections containing HRP-labeled terminals were selected from the rostrocaudal mid-levels of the Vi and of the Vc with a laminated structure. These sections

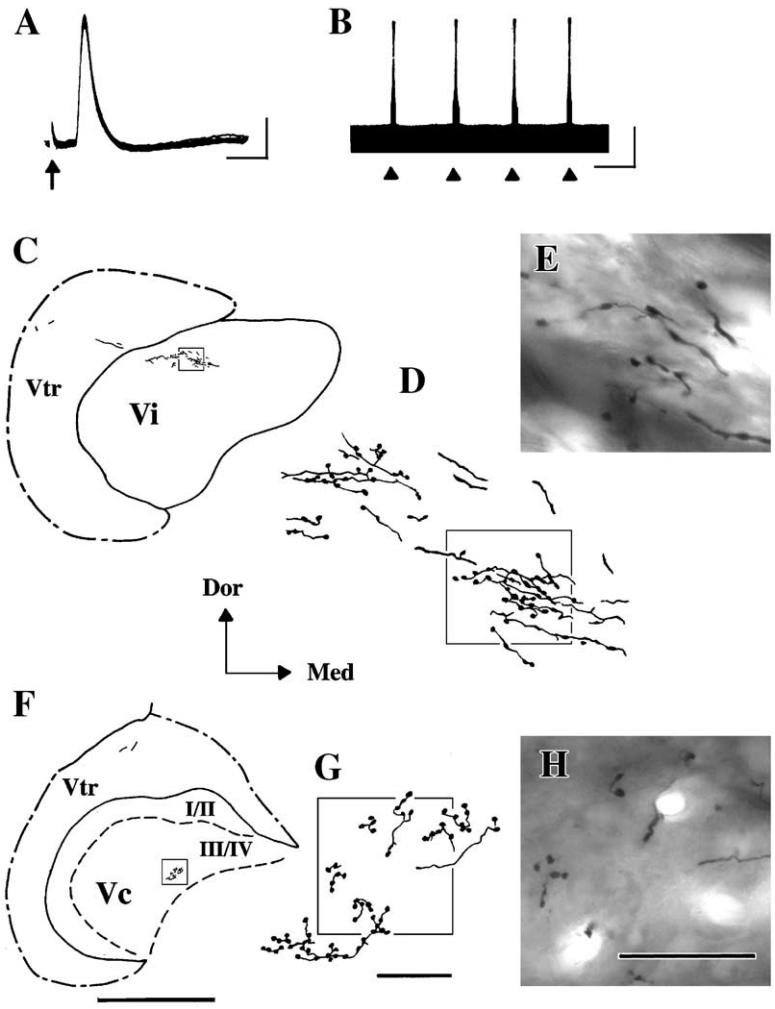

Fig. 1. Electrophysiology and morphology of single fast adapting (FA) vibrissa afferents. (A) An action potential elicited by stimulation of the infraorbital nerve. Arrow indicates an artifact of the electrical stimulus. Vertical and horizontal bars indicate 10 mV and 2 ms, respectively. (B) Discharges evoked by deflection of a vibrissa. Arrowheads denote on-set of the stimulus. Vertical and horizontal bars indicate 10 mV and 2 s, respectively. (C, D, F, G) Camera lucida drawings of horseradish peroxidase (HRP)-labeled single vibrissa afferents in the interpolaris (Vi; C, D) and caudalis (Vc; F, G) at the low (C, F) and high (D, G) magnifications, which were reconstructed from a single section, respectively. The boxed areas in panels C and F are shown in panels D and G, respectively. Note that in this case the two afferents were separately labeled. Scale bars in panels F (applies to C) and G (applies to D) indicate 1 mm and 20 μm, respectively. Dor–Med, dorsal–medial; Vtr, trigeminal spinal tract; I/II, laminae I and II; III/IV, laminae III and IV. (E, H) Photomicrographs taken from the boxed areas in panels D and G. Scale bars in panel H = 20 μm (applies to E).

were treated with 2% OsO_4 in 0.1 M phosphate buffer (pH 7.4) containing 16% sucrose, dehydrated and embedded in Durcupan ACM (Fluka, Chemie AG, Buchs, Switzerland). Serial ultrathin sections were cut with an ultramicrotome and a series of ribbons of 8 to 10 serially cut sections were collected on Formvar-coated, single-slot nickel grids. The grids were contrasted with uranyl acetate and lead citrate before examination in a Hitachi H-600 electron microscope.

3. Results

The detailed physiological and morphological properties of FA vibrissa afferents have been described elsewhere [17,31]. Briefly, the afferents responded to slow or rapid displacement of a single vibrissa in a phasic manner, and they were neither spontaneously active (Fig. 1B) nor directionally sensitive. The terminal arbors formed by single collaterals were generally more compact in the Vi than in the Vc (Figs. 1C–H, compare the boxed areas in D and G or E and H). In the Vc, terminal arbors analyzed in the present study were in laminae III and IV (Figs. 1F–H), mainly in lamina IV because labeled boutons were less dense in lamina III as shown in a previous study [17]. Forty-two and 65 labeled boutons were analyzed in the Vi and Vc, respectively, which were reconstructed from serial sections that completely encompassed the entire bouton. The data obtained from two animals were combined (see above). Consistent with our previous EM studies (slowly adapting (SA) periodontal afferents in Vpv and Vo [2]; SA and FA vibrissa afferents in Vpv [19]; SA lingual afferents in Vpd

and Vo [39]), most labeled boutons in the Vi and Vc contained clear, spherical, synaptic vesicles with few large dense-core vesicles (S terminals; Figs. 2 and 3), and unlabeled axon terminals presynaptic to labeled boutons (p-endings) contained pleomorphic vesicles (a mixture of spherical, oval and flattened vesicles; Figs. 2A and 3A, B). In cases where afferent boutons were not obscured by the presence of the electron-dense HRP reaction product, S terminals and terminals with pleomorphic vesicles were found to establish asymmetric (Figs. 2B and 3C, D) and symmetric (Fig. 2A) contacts, respectively.

3.1. Synaptic microcircuitry in Vi

The labeled boutons were presynaptic to somata or primary dendrites (defined by the presence of rough endoplasmic reticulum and polysomes; Figs. 2C, D), non-primary dendritic shafts (Figs. 2A and 3A, B), or dendritic spines, and they were frequently postsynaptic to p-endings (Figs. 2A and 3A, B; Table 1).

With respect to the postsynaptic elements, of the 42 labeled boutons examined, 7 (17%) and 3 (7%) made a single contact with somata or primary dendrites and dendritic spines, respectively. The remaining boutons (32, or 76%) established synaptic contacts with nonprimary dendritic shafts. Overall, 33 (79%), 6 (14%), and 3 (7%) of the 42 labeled boutons made contacts with 1 or 2, 3 or 4, and 5 to 7 postsynaptic elements, respectively.

Thirty-three (79%) of the 42 labeled boutons were postsynaptic to p-endings such that 10, 14, and 9 received 1 or 2, 3 or 4, and 5 to 8 p-endings, respectively. The remaining labeled boutons (9, or 21%) did not receive any p-endings. Of the 33 labeled boutons that made axoaxonic synapses, 22 boutons participated in triadic synaptic arrangements (triads) where p-endings contacted both labeled boutons and their postsynaptic dendrites (Fig. 3A).

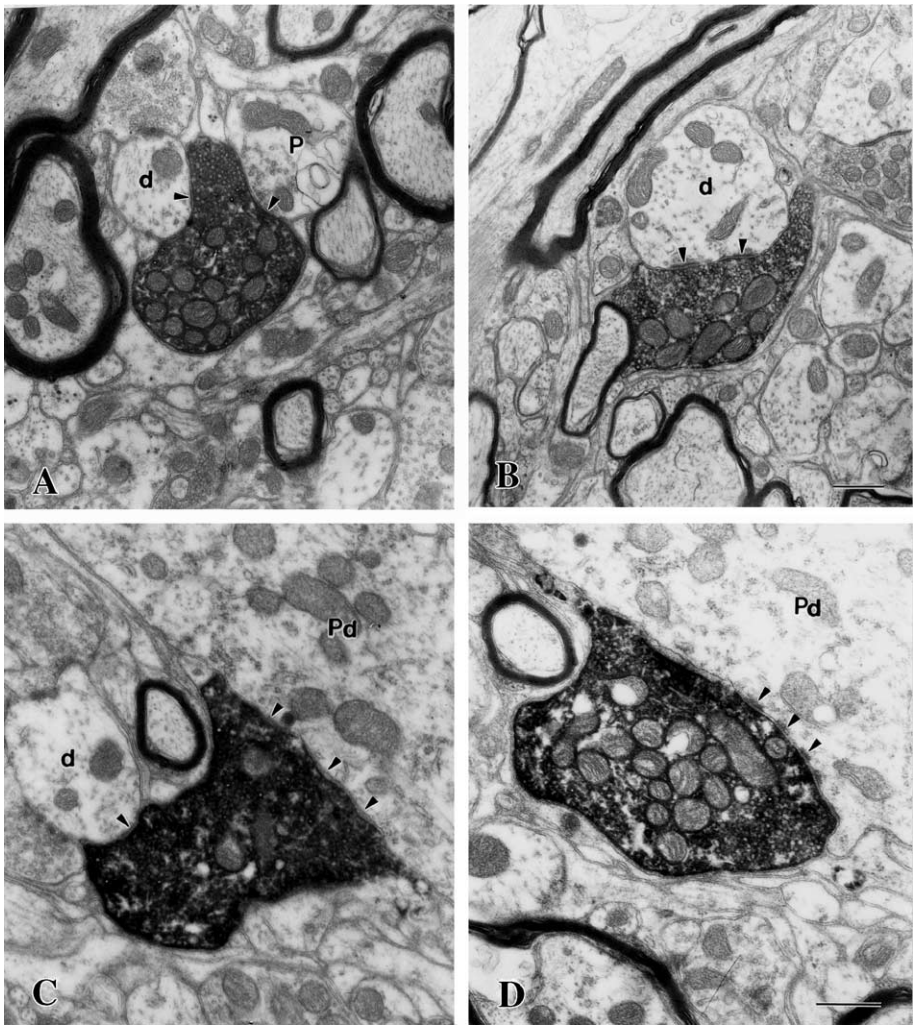

Fig. 2. Electron photomicrographs showing three horseradish peroxidase (HRP)-labeled, fast adapting (FA) vibrissa afferent boutons participating in the simple synaptic arrangement in the interpolaris (A, C, D) and lamina IV (B) of the caudalis. (A) A labeled bouton is presynaptic to a nonprimary dendritic shaft (d) and presynaptic to an unlabeled axon terminal (p) containing pleomorphic vesicles (p-ending). (B) A labeled bouton is presynaptic to a nonprimary dendritic shaft (d). (C, D) A labeled bouton is presynaptic to a primary dendrite (Pd) and a nonprimary dendrite (d) as well. The approximate distance between sections C and D is 320 nm. Arrowheads indicate the sites of synaptic contact. Scale bar = 500 nm in panel D (applies to A–C).

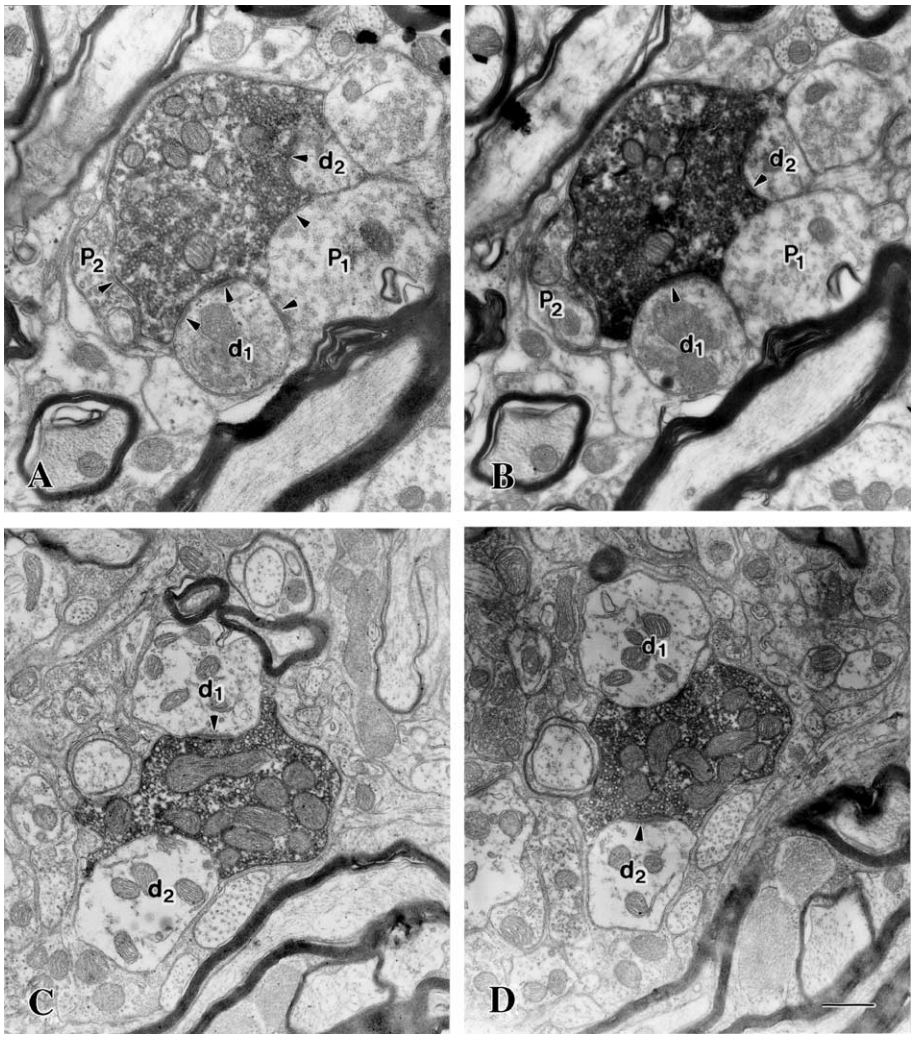

Fig. 3. Electron photomicrographs showing an example of a labeled bouton forming synaptic glomerulus in the interpolaris (A, B) and of a labeled bouton participating in the simple synaptic arrangement in lamina IV of the caudalis (C, D). (A, B) A labeled bouton is presynaptic to two nonprimary dendrites d1 and d2 and postsynaptic to two p-endings p1 and p2. The p-ending p1 contacts both the bouton (A, B) and its postsynaptic dendrite d1 (A; a synaptic triad). Note that this bouton makes synaptic contact with 3 nonprimary dendrites and 5 p-endings. The approximate distance between sections A and B is 160 nm. (C, D) A labeled bouton is presynaptic to two nonprimary dendrites d1 and d2. The approximate distance between sections C and D is 400 nm. Arrowheads denote the sites of contact. Scale bar = 500 nm in panel D (applies to A–C).

On the basis of the total number of contacts over an entire labeled bouton surface, we grouped synaptic arrangements into three categories: simple (1 or 2 contacts), intermediate (3 or 4 contacts), and complex (5 to 15 contacts; Table 2). Of the 42 labeled boutons, 13 participated in simple, 8 in intermediate, and 21 in complex synaptic arrangements. In the simple synaptic arrangements, an entire labeled bouton contacted 1 or 2 postsynaptic targets, or a postsynaptic target and a p-ending. In the intermediate synaptic arrangements, a labeled bouton

Table 1
Frequency of occurrence of different types of contacts per labeled bouton in fast adapting (FA) vibrissa afferents in the interpolar (Vi) and caudal (Vc) nuclei[a]

	No. of boutons examined	Synaptic contacts with				Synaptic triads[b]	Total no. of contacts
		Somata or primary dendrites	Nonprimary dendritic shafts	Dendritic spines	P-endings		
Vi	42	0.2 ± 0.4 (n = 7)[c]	1.6 ± 1.3 (n = 69)	0.1 ± 0.3 (n = 3)	2.8 ± 2.2[d] (n = 118)	1.3 ± 1.2[d] (53)	4.7 ± 3.2[d] (n = 197)
Vc	65	0.0 (n = 1)	2.1 ± 1.5 (n = 138)	0.1 ± 0.4 (n = 8)	1.2 ± 1.5[d] (n = 79)	0.4 ± 0.9[d] (28)	3.5 ± 2.7[d] (n = 226)

[a] Values are mean ± SD.
[b] Numbers in parenthesis indicate number of labeled boutons.
[c] In parentheses, n indicates number of contacts.
[d] Indicates significant difference between Vi and Vc at the 0.05 level (Student's t test).

Table 2
Frequency distribution of FA vibrissa afferents in three types of synaptic arrangements in the interpolar (Vi) and caudal (Vc) nuclei[a]

	No. of boutons examined	Synaptic arrangements[b]		
		Simple	Intermediate	Complex
Vi	42	31 (13)[c]	19 (8)[c]	50 (21)[c]
Vc	65	46 (30)[c]	34 (22)[c]	20 (13)[c]

[a] Values are percentage and the number of boutons (in parentheses).
[b] Labeled boutons making synaptic contacts with 1 or 2, 3 or 4, and 5 or more unlabeled neuronal profiles are subgrouped into three types: simple, intermediate, and complex synaptic arrangements, respectively.
[c] Indicates significant difference between Vi and Vc at 0.05 level (χ^2 test).

contacted 1 or 2 postsynaptic targets and 2 or 3 p-endings. In the complex synaptic arrangements, a labeled bouton contacted 2 to 7 postsynaptic targets and 3 to 8 p-endings. All labeled boutons participating in complex synaptic arrangements were surrounded by two or more postsynaptic targets and three or more presynaptic p-endings invaginating into the labeled bouton (synaptic glomerulus [15,22,23]; Figs. 3A, B).

3.2. Synaptic microcircuitry in Vc

While the general ultrastructural features of labeled boutons observed in the Vi and Vc were similar, differences were noticed in the frequency of occurrence of different types of contacts (Table 1). In contrast to labeled boutons in the Vi, all labeled boutons in the Vc, except for one contacting a soma, made contact with nonprimary dendrites (Figs. 2B and 3C, D); of these boutons, 7 boutons contacted spineheads. Of the 64 boutons contacting nonprimary dendrites, 44, 14, and 6 boutons contacted 1 or 2, 3 or 4, and 5 to 9 postsynaptic targets. The number of postsynaptic targets per labeled bouton was higher in the Vc than in the Vi (2.2 ± 0.6 vs. 1.9 ± 0.7), but the difference was not significant.

Forty-two of the 65 labeled boutons received 1 or 2, 3 or 4, and 5 to 9 p-endings. The remaining 23 boutons were not postsynaptic to any p-endings (Figs. 3C, D). Twenty-eight of the 42 boutons participated in synaptic triads. The number of p-endings and of synaptic triads per labeled bouton was significantly ($P < 0.05$) lower in the Vc than in the Vi (Table 1). The total number of contacts (including postsynaptic targets and p-endings) was also significantly ($P < 0.05$) lower in the Vc than in the Vi (also see Table 1), reflecting a higher number of p-ending contacts on Vi boutons.

Of the 65 labeled Vc boutons categorized, 30 (46%) were found in simple arrangements, 22 (34%) in intermediate, and 13 (20%) in complex synaptic arrangements (Table 2). The frequency of labeled boutons participating in simple or intermediate synaptic arrangements was significantly ($P < 0.05$) higher in the Vc than in the Vi but this trend was reversed for complex synaptic arrangements (also see Table 2).

4. Discussion

The present results reveal both similarities and differences in ultrastructures of low-threshold mechanoreceptive afferent boutons between the Vi and laminae III/IV of the Vc. S terminals (labeled or afferent boutons) in both regions contain clear, spherical, synaptic vesicles and frequently receive unlabeled axon terminals containing pleomorphic vesicles (p-endings). The S terminals are a feature common not only to low-threshold mechanoreceptive afferents with cell bodies located either in the trigeminal ganglion [1,2,19,39] or in the trigeminal mesencephalic nucleus [3,4,16] but also to unmyelinated and myelinated nociceptive afferents in the rat [6,7]. Recent immunocytochemical EM studies in the rat have revealed that myelinated trigeminal primary afferent terminals are immunoreactive for glutamate [5,10], and that the glutamate-immunoreactive boutons are commonly postsynaptic to axon terminals (p-endings). It has been shown that p-endings are frequently immunoreactive to GABA in rats [5] and cats [8] or to both GABA and glycine in cats [8]. Although it is presumed that the cells of origin of p-endings are distributed within the TSNC and receive trigeminal sensory inputs, their real morphology and physiology are not known at present.

Fast adapting (FA) vibrissa afferent boutons in the present study received significantly more contacts of p-endings and synaptic triads in the Vi than in the Vc, highlighting an important difference in organization of the synaptic microcircuitry between the two nuclei. These results are in line with previous EM data on periodontal afferents [2] and lingual afferents [39] indicating that the frequency of axoaxonic contacts and synaptic triads is higher in the Vp than in the Vo. Therefore, it suggests that the synaptic glomerulus, including the triadic synaptic arrangement, is an important biological device that integrates afferent impulses into information exerting particular aspects of sensory discrimination (e.g., the sensory modality, intensity, location, and duration of a stimulus applied to intraoral or facial structure). Regarding FA vibrissa afferents, for instance, the difference in frequency of synaptic glomeruli between the Vi and Vp (50 vs. 15%; see Ref. [19]) may provide the possibility that the main function of sensory discriminative aspects may be different between the two nuclei.

Another important role of the synaptic glomerulus, including afferent boutons with a central axon surrounded by two or more postsynaptic targets, is to amplify the amount of sensory information to higher centers by activating multiple second-order neurons. This idea comes from previous LM studies using intraaxonal and/or intracellular double labeling with HRP or neurobiotin, in which contacts made between a single motoneuron and a single jaw muscle spindle afferent or a single Vo second-order neuron were analyzed [34,37,38]; namely, a bouton never made contact with different postsynaptic profiles of the same motoneuron.

The entire group of low-threshold mechanoreceptive afferents examined in the present and previous studies

[2,19,39] in the cat seems to be distinguishable, to some extent, from muscle spindle afferent terminals in the trigeminal motor nucleus, because group Ia and II afferents frequently make synaptic contacts with somata and primary dendrites [16]. The trigeminal myelinated afferents appear to be, in addition, clearly distinguishable from unmyelinated afferents expressing receptor TRPV1 in the superficial layers of Vc in rats, in that the latter contains many dense-core vesicles as well as clear, round vesicles and receives extremely rare p-endings, axoaxonic synapses [7].

Interestingly, in the Vc and spinal dorsal horn, the total number of contacts per bouton (including postsynaptic targets and presynaptic p-endings) is almost identical between FA vibrissa boutons and rapidly adapting (RA) afferent boutons from the glabrous skin of the cat's hindpaw [24–26]. Also, the types of contacts are similar in the two regions, with the exception of axospinous synapses, which are more frequent for the RA afferent boutons. However, the FA vibrissa boutons appear to have more synaptic contacts than do slowly adapting type 1 (SA 1) or Pacinian corpuscle afferent boutons in the spinal dorsal horn [26]. These observations indicate that the functionally similar fiber group (FA or RA) shares certain characteristics for the organization of synaptic microcircuitry in the Vc and spinal dorsal horn with an analogous structure. However, such common feature is not likely to apply to the synaptic microcircuitry in nonlaminated parts of the rostral trigeminal nuclei.

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