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Investigation of an indirect pathway from spinal trigeminal subnucleus pars oralis to ventrobasal thalamus

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INVESTIGATION OF AN INDIRECT PATHWAY FROM SPINAL TRIGEMINAL SUBNUCLEUS PARS ORALIS TO VENTROBASAL THALAMUS

by

Richard Douglas Hector

THESIS

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INTRODUCTION

Somatosensory information from the face and the anterior two thirds of the head is relayed by way of the brainstem trigeminal nuclear complex (BTNC) to the thalamus and ultimately to the cortex for eventual conscious perception. Although there are numerous studies concerned with the projections from the BTNC to the thalamus, there still remain questions over details, particularly with respect to paths conveying nociceptive information. The BTNC conveys both non-nociceptive and nociceptive-specific information to the thalamus and those studies that are available have concentrated on the non-nociceptive input. Knowledge of nociceptive pathways is derived mostly from direct projections from the BTNC to higher centers (Sessle, 1987; Fields, 1987; Albe-Fessard et al. 1985; Willis, Jr. and Coggeshall, 1991). There are probably indirect pathways by which nociceptive information is relayed, but these are virtually unexplored. The present study will examine the possibility of an indirect pathway conveying nociceptive information from the BTNC to the thalamus in the rat.

Before examining the particular pathway of this study, it will be useful to give an overview of the known connections from the rodent BTNC to the thalamus. The BTNC can be divided into two nuclei, the main sensory nucleus and the subnuclei of the spinal sensory nucleus.

Main sensory nucleus

The main sensory nucleus has been shown to possess a detailed representation of non-nociceptive information from the face, especially whisker representation (Belford and Killackey, 1980; Belford and Killackey, 1979a; Arvidsson, 1982; Kandel et al. 1991; Belford and Killackey, 1979b). Many reports using both retrograde and anterograde tracing experiments have shown projections from the main sensory nucleus to the thalamus (Jacquin et al. 1984; Williams et al. 1994). These projections involve more neurons than do those arising in other BTNC nuclei such as n.interpolaris or n.caudalis of the spinal sensory nucleus (Peschanski, 1984; Fukushima and Kerr, 1979; Patrick and Robinson, 1987; Kemplay and Webster, 1989)

Nucleus Oralis

The spinal nucleus consists of three subnuclei, n.oralis, n.interpolaris and n.caudalis. N.oralis receives input mostly from intraoral and perioral areas of the face (Wall and Taub, 1962; Dallel et al. 1988; Dallel et al. 1987; Raboisson et al. 1991; Dallel et al. 1990; Raboisson et al. 1989; Dallel et al. 1989; Clavelou et al. 1989). Early studies of rat n.oralis by Fukushima and Kerr (1979) suggested that neurons in this nucleus did not project to the thalamus. This was later confirmed by Patrick and Robinson (1987) who also failed to find labeled cells bodies in n.oralis following retrograde tracing experiments from VPM (Fukushima and Kerr, 1979; Patrick and Robinson, 1987). However, Kemplay and Webster (1989) as well as Jacquin et al (1990) reported a few labeled cells bodies in n.oralis following retrograde tracing experiments from VPM to n.oralis (Kemplay and

Webster, 1989; Jacquin et al. 1990). The available data suggest that if n.oralis has projections to the thalamus, they are very sparse.

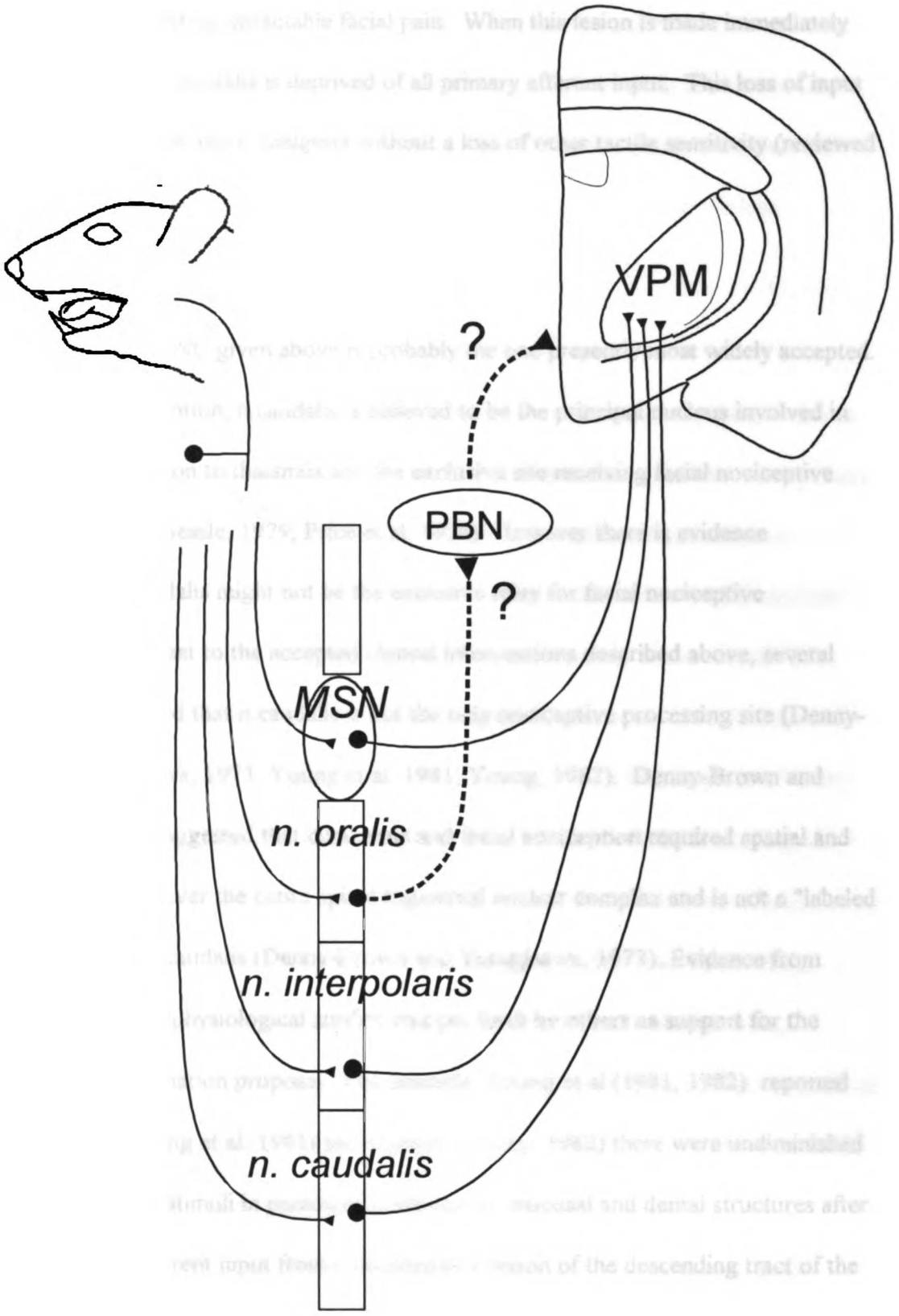
Nucleus Interpolaris

Nucleus interpolaris has been suggested to subserve similar peripheral structures and regions as the main sensory nucleus including vibrissae, guard hairs, facial cutaneous, periorbital and mucosal areas (Jacquin et al. 1989). This nucleus projects to several higher areas including the VPM of the thalamus (Peschanski, 1984; Fukushima and Kerr, 1979; Kemplay and Webster, 1989; Jacquin et al. 1990; Patrick and Robinson, 1987). Williams et al (1994) showed with anterograde tracing that a single n.interpolaris projection neuron terminates on several neurons occupying a small region in VPM (Williams et al. 1994). N.interpolaris seems to process mostly non-noxious stimuli. While several authors have failed to find any cells in n.interpolaris that responded to noxious stimulation (Rhoades et al. 1987; Jacquin et al. 1989), others have reported the presence of nociceptive specific and wide dynamic range neurons (Hayashi et al. 1984).

Nucleus Caudalis

Nucleus caudalis is believed to be the major nucleus for nociceptive input in the BTNC (Sessle, 1987; Amano et al. 1986; Sessle et al. 1986; Anton and Peppel, 1991b). An early report by Nord (1967) suggested that n.caudalis processes information from the entire ipsilateral face by showing that this nucleus contained a somatotopic map of the face (Nord, 1967). Anatomical techniques have shown n.caudalis is continuous with the dorsal

horn of the spinal cord. It is laminated in a manner similar to the dorsal horn and the morphology of its neurons is similar in lamina I and lamina II of the cord (Jacquin et al. 1986). Small myelinated (Aδ) and fine unmyelinated (C) fibers, which convey nociceptive information, are the predominant input to n.caudalis (Mengel et al. 1992; Mengel et al. 1993; Arvidsson and Gobel, 1981). Nociceptive transmitters, including neuropeptides, have also been localized to these fibers (Wiesenfeld-Hallin et al. 1991; Hökfelt et al. 1992; Woolf and Wiesenfeld-Hallin, 1986) (reviewed in Willis and Coggeshall 1991). *Fos-like* imunoreactivity (IR) has been used to show neuronal activity in response to noxious stimulation (Strassman et al. 1993). This assay has been employed in studying nociceptive processing in n.caudalis by several groups who have shown increased *fos-like* IR after noxious compared to non-noxious stimulation of the cornea and facial cutaneous regions (Anton et al. 1991a; Williams et al. 1990; McHaffie et al. 1994). In addition to anatomical observations, electrophysiological experiments have shown neurons in n.caudalis respond to nociceptive stimulation. For example, Maixner et al (1989) have shown that n.caudalis neurons respond to noxious heat stimuli (Maixner et al. 1989). Also, Hamba et al (1992) has successfully elicited an increased response (wind-up) by repeated electrical stimulation of tooth pulp (Hamba et al. 1992) which has significant projections to n.caudalis (see below). Earlier experiments by Fitzgerald and Lynn (1977) demonstrated that wind-up is produced specifically in nociceptive fibers by repeated noxious stimulation (Fitzgerald and Lynn, 1977). Clinical observations have also indicated that n.caudalis plays a significant role in facial nociception. A lesion of the descending tract of the fifth nerve (Figure 1) has


VPM
?
PBN
?
MSN
n. oralis
n. interpolaris
n. caudalis

often been used in treating intractable facial pain. When this lesion is made immediately rostral of the obex, n.caudalis is deprived of all primary afferent input. This loss of input to n.caudalis resulted in facial analgesia without a loss of other tactile sensitivity (reviewed in Sessle 1987).

The description of BTNC given above is probably the one presently most widely accepted. With regard to nociception, n.caudalis is believed to be the principal nucleus involved in nociceptive transmission to thalamus and the exclusive site receiving facial nociceptive information (Hu and Sessle, 1979; Price et al. 1976). However there is evidence suggesting that n.caudalis might not be the exclusive relay for facial nociceptive information. In contrast to the accepted clinical interventions described above, several authors have suggested that n.caudalis is not the only nociceptive processing site (Denny-Brown and Yanagisawa, 1973; Young et al. 1981; Young, 1982). Denny-Brown and Yanagisawa (1973) suggested that cutaneous and facial nociception required spatial and temporal summation over the entire spinal trigeminal nuclear complex and is not a "labeled line" input through n.caudalis (Denny-Brown and Yanagisawa, 1973). Evidence from behavioral and electrophysiological studies was put forth by others as support for the spatial/temporal summation proposal. For example, Young et al (1981, 1982) reported that in monkeys (Young et al. 1981) and humans (Young, 1982) there were undiminished responses to noxious stimuli in paramedian cutaneous, mucosal and dental structures after removing primary afferent input from n.caudalis by a lesion of the descending tract of the fifth nerve. These authors used this data to postulate that the entire spinal sensory nucleus

integrated nociceptive information from orofacial structures (Young et al. 1981; Young, 1982). These results also suggest a more parsimonious explanation. It is possible that nociceptive information from paramedian, mucosal and dental structures is transmitted along different pathways than the nociception treated by the clinical interventions described above.

However, there are also challenges to the idea that the all subnuclei of the spinal nucleus are equally involved in nociception through spatial and temporal summation. Along these lines, an early report by Wall and Taub(1962) suggested that perioral and mucosal nociceptive information seemed to be processed by n.oralis independently of n.caudalis (Wall and Taub, 1962). Dallel and colleagues investigated the role of the n.oralis in processing perioral and mucosal nociceptive information (Dallel et al. 1988; Dallel et al. 1987; Raboisson et al. 1991; Dallel et al. 1990; Raboisson et al. 1989; Dallel et al. 1989; Clavelou et al. 1989). Dallel et al (1990, 1991) found with extracellular recordings that neurons in n.oralis responded to noxious chemical, mechanical and electrical stimulation of the perioral areas (Dallel et al. 1990; Raboisson et al. 1991). They also reported that neurons in n.oralis respond to orofacial noxious mechanical stimuli. Furthermore, responses in the VPM region of the thalamus to noxious mechanical stimulation of perioral and mucosal areas persisted after a tractotomy at the level of the obex (Dallel et al. 1988; Raboisson et al. 1989). Based on these findings, they suggested that nociceptive information is relayed to higher centers independently of n.caudalis. Although these workers pointed to n.oralis as the relay site for nociceptive primary afferent input, their

experiments failed to demonstrate whether n.oralis was the primary input site for perioral nociceptive information or whether it was processing information relayed from n.interpolaris via intersubnuclear projections.

Efforts to clearly determine whether or not n.oralis was the major nociceptive relay site in the rostral spinal subnuclei led to further studies attempting to isolate the role of this nucleus. For example, Broton and Rosenfeld (1986) made sagittal cuts in the brain stem medial to rostral spinal sensory nuclei. The lesions interrupted the ascending tracts from the rostral spinal sensory nuclei of the BTNC. This group reported significantly reduced responses to noxious thermal stimuli delivered to perioral areas. In contrast, responses to innocuous stimuli were not significantly different from pre-cut responses. Cuts in the adjacent reticular formation were not as effective as cuts adjacent to the nucleus (Broton and Rosenfeld, 1986). Similarly, adjacent sagittal cuts made more caudally did not significantly affect perioral nociception (Hu et al. 1981). This is evidence in favor of the position that perioral nociceptive information can be transmitted from the spinal sensory nucleus to higher areas for processing by pathways that do not involve n.caudalis.

Further evidence has been presented by Luccarini et al (1995). Results from a pharmacological study suggested that n.oralis, rather than the adjacent reticular formation, is the primary nociceptive information processing site for perioral nociceptive information. In this study, microinjections of the analgesic opioid morphine restricted to n.oralis were effective in reducing nociceptive responses in rat, while microinjections in adjacent

reticular formation were ineffective in this regard (Luccarini et al. 1995). Microinjections in the adjacent reticular formation were controls for a general inhibition of responses, since the reticular formation has diffuse connections throughout the brain stem.

Taken together, the above evidence strongly suggests that n.oralis is an important site in perioral and mucosal nociception. It is important to show that pain from different regions of the face might be conveyed by distinct pathways to higher centers for processing. Should there be an identifiable pathway from perioral and mucosal areas that involve n.oralis, then it will be pertinent to examine motivational-affective and sensory-discriminatory components of nociception from the perioral and mucosal region along this pathway.

The reports described above provide suggestive but not defining evidence that n.oralis receives primary afferent input. Therefore, there is as yet no direct evidence for the hypothesis that n.oralis is a major input site for perioral nociceptive information to the rostral spinal sensory nucleus since responses recorded in n.oralis may have been relayed from n.interpolaris or other elements of the BTNC. The hypothesis that n.oralis receives primary afferent input can be tested by anatomical studies. Such studies are reviewed below. The goal of this report is to examine this possible pathway for the sensory-discriminatory component of nociception that involve n.oralis.

Although pharmacological manipulations of n.oralis and tractotomies of lateral and medial medullary tracts have demonstrated the involvement of the n.oralis in orofacial pain, they have not determined whether this nucleus receives nociceptive information from primary afferents or from intersubnuclear connections from n.interpolaris. To clarify this issue, investigators have performed anatomical studies to visualize endogenous substances that are characteristic of primary afferents and have been located in specific tissues. For example, several studies have examined the termination of primary afferents believed to be of nociceptive origin using immunohistochemistry for demonstrating calcitonin gene-related peptide (CGRP). CGRP has been shown to be a prominent peptide in Aδ and C fibers (Hökfelt et al. 1992; Woolf and Wiesenfeld-Hallin, 1986; Wiesenfeld-Hallin et al. 1991; Arvidsson et al. 1991) (reviewed in Willis and Coggeshall 1991). Kruger et al (1988) showed that CGRP immunoreactivity labeled 50% of the nociceptive primary afferents to the spinal trigeminal nucleus (Kruger et al. 1988). They reported that while there was little evidence of CGRP-IR fibers entering main sensory nucleus or n.interpolaris, there were densely labeled fascicles leaving the spinal tract of the fifth nerve to terminate in n.oralis and n.caudalis (Kruger et al. 1988). This data supports the idea that the neurons recorded in n.oralis by Dallel et al (see above) were responding to input from primary afferents.

In contrast to the CGRP data, other information suggests quite a different organization of nociceptive input to the spinal sensory nucleus from orofacial areas. In studying nociceptive information processing in the BTNC, workers have taken advantage of the

fact that cornea and tooth pulp are innervated solely or principally by small diameter nociceptive primary afferents (Hamba et al. 1992; Bishop, 1979; Beuerman and Tanelian, 1979; Rozsa and Beuerman, 1982). Several anatomical studies have demonstrated that nociceptive high threshold facial or tooth pulp afferents from the trigeminal nerve terminate in all levels of the BTNC (Jacquin et al. 1983; Jacquin et al. 1986; Jacquin et al. 1988; Arvidsson and Gobel, 1981). For example, Westrum (1981) and Gobel and Arvidsson (1981) using horseradish peroxidase (HRP) injected in tooth pulp and reported that label was distributed to all levels of the spinal sensory nucleus (Westrum et al. 1981; Arvidsson and Gobel, 1981). This report suggests that all subnuclei of the spinal sensory nucleus should respond to noxious stimulation of the tooth pulp.

Marfurt and Del Toro (1987) applied HRP to the cornea and reported terminal field labeling restricted to the n.interpolaris/n.caudalis transition zone and the n.caudalis/cervical dorsal horn transition zone and not elsewhere in the spinal sensory nucleus (Marfurt and Del Toro, 1987). LaVail et al (1990) reported that after inoculation of the cornea with herpes simplex virus (type 1) (HSV-1), immunoreactivity to HSV-1 extended continuously from the level of n.interpolaris to cervical dorsal horn (LaVail et al. 1990).

One explanation for the differences in distribution of nociceptive information between the anatomical data and that from the electrophysiological and behavioral data described above could simply be the methodological differences in these experiments. Alternately,

the spinal sensory nucleus may contain a complex somatotopic map of nociceptive information. In this map, the nociceptive information from the peripheral areas of the face, the cornea and the tooth pulp might be represented predominantly in n.interpolaris and n.caudalis, while nociceptive information from the perioral and mucosal areas might be represented predominantly in n.oralis. This proposal would suggest that projections to higher areas might be small and restricted. The above proposal is different from that of Young (Young et al. 1981) as well as that of Denny-Brown and Yanagisawa (Denny-Brown and Yanagisawa, 1973) in that, the spinal sensory nucleus is not treated as a homogeneous area with a more or less even distribution of nociceptive information.

In addition to any direct input from nociceptive primary afferents, electrophysiological and anatomical evidence of intersubnuclear connections show that there is sharing of information among the subnuclei of the spinal nucleus. Hu et al (1981) showed electrophysiologically that n.caudalis neurons project to n.oralis (Hu et al. 1981). Lovick and Wolstencroft (1983) iontophoresed HRP into n.oralis and n.caudalis to investigate afferent connections from nucleus raphe magnus (NRM) by retrogradely labeling the cell bodies of projection neurons. In addition to finding cell bodies in NRM, this group also reported labeled cell bodies in n.caudalis from HRP iontophoresed into n.oralis and labeled cell bodies in n.oralis from HRP iontophoresed into n.caudalis (Lovick and Wolstencroft, 1983). Recently, connections among the three subnuclei of the spinal sensory nucleus have been investigated in cat by Ikeda et al (1984) and in rat by Jacquin et al (1990). Both groups reported extensive projections between adjacent subnuclei with a greater

predominance of projections from n.caudalis to the rostral subnuclei (Ikeda et al. 1984; Jacquin et al. 1990). Modulatory interactions of spinal sensory subnuclei responses have been recently demonstrated by Ujihara et al (1987). Specifically, they demonstrated that a conditioning stimulus applied to n.caudalis produced a naloxone-reversible inhibition of tooth pulp induced responses in n.oralis (Ujihara et al. 1987). Also, Hallas and Jacquin (1990) made a lesion of the spinal sensory nucleus that interrupted the intersubnuclear connections between n.caudalis and n.interpolaris which produced dramatic changes in the response properties and receptive fields of n.interpolaris neurons (Hallas and Jacquin, 1990). The available evidence suggests that nociceptive information transmitted to higher centers from the spinal sensory nucleus is sculpted by complex facilitatory and inhibitory interconnections among its subnuclei.

The parabrachial nucleus has been shown to have an extensive array of functions and connections subserving input from different sources. It has long been known that parabrachial neurons respond to a variety of inputs including, gustatory input and projects to thalamus, hypothalamus and amygdala (Bernard et al. 1989; Bernard and Besson, 1990; Bernard et al. 1991; Bernard et al. 1994; Alden et al. 1994; Ma et al. 1989). Its connections to the solitary nucleus and physiological responses to taste stimuli have also been shown (van Buskirk and Smith, 1981; Herbert and Saper, 1990a). The parabrachial area is also implicated in visual processing, in that, it has been shown to have projections to the lateral geniculate nucleus (Bickford et al. 1993). It has modulatory effects on respiration. Microinjections of glutamate in subnuclei of the parabrachial nucleus have

elicited dramatic respiratory responses in anesthetized rats (Chamberlin and Saper, 1994). A role in nociception has been demonstrated by showing that electrical and chemical stimulation of the parabrachial area strongly inhibited the responses of n.caudalis neurons to noxious mechanical stimuli (Chiang et al. 1994). Strong support for its role in nociception has also been shown by the anatomical connections of various parabrachial subnuclei and Kölliker-Fuse nucleus with lamina I of n.caudalis and the dorsal horn of the spinal cord (Cechetto et al. 1985; Blomqvist et al. 1989; Kitamura et al. 1989; Slugg and Light, 1994; Hylden et al. 1989; Kitamura et al. 1993; Light et al. 1993; Feil and Herbert, 1995). Parabrachial area neurons also respond to visceral stimulation from intraperitoneal injections of bradykinin and from colorectal dissension (Bernard et al. 1994). Dorsal horn neurons, which stain positive for the nociceptive neuropeptide substance P and n.caudalis neurons which stain for formalin induced Fos-like antigens, have been shown to project to the parabrachial area (Ding et al. 1995; Wang et al. 1994).

The major function of the parabrachial area seems to be a contribution to autonomic functions. It may serve to add autonomic components to the various kinds of peripheral stimuli. It has been shown to project to areas known to regulate autonomic function in the autonomic and limbic forebrain (Krukoff et al. 1993; Alden et al. 1994; Herbert et al. 1990b). Since n.oralis receives input from behaviorally relevant nociceptive orofacial regions (Young et al. 1981; Young, 1982; Raboisson et al. 1991), but does not have significant direct projections to the thalamus (Fukushima and Kerr, 1979), a possible

pathway from n.oralis to the thalamus via the parabrachial area is the topic of this exploration.

Summary

All of the above data indicate that in addition to n.caudalis the rostral nuclei, and particularly n oralis, are involved in nociceptive processes. Whether these rostral nuclei, and in particular n.oralis also play a role in the transfer of information to the thalamus is unclear. Although Sessle's (Sessle, 1987; Dallel et al. 1990) studies suggest such a thalamic relay, the preponderance of anatomic data shows no direct connection to thalamus. It is possible that information is relayed from n.oralis to thalamus either via intersubnuclear connections or via some other brain stem site. One such non-BTNC site that has been proposed is the parabrachial nucleus (Feil and Herbert, 1995). The parabrachial nucleus which surrounds the superior cerebellar peduncle consists of 10 subnuclei and the Kölliker-Fuse nucleus. The parabrachial nucleus extends caudal to rostral from the level of the main sensory nucleus to the n.cuneformis (see Fulwiler and Saper 1984 for a description of nomenclature).

The present study examines possible oralis-parabrachial-thalamus pathway for the sensory discriminatory aspect of nociceptive information from perioral and mucosal regions and structures. Before addressing the specific pathway in this study, it would be helpful to review the function of the parabrachial nuclear complex to be able to put into context any

connections that might exist from the parabrachial nuclear complex to n.oralis or to the somatosensory nuclei of the thalamus.

METHODS

The animals used in this study were adult, male Sprague Dawley rats and all surgeries were performed with the animals under deep barbiturate anesthesia and according to guidelines established by the UCSF animal care committee and the "NIH Guide for Care and Use of Laboratory Animals" (National Institutes of Health Publications No. 80-23, revised '78).

The animals were placed in a stereotaxic frame and microinjections of tracer were made in n.oralis (to examine its afferent connections and efferent projections) and into the VPM nucleus of the thalamus (to examine sources of afferents to VPM). The skin over the skull was reflected and a small burr-hole drilled in the skull above the injection site. The target coordinates were initially taken from the atlas of Paxinos and Watson (Paxinos and Watson, 1986) then adjusted according to the results from previous experiments. Small injections of tracer (0.005-0.1μl) were made using a glass micropipette (<50 μm tip diameter) attached to a picospritzer (a pressure injection device). The micropipettes have been previously calibrated such that know amounts of tracer could be reliably injected. Nine rats received a single injection of biotinlyated dextran amine (BD, 10% in phosphate buffered saline, PBS) into n.oralis and three rats received an injection of BD into n.oralis

and an injection of wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP, 4% in phosphate buffered saline, PBS) in VPM.

Three to seven days after injections, the animals were perfused under deep barbiturate anesthesia with 50 ml PBS (pH 7.4, 37°C) followed by 600 ml of 4% paraformaldehyde, 0.3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for BD-injections or 2% glutaraldehyde, 1% paraformaldehyde in the same buffer for WGA-HRP injections. The brains were removed and left in PBS containing 30% sucrose for two days, then coronal sections were cut on a freezing microtome at 50μm. Sections from BD injected animals were pretreated with 0.1% Triton X100 (Sigma) for one hour and then processed using the Elite ABC kit (Vector) with DAB as the chromogen. Sections from the combined BD and WGA-HRP experiments were divided and one set processed for visualization of the BD (as above) and the other set processed according to the method of Mesulam (1978) using tetramethylbenzidine as the chromagen (Mesulam, 1978). The sections were dehydrated through a graded series of alcohols appropriate to the method used, mounted on slides and cover-slipped with DePeX. The sections were examined under bright field and dark field microscopy and camera lucida drawings were made of selected sections.

RESULTS

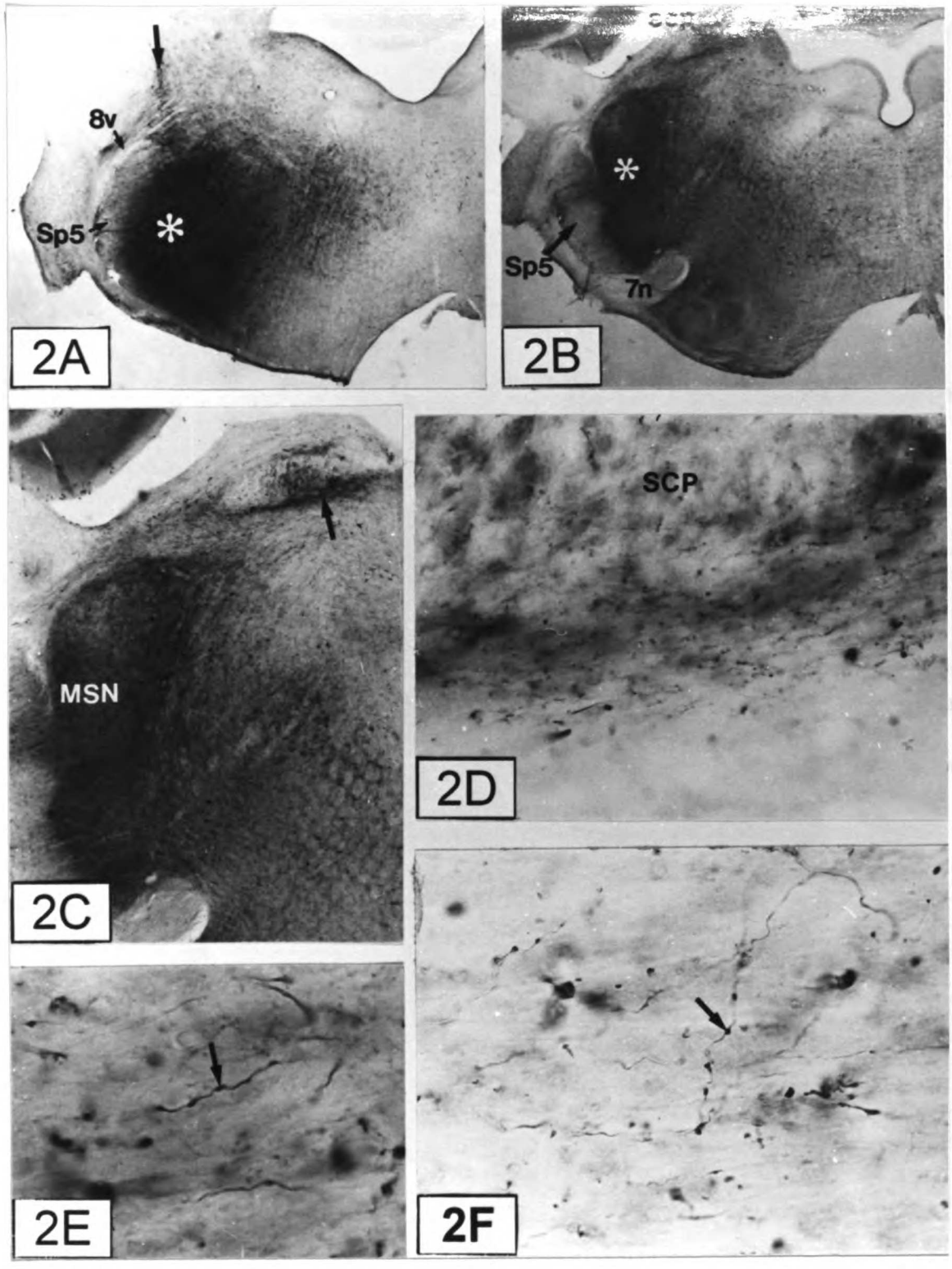
Injections of biotin dextran into n.oralis.

The first series of experiments involved injecting BD into n.oralis to determine whether there are projections to the parabrachial nuclei (PBN). The location of the injection sites were determined using the following criteria. The caudal limit of n.oralis was marked by the appearance of the large neurons of the facial motor nuclei and the concurrent disappearance of the inferior olivary nuclei. An additional criterion was the appearance of the tract of the 8th nerve dorsal to the spinal trigeminal tract. The rostral limit of n.oralis was marked by the tract of the seventh cranial nerve. The lateral border was demarcated by the spinal trigeminal tract and the medial margin by the change from the cell-dense appearance of n.oralis to the more reticulated appearance of the reticular formation. The center of the injection was determined both by the region of heaviest label and by the presence of an electrode tract. Although most labeling with BD probably results from the central core of the injection site, the limit of the injection was taken to be where the density of label dropped off sharply to background level and no densely labeled cells were visible.

Of a total of nine injections aimed at n.oralis. Injections were confined to n.oralis in six animals, two had considerable spread to main sensory nucleus and one was located in the main sensory nucleus. The following results are based on the six injections confined to n.oralis. Figure 2A shows an example of an injection in n.oralis. The dorsal, lateral and ventral spread of the label is confined by the spinal trigeminal tract. There is a slight

medial diffusion of label into the adjacent reticular formation but it is unlikely that label results from this region. The rostral and caudal extent of the injection was within our n.oralis.

The injections that were confined to n.oralis resulted in heavily labeled fibers in the ipsilateral PBN as well a small projection to the contralateral PBN (see below). Figure 2B shows a section through the caudal part of main sensory nucleus rostral to the injection site illustrated in Figure 2A. The main sensory nucleus appears darker than the surrounding tissue in part due to fine diameter labeled fibers and varicosities and a few scattered cell bodies. Dorsal to the main sensory nucleus the amount of label decreases significantly between the dorsal part of main sensory nucleus (Figs. 2B,C) and the area surrounding the brachium (superior cerebellar peduncle). There does not appear to be any well defined tract from n.oralis to the PBN but the many labeled fibers course dorsally and medial to the main sensory nucleus and to a lesser extent laterally adjacent to the spinal trigeminal tract before sweeping laterally to the PBN (Figs. 2C,D). A plexus of fibers was present surrounding the brachium and was most dense in the ventral medial (medial parabrachial nucleus) and dorsal lateral (lateral parabrachial nucleus) areas of the PBN. The labeled fibers in the PBN varied in diameter but many were fine, branched profusely and were studded with multiple varicosities (Figs. 2D,E,F). These fibers and varicosities had an appearance typical of structures that have been found to be preterminal axons and synaptic boutons when examined by electron microscopy. A similar pattern of labeled


8v
Sp5
*
2A
*
Sp5
7n
2B
MSN
2C
SCP
2D
2E
2F

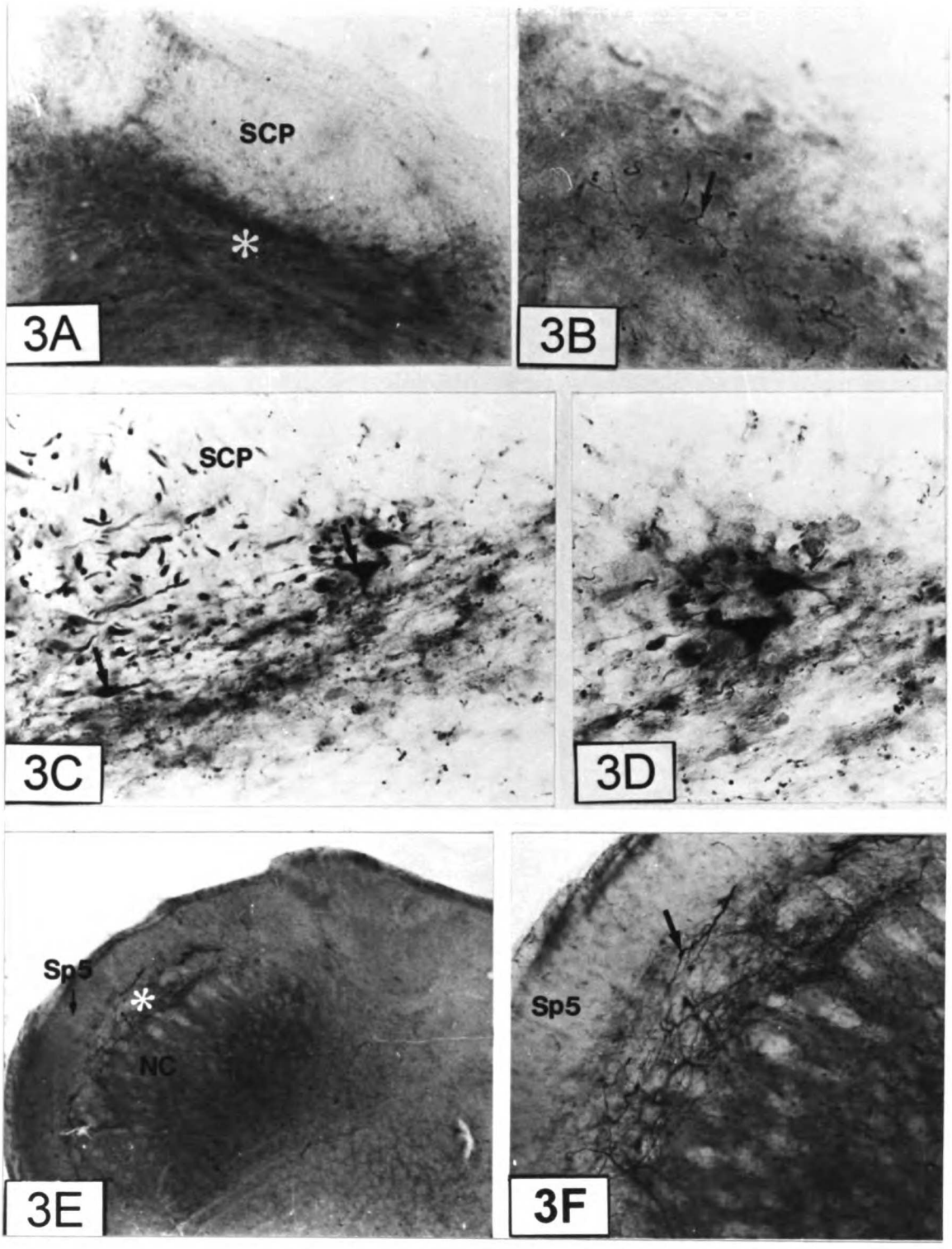
fibers was seen surrounding the contralateral PBN but the fibers were far less numerous than those seen on the ipsilateral side (Figs. 3A,B) Within the brachium on the side ipsilateral to the injection, there were many large diameter, heavily labeled fibers.

In addition to the labeled fibers there were a small number of labeled cells present in the medial nuclei of the ipsilateral PBN and embedded within the plexus of labeled fibers (Figs. 3C,D). Occasionally one or two cells of similar appearance and location were seen in the contralateral PBN.

As there has been discussion in the literature concerning the intersubnuclear connections of the spinal sensory nuclei (see Introduction) we briefly examined the other trigeminal nuclei for labeled elements. Following the n.oralis injections, few labeled cells were found in the main sensory nucleus or n.interpolaris, but a large number or labeled neurons were present in ipsilateral n.caudalis. As shown in Figures 3E, F, the majority of the labeled cell bodies in n.caudalis were located in laminae I and II, with a few scattered labeled neurons in the deeper layers. No labeled neurons were seen in the contralateral n.caudalis.

Injections of WGA-HRP into the thalamus.

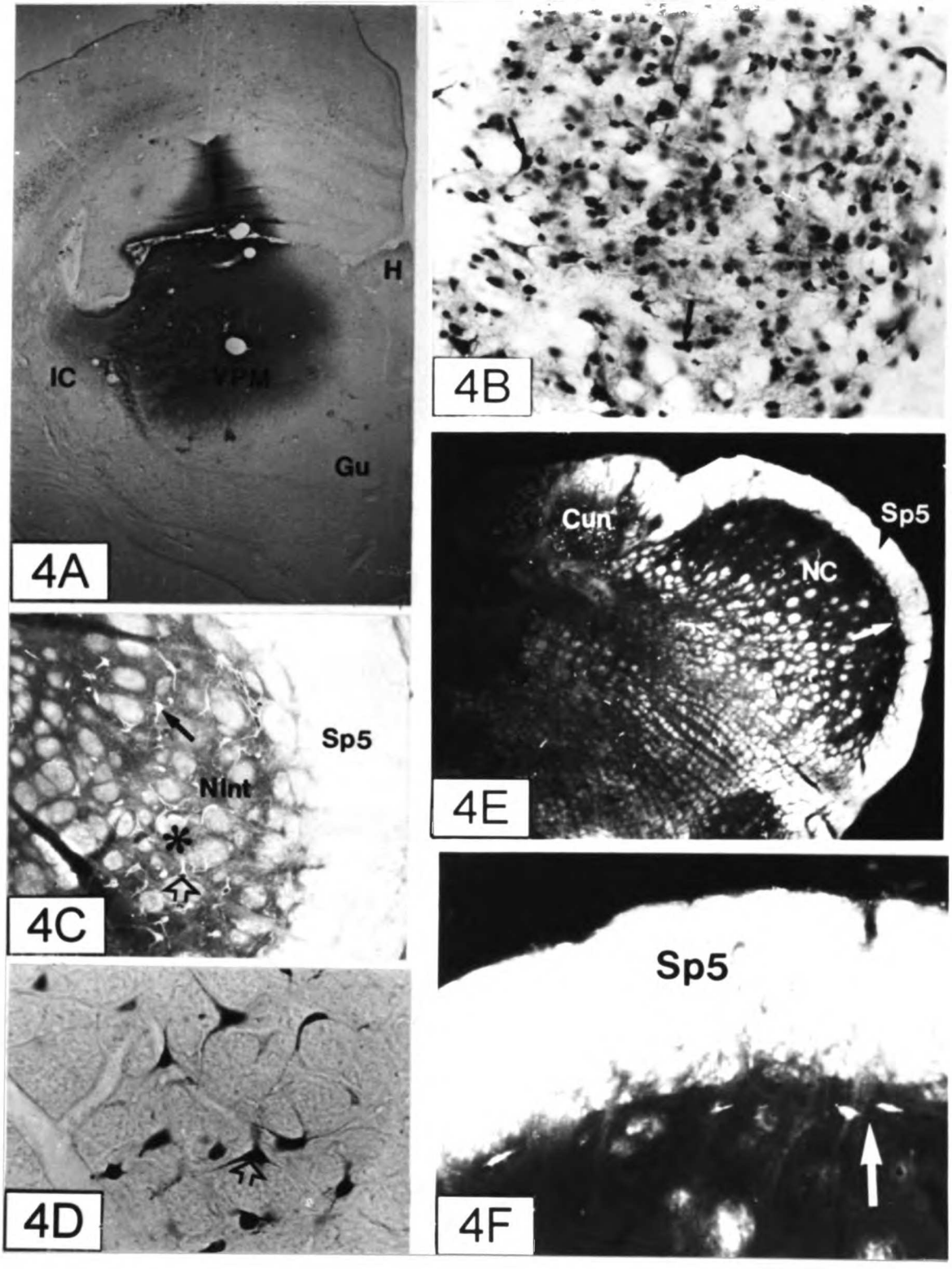
To determine whether or not the region of the PBN receiving n.oralis projections also contained neurons that projected to the thalamus, injections of WGA-HRP were made in the thalamus and the PBN examined for retrogradely labeled neurons. Thalamic injections were confined to the side contralateral to the injection site in n.oralis. The volume of


SCP
3A
3B
SCP
3C
3D
Sp5
NC
3E
Sp5
3F

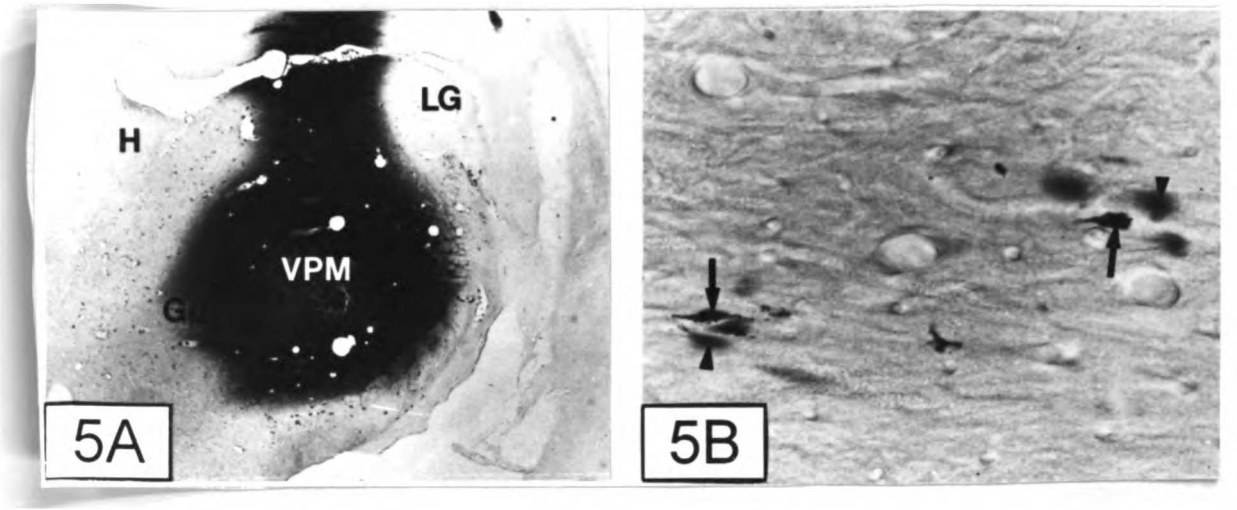
tracer injected into VPM was made large enough to fill most of the ventrobasal thalamus in order to ensure that any parabrachial-thalamic projections would be included in the spread of tracer.

When the thalamic injections were large enough to include all the VPM but did not spread to the most ventral parts of the thalamus (Fig. 4A) no labeled neurons were found in the PBN. After such injections many labeled neurons were found in the main sensory nucleus (Fig. 4B) and n.interpolaris (Figs. 4C,D). A small number of labeled neurons were present in n.oralis but we found dramatically fewer than the number in the main sensory nucleus or n.interpolaris, in that only one or two labeled cells were seen in single sections through n.oralis compared to the number in main sensory nucleus and n.interpolaris. Labeled neurons were also present in the marginal and deep layers of n.caudalis (Figs. 4E,F) but as in the case of n.oralis the number was far less than seen in the main sensory nucleus or n.interpolaris. The presence of labeled neurons in the marginal layers of n.caudalis suggests that the absence of labeled neurons in the PBN was unlikely to be due to methodological artifact such as too short survival time or too small injections. Labeled neurons were also seen in the spinal cord and dorsal column nuclei showing that the injected tracer was in sufficient concentration outside the borders of VPM to result in retrograde transport of tracer.

In one experiment the WGA-HRP spread medially to include some medial nuclei and also into the more ventral parts of the thalamus including the gustatory nucleus and zona


H
IC
VPM
Gu
4A
4B
Cun
Sp5
NC
4E
Sp5
Nint
4C
4D
Sp5
4F

incerta (Fig. 5A). Following this injection several lightly labeled neurons were found in the medial PBN (Fig. 5B) and a lesser number in the dorsal lateral PBN. These were usually small, fusiform cells which often appeared to occur in small groups, which were not systematically studied. In addition to the labeled neurons in PBN, labeled neurons were also present in the nuclei of the brainstem trigeminal complex and dorsal column nuclei as described above.


H
LG
VPM
G
5A
5B

DISCUSSION

Projections from n.oralis to the parabrachial nuclei. A major finding of this study is that there exists a projection from n.oralis to the ipsilateral parabrachial area. This projection appears to include most of the parabrachial nuclei but the amount of anterograde label is far heavier in the caudal portion of the medial parabrachial nucleus. High magnification examination showed that the fibers within the PBN had many varicosities indicative of synaptic boutons and suggests that the fibers seen in the PBN make synaptic contact in the nucleus and are not just fibers of passage. EM analysis will be necessary to verify that these structures do in fact make synaptic contact with neurons in the PBN. In addition to the ipsilateral projection there is also a projection from n.oralis to the contralateral PBN, which is much smaller than the ipsilateral one, the majority of label being found in the caudal portion of the medial nucleus with little label present in the other parabrachial nuclei. In addition to the labeled fibers, a number of labeled cell bodies were also present in the ipsilateral medial parabrachial nucleus. The presence and significance of this PBN projection to the spinal trigeminal nuclei has been previously examined (Feil and Herbert, 1995) and will be discussed below.

This result, outlined above, has recently been confirmed by Feil and Herbert (1995) who examined projections between the spinal trigeminal sensory nuclei and the PBN using anterograde and retrograde tracing methods. These authors report projections from all the spinal trigeminal sensory nuclei to both ipsi- and contralateral PBN with the more dense projections to the ipsilateral side. The major difference between our results and those of

Feil and Herbert (1995) is in the location of label in the PBN. Feil and Herbert (1995) describe the heaviest projection to the Kölliker-Fuse nucleus and a much smaller projection to the medial PBN, where we find our densest projection. There are several possible explanations for the differences in our results. In their paper, Feil and Herbert (1995) only illustrate the results of a representative injection into n.caudalis and state that the projections from the other spinal trigeminal nuclei were similar in location but varied in density. Therefore, it could be that the projections from n.oralis were more dense than illustrated in their paper. Another possible explanation is difference in methodology. We used injections of BD that labeled most of n.oralis while Feil and Herbert (1995) used smaller injections of PHA-L.

Projections from the parabrachial nuclei to the thalamus. The second component of our study involved the injection of WGA-HRP into the thalamus. After thalamic injections which avoided the midline nuclei and gustatory nucleus no labeled neurons were seen in any region of the PBN. This negative finding is unlikely to be due to failure of the method, since labeled neurons were present as far caudal as the cervical spinal cord. In addition, when the thalamic injections involved the more medial thalamic nuclei (central median and central lateral) and the gustatory nucleus, labeled neurons were present in the parabrachial nucleus. The labeled neurons in the PBN following such thalamic injections were principally in the medial nucleus and within the site that received the heaviest terminal label following the n.oralis injections.

The role of n.oralis in somatosensory information processing. However, the lack of projections from the PBN to the VPM nucleus of the thalamus, which is considered the principal thalamic relay nucleus for discriminative somatosensory information, makes it unlikely that the PBN functions as a relay for discriminative sensory information from n.oralis. The overlap in the PBN of projections from n.oralis and neurons that project to the gustatory and intralaminar thalamic nuclei, does suggest a role for an oralis-PBN-thalamic path in the visceral/affective aspects of sensation. Although the PBN has been associated with affective-motivational components of sensation previously (Hylden et al. 1989; Bernard et al. 1994; Kitamura et al. 1993; Light et al. 1993; Bernard and Besson, 1990; Slugg and Light, 1994) there have been few studies examining sources of projections to PBN from the trigeminal spinal nuclei. Our results and those of others (Feil and Herbert, 1995) show that n.oralis along with the other spinal trigeminal nuclei may at least involved be in the affective-motivational components of somatic sensation through connections with the PBN.

The results just described could in part explain the results of experiments reviewed in the introduction of this report (Young et al. 1981; Denny-Brown and Yanagisawa, 1973; Dallel et al. 1990; Raboisson et al. 1991; Broton and Rosenfeld, 1986; Luccarini et al. 1995; Hu et al. 1981) suggesting a role for n.oralis in nociceptive information processing. The continued nociceptive responses in animals which had the descending spinal trigeminal tract cut rostral to n.caudalis could be mediated by an oralis-PBN-thalamic pathway (Hu et al. 1981; Young et al. 1981). However, this last possibility should be distinguished from the suggestion of Dallel et al (1989) that responses recorded in the VPM following a

lesion of the descending spinal trigeminal tract could be mediated by an oralis-thalamic pathway (Raboisson et al. 1989). No projections from the PBN to the VPM were found in the present experiments (also see ref. (Fulwiler and Saper, 1984)) nor was there a significant projection from n.oralis to VPM. Thus, it is unlikely that the thalamic responses recorded by Dallel and colleagues (Raboisson et al. 1989) could be relayed by an indirect path from n.oralis through the PBN or via a direct n.oralis-thalamic path.

With regard to the latter point, the projection from n.oralis to the thalamus, some comments can be made based on the results of our experiments. A review of the literature leads to the conclusion that there is no (Fukushima and Kerr, 1979; Patrick and Robinson, 1987), or only a minor (Kemplay and Webster, 1989; Jacquin et al. 1990) projection from n.oralis to the thalamus. However, some authors have discussed such a pathway to explain some results such as the thalamic responses following tractotomy (Raboisson et al. 1989; Dallel et al. 1988). Our data showed that even after large injections of WGA-HRP into the VPM nucleus of the thalamus there were few labeled neurons within n.oralis. In contrast, large numbers of labeled neurons were present in n.interpolaris and main sensory nucleus. Based solely on the size of the n.oralis-thalamic projection it is hard to imagine that n.oralis plays any significant role in direct trigemino-thalamic discriminative information pathways in the rat.

By the same token the number of neurons found in n.caudalis, particularly in the marginal layers, leads to questions regarding the role of this nucleus in trigemino-thalamic pathways. The experiments in which primary afferent input to n.caudalis is removed

(Raboisson et al. 1989; Young et al. 1981) yet nociceptive responses are retained, and the human studies where pain returns after tractotomy (reviewed in (Denny-Brown and Yanagisawa, 1973)), also leads to questions concerning the role of n.caudalis in nociception. Although outside the scope of this study, it could be that the trigeminal nuclei serve an integrative function and that it is the main sensory nucleus and n.interpolaris are the principal sites for relay of discriminative information of both nociceptive and non-nociceptive origin. For example, Hayashi et al (1984) demonstrated nociceptive relay neurons in n.interpolaris that were antidromically activated from VPM (Hayashi et al. 1984). Taken together with anatomical studies that demonstrate intersubnuclear projections (particularly from caudal to rostral), at least anatomically, the basis for this integration becomes apparent (Panneton and Burton, 1982; Hu and Sessle, 1979; Ikeda et al. 1984; Jacquin et al. 1990). It should be emphasized that this proposal is distinguishable from the Denny-Brown/Yanagisawa (Denny-Brown and Yanagisawa, 1973) proposal that was seconded by Young (Young et al. 1981). These authors suggest that nociception is a quantitative difference in stimuli ranging from the innocuous to the noxious and distributed somewhat homogeneously throughout the spinal sensory nucleus. We prefer a "labeled line" input with modal segregation and information sharing among the spinal sensory subnuclei.

One further finding not yet discussed concerns the occurrence of labeled neurons in the PBN following BD injections into n.oralis. Previous electrophysiological studies by Chiang et al. (1994) have shown that stimulation of the parabrachial area results in inhibition the responses of nociceptive neurons in n.caudalis to noxious stimulation of the

face (Chiang et al. 1994). The labeled neurons found in our study could represent PBN neurons that project to n.caudalis through n.oralis (the route taken by the PBN-n.caudalis fibers is not known) and have been labeled by uptake of tracer by the fibers of passage. Alternately, there could be a parabrachial projection to n.oralis similar to that found for n.caudalis. This last suggestion is encouraged by reports that demonstrated BD is not picked up by fibers of passage (Brandt and Apkarian, 1992; Rajakumar et al. 1993).

This study has concentrated on an oralis-PBN-thalamus relay, but it should be pointed out that there are other sites and connections that are also involved in nociceptive processing and include the PBN. The parabrachial area has direct projections to areas outside of the thalamus like the stria terminalis, forebrain, hypothalamus and amygdala (Bernard et al. 1989; Bernard and Besson, 1990; Bernard et al. 1991; Bernard et al. 1994; Alden et al. 1994; Ma et al. 1989). Several investigators have suggested that these projections, most notably the amygdaloid projection, subserve nociceptive processing that may affect the state of arousal of the animal (Bernard et al. 1989; Bernard et al. 1994; Bernard and Besson, 1990). Thus, many of the other pathways could be involved in the findings discussed and have to be considered when discussing nociceptive processing related to the PBN.

Finally, some comment should be made on one aspect of trigeminal brainstem structure/function that is of general interest, namely, the homology (or lack thereof) between the various trigeminal complex sub-nuclei and spinal cord nuclei. The most obvious spinal cord equivalent of n.oralis is Clarke's column, for in addition to the sensory

components discussed in this paper, the largest projection of n.oralis is to the cerebellum. This n.oralis-cerebellar projection is concerned with the transmission of proprioceptive information from structures of the head and face. The major pathway originating in Clarke's column, the dorsal spinocerebellar tract (DSCT) appears to have no projections to PBN. However, there are fibers from the spinal cord that travel in the ventral spinocerebellar (VSCT) tract and give rise to collaterals that project to the PBN (Kitamura et al. 1989). The neurons of origin of VSCT fibers giving rise to these collaterals appear to include laminae I, V and VII neurons. Many neurons in these laminae (esp. I and V) are specifically associated with the transmission of nociceptive information and thus may play a role in a spinal projection to PBN similar to the oralis-PBN projection. In contrast to the n.oralis-PBN interaction there are no reported projections from the PBN to Clarke's column but this has not been systematically examined. Thus although n.oralis is probably not homologous to Clarke's column alone, it is tempting to speculate that that projections from n.oralis might overlap the functions served by both the DSCT and VSCT.

SUMMARY

The present results show that n.oralis has significant projections to several nuclei of the PBN and the same area of the PBN projects to the midline and gustatory nuclei of the thalamus. This data supports the idea that n.oralis may be involved in the relay of nociceptive information involved in affective aspects of sensation through the PBN. This

study also confirms that the projection from n.oralis to the VPM of the thalamus is minimal and that the projection from n.caudalis to VPM is less than the impression often obtained from the literature. These findings support the view that the individual spinal trigeminal nuclei might be better regarded as a complex for integration of information of trigeminal nerve origin rather than as a several separate nuclei each relying a single component of sensory information.

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FIGURE LEGENDS

All micrographs are brightfield unless otherwise noted. Where possible the terminology and abbreviations used are from the atlas of Paxinos and Watson (1986).

Figure 1. Transmission of sensory information from orofacial and mucosal regions to higher centers. Primary afferents from the trigeminal ganglion synapse on second-order neurons in sensory subnuclei of the brainstem trigeminal nuclear complex. Relay neurons in the sensory subnuclei project to the various thalamic nuclei. The dashed line indicate the pathway investigated in this study that looks for neurons from n.oralis conveying information to thalamic nuclei via indirect projections through parabrachial nuclei.

Figures 2and 3 are all taken from the same experiment in which a single injection of biotin dextran was injected into n.oralis.

Figure 2A. Low magnification micrograph showing an injection of biotin dextran into n.oralis. The most dense region of label is centered in n.oralis (asterisk) and there is only minimal spread medially into the reticular formation. Sp5, spinal trigeminal tract; 8v vestibular root of the 8th nerve; 7n, 7th nerve; SCP superior cerebellar peduncle; arrow, electrode tract.

Figures 2B, C. Low (B) and medium (C) power micrographs of the brainstem at the level of the MSN. The above background staining of the MSN (asterisk) is mainly due to labeled fibers (see text). The arrow in 2C indicates the location of micrographs D-F.

Figure 2D. Medium power micrograph of the heavily labeled region just ventral to the brachium indicated by the arrow in 2C. SCP superior cerebellar peduncle.

Figures 2E, F. High magnification micrographs of labeled fibers and varicosities (arrows) taken from the region indicated by the arrow in 2C.

Figures 3A, B. Medium (A) and high power (B) micrographs of the PBN contralateral to that shown in Figure 2C. Although the region ventral to the superior cerebellar peduncle (SCP) is labeled above background the number of fibers is far less than seen on the side ipsilateral to the injection. The arrow in 3B indicates a labeled fiber with varicosities and is taken from the region marked with the asterisk in Figure 3A.

Figures 3C, D. Medium (C) and high power (D) micrographs of the region of the PBN ipsilateral to the injection site indicated by the arrow in Figure 2C. The region ventral to the superior cerebellar peduncle (SCP) contains several labeled cell bodies indicated by the arrows. The two neurons on the right of the micrograph are shown at a higher magnification in 3D.

Figures 3E, F. Low (E) and medium (F) magnification micrographs of the brainstem at the level of nucleus caudalis (NC). A plexus of fibers and neurons can be seen in the outer laminae of nucleus caudalis. 3F shows some of the neurons (arrows) at a higher magnification taken from the area indicated by the asterisk in 3E. Sp5, spinal trigeminal tract.

Figures 4 and 5 show the results of two injections of WGA-HRP into the thalamus.

Figure 4A. Low power micrograph showing a large injection of WGA-HRP which includes the majority of the VPM. IC, internal capsule; H, Habenula; Gu, gustatory nucleus.

Figure 4B. Medium power micrograph from the center of MSN contralateral to the thalamic injection site. Note the large number of densely labeled neurons (arrows)

Figure 4C, D. Darkfield (4C) and (4D) brightfield micrographs from n.interpolaris (Nint) contralateral to the thalamic injection site. Although the number of labeled neurons (e.g.

solid arrow) is not as great as that found in the MSN (see 4B) they are still far more numerous than in n.caudalis (see 4E, F) or n.oralis (see text). The open arrows in 4C and 4D indicate the same neuron.

Figures 4E, F. Low and high magnification dark field micrographs of n.caudalis contralateral to the thalamic injection site. Within n.caudalis there are a small number of labeled cells scattered throughout the superficial and deep laminae. The white arrow in 4E indicates the location of the two labeled marginal layer neurons indicated by the arrow in 4F. Note that Figure 4F is rotated 90 degrees counterclockwise from figure 4E. Even at low magnification a large number of labeled neurons can also be seen in the cuneate nucleus (Cun). NC, nucleus caudalis; Sp5, spinal trigeminal tract.

Figure 5A. Low magnification micrograph showing a large injection of WGA-HRP into the thalamus slightly more caudal to that shown in Figure 4A. This injection includes the gustatory nucleus (Gu). H, Habenula, LG, dorsal lateral geniculate nucleus.

Figure 5B. High magnification of the ventral PBN from a region equivalent to that arrowed in Figure 2C. Note that in addition to the WGA-HRP labeled neurons that are in focus (arrow) there are other labeled neurons in the same areas out of the plane of focus (arrowheads).