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Primary afferent projections in a diver, the muskrat

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PRIMARY AFFERENT PROJECTIONS IN A DIVER, THE MUSKRAT

by

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PRIMARY AFFERENT PROJECTIONS IN A DIVER, THE MUSKRAT

A THESIS

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By
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ABSTRACT

In a preliminary search for primary afferent connections involved in the diving response, cutaneous afferents from the nose were traced in muskrats and compared with those in rats, and with projections from the soft palate, posterior pharynx and larynx. Horseradish peroxidase (HRP) was injected into the skin or mucosa, under anesthesia. After 48 h survival, the deeply anesthetized animal was transcardially perfused and the brain was frozen and sectioned transversely in a cryostat. The sections were reacted for HRP according to standard techniques, using tetramethylbenzidine; alternate sections were Nissl stained. HRP-labeled structures were mapped using darkfield photomicrographs and camera lucida drawings.

Cutaneous afferents from the nose in the muskrat project densely to layers I-II of the ventral and dorsolateral parts of the caudal subnucleus of the spinal trigeminal nucleus (Sp5C) and sparsely to layers V-VI of Sp5C, sparsely to the ventromedial part of the interpolar subnucleus of the spinal trigeminal nucleus (Sp5I), moderately to the oral subnucleus of the spinal trigeminal nucleus (Sp5O) - particularly the dorsomedial part, possibly overlapping with the nucleus of the solitary tract, and with processes of labeled cells of the lateral facial nucleus extending into ventromedial Sp5O, - moderately to the principal trigeminal nucleus (Pr5); and to the paratrigeminal nucleus (Pa5). Projections in the rat were the same,

except that little or no labeling of layers V-VI of Sp5C, dorsomedial Sp5O, or Pa5 was present. Projections from the soft palate to layers I-II of rostral Sp5C, Sp5O, Sp5I, and Pr5 were similar to those from the nose in the muskrat. Heavy projections from the soft palate, and less dense projections from the posterior pharynx and larynx, to Pa5 also were found. Those regions receiving dense projections from the nose, overlapping projections from the various sites, and more highly developed projections from the nose in the muskrat than in the rat, are of particular interest for further investigation of the neural substrate underlying the diving response. The projections traced from the nose correspond particularly with nociceptive and thermoreceptive projections, which suggests that thermoafferent function may be involved in the elicitation of the diving response.

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INTRODUCTION

The circuitry of the central nervous system underlies its integrative function. Understanding central nervous functions requires precise localization of their morphological substrates, the pathways and connections involved. The classical discipline of neuroanatomy forms the basis for much of our knowledge in neuroscience. Recent advances in neuroanatomical tract-tracing technique have revolutionized the discipline by allowing the precise determination of axon trajectories, interspecific differences in circuits, and their ontogenetic development (Nauta and Ebbesson 1971; Heimer and RoBards 1981). In particular, techniques employing the axonal transport of tracers such as horseradish peroxidase (HRP), have become powerful research tools in defining the organization of functional systems in the brain. In this study, the HRP method was used to investigate the neural substrate of the diving response.

The diving response comprises a triad of reflex adjustments: peripheral and visceral vasoconstriction, slowing of heart rate (bradycardia), and cessation of breathing (apnea). Reapportionment of a reduced cardiac output in favor of the cerebral circulation conserves oxygen stores for the use of the brain and allows the diver to resist asphyxia and prolong breath-holding. Control of the diving response involves not only the integration of sensory inputs with effector pathways of the component reflexes, but also their precise regulation by a complex system of interactions. The diving response is

qualitatively similar to the responses of nondiving animals when confronted with asphyxia in a variety of situations, and appears to be a specialization of a general defense mechanism against asphyxia. Physiological studies of diving animals have helped to elucidate this basic mechanism. Since asphyxia is a complication of numerous pathological conditions, and since incidental stimulation of the face and upper airways similar to that involved in the elicitation of the diving response occurs in many situations, these comparative studies also have implications for clinical medicine. Knowledge of the neural substrate underlying the diving response may enhance our understanding of the central nervous mechanisms underlying general defenses against asphyxia and certain pathological conditions.

The diving response is triggered by immersion of the nose, and similar responses can be elicited from the soft palate, posterior pharynx and larynx, in muskrats (Drummond and Jones 1972, 1979; Drummond 1980). Therefore, the projections of sensory fibers from these areas to the brain include initial connections involved in the elicitation of the diving response and related upper respiratory tract reflexes. The HRP method was used in this study to describe projections to these areas in the muskrat, to test the hypothesis that unique or specialized projections may be present in the diving animal, by comparison with projections in the rat, and to define such differences, if any. This, and a similar study reported in a recent abstract (Panneton 1989), are the first neuroanatomical investigations concerning the diving response. Cutaneous afferents from the nose have not previously been traced in any species. The results of this study

provide a preliminary indication of primary afferent connections involved in the diving response, which may guide more detailed neuroanatomical and neurophysiological investigations. The comparative approach used in this study may also provide information relevant to the general question of how brains have evolved specializations for specific functions.

Further information on the diving response, its peripheral innervation and the known central terminations and origins of these pathways in terrestrial mammals, and the organization of the trigeminal brainstem sensory complex, to which cutaneous facial afferents project, are presented in the following chapter in order to provide a background for understanding the significance of the diving response and the relevance of the results of this study to its integration and control.

BACKGROUND

I. THE DIVING RESPONSE: HISTORICAL OVERVIEW

The most insistent requirement for life in higher animals is oxygen. Obstruction of ventilation of the organism, or of perfusion of the tissues, results in progressive hypoxia, hypercapnia and acidosis, and the eventual disruption of cellular processes. Defense against asphyxia, therefore, is a fundamental need. Resistance to asphyxia varies with species and age. Aquatic representatives of many divergent groups have become breath-holding specialists. These animals respond to immersion with a characteristic set of reflexes, the "diving response." Qualitatively similar, but quantitatively less pronounced responses are exhibited by terrestrial animals when confronted with the threat of asphyxia under various conditions. The diving response appears to be a specialization of a general mechanism of defense against asphyxia, the "master switch of life" (Scholander 1963). Study of the responses of diving animals has illuminated basic mechanisms for coping with asphyxia.

Robert Boyle (1670), of Boyle's gas law fame, was perhaps the first to experiment with diving animals. He found that a duck succumbed as quickly in a vacuum as did a hen, but was more resistant than the hen to submersion. Boyle subscribed to the current theory that waterfowl are endowed with "a peculiar structure of some vessels about the heart" which somehow enables them to survive without

respiring while underwater. Unusual vascular structures in a seal were described nearly two centuries later, by Burow (1838). He noted an unusually large and complex abdominal venous system and enlargement of the ascending aorta, and the presence of a muscular sphincter around the thoracic posterior vena cava. This sphincter contracts during diving, he suggested, restricting blood to the abdominal venous reservoir and solving "the problem of the circulation of blood in diving animals" by reducing venous return to the heart and pulmonary blood flow (Burow 1838; cited by Harrison and Kooyman 1968). Gratiolet (1860; cited by Bert 1870) described similar structures in a hippopotamus and suggested a similar function for the sphincter. He further speculated that this function combines with compression of the external carotid arteries, a faculty he also attributed to the diving hippopotamus, to prevent engorgement of the brain, which he considered the principal cause of death by asphyxia. Gratiolet's conclusion, which he claimed was "at first paradoxical, but necessary, and therefore certain," although erroneous, was the first attempt to address the problem of diving asphyxia. His statement, "the flame therefore becomes smaller to last longer in a limited atmosphere," contained a key to the solution.

Paul Bert (1870), rejecting Gratiolet's theory on the basis that it lacked experimental evidence, performed the first experiments to determine how diving animals withstand asphyxia. He found that the duck has a greater blood volume than the chicken and that, when this increment of blood was eliminated by bleeding a duck, its endurance

when submerged was reduced almost to that of a large rooster. This clearly demonstrated, he claimed, that the principal cause of the duck's resistance is its enhanced oxygen supply due to its greater blood volume. Bert also noted bradycardia on submersion, for which he is most often cited, but did not consider the significance of this finding.

Bert's conclusion was generally accepted for a quarter of a century, until another French physiologist, Charles Richet, presented the results of a remarkable series of studies (see Richet 1899). Richet pointed out that Bert's single experiment was insufficient basis for his conclusion and that the bled duck, despite its disadvantage, had still outlasted the chicken. Richet performed similar experiments and found that reducing blood volume does not greatly affect the duck's endurance. By calculating how long the oxygen stores would support oxygen consumption at the air-breathing rate, he further showed that the duck's increased blood volume does not nearly account for its increased resistance. Richet demonstrated that submersion bradycardia is due to vagal action, and that section of the vagi or atropine treatment abolishes the duck's increased resistance. He observed that this reflex depends on water contact, probably through trigeminal pathways from the beak and nares. Richet concluded that the duck's resistance to asphyxia is due to reduction of metabolism and conservation of oxygen stores during submersion. He attributed this to: apnea, signaled by water contact; retention of air in the lungs; and bradycardia, produced by water contact and perhaps, in part, by chemoreceptor input. At the same time in Denmark, Christian Bohr

(1897; cited by Scholander 1940) also repeated Bert's experiment, with puffins and guillemots, and made calculations similar to Richet's. He concluded, however, that an increase in anaerobic metabolism makes up the difference between oxygen stores and metabolic rate in air, during a dive.

Richet contributed greatly toward solving the puzzle of how divers resist asphyxia. But he could not explain how metabolic rate is reduced and oxygen conserved, other than by the reduced metabolism of the lungs and heart with apnea and bradycardia. The first clear formulation of this key element of the diving response appeared a decade later, in a note on the viscera of a walrus. Burne (1909) observed structures similar to those previously described by Burow and Gratiolet, and offered this explanation of their function:

> There seems little reason to doubt that the venous reservoir and the sphincter of the vena cava forms parts of one mechanism, the use of which is possibly to restrict the flow of venous blood to the heart and so to keep up the average purity of the blood when the animal is immersed. For it is clear that the more the aeration of the blood is confined to that necessary for the action of the central nervous system and the voluntary parts of the animal, the further the oxygen stored in the lungs will go in carrying on the absolutely necessary activities of the body and the longer the animal will be able to stay immersed....during immersion the action of the vegetative as opposed to the voluntary organs might without much disadvantage be held more or less in abeyance and their circulation be stopped or retarded for the time.

Burne's anatomical note apparently went unnoticed by physiologists studying diving animals for several decades. Huxley (1913a,b,c), Lombroso (1913), Orr and Watson (1913) and Paton (1913;

1927) described the control of apnea and bradycardia in diving birds, but were unable to add to Richet's explanation of how these adjustments protect the animal against asphyxia. The three elements of the diving response were evident in studies by Koppanyi and Dooley (1928; 1929) in which submersion or postural apnea, bradycardia and increased blood pressure were observed in muskrats and ducks, but the functional significance of their integrated action was not yet recognized.

In a lecture at Woods Hole, Laurence Irving (1934) argued that the ability of diving animals to resist asphyxia, which cannot be sufficiently explained by increased vital capacity, blood volume or muscular anaerobic capacity, might rather be due to the way in which organs that are essentially similar to those of nondiving animals are made to cooperate, through the integration of reflex adjustments. In subsequent studies, he found that, with apnea or the addition of carbon dioxide to inspired air, blood flow to the brain increases while muscular circulation decreases and that these changes are more prominent in the muskrat and beaver than in terrestrial mammals, and that during apneic pauses in the intermittent breathing of the seal, heart rate slows by about 50% while arterial blood pressure rises, suggesting peripheral vasoconstriction (reviewed by Irving 1939). Irving proposed that the principal defense against asphyxia is a redistribution of oxygen, favoring the brain and heart at the expense of less sensitive tissues. Diving mammals may provide a natural experiment, Irving suggested, to study basic physiological adjustments to asphyxia and the integration of breathing and cardiovascular functions.

Extensive studies were already being conducted in Oslo by Per Scholander, who presented abundant experimental confirmation of Irving's theory in his landmark monograph of 1940. Scholander charted blood oxygen, carbon dioxide and lactate changes during simulated diving and recovery in a variety of diving mammals and birds, indicating peripheral vasoconstriction and reduced oxygen consumption during diving. The changes in blood flow during diving have since been shown using ultrasonic flow-transducer and radioactive microsphere techniques. Flow to the muscles, skin and viscera is drastically reduced, while the brain remains perfused and flow to the myocardium is reduced in proportion to its reduced work load (Elsner et al. 1966; Zapol et al. 1979; Jones et al. 1979, 1982). Cardiac output is reduced accordingly by bradycardia, and mean arterial blood pressure remains unchanged (Irving et al. 1942; Elsner et al. 1966). Through reflex interactions, apnea promotes bradycardia (Anrep 1936; Drummond and Jones 1979; Angell-James et al. 1981). These adjustments are initiated by stimulation of trigeminal receptors (Andersen 1963; Dykes 1974; Blix et al. 1976; Drummond and Jones 1979), and reinforced by chemoreceptor input as apneic asphyxia progresses (Daly et al. 1977), as Richet (1899) hypothesized. A schematic view of the control of the diving response is presented in Fig. 1.

The specialized vascular structures of marine mammals described by Burow (1838), Gratiolet (1860) and Burne (1909) participate in these adjustments, but their functions are complex and, in some cases, are still poorly understood (Harrison and Kooyman 1968; Elsner and Gooden

1983; Blix and Folkow 1983). Oxygen stores are elevated in many diving species, due primarily to elevated blood volume, as Bert (1870) found, and hemoglobin and myoglobin concentrations (Scholander 1940; Blessing and Hartschen-Niemeyer 1969; Lenfant et al. 1970). Specializations supporting anaerobic processes and tolerance of low oxygen tensions exist in some (Elsner et al. 1970; Kerem and Elsner 1973; Kerem et al. 1973). The latter adaptations are insufficient to support prolonged submergence, as Richet (1899) concluded, and play a supporting role to the reflex adjustments of the diving response.

As Irving suggested, elucidation of the diving response has enhanced our understanding of the common vertebrate response to asphyxic conditions, for example, the response of the fetus to reduced umbilical circulation (Bauer 1937; Dawes and Mott 1964). A "diving response" has been implicated in the survival of cold water near-drowning victims (Gooden 1972; Nemiroff 1979; Elsner and Gooden 1983) and in sudden underwater death and sudden infant death syndrome (Daly and Angell-James 1979; Gooden 1982; Elsner and Gooden 1983). Facial immersion has been used as a clinical tool for the treatment of paroxysmal atrial tachycardia (Wildenthal et al. 1975; Sperandeo et al. 1983), as a test of parasympathetic function (Sturani et al. 1982), a pressor test (Arnold 1986) and in the assessment of trigeminal-brainstem-vagal function (Khurana et al. 1980). Knowledge of the central nervous substrate underlying the diving response may similarly enhance our understanding of common mechanisms of defense against asphyxia, and have implications for clinical medicine.

II. MECHANISMS OF THE DIVING RESPONSE

The apnea, bradycardia and peripheral and visceral vasoconstriction of the diving response are generated and modulated by a complex system of interacting factors (Fig. 1). The response is initiated naturally by immersion of the face in water. In seals and muskrats, the nares close immediately on contact with water, preventing water from entering the nasal cavity. Drummond and Jones (1972; 1979; Drummond 1980) observed that the response in muskrats is triggered specifically by water lapping the nares and is eliminated by covering the nares with vaseline and excluding water from the nasal cavity. They refer to the response triggered by wetting the skin of the nose as the "external narial reflex" or "narial reflex." The narial reflex presumably is mediated by branches of the maxillary and ophthalmic divisions of the trigeminal nerve (V). Drummond (1980) found that section of the maxillary nerve at the level of the zygomatic arch did not affect this response, but did not rule out the involvement of less accessible maxillary branches or of the ophthalmic division of the trigeminal nerve. In seals, trigeminal neurotomy slows but does not eliminate the response to water immersion (Harrison and Kooyman 1968; Dykes 1974). Andersen (1963a,b) showed that the stimulation of water on the nares, transmitted by the trigeminal nerve, elicits the diving response in ducks. Wetting of trigeminal receptors around the nose and mouth, in combination with breath-holding, also triggers bradycardia

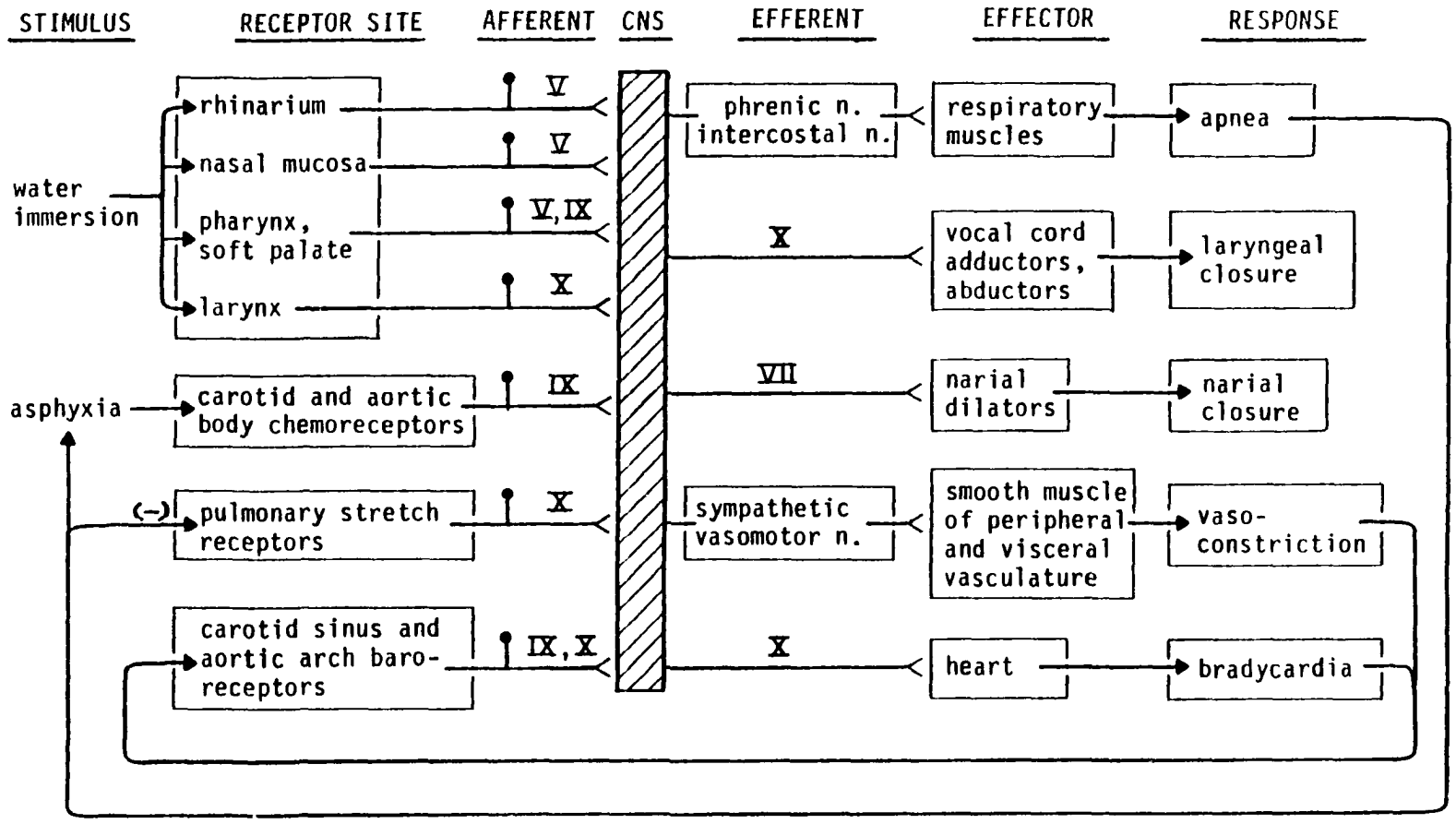

Fig. 1. Diagrammatic representation of the afferent and efferent pathways mediating the diving response and related upper respiratory tract reflexes. The central nervous system (CNS) is depicted as a "black box."

and peripheral vasoconstriction in humans (Heistad and Wheeler 1970). The response in humans is dependent on a cold stimulus (Craig 1963; Hong et al. 1967; Kawakami et al. 1967; Whayne and Killip 1967; Paulev 1968), whereas altering water temperature does not affect the response in muskrats (Drummond 1980) or seals (Dykes 1974). It has been shown in seals that stimulation of trigeminal receptors not only triggers the onset of the diving response but also participates in sustaining apnea by overriding the increasing inspiratory drive mediated by peripheral chemoreceptors as diving asphyxia progresses (Daly et al. 1977).

In muskrats anesthetized with urethane (see Daly et al. 1977), the immediate narial response to submersion was lost and water was drawn into the nasal cavity, which then triggered a similar response, the "internal nasal reflexes" (Drummond and Jones 1979; Drummond 1980). Passing water anteriorly through the nasal passages, perfusion of the pharynx and anterior glottis, or probing of the dorsal anterior soft palate also produced a response similar to the diving response (Drummond 1980). The internal nasal reflexes were abolished by section of both the maxillary and inferior laryngeal nerves; stimulation of these nerves, and of the glossopharyngeal nerve (IX), produced these reflexes (Drummond 1980). Perfusion of the nasal mucosa in dogs also produces a response qualitatively similar to the diving response, which is abolished by trigeminal neurotomy (Angell-James and Daly 1969, 1972). Kratschmer (1870) discovered, and numerous investigators since have studied, similar responses in rabbits exposed to noxious gases, which are mediated by trigeminal and not by olfactory pathways

(McRitchie and White 1974). Upper respiratory tract reflexes similar to the diving response appear to be universal. They protect the airways against inspired foreign particles, noxious gases or vomitus, and may be elicited by stimulation of the nasal mucosa, nasopharynx, and larynx or the superior laryngeal nerve (see Daly 1986 for review). The sites from which the diving response and similar upper respiratory tract reflexes are elicited are illustrated in Fig. 2.

Prolonged breath-holding leads to progressive arterial hypoxia and hypercapnia. Stimulation of arterial chemoreceptors is primarily responsible for the maintenance of bradycardia in prolonged dives. In harbor seals, removal of chemoreceptor drive by perfusion of the carotid sinus and carotid body regions (Fig. 2) with oxygenated blood had no effect on bradycardia at the beginning of a simulated dive, but eliminated bradycardia (without affecting apnea) later, as asphyxia developed; subsequent re-establishment of chemoreceptor drive reinstated diving bradycardia (Daly et al. 1977). In the domesticated duck (Jones and Purves 1970) and rat (Huang and Peng 1976), previous removal or denervation of the carotid bodies largely eliminated diving bradycardia. Stimulation of peripheral and central chemoreceptors also contributes to the increase in peripheral vascular resistance during diving in ducks (Jones et al. 1982).

The effects of arterial baroreceptor activity on heart rate and vascular resistance are modulated during diving by trigeminal, carotid chemoreceptor and central respiratory neuron activities. These reset the baroreceptor-cardiac reflex toward bradycardia, and the baroreceptor-vasomotor reflex toward increased tone, at the same level

of blood pressure as in the nondiving state (Angell-James et al. 1978).

Most divers submerge on expiration or exhale early in the dive (Bamford and Jones 1976; Elsner et al. 1977; Drummond and Jones 1979). Apnea in the expiratory position is required for the full development of the cardiovascular responses to diving. The excitability of cardiac vagal motoneurons, which produce bradycardia, is modulated by respiration. Their excitability is reduced during inspiration through the 'irradiation' of impulses from central inspiratory neurons and the activity of pulmonary stretch receptor afferents (Anrep et al. 1936). The cessation of this activity with diving apnea in expiration therefore allows full expression of bradycardia produced by trigeminal, chemoreceptor and baroreceptor inputs to cardiac vagal motoneurons during diving (Elsner et al. 1977; Daly et al. 1978; Drummond and Jones 1979). Changes in respiration modulate sympathetic efferent activity, as well as vagal activity. Since lung inflation produces reduced vascular resistance as well as tachycardia (Daly et al. 1967; Daly and Robinson 1968), apnea in expiration may also potentiate the vasoconstrictor responses to trigeminal and peripheral arterial chemoreceptor inputs during diving (Daly 1984).

From this summary of the mechanisms involved in the production of the diving response, it is apparent that this response represents a set of reflexes precisely regulated and integrated by complex interactions within the central nervous system. The popular term, "diving reflex," therefore is clearly inappropriate. The above description is probably also an oversimplification of the responses to diving. Denervation

experiments reveal that the pattern of stimuli involved in the elicitation of the diving response may be complex. Andersen (1963b) found that denervation of the ophthalmic division of the trigeminal nerve abolished the diving response in ducks only when blindfolded animals were submerged very carefully, without audible splashes or touching the beak to the bottom of the pool. Following electrocoagulation of the receptor areas for the diving response in ducks, Florin-Christensen et al. (1986) found no response in animals submerged in 36^{0}C water in the dark or blindfolded, but a normal diving response in 30% of animals able to see in 36^{0}C water, 70% of blindfolded subjects in 15^{0}C water, and 90% of animals able to see in 15^{0}C water. Whereas "normal" diving responses have been observed in the decerebrated duck (Andersen 1963b; Djojosugito et al. 1969) and muskrat (Jones et al. 1982), it has long been known that higher nervous influences can both elicit bradycardia without immersion and modify the expression of diving bradycardia. In their pioneering studies, Irving and Scholander (Scholander 1940; Irving et al. 1942) observed that an abrupt noise or gesture could elicit bradycardia in a seal. The onset or termination of diving bradycardia in anticipation of immersion or emersion, respectively, has been noted by numerous investigators (e.g., Jones et al. 1973). Since Scholander (1940) observed that bradycardia may fail to develop in a freely diving seal, numerous studies have shown variability in the responses to forced, voluntary or trained diving. The absence of bradycardia, and presumably peripheral vasoconstriction, has been observed in freely diving birds during short dives (Butler and Woakes 1979; Kanwisher et al. 1971). Profound

bradycardia, on the other hand, was recently observed in a freely diving seal (Elsner et al. 1989). Variations in timing and intensity of the responses to diving produced by the mechanisms described above appear to depend on the degree of stress imposed by the situation and the influence of higher cortical function (Elsner and Gooden 1983).

III. INNERVATION FOR THE DIVING RESPONSE

A. SENSORY RECEPTORS

The initiation, reinforcement and modulation of the diving response involves a complex variety of stimuli, as discussed in the previous section (Fig. 1). The initial response to immersion in the muskrat is mediated by cutaneous receptors in the rhinarium (Fig. 2) (Drummond and Jones 1979; Drummond 1980). Thermoreceptors and tactile receptors may be involved. Receptors in the nasal mucosa responding to water flow, and receptors responding to probing, suction or electrical stimulation of the soft palate and anterior larynx (Fig. 2), mediate similar responses in muskrats (Drummond 1980). Mechanical, electrical and noxious chemical stimulation of these areas commonly produces apnea, bradycardia and hyper- or hypotension in mammals, particularly the immature animal (revieved by Daly 1986; Widdicombe 1986).

Arterial chemoreceptors responding to diving hypoxia and hypercapnia include those in the carotid bodies, near the bifurcation of the external carotid artery (Fig. 2) in muskrats (Drummond and Jones 1979) and seals (Daly et al. 1977). It is not known whether functional

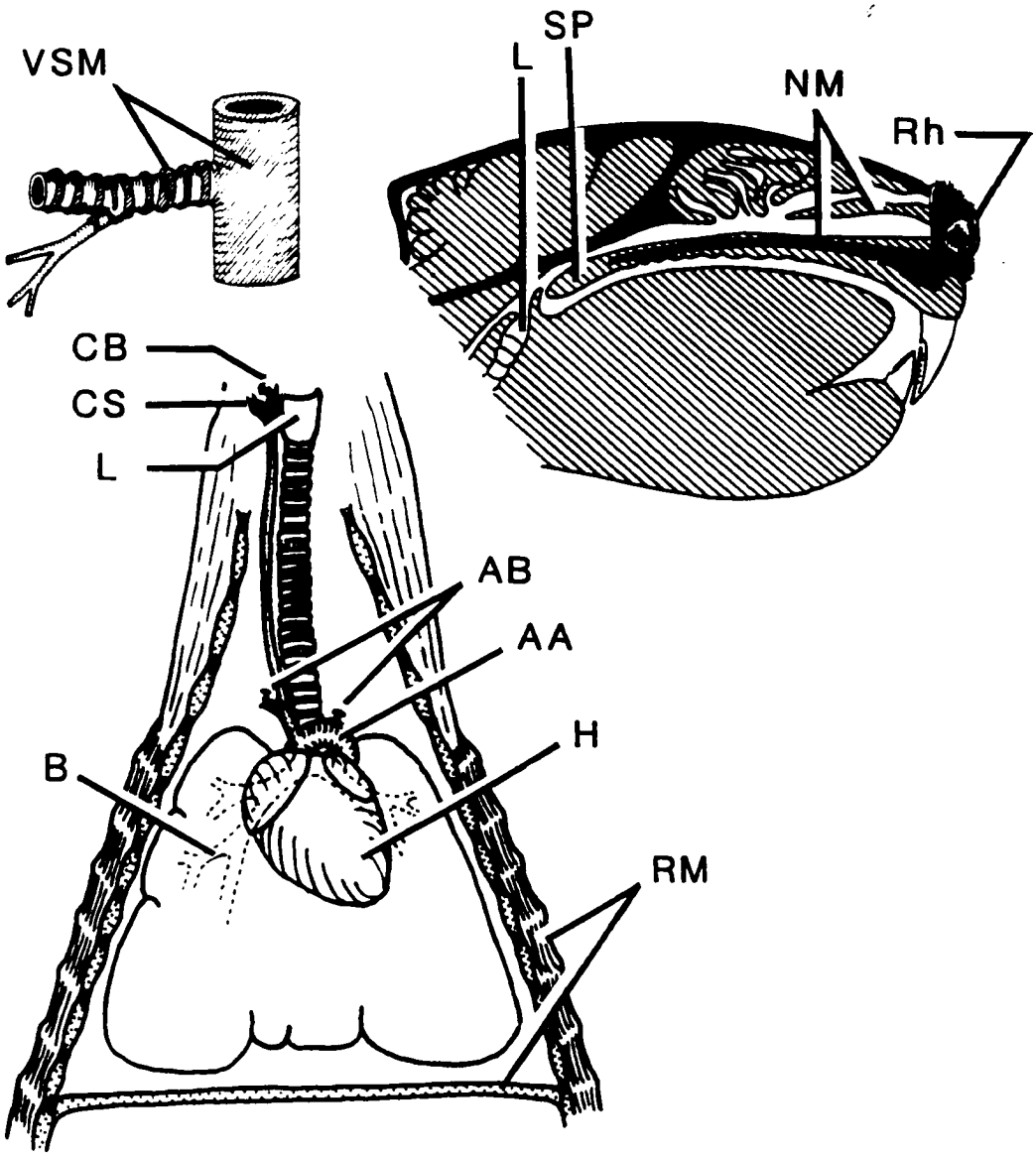

Fig. 2. Sensory sites and effectors involved in the diving response and related upper respiratory tract reflexes. (AA = aortic arch, AB = aortic bodies, B = bronchioles, CB = carotid bodies, CS = carotid sinus, H = heart, L = larynx, NM = nasal mucosa, Rh = rhinarium, RM = respiratory muscles, SP = soft palate, VSM = vascular smooth muscle.)

aortic bodies exist in diving mammals, as are found in the aortic arch region (Fig. 2) in the dog and cat (Comroe 1939), but not in the rabbit and rat (Neil et al. 1949; Sapru and Krieger 1977). Central chemosensitive structures have been found in the ventral superficial layer of the rostral medulla in the rat (Hori et al. 1970; Laha et al. 1977; Fukuda and Loeschcke 1979; Loewy 1981). These include an area at the lateral border of the pyramidal tract, corresponding to the most ventral part of the paragigantocellular nucleus, and an area ventral to the lateral reticular nucleus.

The arterial baroreceptors responding to mean arterial pressure, pulse pressure and pulse frequency, are located in the carotid sinus, just above the bifurcation, and in the wall of the aortic arch (Fig. 2). Pulmonary stretch receptors responding to lung inflation are located in the mucosa of the broncho-pulmonary tree (Fig. 2).

B. AFFERENT PATHWAYS

1. Afferents from the Nose

In the rat, the skin of the nose is innervated by the ophthalmic division of the trigeminal nerve, via the external nasal branch of the anterior ethmoidal branch of the nasociliary nerve, and by the maxillary division via external nasal branches of the infraorbital nerve. The nasal mucosa is supplied by internal nasal branches of the anterior ethmoidal nerve and by the maxillary division via the nasopalatine branch and a minute nasal branch of the anterior superior alveolar nerve. (Greene 1935)

TABLE 1
Abbreviations of neuroanatomical structures

Amb	=	ambiguus nucleus
g7	=	genu of facial nerve
I5	=	intertrigeminal nucleus
m5	=	motor root of the trigeminal nerve
Me5	=	mesencephalic trigeminal nucleus
Mo5	=	motor trigeminal nucleus
Pa5	=	paratrigeminal nucleus
Pr5	=	principal trigeminal sensory nucleus
rAmb	=	retroambiguus nucleus
s5	=	sensory root of trigeminal nerve
sol	=	solitary tract
Sol	=	nucleus of solitary tract
sp5	=	spinal trigeminal tract
Sp5C	=	spinal trigeminal nucleus, pars caudalis
Sp5I	=	spinal trigeminal nucleus, pars interpolaris
Sp5O	=	spinal trigeminal nucleus, pars oralis
Su5	=	supratrigeminal nucleus
6	=	abducens nucleus
7	=	facial nucleus
7n	=	facial nerve or its root
10	=	dorsal motor nucleus of vagus
12	=	hypoglossal nucleus
V	=	trigeminal nerve
IX	=	glossopharyngeal nerve
X	=	vagus nerve
XII	=	hypoglossal nerve

The cell bodies of trigeminal afferent fibers (except proprioceptive ones) are located in the trigeminal ganglion, and their centrally directed processes terminate in the trigeminal brainstem sensory complex (Fig. 3; abbreviations for these and other neuroanatomical structures are listed in Table 1). The general organization of trigeminal projections to this complex is discussed in section IV of this chapter. Maxillary and ophthalmic divisions of the trigeminal nerve terminate in the lateral and ventral parts, respectively, of this complex in mammals including the rat (Torvik 1956). Projections of the ethmoidal nerve have been traced to layers I-II of the ventral part of the caudal subnucleus (Sp5C) and to the interpolar subnucleus (Sp5I) of the spinal trigeminal nucleus in the cat (Lucier and Egizii 1986). Projections of the ethmoidal nerve in the muskrat to these areas and to layer V of Sp5C, the oral subnucleus of the spinal trigeminal nucleus (Sp5O), the principal sensory trigeminal nucleus (Pr5) and the paratrigeminal nucleus (Pa5) (Fig. 4) were recently reported by Panneton (1989). Similar, more lateral projections of the infraorbital nerve have been reported in the cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986a,b).

2. Afferents from the Palate, Pharynx and Larynx

Sensory innervation of the palate, pharynx and larynx is more complex, and less well described in the rodent. In the human, the pterygopalatine ganglion distributes maxillary fibers to the nasopharynx via a pharyngeal branch to the hard palate via the

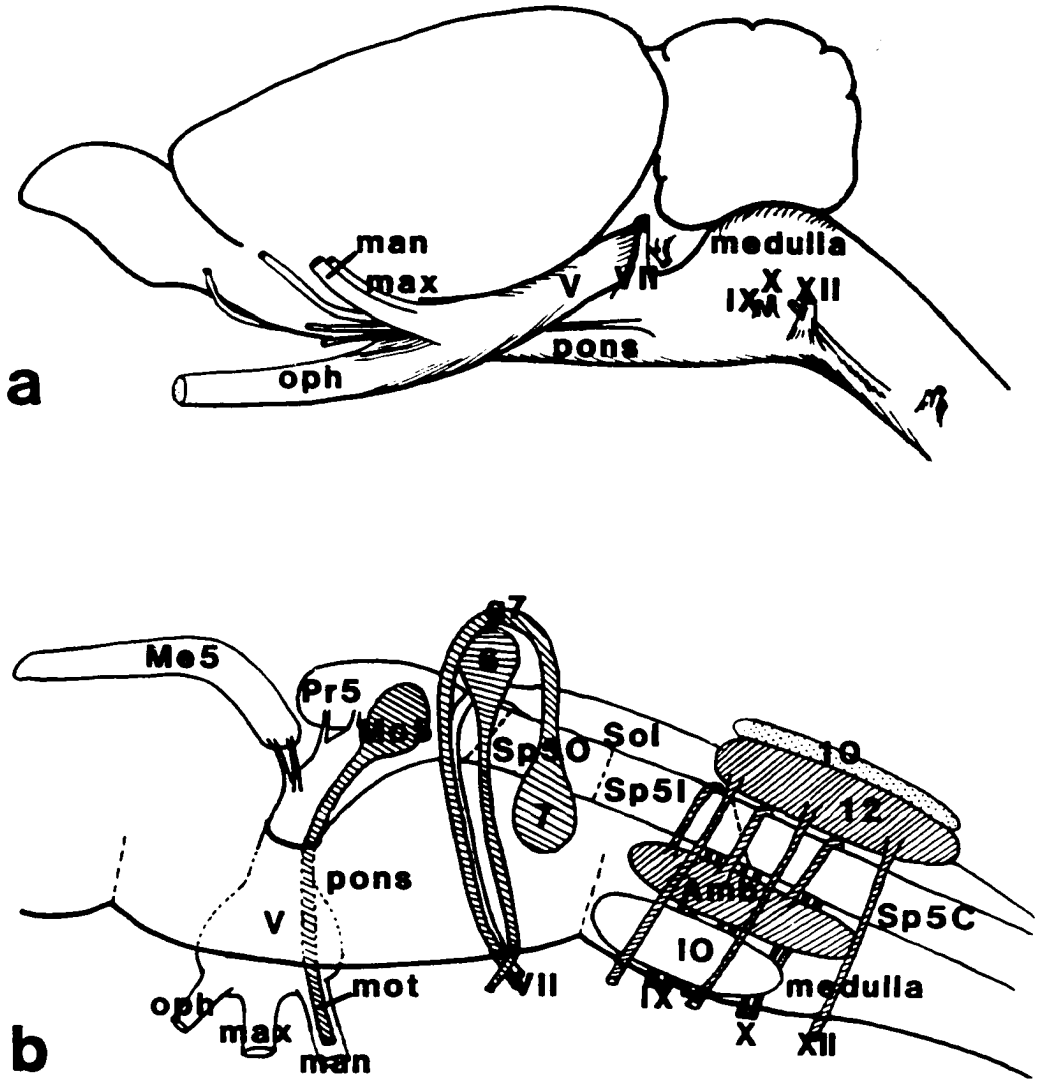

Fig. 3. (a) Lateral aspect of rat brain, showing brainstem areas and roots of cranial nerves involved in this study (after Greens 1963). (b) Schematic diagram of the course of these nerves and their nuclei, in midsagittal view (after Carpenter 1978). Oph, max, man, mot = ophthalmic, maxillary, mandibular and motor divisions, respectively, of the trigeminal nerve. See Table 1 for abbreviations of brain structures.

nasopalatine nerve and greater palatine nerve, and to the soft palate via the lesser palatine nerves. The greater and lesser palatine nerves subserve taste as well as general sensation. Taste fibers from the palate pass through the pterygopalatine ganglion to the greater petrosal branch of the seventh cranial nerve, the facial nerve. In addition, the ninth cranial nerve, the glossopharyngeal (IX), supplies general visceral afferent (GVA) fibers from the pharynx and soft palate via branches to the tonsillar plexus and pharyngeal plexus, which passes some fibers to a pharyngeal branch of the tenth cranial nerve, the vagus (X). The vagus also supplies GVA fibers from the larynx, via the internal laryngeal branch of the superior laryngeal nerve and the recurrent laryngeal nerve. The superior laryngeal nerve innervates the larynx above the vocal folds, the piriform fossa and vallecula epiglottica. The recurrent laryngeal nerve, via its termination as the inferior laryngeal nerve, supplies the laryngeal mucosa below the folds. Taste from the epiglottis also is provided by the internal laryngeal nerve. (Craigmyle 1985)

The inferior glossopharyngeal ganglion contains pseudo- unipolar cells of IX GVA fibers from the pharyngeal palate. The central termination of these fibers is described in most texts, e.g. Craigmyle (1985), as the caudal part of the nucleus of the solitary tract (Sol) in the medulla (Figs. 3,4). This nucleus also receives GVA fibers contained in the pharyngeal branch of X from these regions and X GVA fibers from the larynx and laryngopharynx, whose cells are located in the inferior vagal ganglion. Palatal taste fibers are the dendrites of

pseudounipolar neurons of the facial, or geniculate, ganglion whose axons terminate in the rostral part of Sol. This nucleus also receives central processes of cells in the inferior vagal ganglion whose distal processes convey taste sensations from the epiglottis. (Craigmyle 1985)

Pharyngeal, laryngeal and palatal afferents have recently been traced in the rat by Altschuler et al. (1989). Pharyngeal and laryngeal projections were traced specifically to the interstitial and intermediate subnuclei of Sol, and palatal fibers were traced to the lateral, interstitial and medial subnuclei. Palatal fibers projected most heavily to the spinal trigeminal and paratrigeminal (Pa5) nuclei (Fig. 4), however, and heavy projections to Pa5 from the pharynx and larynx were also seen. Panneton (1989) recently reported projections of the pharyngeal branch of IX to all levels of the trigeminal sensory nuclei and to Pa5. Projections to the trigeminal sensory nuclei from the pharyngeal branch of IX in the rat (Hamilton and Norgren 1984), cat (Mizuno and Nomura 1986), rabbit (Hanamori and Smith 1989) and lamb (Sweazey and Bradley 1986), the palatine branch of the trigeminal nerve in the cat (Shigenaga et al. 1986a,b) and the superior laryngeal nerve in the cat (Nomura and Mizuno 1983), rabbit (Hanamori and Smith 1989) and lamb (Sweazey and Bradley 1986), and to Pa5 from the pharyngeal branch of IX in the rat (Hamilton and Norgren 1984), the palatine branch of the trigeminal nerve in the cat (Shigenaga et al. 1986a,b) and the superior laryngeal nerve in the cat (Nomura and Mizuno 1983) and rabbit (Hanamori and Smith 1989) have also been shown in recent studies employing the HRP technique.

3. Afferents from Chemoreceptors, Baroreceptors and Pulmonary Stretch Receptors

Arterial chemoreceptor and baroreceptors are innervated by carotid branches of the glossopharyngeal and vagus nerves, carried in the carotid sinus nerve. Pulmonary stretch receptors are innervated by thoracic vagal fibers. Cell bodies of the glossopharyngeal and vagus nerves are located in the petrous and nodose ganglia, respectively. Carotid sinus nerve afferents project widely to subnuclei of Sol (Figs. 3,4) in the cat (Berger 1979; Panneton and Loewy 1980; Ciriello et al. 1981; Davies and Kalia 1981; Nomura and Mizuno 1982) and rat (Seiders and Stuesse 1984; Housley et al. 1987). Pulmonary stretch receptor activity has been mapped (Berger and Averill 1983) and pulmonary afferents have been traced (Kalia and Mesulam 1980) to Sol, particularly the ventrolateral and dorsolateral subnuclei in the cat.

C. Efferent Pathways

1. Bradycardia

On the efferent side, negative chronotropic and perhaps inotropic effects during diving are mediated by general visceral efferent fibers in cardiac branches of the vagus in ducks (Richet 1899; Huxley 1913c; Lombroso 1913; Butler and Jones 1968), seals (Harrison 1960; Van Citters et al. 1965) and the muskrat (Drummond and Jones 1979). Cardioinhibitory motoneurons are located in the nucleus ambiguus (Amb) Figs. 3,4) in the cat (Kerr 1965; Calarescu and Pearce 1965; McAllen and Spyer 1976) and also in the dorsal motor nucleus of the vagus (10)

Fig. 4. Nissl-stained coronal section of muskrat brain, at -0.5 mm from the obex, showing some of the structures involved in this study. (Amb = ambiguus nucleus, Pa5 = paratrigeminal nucleus, sol = solitary tract, Sol = nucleus of the solitary tract, sp5 = spinal trigeminal tract, Sp5C = caudal subnucleus of spinal trigeminal nucleus, 10 = dorsal motor nucleus of the vagus, 12 = hypoglossal nucleus.)

(Figs. 3,4) in the dog (Gunn et al. 1968), rabbit (Schwaber and Schneiderman 1975) and monkey (Mitchell and Warwick 1955).

2. Peripheral Vasoconstriction

Postganglionic fibers innervating the smooth muscle of the peripheral blood vessels (Fig. 2) arise from cells in the sympathetic ganglia. Preganglionic fibers arise from cells in the intermediolateral column of the thoracolumbar spinal cord. Pressor activity is associated with descending pathways from the lateral and ventrolateral reticular nuclei, parvocellular nucleus, and lateral paragigantocellular nucleus in the medulla, and regions in the hypothalamus (see Daly 1986, Abboud and Thames 1983 for reviews).

3. Apnea

The ability to breath-hold in the face of progressive diving asphyxia cannot be explained by insensitivity to blood gases. Apnea in expiration is engendered reflexly and chemoreceptor drive is overridden by sustained inhibitory input to central inspiratory neurons from trigeminal receptors. Reflex laryngeal (Banting et al. 1938) and narial closure, as well as cessation of lung inflation, occurs. The premotor neurons governing respiration include brainstem populations in a longitudinal column of the ventrolateral medulla called the ventral respiratory group, and a dorsal respiratory group in the ventrolateral Sol (Fig.4). The ventral respiratory group is divided into rostral, intermediate and caudal parts involving the retrofacial nucleus, para-

ambigual nucleus and retroambigual nucleus, respectively. The motor elements include the following:

a. Lungs: The principal inspiratory motoneurons are carried by the phrenic nerve to the diaphragm and by intercostal nerve branches to the external intercostal muscles; branches to the internal intercostal muscles are involved in active expiration (Figs. 1,2). Cell bodies of these fibers are located in the cervical and thoracic spinal cord.

b. Larynx: Laryngeal closure is effected by the contraction of vocal cord adductor muscles and the relaxation of abductors (Fig. 1). These muscles are innervated by recurrent laryngeal nerve branches with cell bodies in the nucleus ambiguus (Amb) (Figs. 3,4). Some cells of Amb identified as laryngeal motoneurons fire spontaneously with a respiratory rhythm and may themselves be part of the central pattern generator, or they may be driven as are the spinal motoneurons serving respiratory muscles (Bartlett 1986).

c. Nares: The few muscles around the nose are involved in dilating the nares and drawing back the upper lip. They are innervated by facial nerve fibers, with cell bodies in the facial nucleus (7) of the pons (Fig. 3). Those innervating the naris dilator in the rat are located in the lateral subdivision of the facial nucleus (Travers 1985). The rhinarium of seals is described as elastic and valvular, and the nares are opened by voluntary effort (Scheffer 1958); narial closure is effected by relaxation of the nasolabialis and maxillonasolabialis muscles (King 1964). The nares of rats and

maxillonasolabialis muscles (King 1964). The nares of rats and muskrats, too, are slitlike and their closure may also be a passive process.

IV. The Trigeminal System

The trigeminal nerve complex is thought to have been associated in the primitive condition with two anterior gill slits that have been eliminated by jaw development and expansion of the mouth region (Romer 1970). The ophthalmicus profundus nerve served the more anterior region. In lower vertebrates, it may emerge from the brain separately from the trigeminus and have a separate ganglion. In mammals, it is intimately associated with the trigeminus and considered the ophthalmic trunk of that nerve. The trigeminal nerve proper served the second primitive gill slit and comprises a maxillary ramus to the upper jaw region and a mandibular ramus to the lower jaw. Both trunks include somatosensory elements supplying the anterior oral cavity and much of the surface of the head. Phylogenetically, the facial branches of the trigeminal nerve increase in number while those of the other branchial arch nerves decrease.

A. Trigeminal Nerve, Ganglion and Root

In mammals, the ophthalmic division of the trigeminal nerve carries touch-pressure, thermal and nociceptive fibers from part of the scalp, forehead, upper eyelid, cornea, sclera, conjunctiva, dorsum of the

information from the dura, skin and mucosa of the cheek and side of the head, lower lid, nose and nasal mucosa, upper lip and oral mucosa, and teeth of the upper jaw. The mandibular carries similar information from the skin of the lower jaw, chin and upper lip, oral mucosa, pinna, acoustic meatus, teeth and gums of the lower jaw, and anterior tongue. In addition, it carries proprioceptive afferents from the muscles of mastication, temporomandibular joint, and periodontal ligaments, and provides motor innervation to the muscles of mastication and the mylohyoid, anterior belly of the digastric, tensor veli palatini and tensor tympani muscles.

Pseudounipolar cell bodies of afferent fibers, other than proprioceptive ones and somata of tendon organs, from the three trigeminal divisions lie in the trigeminal (semilunar, or Gasserian) ganglion and enlargement of the sensory root (Fig. 3). Proprioceptive and motor fibers of the mandibular division bypass the ganglion to form the smaller motor root. A somatotopic arrangement of cells and fibers exists in the peripheral nerves, ganglion and root, e.g. lateral orofacial tissues are represented laterally in the ganglion in the rat (Gregg and Dixon 1973). Representation of the individual rows of vibrissae is somatotopically organized in the ganglion (Zucker and Welker 1969; Erzurumlu and Killackey 1983). These topographic relationships are maintained through the system to the cerebral cortex.

B. Trigeminal Brainstem Sensory Complex

1. Principal Sensory Trigeminal Nucleus

On entering the pons, most sensory fibers bifurcate into ascending and descending branches. Ascending fibers give off side branches which run medially to terminate in the principal sensory trigeminal nucleus (Pr5) in the lateral rostral pontine tegmentum, from slightly rostral to the rostral pole of the facial nucleus, to the rostral pole of the motor trigeminal nucleus (Fig. 3). Ventrolateral (VL) and dorsomedial (DM) subdivisions are recognized. At its caudal extent, VL overlaps with pars oralis of the spinal trigeminal nucleus. A segmented pattern of termination of afferents from the vibrissae is present in VL of the rat (Belford and Killackey 1979; Arvidsson 1982). In the rat, VL receives projections from corneal, supraorbital, infraorbital, infratrochlear and lacrimal nerves (Marfurt 1981; Panneton and Burton 1981). Electrophysiologic studies demonstrate additional projections from the tooth pulp, periodontium, and superior laryngeal and glossopharyngeal nerves (Eisenman et al. 1963; Sessle and Greenwood 1976). Most primary afferents to DM are from the oral cavity, including mental and inferior alveolar nerve and maxillary and mandibular dental pulp and periodontium projections (Westrum et al. 1980; Arvidsson and Gobel 1981; Marfurt 1981; Johnson et al 1983). Infraorbital, lacrimal, auriculotemporal, chorda tympani, glossopharyngeal and superior laryngeal afferents may also project to DM (Sessle and Greenwood 1976; Panneton and Burton 1981; Nomura and Mizuno 1983). VL projects contralaterally and DM projects

ipsilaterally to the ventrobasal thalamus (Torvik 1957; Fukushima and Kerr 1979; Burton and Craig 1979; Matsushita et al. 1982; Shigenaga et al. 1983). Pr5 also projects to the posterior thalamic nuclei and ventral zona incerta (Smith 1973), Mo5 (Travers and Norgren 1983), and the superior colliculus (Huerta et al. 1983).

2. Spinal Trigeminal Tract and Nucleus

Descending branches from the sensory root form the spinal trigeminal tract in the dorsolateral brainstem from the pons to the level of the second cervical spinal segment (C2). The facial, glossopharyngeal, and vagal nerves also contribute general somatic afferent fibers to the tract. Larger, myelinated fibers predominate superficially. Fiber diameter decreases caudally, as larger fibers terminate at more rostral levels and as the branching off of collaterals reduces the remaining fiber diameter; below the obex most fibers are unmyelinated (Tracey 1985). In the rat, a somatotopic arrangement similar to that in the sensory root continues with medial rotation of the root, such that ophthalmic fibers are situated ventrolaterally, mandibular fibers dorsomedially and maxillary fibers in between. Within the area occupied by the maxillary fibers, the dorsal rows of vibrissae are represented ventrally and the ventral rows are represented dorsally (Erzurumlu and Killackey 1983).

Near their points of termination, fibers descending in the spinal trigeminal tract turn abruptly inward and ramify in the underlying nucleus of the spinal trigeminal tract (Sp5), which is continuous with

Pr5 rostrally and with the cervical dorsal horn caudally. On the basis of cytoarchitecture, Sp5 is divided rostrocaudally into three subnuclei: oralis (Sp5O), interpolaris (Sp5I) and caudalis (Sp5C) (Olszewski 1950; Aström 1953; Torvik 1956) (Fig. 3).

Sp5O extends from Pr5 to the rostral extent of the inferior olive, coinciding with the level of the facial nucleus. It contains small and medium size cells, loosely distributed individually and in clusters. Sp5I extends from the rostral inferior olive to the obex. It contains mostly small and medium cells, with larger neurons concentrated in the rostral half of the nucleus in the rat (Phelan and Walls 1983) and cat (Taber 1961), and a marginal plexus of larger cells reported in the mouse (Aström 1953). Sp5C extends from the obex to C2 and exhibits three layers. Underlying the tract the narrow marginal layer contains a few large cells and many smaller ones oriented horizontally, and corresponds to Rexed's (1952) layer 1 of the dorsal horn. Next is the broader gelatinous layer with small, spindle shaped cells. A feltwork of unmyelinated fibers in the outer part of this layer corresponds to Rexed's layer 2 or substantia gelatinosa Rolandi of the dorsal horn, whereas the deeper part of the gelatinous layer corresponds to layer 3 of the dorsal horn. The deepest Sp5C layer, the magnocellular, contains variably sized neurons and corresponds to layer 4 of the spinal cord.

3. Somatotopic Organization and Projections

The trigeminal sensory nuclear complex receives information

from mechanoreceptors, thermoreceptors and nociceptors. In Sp5C, vibration sensitive afferents terminate extensively throughout the nucleus except in the marginal layer, whereas rapidly and slowly adapting vibrissal afferents have circumscribed terminations in the deep layers (Jacquin et al. 1983). The marginal layer contains cells that selectively respond to noxious and thermal stimuli.

Since fine fibers, presumably mediating pain and thermal sense, terminate selectively in the caudal region, it has been reasoned that this region mediates nociception whereas more rostral regions subserve other modalities. Indeed, interrupting the spinal trigeminal tract just rostral to Sp5C - the Sjöqvist (1938) tractotomy procedure - alleviates trigeminal neuralgia, or tic douloureux, while preserving facial tactile sensibility. However, nociceptive afferent inputs to Sp5O (Azerad et al. 1982) and Sp5I (Hayashi et al. 1984) have also been reported. A similarly complex and controversial situation exists with regard to rostrocaudal somatotopic organization. Déjerine (1914) proposed such an arrangement based on the analogy of peeling an onion, with oral and perioral structures represented rostrally and posterior facial structures represented caudally. Numerous clinical, physiological and anatomical studies support similar conclusions. Others, however, show that each of the three trigeminal divisions projects to all levels of the sensory complex. Marfurt (1981) concluded that although projections are extensive, quantitative differences exist among the rostrocaudal components in the cat. For example, the oralis subnucleus receives significant projections from

the nasal and oral cavities, but not from the vibrissae (Wall and Taub 1962).

There is general agreement, on the other hand, on a dorsoventral somatotopic organization of the trigeminal sensory nuclear complex similar to that of the tract. The mandibular, maxillary and ophthalmic divisions project in an ipsilateral dorsoventral sequence, such that the face is represented in an inverted, medially facing fashion. This was originally shown in the electrophysiological experiments of Harrison and Corbin (1942). Similar results have been reported for the rat by Nord (1967), and confirmed in degeneration studies by Torvik (1956). Individual, horizontal rows of vibrissae are also represented in an inverted fashion from dorsal to ventral, in all trigeminal sensory nuclei in the rat (Arvidsson 1982).

A less distinct mediolateral pattern also exists in the trigeminal sensory complex, such that the oral cavity tends to be represented medially and the facial skin more laterally (Nord 1967). More anterior, vertical rows of vibrissae are represented more deeply in the nonlaminated nuclei, but the opposite pattern occurs in laminated Sp5C (Arvidsson 1982).

In Sp5C, cells of the marginal and gelatinous layers project to more rostral trigeminal nuclei and to the thalamus (Kruger et al. 1977; Shigenaga et al. 1979; Fukushima and Kerr 1979); the magnocellular layer projects to the lateral facial nucleus (Erzurumlu and Killackey 1979). Some cells of Sp5I also project to the thalamus (Kruger et al. 1977; Fukushima and Kerr 1979; Erzurumlu and Killackey 1980), but most project to the cerebellum (Watson and Switzer 1978). Some Sp5O cells

also project to the thalamus (Kruger et al. 1977; Fukushima and Kerr 1979); others, to brainstem regions, including the facial nucleus (Erzurumlu and Killackey 1979). Pr5 sends the strongest projection of the trigeminal sensory complex to the thalamus, and projects to the ventral zona incerta, the motor trigeminal nucleus, and the superior colliculus (Smith 1973; Fukushima and Kerr 1979; Travers and Norgren 1983; Huerta et al. 1983).

All levels of the trigeminal sensory complex send tactile inputs to the ventrobasal complex of the thalamus, which projects to areas I and II of the somatosensory cortex. Facial representation in these structures in the rat appears to be largely concerned with the vibrissae, which project specifically to groups of cells called "barrels" (Woolsey and Van der Loos 1970). The submedius thalamic nucleus receives input from the marginal zone of the spinal cord and Sp5C, and probably projects to the orbitofrontal cortex, mediating nociception (Craig and Burton 1981).

4. Mesencephalic Trigeminal Nucleus

The monopolar cell bodies of proprioceptive trigeminal fibers form the mesencephalic trigeminal nucleus (Me5) (Fig. 3), a narrow band along the lateral margins of the periaqueductal gray matter, from the level of the superior colliculus to Mo5. These are the only somata of primary afferents present in the central nervous system.

C. Motor Trigeminal Nucleus

In the rat, motor fibers to the jaw-closing muscles pass ventral to Pr5 from multipolar cell bodies in the dorsolateral division of the motor trigeminal nucleus (Mo5) (Fig. 3). Fibers to most jaw-opening muscles pass from the ventromedial division of Mo5 dorsally, then ventrolaterally, forming a genu rostral to the genu of the facial nerve. Mo5 lies in the posterolateral part of the middle pontine region just medial to Pr5. The dorsolateral division extends through the rostrocaudal length of the nucleus. The smaller ventromedial division is situated in the caudal half only. (Travers 1985)

D. Related Structures

The supratrigeminal nucleus (Su5) caps the dorsal aspect of Mo5 in the rat (Torvik 1956), mouse (Aström 1953) and cat (Torvik 1957). Torvik (1956) describes this as a dorsomedial extension of Pr5, with loosely arranged cells slightly larger than those of Pr5. It receives trigeminal primary afferent input and afferent projections from Pr5, projects to the hypoglossal nucleus, and has reciprocal connections with Mo5 (Travers and Norgren 1983; Vornov and Sutin 1983). The intertrigeminal nucleus or area (I5) is interposed between Pr5 and Mo5 (Taber 1961). Together with Su5, it may provide cholinergic input to Mo5 (Wamsley et al. 1981; Travers 1985).

The accessory trigeminal nucleus is one of three accessory motor neuron groups in the lateral tegmentum. It also has been called the retrotrigeminal nucleus, the posterior trigeminal nucleus, and the

alpha division of Mo5. It forms a medial bulge on Mo5 and merges caudally with the facial accessory nucleus. Axons from this group follow a distinct route in the brainstem and segregate into a separate branch of the mandibular nerve, innervating the suprahyoid muscles. (Szekely and Matesz 1982)

Ramon y Cajal (1909) first described groups of cells in the spinal trigeminal tract, which he referred to as the interstitial nucleus. Similar islands of cells were described in human (Olszewski and Baxter 1954) and cat brain (Taber 1961) and termed "insulae nuclei cuneati lateralis." Ström (1953) called a similarly located nucleus the "promontorium" and hypothesized that it receives tactile impulses from the external ear via the glossopharyngeal, vagal and facial nerves. This was considered unlikely by Torvik (1956), who described homologous structures in the rat as gelatinous substance detached from Sp5C and intercalated as "cell nests" in the dorsal trigeminal tract at that level. Gobel and Purvis (1972) described similar structures in the cat at the levels of Sp5I and Sp5O. Gobel and Hockfield (1977), on the other hand, described these as interstitial extensions of cells from the marginal layer, in the cat. They reported that, following HRP injection into the thalamus and midbrain tegmentum, labeling was restricted to the marginal layer of subnucleus caudalis and these interstitial extensions. Similar structures in the opposum were referred to as "nucleus paratrigeminalis" (Oswaldo-Cruz and Rocha-Miranda 1968).

Chan-Palay (1978a,b) describes the synaptic organization and presence of neurotransmitters in the paratrigeminal nucleus (Pa5) of

the rat, human and rhesus monkey. Hayashi et al. (1984) recorded nociceptive neurons in the lateral margin of subnucleus interpolaris and within the tract at this level in the cat, which they suggest may be remnants of the marginal layer or Pa5 from caudal levels. Projections to Pa5 include corneal, periocular, infraorbital and lingual branches of the trigeminal nerve (Panneton and Burton 1981; Shigenaga et al. 1986a,b) and glossopharyngeal and vagal afferents in the cat (Ciriello et al. 1981; Nomura and Mizuno 1982; Panneton and Loewy 1979). Pa5 projects to the cerebellum (Somana and Walberg 1979), dorsolateral pons and thalamus (Panneton and Burton 1985).

MATERIALS AND METHODS

I. ANIMALS

Muskrats (Ondatra zibethicus) used in this study were mostly juvenile animals of both sexes, weighing 480-780 g. Younger animals were preferred, as their cells may take up and transport horseradish peroxidase more readily (LaVail and LaVail 1972). They were obtained by trapping at various locations in interior Alaska, with Tomahawk wire live-traps. Most were trapped by open water in the summer and fall, but I also obtained some in early spring by annexing traps onto the dome-shaped "push-ups" that cover holes in lake ice maintained by muskrats during the winter. The trapped animals were kept indoors in wooden nesting boxes, or in large cages with wooden platforms, with access to a tub of running water, or in similarly equipped outdoor runs. They were fed carrots, Mouse Chow and Quality Texture (Purina), and barley.

Three feral (pigmented) Norway rats (Rattus norvegicus), a male and 2 female adults weighing 180-280 g, were live-trapped at the Fairbanks landfill. They were kept in small-animal cages and fed Mouse Chow.

II. THE HRP METHOD

The development in the 1970s of techniques based on the axonal transport of tracers, such as radiolabeled amino acids and horseradish

peroxidase (HRP), began a revolution in neuroscience. The HRP method has become particularly popular because of its sensitivity, brevity, ability to show morphological detail, and ability to localize specific cells of origin. Peroxidases are enzymes that display oxidizing properties in the presence of peroxides. They are ubiquitous in tissues, especially in plants; the root of the horseradish is one of the richest sources. As a macromolecule, extracellular HRP is taken up into neurons by endocytosis and transported from one part of the cell to another. The peroxide-HRP complex can catalyze the oxidation of chromogens, which change color and precipitate at the site, serving as a visible marker. In addition to this direct demonstration of HRP activity, HRP can be attached to anti-HRP, radioactive labels, or fluorescent substances and subsequently demonstrated by immunohistochemical or immunofluorescent techniques, autoradiography, or fluorescent microscopy, respectively.

HRP was first used as a histochemical marker by Straus (1959), who demonstrated HRP in endocytotic vesicles in liver and kidney following its intravenous administration, using the chromogen benzidine. The first demonstration of endocytotic uptake and transport by nerve cells was by Kristensson and Olsson (1971). They showed HRP in the spinal cord, using diaminobenzidine, following intramuscular administration. Similar, anterograde and transganglionic transport of HRP were demonstrated later, with modifications of the HRP method which increased its sensitivity. These include the use of the chromogen tetramethylbenzidine (TMB) and conjugation of HRP to wheatgerm

agglutinin (WGA), which enhances its uptake. The procedures used in this study generally follow the TMB method described by Mesulam (1982).

III. INJECTION OF TRACER

HRP and HRP-WGA were administered by pressure injection in anesthetised animals. The animal was placed in a small plexiglas chamber, into which halothane in oxygen was delivered from an anesthesia machine and scavenged via a central vacuum system. When anesthesia was induced, the animal was removed from the chamber and an endotracheal catheter was inserted, using a laryngoscope with an '0' neonatal blade. Anesthesia was maintained by insufflation through the catheter, connected to a line from the anesthesia machine to the scavenging system. Body temperature was maintained with a heating pad.

Three experimental groups were used, according to injection site. In one group, cutaneous injections were made in the wing and tip of the nose, around the nares. Of 7 muskrats, 3 (HM 27, HM 28 and HM 29) were injected lateral to the left naris. HM 15 was injected lateral to the left naris and within the entrance to the naris, in the head of a pouch-like fold of the lateral wall of the nasal cavity. Two muskrats, HM 25 and HM 26, were injected similarly to HM 15, with tracer also injected medial to the left naris. HM 16 was injected medial and lateral to the right naris. Three rats (HR 4, HR 5 and HR 6) also were injected lateral to the left naris. In 3 muskrats (HM 20, HM 21 and HM 24), shallow injections were made in the ventral surface of the soft palate, on the right side. In the third group, one muskrat (HM 22) was

injected in the posterior pharynx, from an oral approach, and a second (HM 30) was injected in the larynx under the rostral end of the thyroid cartilage, following ventral neck dissection.

Solutions of HRP (Boebringer Mannheim, Grade II) and HRP-WGA (Sigma) in saline (10-15 mg/100 ul and 0.1-0.6 mg/100 ul, respectively) with 2% dimethyl sulfoxide were injected. A total volume of 50-150 ul was injected slowly at several points in the areas described, using a 100-ul Hamilton syringe and 22-gauge needle or tuberculin syringe and 25-gauge needle. The needle was left in place for several minutes after each injection, before withdrawing. A combination of 10-25 mg HRP and 0.1-0.4 mg HRP-WGA was injected. At 24 h after the first injection, muskrats were reinjected. Swelling in the region of the mystacial pad occurred immediately after injecting the 3 rats. This swelling disappeared within a few hours. The rats were not reinjected.

IV. PERFUSION AND FIXATION

At 48 h after the initial injection, brains were fixed <u>in situ</u> by transcardial perfusion (Mesulam 1982). Animals were deeply anesthetized by intramuscular injection of ketamine followed by intraperitoneal injection of pentobarbital sodium. The perfusate was delivered by gravity flow from about 120 cm, through a 15-gauge needle, into the left ventricle at the apex, with the right atrium cut. An initial bolus of 1 ml heparin (1,000 U/ml) and 1 ml lidocaine (2%), to prevent clotting and vasoconstriction, was followed by 1 l 0.9% NaCl at pH 7.4, with 10,000 U heparin, until the blood had been washed out.

Then the animal was perfused with 1-1.5 l fixative: 1.5% glutaraldehyde and 4% sucrose in 0.1 M phosphate buffer, pH 7.4, given over a period of 20-30 min. The brain and trigeminal ganglia were dissected from the cranium and the injected tissues excised, and post-fixed for about 4 h in cold fixative.

V. SECTIONING

The fixed brains were dissected by transverse cuts, transferred with the other tissues to chilled, buffered 30% sucrose and kept refrigerated until the tissues sank, in 24- 48 h, to afford cryoprotection and remove excess fixative. The tissue was then frozen on dry ice or in hexanes cooled in a bath of acetone on dry ice (-30 to -60^{0}C) and mounted on a microtome chuck and encased using either 2% gelatin, Tissue Tek O.C.T. compound or Lipshaw M-1 embedding matrix. Frozen tissue was stored, if necessary, at -50 to -80^{0}C.

Coronal brain sections were cut serially at 32-34 um in a Hacker Instruments-Bright microtome cryostat (Ebbesson et al. 1981). Sections were mounted by thawing onto room-temperature slides previously coated with 1% gelatin (300 bloom-porcine, Sigma)-0.1% chromium potassium sulfate. Slides were filled three at a time in an alternating sequence, such that each slide held sections at about 100-um intervals and three sets of such slides resulted, representing the brain in triplicate along its rostrocaudal axis. Trigeminal ganglia were sectioned horizontally in a similar manner. Slides were refrigerated until reacting for HRP as soon as sectioning was completed, or stored

frozen in plastic-wrapped trays.

VI. STAINING

One set of sections from each brain was stained for HRP, by a procedure that combines the attributes of an on-the-slide modification by Ebbesson et al. (1981) of standard HRP methods, and Mesulam's (1978; 1982) procedure using the chromogen TMB. Slides were soaked for 20 min in cold sodium acetate buffer at pH 3.3 (25 mM sodium acetate, 9.5 mM HCl, titrated) with 3.4 mM sodium nitroferricyanide, to which TMB dissolved in absolute ethanol had just been added (5 mg/100 ml soaking solution). The enzymatic reaction was initiated by adding 1-5 ml 0.3% hydrogen peroxide per 100 ml solution. After 20-45 min incubation, the slides were rinsed by soaking for 5 min in each of 6 changes of cold acetate buffer. All solutions were kept in an ice-water bath. In some cases, a set of sections also was reacted for HRP with benzidine dihydrochloride, according to Ebbesson et al. (1981). Some HRP-stained sets were counterstained for 2-5 min in buffered (0.1 M acetic acid-sodium acetate, pH 3.5-4.0) 1% neutral red.

Initially, HRP-stained slides were dehydrated at this point by transferring to a graded series of cold acetones, cleared in several changes of cold xylene, and coverslipped with Permount or Histoclad. Later in the project, slides were air-dried overnight, cleared in xylene and coverslipped. This procedure was simpler, avoided fading of the stain that occurs in acetone or alcohol, and resulted in a more permanent stain. HRP-stained slides were stored in a refrigerator.

A second set of sections from each brain was stained for Nissl substance by rehydrating in acetic acid-sodium acetate buffer, staining in buffered 0.5-1% cresyl echt violet, rinsing in water, dehydrating and differentiating in a graded ethanol series, and clearing in xylene. Often, the sections were first dehydrated and cleared, then rehydrated in the alcohol series, to enhance subsequent staining.

VII. MAPPING

HRP-stained material was examined with an Olympus Vanox photomicroscope, primarily with darkfield illumination. Representative areas with labeled neuronal elements were photographed, usually at 25X magnification. The color prints, reconstructed into montages where appropriate, were used to record detail in drawings of the same sections from projected images. Drawings of neuroanatomical landmarks in the adjacent, Nissl-stained sections were made from images projected onto tracing paper, and superimposed on the drawings of HRP-labeled structures. Rostrocaudal locations were calculated, with the obex as reference point '0'. The principal reference used in mapping was The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson 1986).

RESULTS

The preparations provided a clear view of the HRP-labeled fibers, terminals and cell bodies, especially when examined under darkfield illumination. All cases within an experimental group showed similar trajectories. The case exhibiting the most intense staining, within each experimental group, was used for illustration (Figs. 5-14; abbreviations listed in Table 1).

I. PROJECTIONS FROM THE NOSE

Projections were traced from the skin around the nares in 7 muskrats and 3 rats. The results were similar for the two species, except that labeling of layers V-VI of the medullary dorsal horn, the dorsomedial part of the oral subnucleus of the spinal trigeminal nucleus and the paratrigeminal nucleus was present in the muskrat but absent or very faint in the rat.

A. EXPERIMENTS ON MUSKRATS

Cell bodies of the trigeminal ganglia and fibers of the trigeminal sensory roots (s5) and spinal trigeminal tract (sp5) were labeled. Primary afferent fibers were traced to terminals in the principal sensory trigeminal nucleus (Pr5), all levels of the spinal trigeminal nucleus, cells of the mesencephalic trigeminal nucleus (Me5), and the paratrigeminal nucleus (Pa5). Retrogradely labeled fibers and cells

were found in the motor trigeminal root (m5) and nucleus (Mo5) and in the facial nerve root (7n) and nucleus (7). In HN 27, the most intensely stained case, terminal labeling of the dorsomedial part of the oral subnucleus of the spinal trigeminal nucleus (Sp5O) may overlap with the solitary nucleus (Sol). (Figs. 5a-14a)

1. Level of the Principal Sensory Trigeminal Nucleus

In the pons, HRP-labeled fibers were seen in the ventral sensory trigeminal root (s5) and labeled terminals were seen in the principal sensory trigeminal nucleus (Pr5) (Figs. 5a, 6a). In the rostral part of Pr5, the intensity of label in the ventrolateral and dorsomedial portions was fairly uniform or, in some cases, more intense in the dorsomedial portion (Fig. 5a). Rare cells in the mesencephalic trigeminal nucleus (Me5) were labeled at this level (Fig. 5a). Labeling of the motor trigeminal nucleus (Mo5), both extensive granular staining and labeling of scattered cells, also was apparent at this level (Fig. 5a). Faintly labeled fibers were identified in the motor trigeminal root (m5). Labeling of m5 and Mo5 may be due to diffusion of HRP from the injection site into adjacent muscles. The intensity of labeling of Pr5 at its caudal pole, just rostral to the facial nucleus, in most cases was moderate throughout the ventrolateral portion and faint throughout the dorsomedial portion (Fig. 6a). Heavily labeled fibers of the facial nerve (7n) appeared at this level, exiting the brain (Fig. 6a). Labeling was bilateral, but weak contralateral to the injection site.

In 2 cases (HH 27 and HH 28), rare fine-caliber fibers were labeled in more rostral sections, in the dorsal raphe nucleus, central gray matter, dorsal tegmental nucleus and predorsal bundle.

2. Level of the Spinal Trigeminal Nucleus, Pars Oralis

Labeling of primary afferent fibers continued in more caudal sections, in the ventral and lateral portions of the spinal trigeminal tract (sp5). Terminals were labeled in the most rostral division of the spinal trigeminal nucleus, subnucleus oralis (Sp5O), at the level of the facial nucleus (7) (Fig. 7a). Labeling of Sp5O was bilateral and extensive, of slight to moderate intensity, and included the dorsomedial portion, corresponding to the dorsomedial spinal trigeminal nucleus (DMSp5) distinguished by Paxinos and Watson (1986) in the rat. Labeling of Sp5O was denser in the dorsal portion, where cell density is greater. In HH 27, terminal labeling of dorsomedial Sp5O appears to overlap dorsomedially with the nucleus of the solitary tract (Sol) (Fig. 7a).

Intense bilateral labeling of cell bodies and axons of the facial nerve (7n), and granular labeling of the facial nucleus (7), were evident at this level (Fig. 7a). Labeling of the facial nucleus occurred in the lateral and intermediate portions. In addition, the reticulum of "channels" of neuropil between the trigeminal and facial nuclei was filled with a granular deposition of HRP. Labeled dendrites of neurons in the facial nucleus appeared to extend into the ventromedial part of Sp5O. A few labeled triangular or spindle-shaped

somata were noted along the ventromedial border of Sp5O.

3. Level of the Spinal Trigeminal Nucleus, Pars Interpolaris

Caudal to the nucleus of the facial nerve and rostral to the obex, at the level of the inferior olivary and external cuneate nuclei, labeling of the spinal trigeminal nucleus continued in the interpolar subnucleus of the spinal trigeminal nucleus (Sp5I) (Fig. 8a). Projections to Sp5I were faint and located in the medial or deep region of the nucleus. The spinal trigeminal tract at this level contained many labeled fibers in the ventral portion and in the dorsolateral portion, around the paratrigeminal nucleus (Pa5). At the rostral pole of Sp5I (not illustrated), labeling included the dorsal tip, corresponding to DMSp5 of the rat (Paxinos and Watson 1986). In the clearest case, HM 27, terminal labeling of this region appeared to extend dorsomedially, possibly overlapping with the lateral Sol, as at the level of Sp5O (Fig. 7a). Labeling of structures contralateral to the side of the injection was faint or absent.

At mid- to caudal-Sp5I levels, labeled terminals were noted in restricted portions of Pa5 (Figs. 8a, 9a).

4. Level of the Spinal Trigeminal Nucleus, Pars Caudalis

Labeled fibers continued caudally in the descending tract and terminal labeling of the spinal trigeminal nucleus continued at the level of subnucleus caudalis (Sp5C). At its rostral pole, Sp5C is alaminar and overlaps with Sp5I. Sp5C occupies the dorsomedial

portion, and Sp5I the ventrolateral portion, of the nucleus. Labeling of the nucleus at this level was faint and homogeneous, and restricted to the medial portion (Fig. 9a). In more caudal sections within this transition zone, Sp5C displayed a laminar organization and intense labeling of the nucleus was seen in the peripheral layers of Sp5C, the marginal and gelatinous layers, or layers I-II of the medullary dorsal horn (Fig. 10a). Terminals and rare cells were faintly labeled medially in the deepest region of the nucleus and in the adjacent reticular formation, layers V-VI, at this level (Fig. 10a). The ipsilateral tract was densely populated with labeled fibers, especially in the ventral portion (Figs. 9a, 10a); decreasing numbers of fibers were labeled at more caudal levels. Terminal labeling of Pa5 was present in the dorsolateral and ventral portions of the tract (Figs. 9a, 10a).

At about 1 mm caudal to the obex, dense ipsilateral labeling appeared in the peripheral layers of dorsolateral Sp5C, occupying the interconnecting islands of neuropil among prominent axon bundles (Fig. 7a). Presumed, weak labeling of the deep magnocellular zone, layer V, and of terminals and cell bodies in the adjacent reticular formation, layer VI, continued.

At a more caudal level, labeling of the nucleus was restricted to a thin, marginal-gelatinous "rind" in the dorsolateral portion, and labeling of the deep region was very faint (Fig. 8a). Terminal labeling appeared in the ventral part of the descending tract. These narrow clusters of cells appear to represent a ventral extension of the

Pa5.

At the level of the pyramidal decussation, labeling of the ipsilateral marginal and gelatinous layers was very dense and continuous through the dorsolateral and ventral portions (Fig. 9a). A wedge-shaped extension of labeling from the dorsal part of the margin into the deeper layers was seen. Faint presumed label extended into the magnocellular region of the nucleus from the dorsolateral and ventral margins and into the dorsal medullary reticular field.

At the most caudal level of Sp5C, labeling was sparsely and evenly distributed in the ventral and, more prominently, dorsolateral portions, in the marginal and gelatinous layers. In HM 16, however, the most caudal label was restricted to the ventral portion. In HM 26, labeling at this level was restricted to the marginal layer. These variations may relate to slight variability in location of the injection. The dorsomedial portion of the laminated nucleus, at all levels, bore little or no tracer.

Labeling of structures contralateral to the side of the injection was present in all cases. This generally did not extend as far caudally, first appearing at the level of the pyramidal decussation as terminal labeling of the marginal and gelatinous layers of the ventral portion of the subnucleus (Fig. 9a). At more rostral levels, weak contralateral labeling was present in the periphery of the dorsolateral portion of Sp5C, the dorsal medullary reticular field, Pa5, and fibers of the spinal trigeminal tract, mirroring but less intense than the ipsilateral projections.

5. Cervical Spinal Cord

In some cases, projections extended into the dorsal horn of the first cervical segment. The pattern of labeling of Sp5C in the caudal medulla was continued in laminae I and II at this level (Fig. 10a).

B. EXPERIMENTS ON RATS

Somata of the trigeminal ganglia and fibers of the trigeminal sensory roots (s5) entering the pons and continuing in the spinal trigeminal tract (sp5), were labeled. Primary afferent fibers were traced to terminals in the principal sensory trigeminal nucleus (Pr5), all levels of the spinal trigeminal nucleus, and to rare cells in the mesencephalic trigeminal nucleus (Me5). Labeling of the paratrigeminal nucleus (Pa5) was absent in two cases and very faint in the third. Fibers and cells of the motor trigeminal root (m5) and nucleus (Mo5) and of the facial nerve root (7n) and nucleus (7) were retrogradely labeled. (Figs. 5b-14b)

1. Level of the Principal Sensory Trigeminal Nucleus

At its rostral pole, Pr5 was labeled homogeneously. At a more caudal level, ipsilateral Pr5 was labeled with moderate intensity in the ventrolateral subdivision and faintly in the dorsal portion (Fig. 5b). Rare cells were labeled in Me5 (Fig. 5b). Bilateral labeling of fibers of the motor root and terminals and scattered cells of Mo5 were

seen at these levels. Labeling of Pr5 at its caudal pole was weak and somewhat concentrated in the ventromedial portion (Fig. 6b). Labeled fibers were distinct throughout the sensory root, particularly the ventral part, and in the exiting facial nerve, at this level. Labeling was bilateral but weak on the contralateral side.

2. Level of the Spinal Trigeminal Nucleus, Pars Oralis

Labeling of the trigeminal brainstem sensory complex continued caudally into the spinal trigeminal tract and nucleus. At the level of the facial nucleus, Sp5O was faintly labeled (Fig. 7b). The ventral and lateral portions of the trigeminal tract were filled with labeled fibers. Densely labeled perikarya appeared in the lateral and intermediate zones of the facial nucleus (7). Dendritic processes of labeled cells in lateral 7 extended into the ventromedial portion of Sp5O, as seen in the muskrat.

3. Level of the Spinal Trigeminal, Pars Interpolaris

Caudal to the facial nucleus, the ventral and lateral portions of the spinal trigeminal tract continued to be filled with labeled fibers, and labeling of the spinal trigeminal nucleus continued in Sp5I. Labeling was faint in the ventral portion of the nucleus and slightly concentrated at the ventromedial apex (Fig. 8b). Labeling was bilateral, but weak on the contralateral side. Caudally, at the level of the obex, ipsilateral Pa5 was faintly labeled in the dorsal trigeminal tract in HR 4 (Fig. 9b), but not in the other two cases.

4. Level of the Spinal Trigeminal Nucleus, Pars Caudalis

Caudal to the obex, labeling of the spinal trigeminal tract and nucleus continued. Fibers were labeled in the ventral part of the tract and terminals were labeled throughout the rostrocaudal extent of Sp5C. At its rostral extent, where Sp5I occupies the ventrolateral portion of the nucleus, intense labeling occurred within the periphery of Sp5C, which occupied the ventromedial portion of the nucleus (Fig. 10b). Labeling of Pa5 in the dorsal part of the tract was absent or very faint.

More caudally, just above the level of the pyramidal decussation, labeling was present in the ventral and dorsolateral portions of the marginal and gelatinous layers of Sp5C, or laminae I-II of the medullary dorsal horn (Fig. 11b). At the level of the pyramidal decussation, labeling continued in the marginal and gelatinous layers of the dorsolateral and ventral portions of the nucleus (Fig. 12b). Labeling at these levels was bilateral, but weak on the contralateral side.

Caudal to the level of the pyramidal decussation, bilateral labeling of the peripheral layers of the nucleus continued (Fig. 13b). Bilateral labeling of the ventral and dorsolateral portions of the superficial layers of Sp5C continued to the caudal pole of the nucleus, with a gap in labeling between these two portions (Fig. 14b).

5. Cervical Spinal Cord

The caudal extent of labeling varied among the three cases. In HR 4, HRP-bearing terminals were evident in the second cervical segment (C2) in laminae I and II of the dorsal horn and medially in lamina III, ipsilateral to the side of the injection. Label extended to C2 in HR 6, and to the caudal medulla in HR 5.

II. PROJECTIONS FROM THE SOFT PALATE

Projections from the soft palate were investigated in 3 muskrats. Cells of the trigeminal ganglion and fibers of the sensory root and spinal tract were labeled. Primary afferent fibers were traced to the principal sensory trigeminal, spinal trigeminal, mesencephalic trigeminal, supratrigeminal, intertrigeminal and paratrigeminal nuclei. Retrograde labeling was seen in the motor roots and nuclei of the trigeminal, facial and hypoglossal nerves, and in ventral horn cells of the cervical spinal cord. Glossopharyngeal and/or vagal fibers also were labeled. Projections were traced to the solitary tract and nucleus, the retroambiguus and ambiguus nuclei, and periambigual area. (Figs. 5c-14c)

A. Level of the Principal Sensory Trigeminal Nucleus

Labeled fibers appeared in the trigeminal sensory roots (s5) and labeled terminals appeared in Pr5. In rostral Pr5, labeling was relatively intense in the dorsomedial portion and appeared in regularly

arranged patches in the ventrolateral division (Fig. 5c). In some cases, label was particularly intense at several points around the margin of the dorsomedial subdivision. At the caudal pole of Pr5, lateral to the exiting facial nerve (7n), terminal labeling was faint and restricted within the dorsomedial subdivision, where a few faintly labeled triangular or multipolar neurons also appeared (Fig. 6c).

Granular labeling of the intertrigeminal (I5) and supratrigeminal (Su5) nuclei, and labeling of cell bodies of Me5 appeared at this level (Fig. 5c). Adjacent to the caudal pole of Pr5, fibers of the facial nerve root were labeled (Fig. 6c). Labeling at the level of Pr5 was bilateral, but weak on the contralateral side. Labeled fibers were present in the trigeminal motor root, cells and processes of Mo5 were intensely labeled, and granular label appeared throughout Mo5 (Fig. 5c).

B. Level of the Spinal Trigeminal Nucleus, Pars Oralis

Labeling of primary afferent fibers continued to more caudal levels, in the dorsolateral part of the spinal trigeminal tract. Terminal labeling continued caudally into the spinal trigeminal nucleus in Sp5O, coincident with the facial nucleus (Fig. 7c). Labeling of Sp5O was extensive and more intense in the dorsal portion. In the caudal part of this region, labeling of the dorsomedial spinal trigeminal nucleus appears to overlap with the lateral nucleus of the solitary tract (Sol). Fibers were labeled in the solitary tract (sol). In Sol, granular labeling was apparent and rare cells, both small oval

and large multipolar, were labeled. Labeled fibers of the glossopharyngeal and/or vagal nerves were seen traversing the lateral brainstem.

Labeling of nucleus ambiguus (Amb) and the periambigual area was intense and extensive. Large multipolar cells, smaller cells in the ventral portion, and processes were intensely labeled. Granular labeling occurred in and around Amb. Labeled fibers extended between Amb and the Sol, and multipolar cells within this region were faintly labeled.

In the rostral third of the facial nucleus (7), cells of the dorso-intermediate subnucleus were intensely labeled (Fig. 7c). This subnucleus is proximal to Amb and processes of cells in each nucleus appear to extend to the other. In one case, cells of the dorsomedial and ventromedial subdivision of the facial nucleus also were labeled. Labeling of all structures was bilateral, but was weak on the contra-lateral side.

C. Level of the Spinal Trigeminal Nucleus, Pars Interpolaris

Caudal to the facial nucleus and rostral to the obex, labeled fibers continued to appear in the dorsal part of the spinal trigeminal tract and labeling of the spinal trigeminal nucleus continued in Sp5I (Fig. 8c). Labeling of Sp5I was diffuse, faint to moderate on the ipsilateral side and faint on the contralateral side, throughout its rostrocaudal extent. Near the obex, in the dorsal part of the descending tract, labeled terminals of Pa5 appeared (Fig. 8c).

The hypoglossal root and nucleus (12) were labeled through their oral extent. The ambigual and periambigual areas were intensely labeled throughout this level. In HM 21, a few large multipolar cells in the intermediate reticular nucleus, dorsomedial to the linear nucleus of the medulla, were labeled (Fig. 8c). Fibers in the solitary tract (sol) were labeled and granular labeling of Sol appeared through its rostral pole. Labeling of Sol was seen both medial and lateral to the solitary tract. A few small, oval cells medial to the solitary tract were labeled (not illustrated). Some labeled cells may be included in the intercalated nucleus of the medulla. Larger triangular cells in the reticular formation, within or adjacent to ventromedial Sol were faintly labeled.

Fascicles of labeled fibers of the glossopharyngeal and/or vagal nerves were prominent as they cross the inferior cerebellar peduncle, spinal trigeminal tract and nucleus, and dorsal reticular formation. Some appeared to turn ventrally and enter Amb. Labeled fibers in more dorsal fascicles entered the region of the solitary tract and its nucleus.

D. Level of the Spinal Trigeminal Nucleus, Pars Caudalis

Caudal to the obex, labeling continued in the descending tract and throughout the rostrocaudal extent of Sp5C. Just caudal to the obex, where the caudal pole of Sp5I occupied the ventrolateral portion of the nucleus, diffuse labeling was restricted to the deep part of dorsomedial portion of the nucleus occupied by Sp5C (Fig. 9c). The

paratrigeminal nucleus, which occupies much of the dorsolateral trigeminal tract at this level, was intensely labeled. Terminal labeling was also seen in the ventral tail of the tract, in the ventral part of Pa5. Fibers were labeled in the dorsolateral part of the tract.

At a more caudal level, where Sp5I still occupies the ventrolateral margin of the nucleus, intense labeling of the nucleus followed the periphery of Sp5C (Fig. 10c). Labeling of the medial tip of the nucleus at this level was continuous with more medial labeling, ventral to the external cuneate nucleus. Labeling of fibers continued in the dorsal part of the tract. Terminal labeling continued in the dorsal and ventral parts of the tract, in Pa5.

Just rostral to the level of the pyramidal decussation, labeling was seen along the dorsal edge of Sp5C and in the extensions of neuropil from this region into the descending tract (Fig. 11c). At the level of the pyramidal decussation, fibers were faintly labeled in the dorsal trigeminal tract. Adjacent to it, terminals were labeled in the marginal layer of the mid-dorsal portion of Sp5C (Figs. 12c, 13c). In HH 21, terminal labeling also extended into the gelatinous and magnocellular layers, in a narrow band across the short axis of the nucleus (Fig. 13c).

At these levels, bilateral labeling of somata of the dorsal and ventrolateral subdivisions of the hypoglossal nucleus, and of fibers of the hypoglossal nerve, was seen. Granular labeling was present in the hypoglossal nucleus, Sol, and in the reticular formation extending between Sol and the medial aspect of Sp5C (Fig. 9c). Rostral to the

level of the pyramidal decussation, labeled cells and granular labeling appeared in the retroambiguus nucleus, Amb and the periambigual area. Near the obex, labeled fibers of the glossopharyngeal and/or vagal nerves appeared, crossing the spinal trigeminal nucleus and tract, and dorsal reticular formation (Fig. 9c). Fibers were labeled in the solitary tract.

E. Cervical Spinal Cord

Sensory projections continued into the cervical spinal cord. Terminals of lamina I of the ipsilateral dorsal horn, and fibers turning inward from the Zone of Lissauer immediately dorsal to it, were labeled (Fig. 14c). These projections extended to 4.7 mm below the obex in HH 20, in C1, and to 5.8 mm below the obex in HH 21, in C2.

Neurons of the lamina IX motor nuclei of the cervical spinal cord were also labeled bilaterally. In HH 21, this occurred as far caudally as C6, in a medial ventral horn nucleus containing large, elongate cells with long dendrites. Rostrally, from C4, additional lamina IX nuclei in the ventral apex of the ventral horn were labeled. These contained very large, multipolar cells with long dendrites. In the upper cervical segments, a few cells in the lateral lamina IX nuclei were labeled, and labeled processes extended medially into the ventral gray commissure (Fig. 14c).

Sensory projections also extended caudally into the cervical spinal cord: in HH 20 at 4.7 mm below the obex, in C1; and in HH 21 at 5.8 mm below the obex, in C2. Terminals of lamina I of the right

dorsal horn, and fibers turning inward from the Zone of Lissauer immediately dorsal to it, were labeled (Fig. 14c).

III. PROJECTIONS FROM THE POSTERIOR PHARYNX AND LARYNX

Projections were traced from the larynx in one muskrat (HM 30) and from the posterior pharynx in one (HM 22). Labeling was bilateral and very weak in comparison to the other experimental groups. Fiber labeling was absent. Terminal labeling was seen in the spinal trigeminal nucleus, paratrigeminal nucleus and nucleus of the solitary tract. Cells were labeled in the motor trigeminal nucleus, facial nucleus, hypoglossal nucleus and nucleus ambiguus. (Figs. 5d,e-14d,e)

A. Level of the Principal Sensory Trigeminal Nucleus

No labeling of rostral Pr5 was observed in either case (Figs. 5d,e). Caudally, just above the level of the exiting facial nerve root, very faint and diffuse questionable labeling of Pr5 in HM 30, and no labeling of Pr5 in HM 22, was observed (Figs. 6d,e). Rare cells of Me5 were labeled in HM 22 (Fig. 5d). Cells of Mo5 were labeled in both cases (Figs. 5d,e). Large multipolar cells in the reticular formation, just medial to the exiting facial nerve, were also labeled (Figs. 6d,e). No clear labeling of fibers was evident in the sensory or motor trigeminal roots.

B. Level of the Spinal Trigeminal Nucleus, Pars Oralis

At the level of the facial nucleus, faint questionable staining of Sp5O was seen in both cases (Figs. 7d,e). Faint granular staining of Sol was also present in both cases. In HM 22, cells were labeled in Sol (Fig. 7d). In both cases, the granular staining extended medially from Sol to the dorsomedial spinal trigeminal nucleus. Both cases exhibited labeling of cells in Amb. Cells of the medial facial nucleus were labeled in HM 22. Cells labeled in the ventral medulla at this level in HM 30 occurred dorsal to the facial nucleus, in a position corresponding to that of Amb at more caudal levels. These are large multipolar cells which have long dendrites with a mediolateral orientation. No clear labeling of fibers, in the spinal trigeminal tract or facial, glossopharyngeal or vagal nerve roots was apparent at this or more caudal levels.

C. Level of the Spinal Trigeminal Nucleus, Pars Interpolaris

Below the level of the facial nucleus and above the level of the obex, faint questionable staining of Sp5I was seen in both cases (Figs. 8d,e). Labeling of Pa5 in the dorsolateral trigeminal tract was seen in HM 22 (Fig. 8d). Cells and processes of Amb were clearly labeled, and granular staining was observed in this nucleus and in the periambigual area, in both cases. Granular labeling was present in Sol in both cases, and rare cells were labeled in the ventrolateral portion of this nucleus in HM 22 (not illustrated). Labeled cells were seen in the hypoglossal nucleus, in both cases.

D. Level of the Spinal Trigeminal Nucleus, Pars Caudalis

No labeling of the spinal trigeminal nucleus caudal to the level of the obex was visible in either case. In both cases, labeling of terminals in Pa5 in the dorsolateral spinal trigeminal tract was seen at this level (Figs. 9d,e, 10e). In HM 22, terminal labeling was identified in Sol, medial and lateral to the solitary tract (Fig. 10d). Labeling of cells and granular labeling in the retroambiguus nucleus and its continuation as Aab was seen as far caudally as the pyramids (Figs. 9e, 10e). At the level of the pyramidal decussation, cells of the hypoglossal nucleus were labeled in both cases (Figs. 10d,e).

E. Cervical Spinal Cord

As in soft palate-injected animals, labeled cells were located bilaterally in the ventral horn of the upper cervical segments, in the lamina IX motor nuclei. Cells were labeled in medial, ventral and lateral nuclei of lamina IX in HM 30, and medial and ventral nuclei in HM 22.

Fig. 5. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

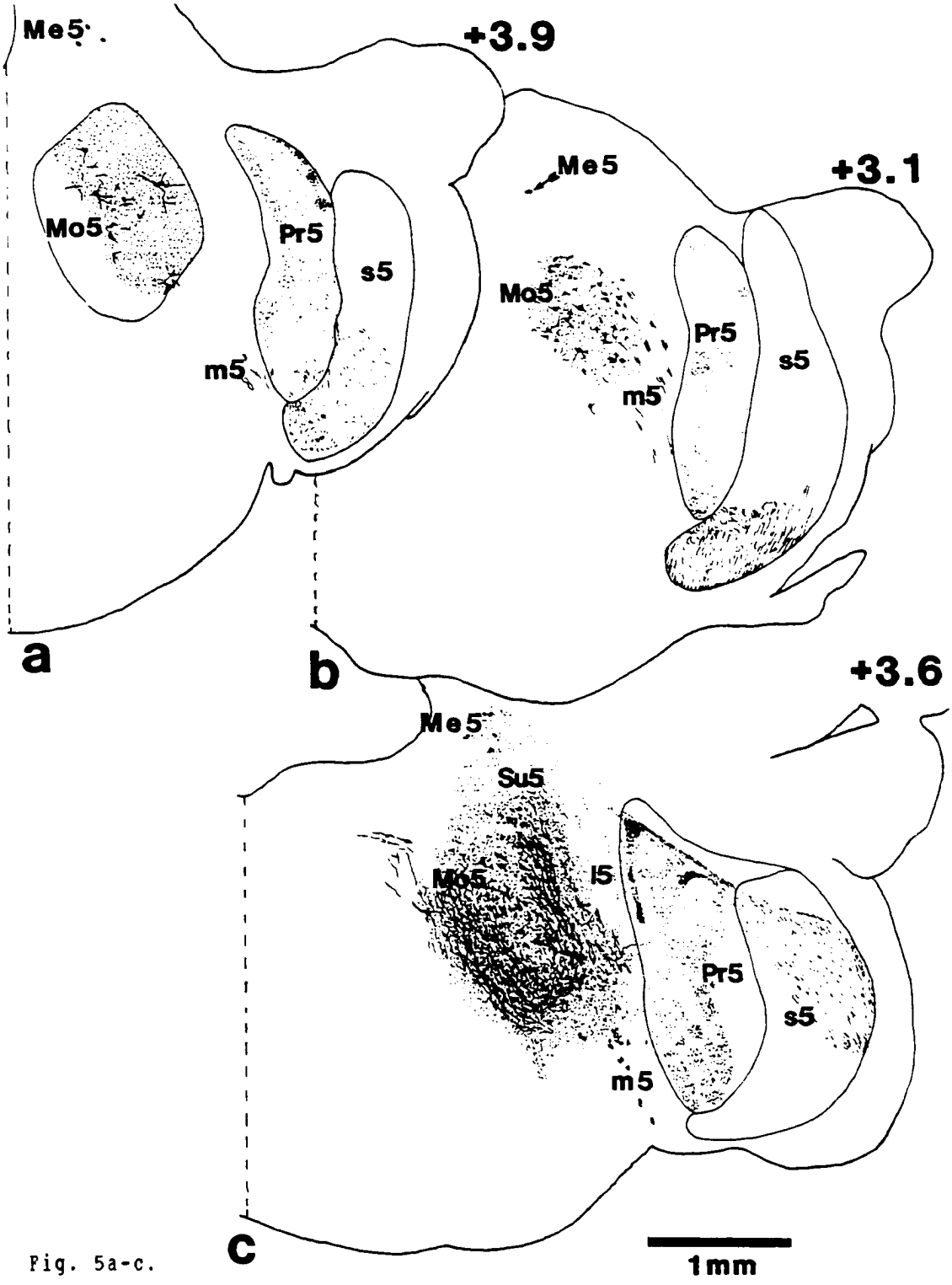

Fig. 5a-c.

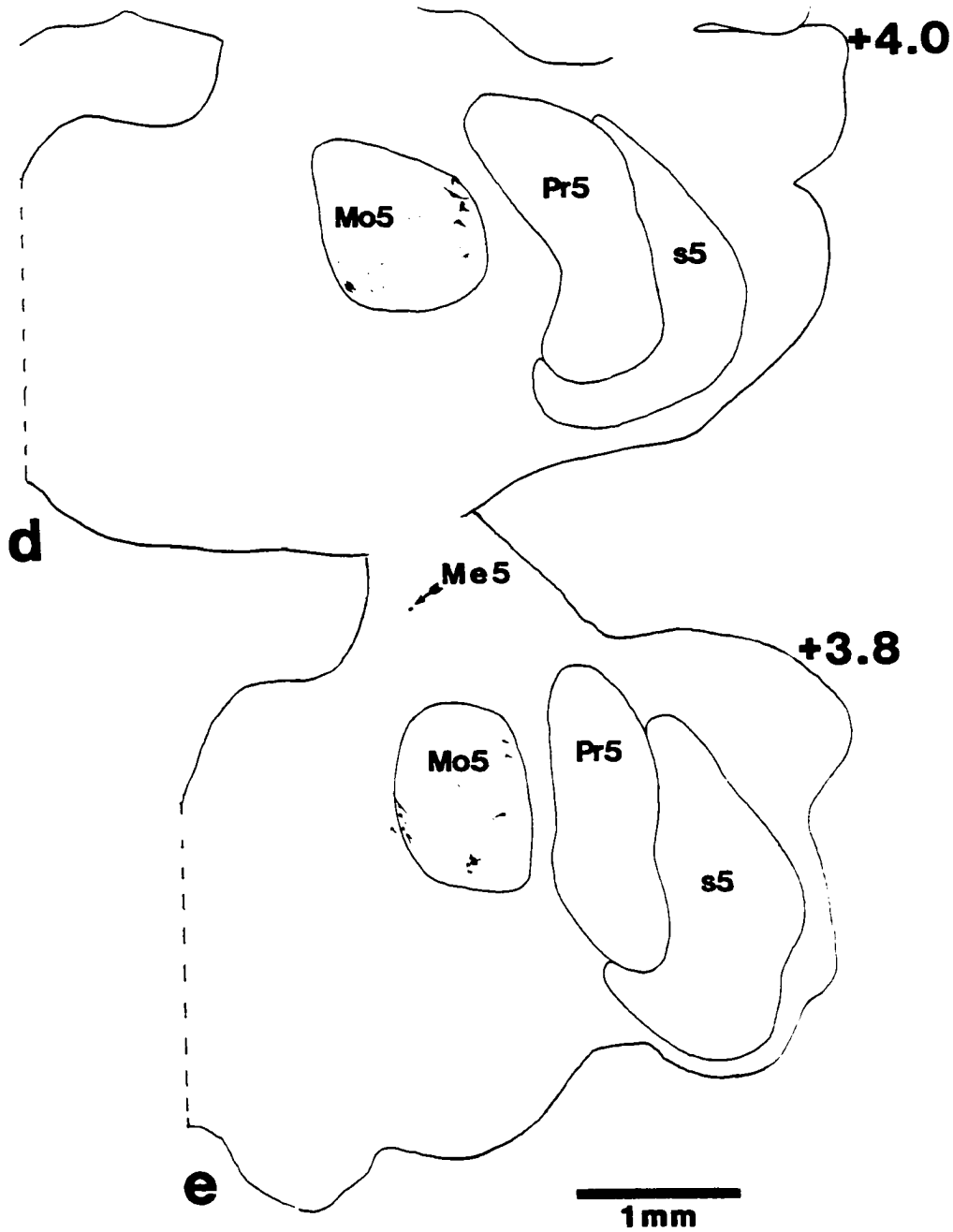

Fig. 5d,e.

Fig. 6. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

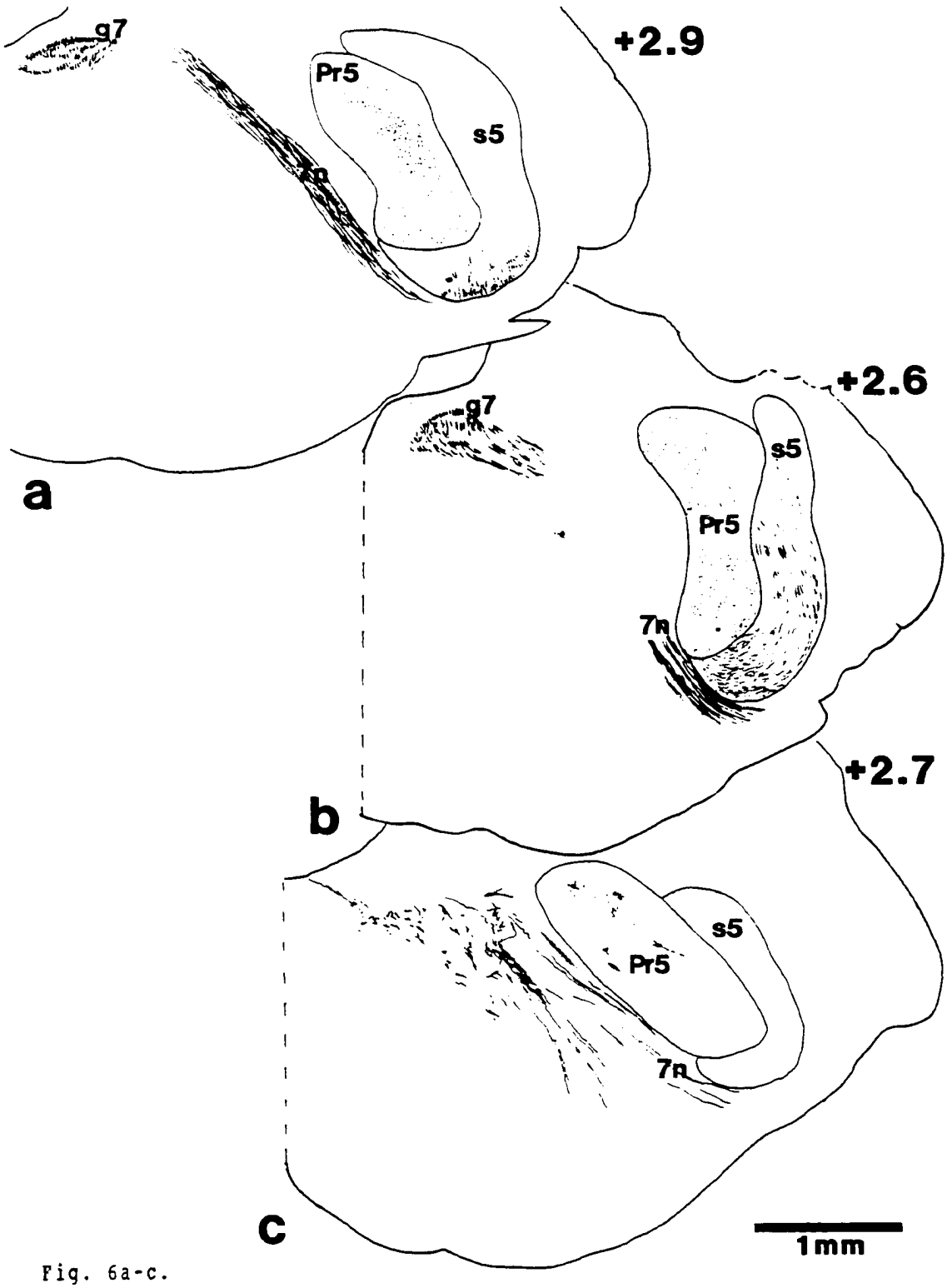

Fig. 6a-c.

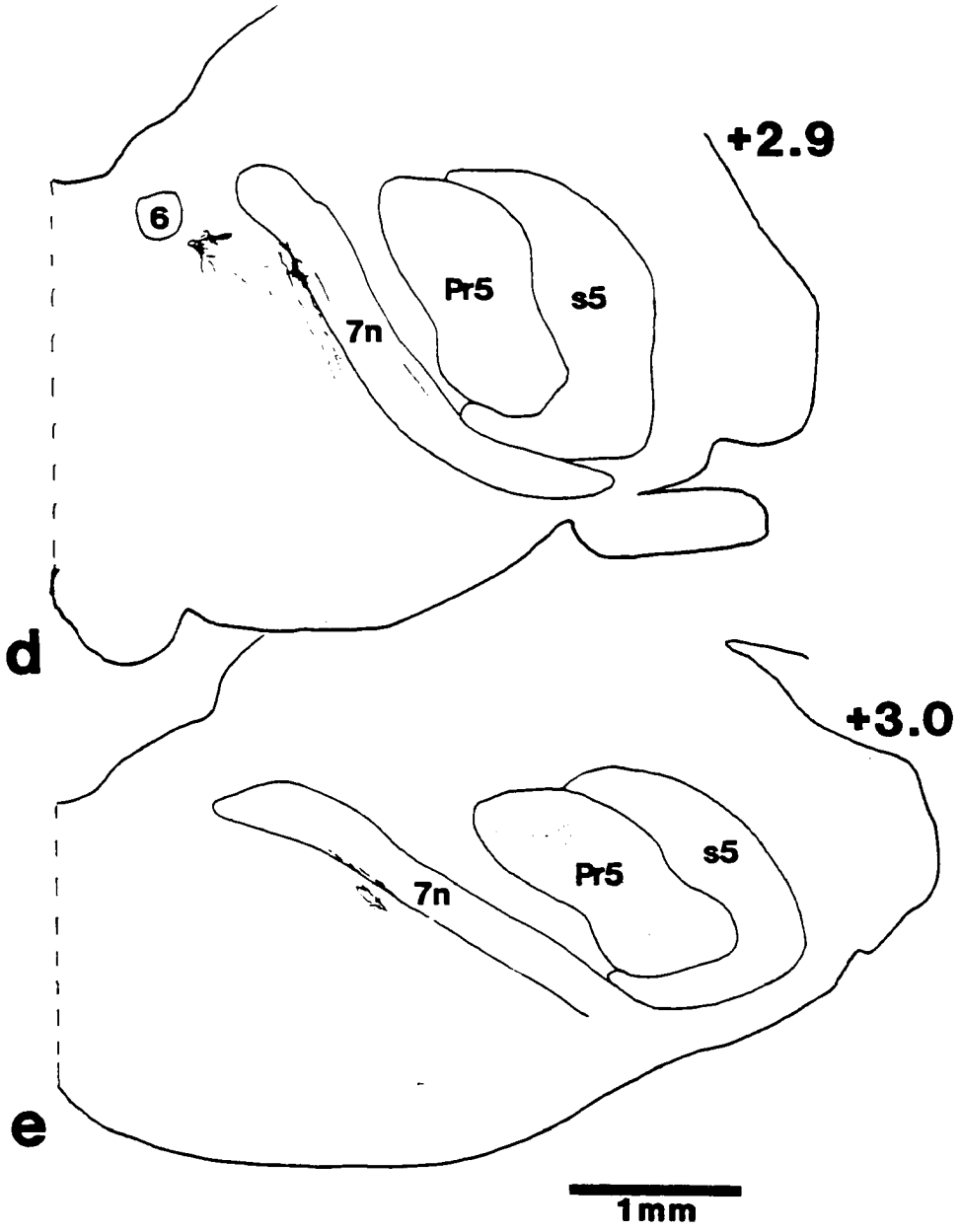

Fig. 6d,e.

Fig. 7. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

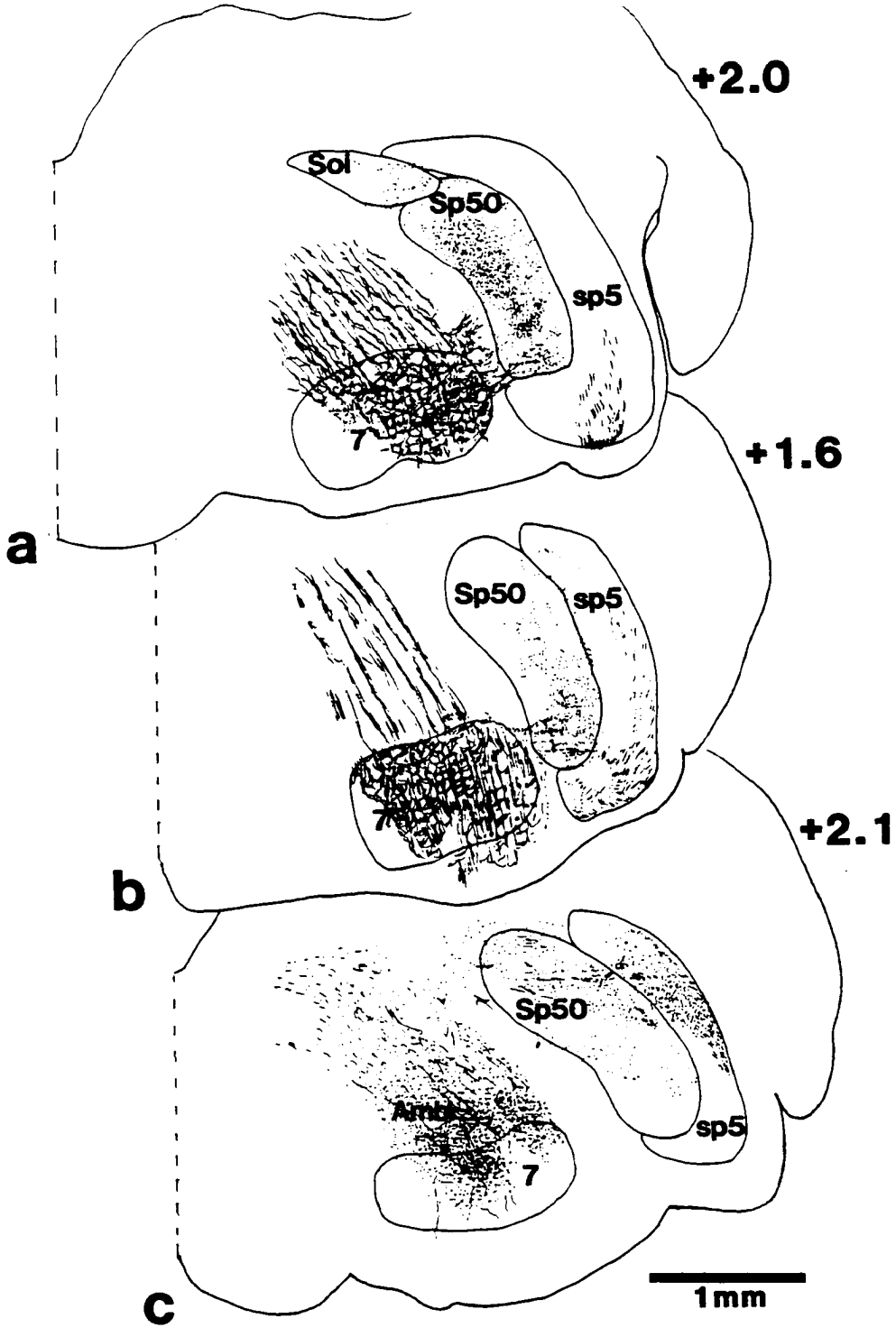

Fig. 7a-c.

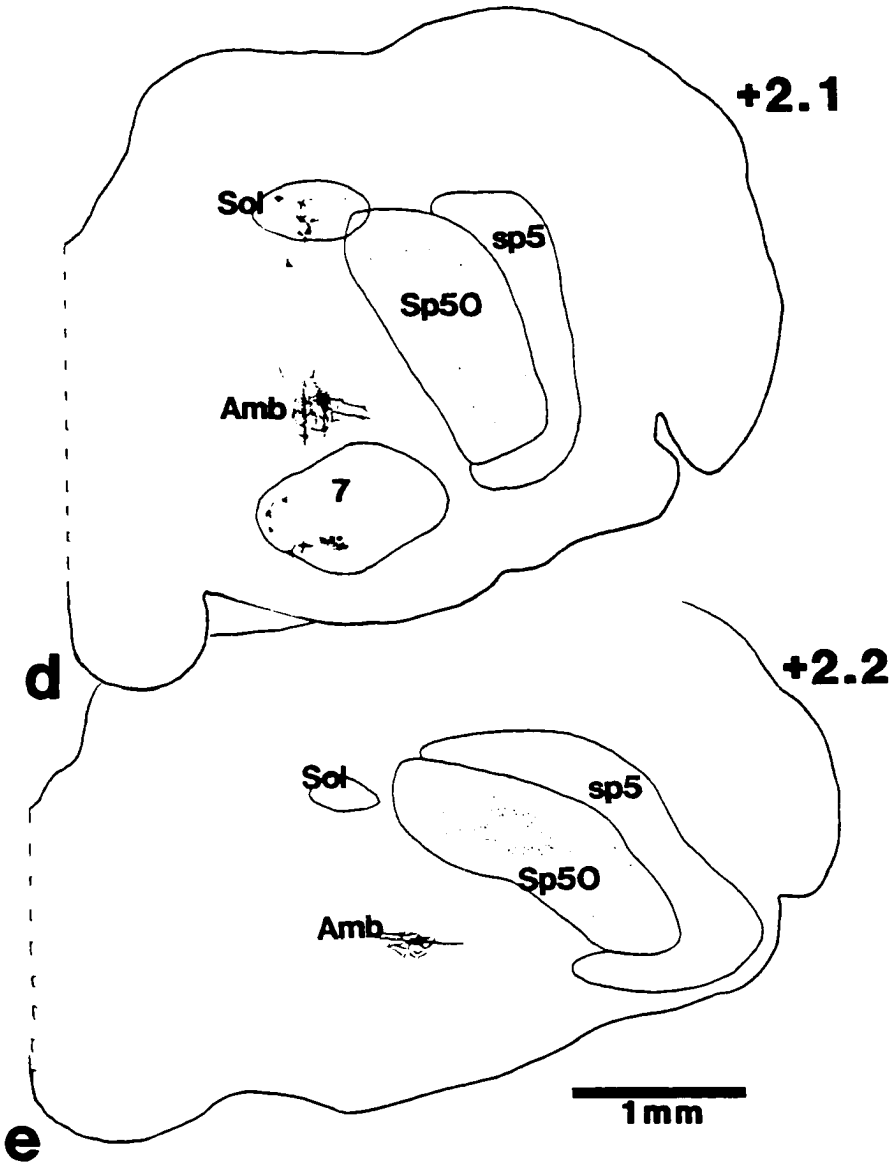

Fig. 7d,e.

Fig. 8. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

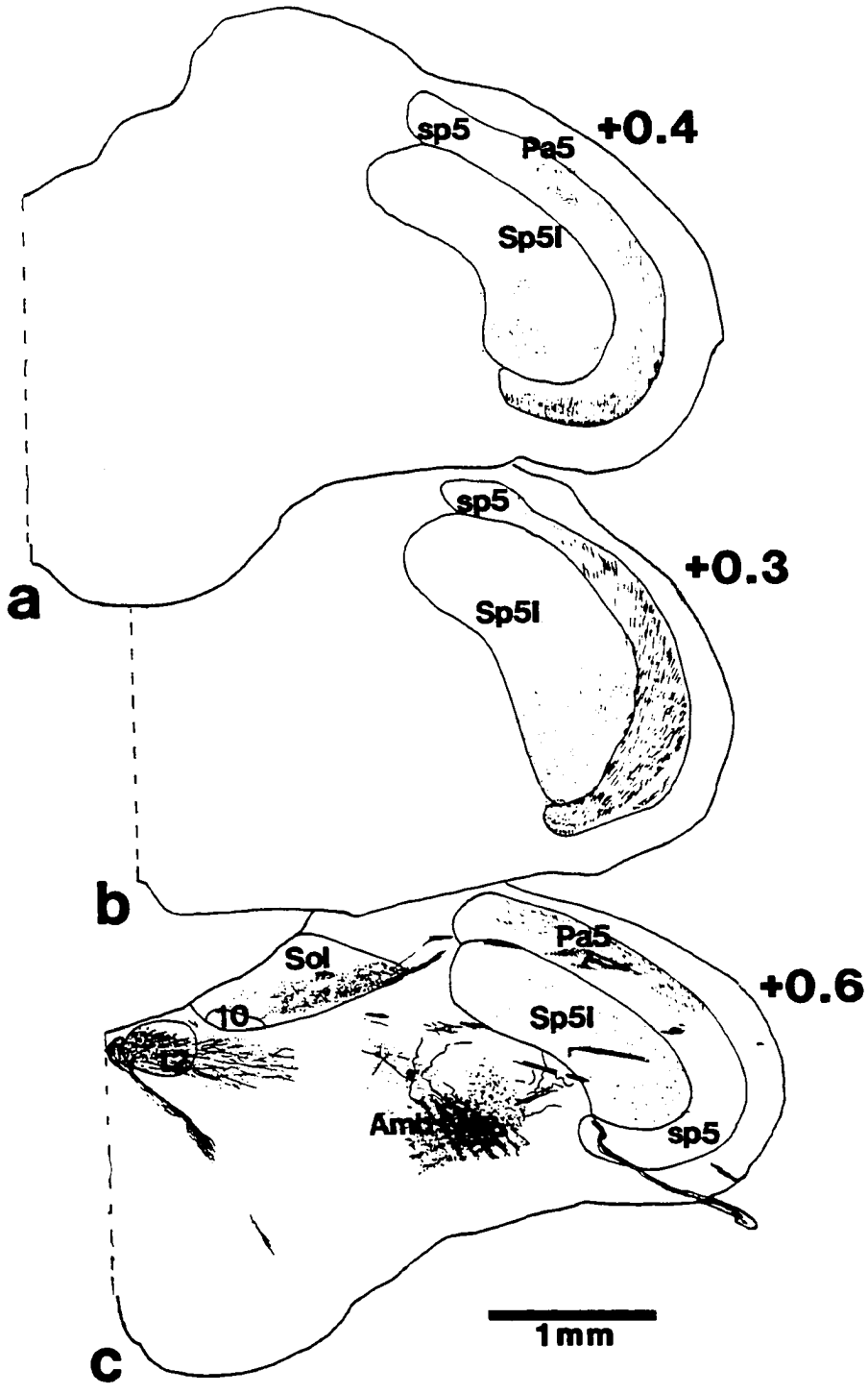

Fig. 8a-c.

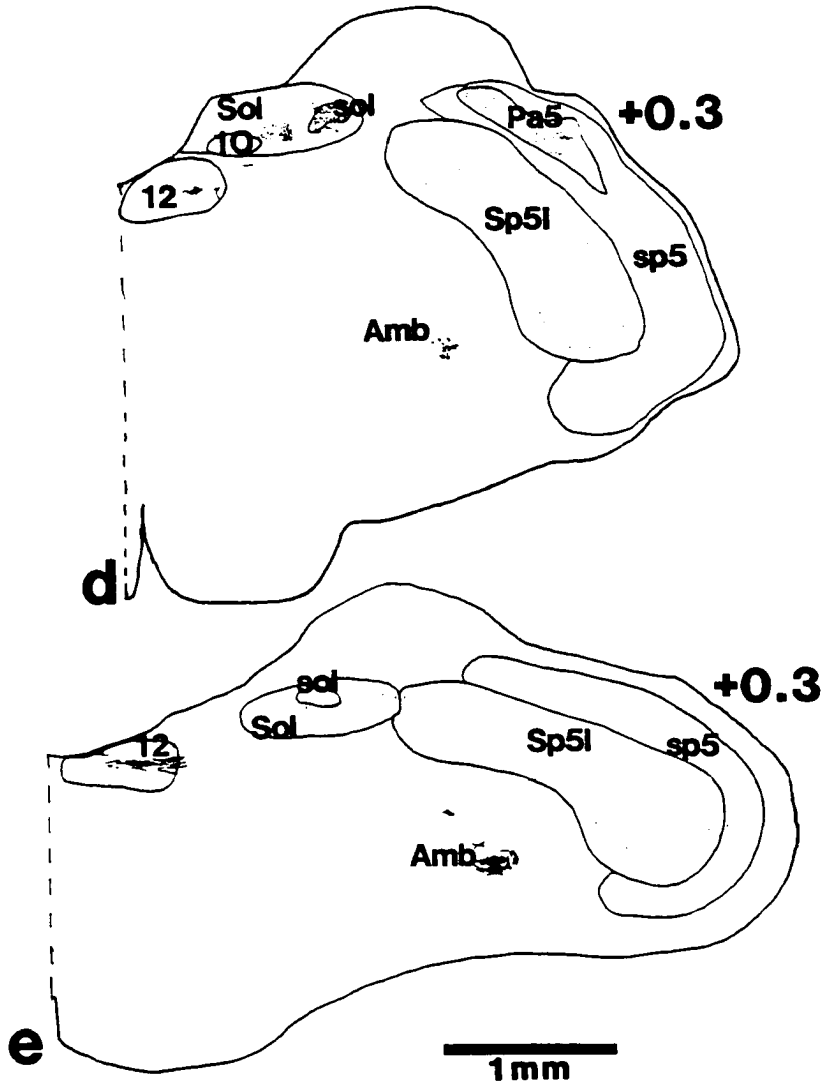

Fig. 8d,e.

Fig. 9. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

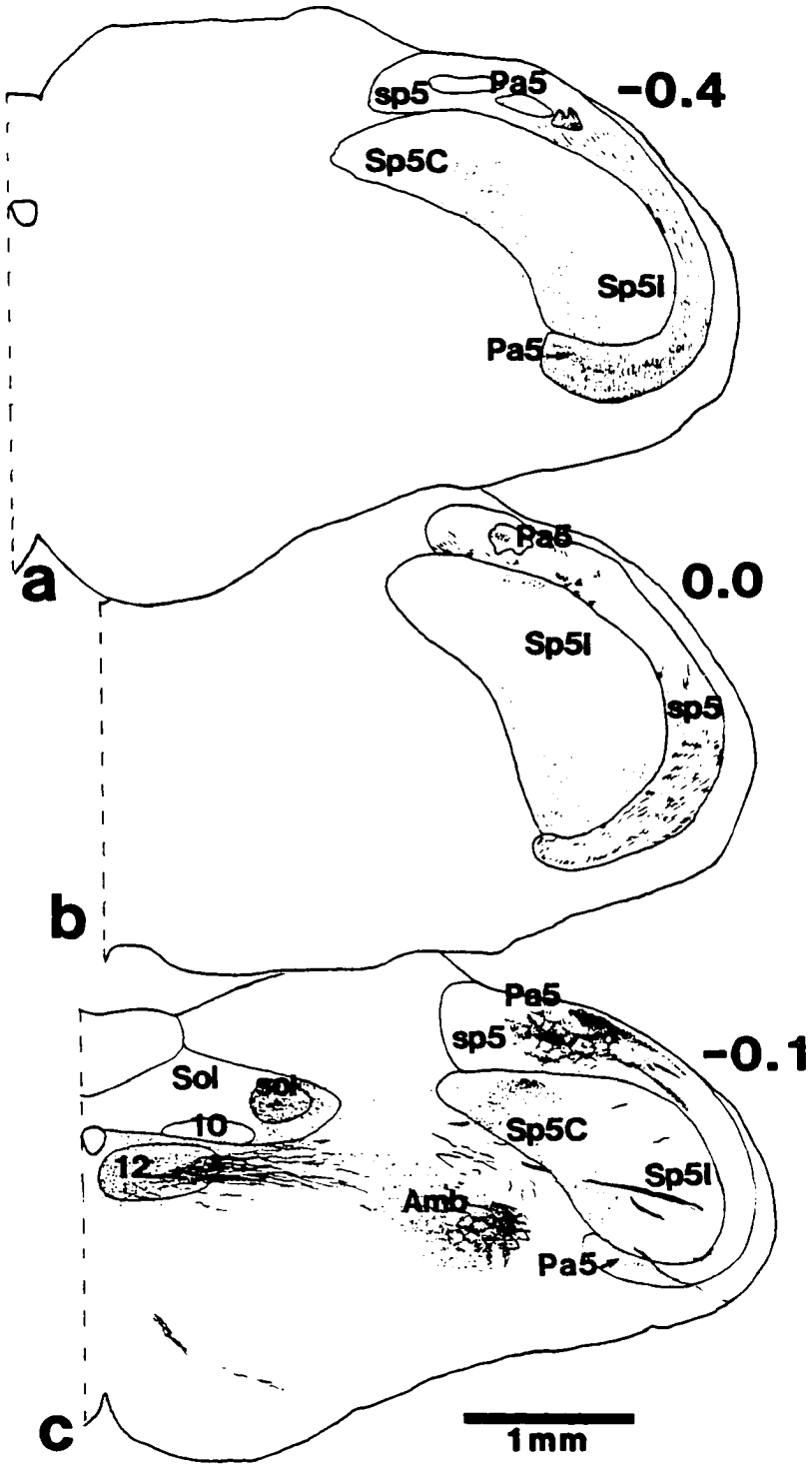

Fig. 9a-c.

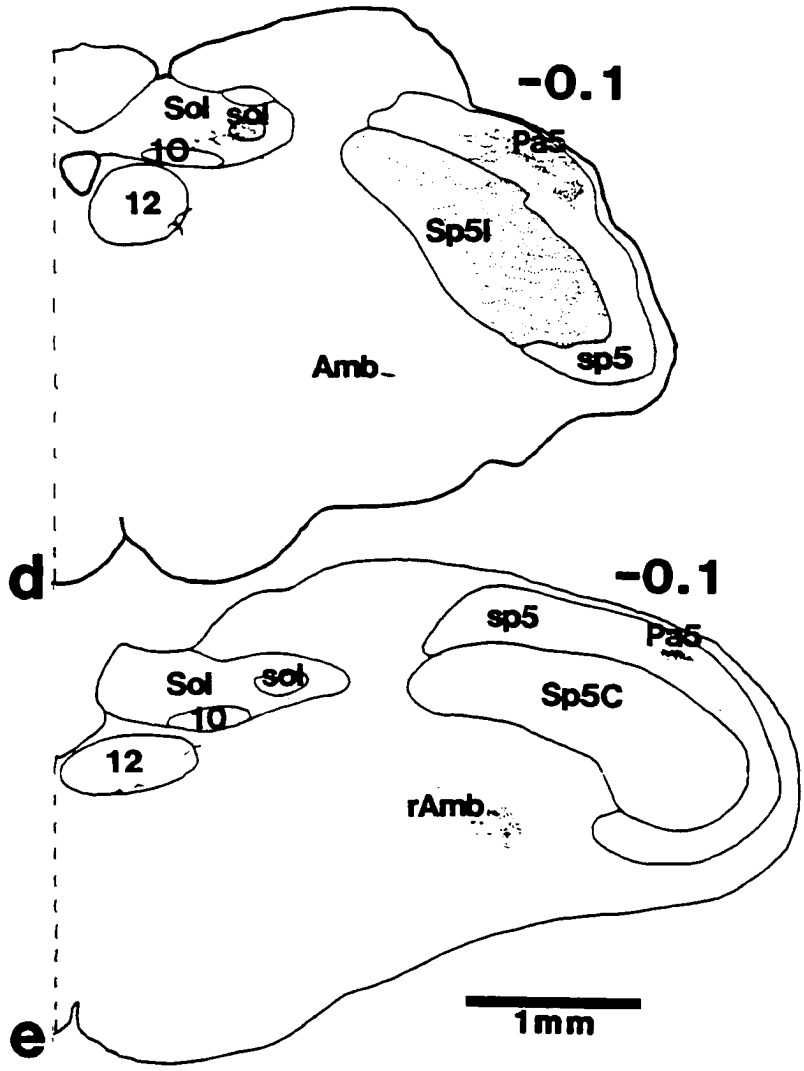

Fig. 9d,e.

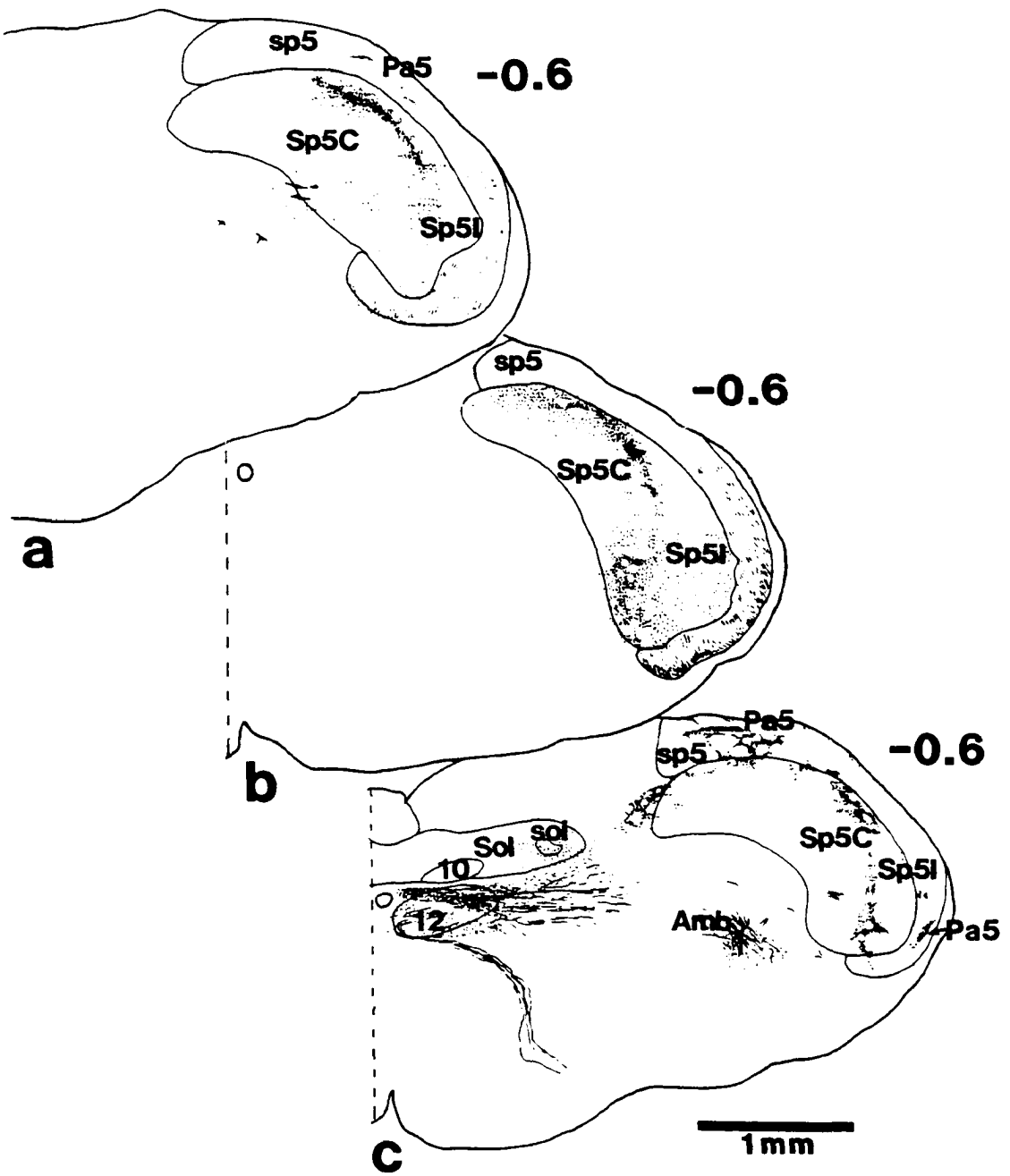

Fig. 10. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat, (d) posterior pharynx of the muskrat, and (e) larynx of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

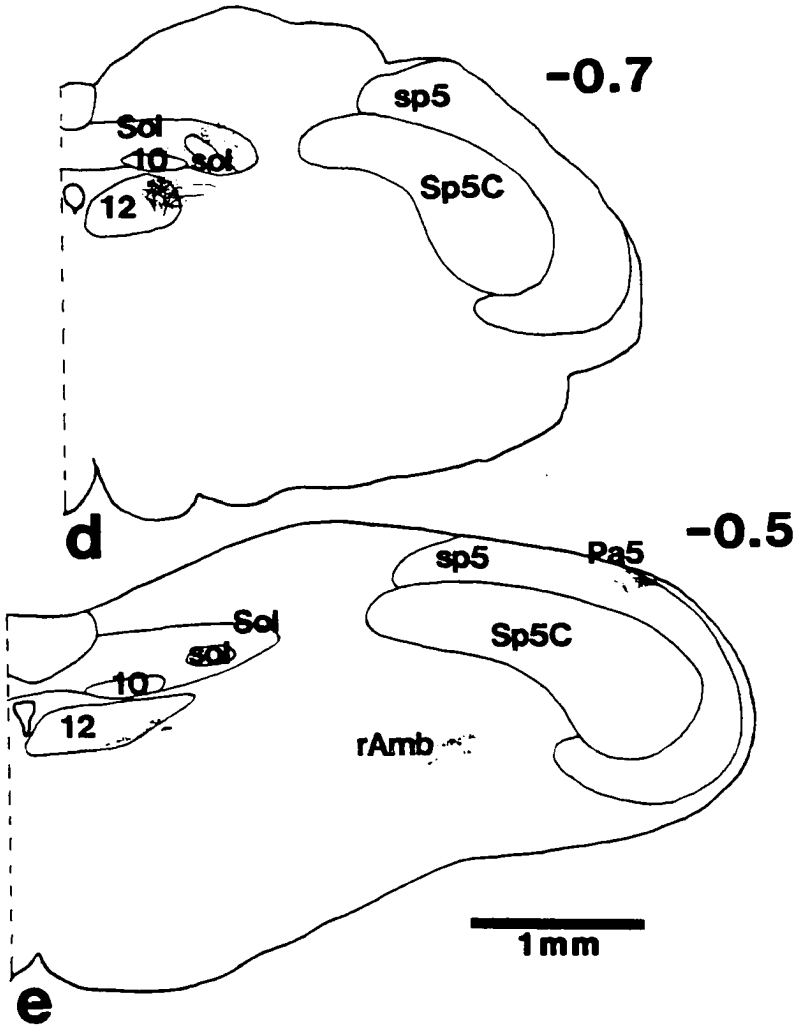

Fig. 10d,e.

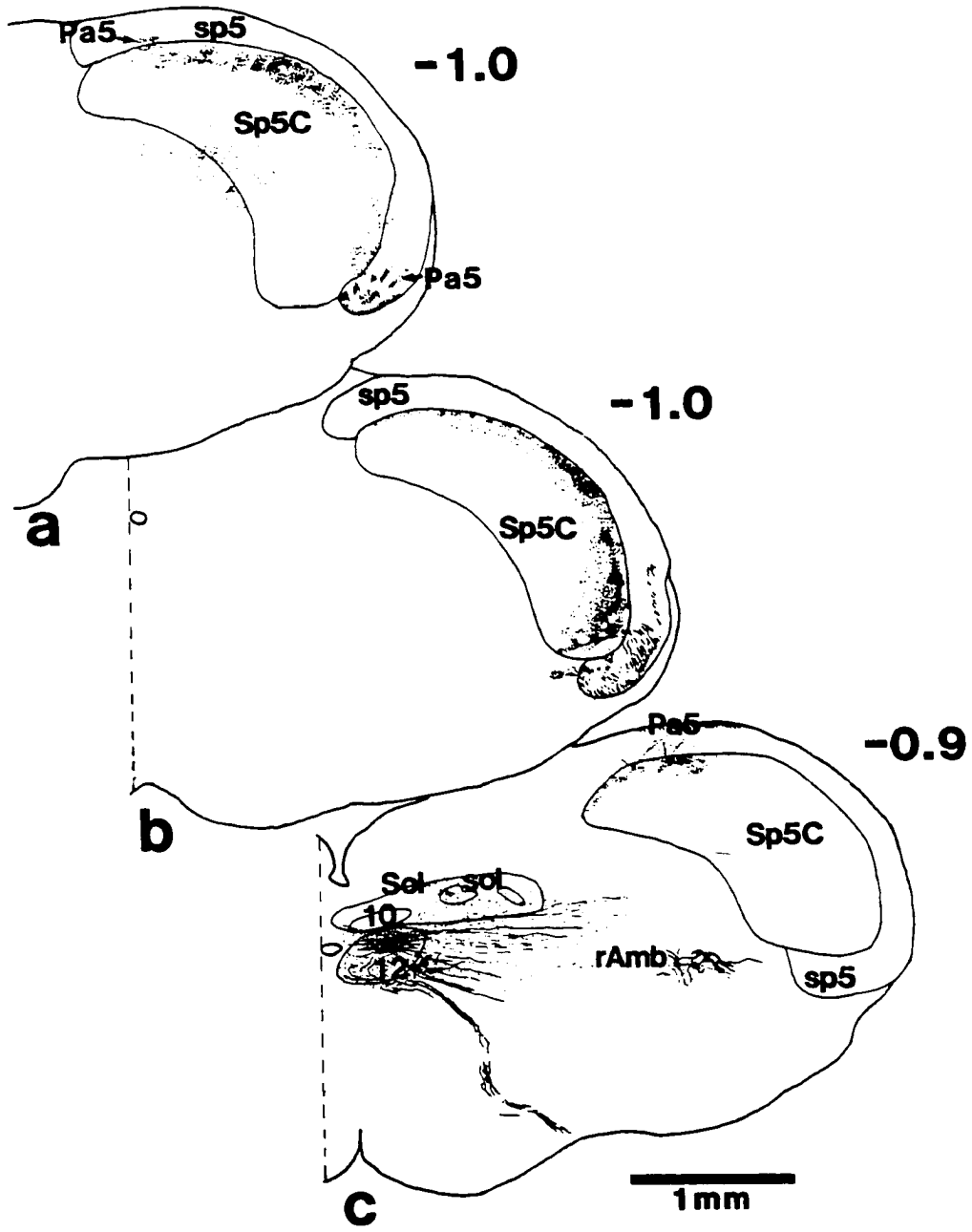

Fig. 11. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

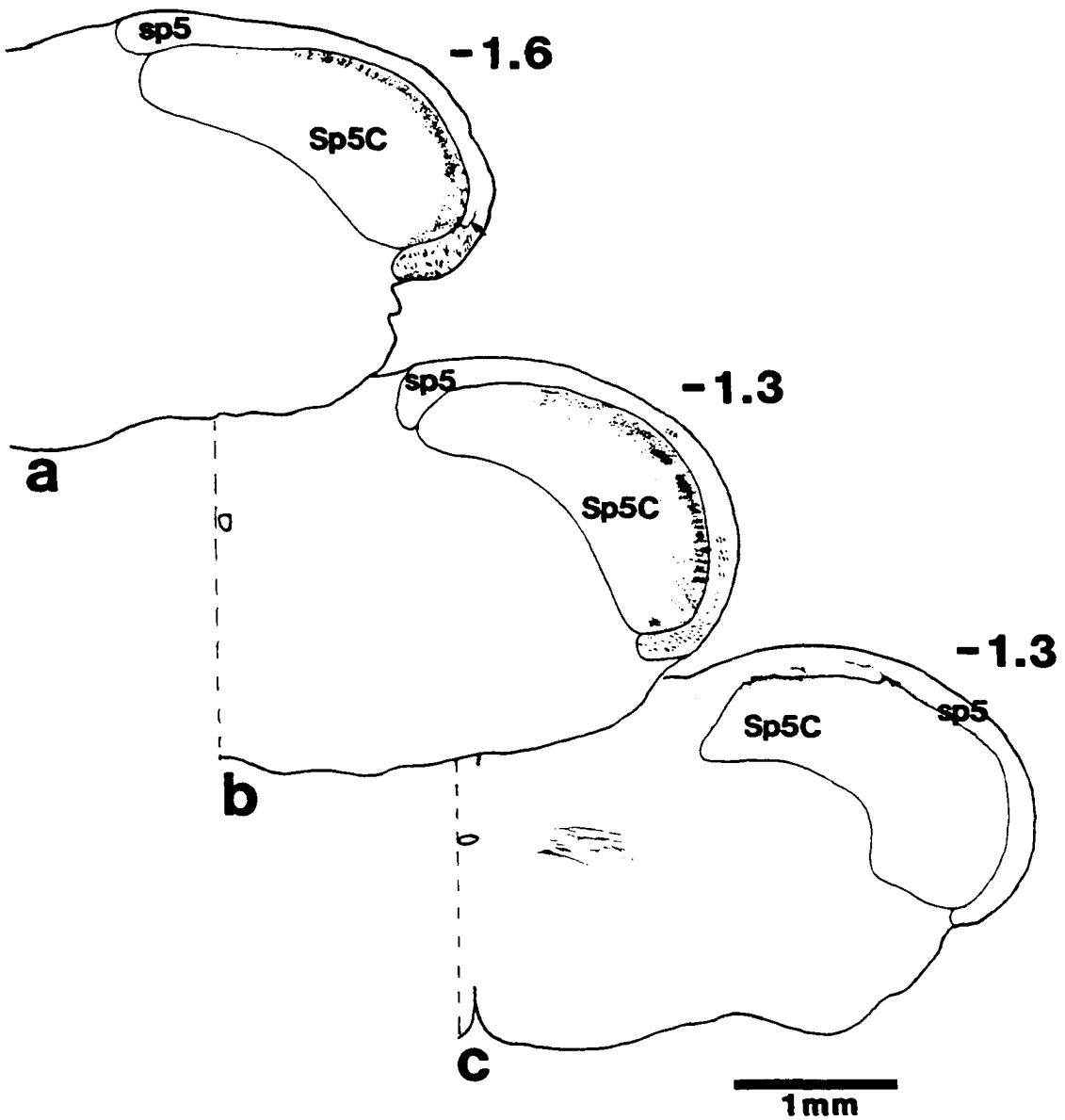

Fig. 12. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

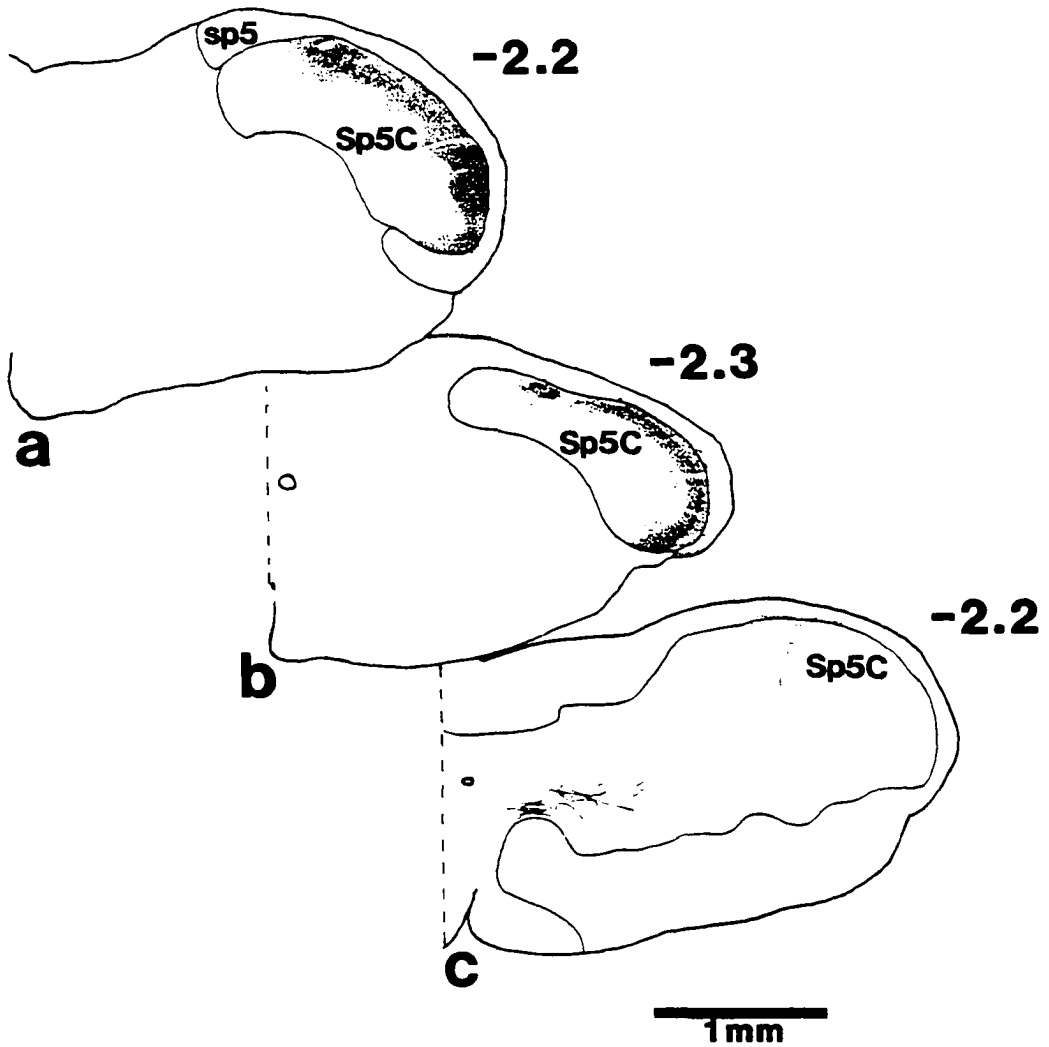

Fig. 13. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

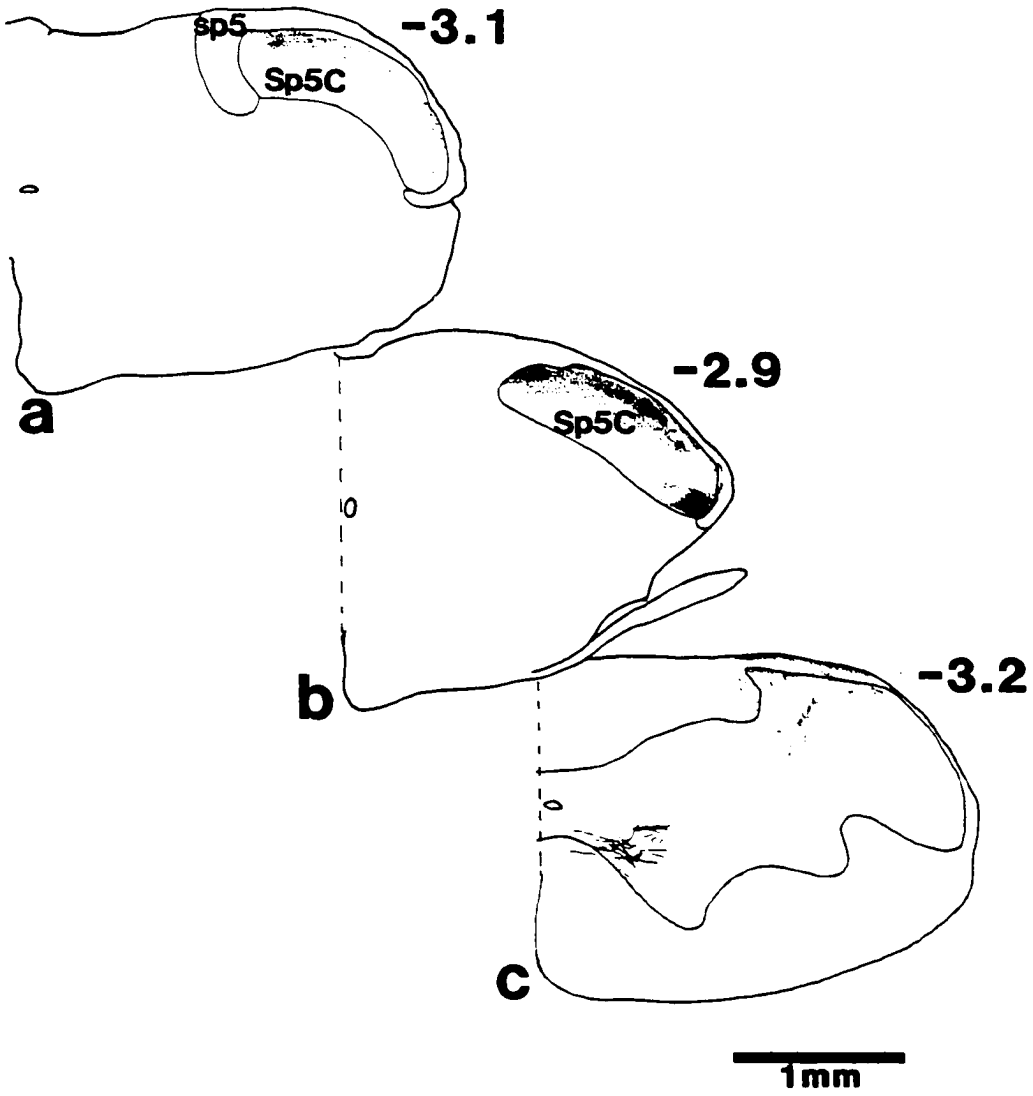

Fig. 14. Projection drawings of coronal sections illustrating the distribution of HRP reaction product, following injections in (a) the nose of the muskrat, (b) nose of the rat, (c) soft palate of the muskrat. Distance in mm from the obex is indicated. See Table 1 for abbreviations.

DISCUSSION

The results of this study provide a preliminary indication of primary afferent connections that may be involved in the diving response and related upper respiratory tract reflexes. The results reported from this study (Jarrell and Ebbesson 1988; DeLisa and Ebbesson 1989) and those of Panneton (1989) are the first concerning primary afferent projections in a diving species. This is the first study in which cutaneous afferents from the nose, by which the diving response is triggered under natural conditions, have been specifically traced. In a recent abstract, Panneton (1989) reported projections in the muskrat of the ethmoidal nerve, which innervates the nasal cavity as well as the skin of the wing and tip of the nose, and the superior laryngeal nerve and pharyngeal branch of the glossopharyngeal nerve, which innervate the mucosa of the anterior larynx and epiglottis, and the mucosa and muscles of the palate and pharynx, respectively (Greene 1935; Craigmyle 1985). Fibers of the ethmoidal nerve have previously been traced in the cat (Lucier and Egizii 1986). Projections of the infraorbital nerve, which innervates skin of the side of the nose as well as the cheek, upper lip and lower eyelid (Craigmyle 1985), have been studied in the rat (Arvidsson and Grant 1979) and cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986a,b).

Since large injections of HRP were made in order to assure adequate uptake and transport, labeling may include projections from

areas where HRP diffused from the injection site. Therefore, the results of this study should be regarded as preliminary information that may serve as a guide for more refined anatomical tracing and for neurophysiological studies linking structure and function.

Clear labeling of fibers and terminals was observed in the brainstem, and the distribution of label was consistent in all cases with similar injection sites, once the parameters of survival times, fixation and staining had been determined. The findings are also consistent with those of other studies, involving small injections, but provide greater detail than reported in some previous studies. These aspects of the results argue against spurious labeling. Differences between muskrat and rat projections from the nose, and overlap with projections from the other areas, also serve to indicate regions relevant to these reflexes.

I. AFFERENT PROJECTIONS

Labeling of terminal fields included all levels of the trigeminal sensory complex, Pa5 and Sol. Sensory projections of the experimental groups are compared with each other, and with those of other studies, below.

A. PROJECTIONS FROM THE NOSE

Sensory projections from the nose to the classical trigeminal sensory complex were similar in the muskrat and rat. The projections

generally correspond to previously reported projections of the ophthalmic and maxillary divisions. Labeled cells in the trigeminal ganglia were concentrated in the medial and central portions, corresponding to the representation of ophthalmic and maxillary divisions, respectively, in the rat (Darian-Smith et al. 1965; Gregg and Dixon 1973), cat and monkey (Kerr 1963). Labeled fibers were visible in the ventral and lateral portions of the trigeminal sensory root and spinal tract (Figs. 5a,b-14a,b), corresponding to ophthalmic and maxillary representations, respectively (Kerr 1963). Label was present at all levels of the trigeminal nuclear complex in both groups. Labeling of Pr5 was similar in the muskrat and rat, and consistent with ophthalmic-maxillary projections previously reported in the rat (Torvik 1956). Projections to Pr5 in the muskrat also have been reported recently by Panneton (1989), following HRP injection of the ethmoidal nerve. Neurons responsive to electrical stimulation of the ethmoidal nerve have been located in Pr5 in the rat (Van Buskirk and Erickson 1977). Lucier and Egizii (1986), on the other hand, did not find ethmoidal projections to Pr5 in the cat. Infraorbital nerve projections to Pr5 have been reported in the cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986b). A few cells of Me5 also were labeled at this level, in the muskrat and rat. This may have been due to diffusion of HRP and uptake by muscle afferents. Projections from the nasal region to Me5 via the ethmoidal nerve, however, have been found in the muskrat (Panneton, pers. comm.) and cat (Lucier and Egizii 1986). It has been proposed that these subserve nasal proprioception (Lucier and Egizii 1986). Tactile receptors of the

teeth, mucosa and palate also project to Me5 (Dubner et al. 1978).

In this study, cutaneous afferent projections from the nose to Sp5O were concentrated ventrolaterally in the rat, but extended dorsomedially in the muskrat, including an area corresponding to DMSp5 of the rat (Paxinos and Watson 1986); labeling in this area appeared to overlap with Sol in the muskrat (Figs. 7a,b). Similar projections have previously been shown from ophthalmic-maxillary, as well as mandibular, divisions of the trigeminal nerve in the rat and other mammals (Torvik 1956; Beckstead and Norgren 1979; Contreras et al. 1982; Jacquin et al. 1982). Panneton (1989), on the other hand, found that ethmoidal projections to Sp5O were dense ventromedially and sparse dorsomedially in the muskrat. Neurons responsive to electrical stimulation of the ethmoidal nerve have been located in the dorsomedial as well as ventral Sp5O in the rat (Van Buskirk and Erickson 1977). Panneton (1989) did not report ethmoidal projections to Sol, but identified a projection medial to Sp5 as Probst's tract (Panneton, pers. comm.). This area lies further medial, however, than the projection I have assigned to Sol (Paxinos and Watson 1986). Lucier and Egizii (1986) described only a very light ethmoidal projection to Sp5O in the cat. In an earlier electrophysiological study, ethmoidal projections to all 3 levels of Sp5 were reported in the cat (Beuerman 1975). Infraorbital projections to Sp5O are concentrated ventrolaterally in the cat (Panneton and Burton 1981; Shigenaga et al. 1986b). These patterns continue caudally into rostral Sp5I.

Further caudally, labeling of Sp5I was faint and extended

throughout the ventral half of the nucleus in both the muskrat and rat in this study (Figs. 8a,b), in comparison to dense ventromedial projections following HRP application to the ethmoidal nerve in the muskrat and cat (Panneton 1989; Lucier and Egizii 1986). Infraorbital nerve applications resulted in faint to moderate labeling that extended further dorsolaterally in Sp5I, in the cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986b).

Labeling of the ventromedial portions of Pr5, Sp5O and Sp5I following nose injections is consistent with a topographic organization of these nuclei, with the face represented in an inverted medially-facing position. More dorsal projections to these nuclei from the soft palate are also consistent with this arrangement. Projections to Pr5 and the rostral spinal trigeminal nucleus were more extensive in this study than in others involving single nerve injections, however, and do not exhibit a clear somatotopy. This may be due to mixed innervation of the structures injected, or diffusion of HRP.

The most dense projections seen in this study were to Sp5C, in both the muskrat and rat (Figs. 10a,b-14a,b). Dense projections to Sp5C also were recorded in the studies cited above. Consistent with these studies, labeling was concentrated in layers I-II. Additional terminal labeling and rare labeled cells were seen in this study in the muskrat, in layers V-VI (Figs. 10a, 11a). Panneton (1989) also found ethmoidal projections to layer V in the muskrat. Beuerman (1975) found ethmoidal primary afferent-driven cells in the reticular formation adjacent to Sp5C in the cat. At caudal levels, a wedge-shaped extension of labeling from the dorsolateral rim of the nucleus into the

deeper layers was seen in the muskrat and rat, in this study (Figs. 13a, 14b), and following infraorbital applications in the cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986a). Labeling of layers I-II extended from the ventromedial corner of the nucleus to a dorsolateral position. Ethmoidal nerve projections are restricted to the ventromedial portion of Sp5C in the muskrat and cat (Panneton 1989; Lucier and Egizii 1986), whereas the infraorbital nerve has a more extensive, ventral-to-dorsolateral projection in the cat (Panneton and Burton 1981; Shigenaga et al. 1986a). These patterns are consistent with those demonstrated for ophthalmic and maxillary projections, respectively, to the substanstia gelatinosa of the medullary dorsal horn in the rat (Rustioni et al. 1971). Dense projections to layers I-II extended caudally into the upper cervical dorsal horn in both the muskrat and rat, in this study. Panneton (1989), however, reports dense ethmoidal projections extending only to the rostral medullary dorsal horn in the muskrat. Lucier and Egizii (1986), on the other hand, report ethmoidal projections through the caudal extent of Sp5C in the cat, and infraorbital projections to the deeper laminae extend into the upper cervical cord (Panneton and Burton 1981; Shigenaga et al. 1986a).

Projections to Pa5 also were seen in the ventral and dorsolateral parts of sp5 at the levels of Sp5I and Sp5C, in the muskrat (Figs. 8a-11a). Similar, but more faint and restricted, projections were seen in one rat (Fig. 9b). Panneton (1989) found ethmoidal projections to ventral Pa5 in the muskrat. Infraorbital projections to Pa5 in the

cat have been found ventrally by Shigenaga et al. (1986a), and both ventrally and dorsolaterally by Panneton and Burton (1981). Lucier and Egizii (1986) do not report ethmoidal projections to Pa5, but ventral Pa5 appears to be labeled, in their photomicrographs of this region.

Cutaneous projections from the nose are similar in the muskrat and rat, and consistent with projections traced from the ethmoidal nerve in the muskrat (Panneton 1989) and cat (Lucier and Egizii 1986), and from the infraorbital nerve in the cat (Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986a,b). As in the other studies, the heaviest projections were to layers I-II of the medullary dorsal horn. Possible differences are a greater projection in the muskrat to layer V, Pa5 and dorsomedial Sp5O (possibly overlapping with Sol). This agrees with the findings of Panneton (1989), in the muskrat.

B. OTHER PROJECTIONS

Responses similar to the diving response also can be elicited by stimulation of the soft palate, larynx and posterior pharynx. Projections were traced from these areas in the muskrat in order to find overlapping projections, which may include common connections of these reflex pathways. The following discussion concerns these overlapping projections. Dense, extensive projections were seen following soft palate injections. In the single posterior pharynx and larynx cases, however, labeling was very faint. Therefore, negative results in these cases should not be considered conclusive.

Following palatal injections, labeled fibers were seen

dorsolaterally in the sensory root and the spinal tract of the trigeminal nerve (Figs. 5c,7c,8c), consistent with innervation by the maxillary division of the trigeminal nerve (Kerr 1963), and by the glossopharyngeal and vagus nerves (Aström 1953; Kerr 1962; Rhoton et al. 1966). Labeled fibers were not seen caudally, at the level of Sp5C, as in nose-injected cases. No labeling of the root or tract was seen in larynx- or pharynx-injected cases. Palate injections resulted in distinct labeling of rostral Pr5 that appears to overlap with nose projections, and exhibits a barreloid-like pattern ventrally (Fig. 5c). Very faint, questionable labeling of Pr5 was seen following injection of the larynx. In a recent study by Altschuler et al.(1989) of projections from the mucosa of the soft palate, larynx and pharynx of the rat, projections to Pr5 were not found. Kalia and Mesulam (1980) also did not find projections to Pr5 following HRP injections into the larynx of the cat. Projections to Pr5 have been traced, however, from fibers of the following: the pharyngeal or lingual-tonsillar branch of the glossopharyngeal nerve in the muskrat (Panneton 1989), rat (Hamilton and Norgren 1984), cat (Mizuno and Nomura 1986), rabbit (Hanamori and Smith 1989), and lamb (Sweazey and Bradley 1986); the palatine branch of the trigeminal nerve in the cat (Shigenaga et al. 1986b); and the superior laryngeal nerve in the cat (Nomura and Mizuno 1983) and lamb (Sweazey and Bradley 1986). In most cases, these nerves serving the palate, pharynx and larynx were represented in the dorsomedial Pr5.

Projections from the soft palate to the dorsomedial Sp5O and DMSp5 also appear to overlap with those from the nose, in the muskrat (Fig.

7c). Faint, questionable labeling was seen in this region following pharynx and larynx injections. Altschuler et al. (1989) observed a small patch of labeling in the dorsomedial Sp5O following HRP injection into the soft palate, but not the pharynx and larynx, in the rat. Projections to this area have been traced from the pharyngeal branch of the glossopharyngeal nerve in the muskrat (Panneton 1989) and cat (Mizuno and Nomura 1986), and the palatine branch of the trigeminal nerve in the cat (Shigenaga et al. 1986b). Granular reaction product also was observed at this level, just dorsomedial to Sp5O, following soft palate injections in this study. These projections appear to overlap with similar projections from the nose in the muskrat, but not in the rat, and may include the Sol (Figs. 7a-c). Faint, questionable terminal labeling also was seen in this region following pharyngeal injection in the muskrat. Afferent projections to lateral Sol have been demonstrated from the soft palate in the rat (Altschuler et al. 1989) and from the larynx in the cat (Kalia and Mesulam 1980).

Sparse projections from the soft palate to ventral Sp5I also overlap with those from the nose (Figs. 8c,9c). Faint, questionable labeling of rostral Sp5I was seen following injections of the pharynx and larynx (Figs. 8d,e). Dense reaction product was observed in caudal Sp5I following pharyngeal injection, but this appears to be at least partly artifactual (Fig. 9d). Altschuler et al. (1989) observed projections to the dorsolateral margin of Sp5I from the soft palate, pharynx and larynx in the rat. Panneton (1989) observed sparse projections to Sp5I from the pharyngeal branch of the glossopharyngeal

nerve, and a sparse projection to the medial edge of Sp5I from the superior laryngeal nerve, in the muskrat. A sparse projection to the dorsolateral Sp5I was seen from the lingual-tonsillar or pharyngeal branch of the glossopharyngeal nerve in the cat (Mizuno and Nomura 1986) and lamb (Sweazey and Bradley 1986), and from the superior laryngeal nerve in the cat (Nomura and Mizuno 1983), rabbit (Hanamori and Smith 1989) and lamb (Sweazey and Bradley 1986). A projection from the palatine branch of the trigeminal nerve to the dorsomedial Sp5I was seen in the cat (Shigenaga et al. 1986b).

At the level of transition between Sp5I and Sp5C, projections from the soft palate became more dense, following layers I-II of Sp5C in a pattern similar to that of the nose projections (Fig. 10). Labeling of Sp5C occupied a more dorsolateral position, and was relatively sparse at lower levels, following injection of the soft palate (Figs. 10-14). This pattern is consistent with the projection of the maxillary division to the substantia gelatinosa (Rustioni et al. 1971). Sp5C was not labeled following larynx or posterior pharynx injections. The pharyngeal branch of the glossopharyngeal nerve projects heavily to layers I and V of the medullary dorsal horn, and the superior laryngeal projects to these regions, in the muskrat (Panneton 1989). Altschuler et al. (1989) also report heavy labeling of the spinal trigeminal nucleus just caudal to the obex, but do not describe the pattern of labeling, following soft palate injections in the rat. Projections to layers I-II and IV-V from the palatine branch of the trigeminal nerve have been found in the cat (Shigenaga et al. 1986a). Projections to the dorsal margin of rostral Sp5C from the lingual tonsillar branch of

the glossopharyngeal nerve and the superior laryngeal nerve have been traced in the rabbit (Hanamori and Smith 1989) and lamb (Sweazey and Bradley 1986).

The most intense terminal labeling following soft palate injections was seen around the level of the obex in Pa5, in dorsolateral and ventral locations in sp5 similar to those of nose projections to Pa5 (Figs. 8c-11c). Labeling of dorsal Pa5 was more extensive than that following nose injections. Projections to dorsal Pa5 also were seen from the larynx and posterior pharynx (Figs. 8d,e-10d,e). Panneton (1989) traced dense projections to dorsal and ventral Pa5 from the pharyngeal branch of the glossopharyngeal nerve and the superior laryngeal nerve in the muskrat. Altschuler et al. (1989) also found the heaviest anterograde labeling after soft palate injections in Pa5, in the rat; extensive labeling of dorsal Pa5 also was seen following pharynx and larynx injections. Kalia and Mesulam (1980) report that a few sensory fibers terminate in sp5 following laryngeal injections in the cat. Projections to dorsal Pa5 have been demonstrated from the superior laryngeal nerve in the cat (Nomura and Mizuno 1983), and rat (Hamilton and Norgren 1984), and from the pharyngeal branch of the glossopharyngeal nerve in the rat (Hamilton and Norgren 1984). Shigenaga et al. (1986a) also found a dense projection from the palatine branch of the trigeminal nerve to ventral and medial parts of Pa5 in the cat.

Overlapping projections from the nose soft palate, which may contain convergent projections, include: Pa5; layers I-II of the

rostral medullary dorsal horn; dorsomedial Sp5O, possibly overlapping with Sol; Pr5; and Sp5I. These areas are of particular interest for further investigation of the neural substrate of the diving response and related upper respiratory tract reflexes.

II. EFFERENT PROJECTIONS

The aim of this study was to identify primary afferent projections that would include connections involved in triggering the diving response. I attempted to make shallow injections of HRP into cutaneous or mucosal surfaces. Retrograde labeling of motor neurons was incidental and difficult to interpret. These projections may reflect inadvertent injection or diffusion of HRP into the underlying musculature, or transynaptic transport of HRP. Efferent projections are briefly compared below.

A. PROJECTIONS FROM THE NOSE

Fibers of the motor root of the trigeminal nerve and the facial nerve, and cells of Mo5 and 7, were similarly labeled in the muskrat and rat following injections into the skin of the nose (Figs. 5-7) . Labeling of 7, serving the muscles of facial expression, presumably derives at least in part from uptake of HRP by the naris dilator. Motoneurons innervating the naris dilator of the rat are located in lateral 7 (Travers 1985), the region labeled in this study. Of interest concerning the labeling seen in 7, is the extension of labeled dendrites from lateral 7 into the labeled terminal field of the

ventromedial spinal trigeminal nucleus, in both the muskrat and rat. It is possible that these form the basis of a monosynaptic reflex effecting narial closure in response to wetting of the nose, which occurs during the diving response.

Labeling of Mo5 is more difficult to interpret. If there was significant spread of HRP to muscles of mastication, then anterograde transport from these muscles may also have occurred and the afferent labeling seen in this study may reflect these projections as well as the cutaneous projections.

B. OTHER PROJECTIONS

Injection of the soft palate, posterior pharynx and larynx also resulted in labeling of Mo5 (Fig. 5c,d,e). A few cells in dorsomedial 7 were labeled following injections into the soft palate, and cells in ventromedial 7 were labeled from injection into the posterior pharynx (Figs. 7c,d). Fibers and cells of 12 and Amb were labeled after the 3 types of injection (Figs. 8-11). Labeling of the solitary tract was seen following soft palate and pharynx injections (Fig. 9c,d), and the latter resulted in labeling of occasional cells in Sol (Fig. 7d). Motor innervation of the soft palate involves Amb, Mo5, 12 and occasional 7 neurons, in the guinea pig and monkey (Strutz et al. 1988) and cat (Holstege et al. 1983). Laryngeal motoneurons are located primarily in Amb in the cat and rat (Kalia and Mesulam 1980; Hinrichsen and Ryan 1981; Bieger and Hopkins 1987). Pharyngeal motoneurons have been demonstrated in Amb and 7 in the cat (Holstege et al. 1983).

III. IMPLICATIONS FOR THE DIVING RESPONSE

Under natural conditions, the diving response is triggered by immersion of the nose. The nares close, and apnea, bradycardia and peripheral vasoconstriction occur immediately on contact of the nose with water. Thus, cutaneous afferents from the nose trigger the initiation of the diving response. These afferents also signal continuing inspiratory inhibition, which overrides the increasing inspiratory drive mediated by carotid chemoreceptors as the dive progresses (Elsner and Daly 1988). The projections of these afferents, specifically traced for the first time in this study, therefore include primary afferent connections involved in the initiation and maintenance of the diving response. More highly developed or unique projections of these afferents in the muskrat, compared with the rat, are of particular interest because they may reflect specializations for the diving response. Stimulation of the nasal cavity, soft palate, pharynx and larynx produces apneic and cardiovascular responses resembling the diving response. Overlapping projections of primary afferents from these structures may include common connections in the pathways mediating these reflexes. This study complements a recent abstract reporting the results of HRP injections into the ethmoidal, glossopharyngeal and superior laryngeal nerves in the muskrat (Panneton 1989). These are the first neuroanatomical investigations concerning the diving response. They provide an indication of both structural and

functional aspects of primary afferent mechanisms of the diving response, in relation to the functional somatotopic organization of the trigeminal sensory complex.

A. STRUCTURAL CONSIDERATIONS

In discussions of the control of the diving response, the brain has been treated as a black box, or as a group of respiratory, cardiac and vasomotor centers, with inputs from peripheral afferents and outputs to peripheral efferent pathways. The actual pathways involved in the diving response, however, are unknown. Laryngeal, carotid sinus and, to a lesser extent, pulmonary primary afferent projections have been studied in common laboratory species (reviewed by Jordan and Spyer 1986). Ethmoidal and infraorbital projections have previously been studied in the cat and rat (Arvidsson and Grant 1979; Marfurt 1981; Panneton and Burton 1981; Lucier and Egizii 1986; Shigenaga et al. 1986a,b). In recent reviews, it has been suggested that Sol is the site of primary afferent connections of the diving response (Daly 1984; Elsner and Daly 1988). Arterial baroreceptors and chemoreceptors, pulmonary stretch receptors, cardiac receptors, laryngeal receptors and some trigeminal receptors project to Sol (Jordan and Spyer 1986). The dorsal respiratory group of neurons is located in the ventrolateral subdivision of Sol (Baumgarten et al. 1957), and efferent projections from Sol have been traced to Amb, which includes cardioinhibitory motoneurons. Facial and nasal sensory fibers, however, project primarily to the trigeminal sensory brainstem complex. Laryngeal

afferents also project to the trigeminal system, including Pa5 (Kalia and Mesulam 1980; Nomura and Mizuno 1983; Hamilton and Norgren 1984; Sweazey and Bradley 1986; Altschuler et al. 1989; Hanamori and Smith 1989). Secondary projections from the trigeminal spinal nucleus and Pa5 to Sol have been reported in the rat, and it has been suggested that these participate in the integration of somatic and visceral afferents and the production of somatovisceral reflexes (Menetrey and Basbaum 1987). Projections from Pa5 to the parabrachial nucleus, which participates in respiratory and cardiovascular control, also have been demonstrated (Panneton and Burton 1985; Nasution and Shigenaga 1987). Multiple primary afferent projections may be involved in the diving response, which may include connections in the trigeminal sensory complex and Pa5. This study demonstrates that cutaneous afferents from the nose in the muskrat project most heavily to layers I-II of the ventral and dorsolateral regions of Sp5C, and to more rostral trigeminal nuclei. Projections to layer V of Sp5C, Pa5, and dorsomedial Sp5O, possibly extending into Sol, were present in the muskrat but absent, or present to a lesser degree, in the rat. Pharyngeal and laryngeal projections to Pa5 were also found in the muskrat, and projections from the soft palate included Pa5, layers I-II of the dorsolateral region of Sp5C, and dorsomedial Sp5O. These projections are of particular interest for further investigation of primary afferent connections of the diving response. The projections highlighted in this study are similar to those in Panneton's (1989) study of ethmoidal, superior laryngeal and glossopharyngeal projections in the muskrat. Panneton (pers. comm.) has found that lesion of ventral

Sp5C blocks the diving-like response to nasal perfusion with water or ammonia vapors in muskrats.

Cells were labeled in lateral 7, with dendritic processes extending into labeled terminal fields in ventromedial Sp5O, in both the muskrat and rat. These may be motoneurons innervating narial dilators, which project to this region (Travers 1985), labeled by incidental uptake of HRP. It is possible that these projections subserve a monosynaptic reflex effecting narial closure in response to wetting of the nose, which occurs in these species and may participate in the diving response.

B. FUNCTIONAL CONSIDERATIONS

Since trigeminal afferent projections exhibit a functional somatotopic organization, the results of this study also may be suggestive of the modalities involved in initiation of the diving response. The receptors concerned have not been identified. The external narial reflexes would appear to involve general cutaneous sensation, whereas the internal nasal reflexes and reflexes elicited from the soft palate, pharynx and larynx may involve chemoreception as well as general sensation. The external narial reflexes are produced by immersion in saline as well as water in muskrats, and are blocked by covering the nares with vaseline or by local anesthesia in muskrats and seals (Dykes 1974; Drummond and Jones 1979; Drummond 1980). Immersion in mercury produces a normal diving response in ducks (Blix et al. 1976). The internal nasal reflexes of muskrats, on the other hand, are

less pronounced with saline than with water; these depend on trigeminal and vagal, but not olfactory, innervation (Drummond and Jones 1979). Responses similar to the diving response are elicited from water-sensitive units in the laryngeal mucosa of newborn mammals (Harding et al. 1978). In many mammals, chemical as well as electrical and mechanical stimulation of the nasal passages, pharynx and larynx produces a diving-like response. For example, rabbits respond to inhalation of noxious gases with apnea in expiration, marked bradycardia and mild hypertension, signaled by trigeminal, and not by olfactory, afferents (McRitchie and White 1974).

It is not known whether the external narial reflexes of diving animals are mediated by tactile or thermal afferents, or both. The rat rhinarium contains both corpuscular and hederiform sensory nerve endings, and free intraepidermal endings (MacIntosh 1975), which may mediate both tactile and temperature or pain sensation. The densest concentrations of cold receptors are found in the rhinarium in cats (Kenshalo et al. 1971). In humans, cold is clearly the predominant stimulus for immersion bradycardia. A cold, damp cloth (Whayne and Killip 1967) or cold air (LeBlanc 1975; Hayward et al. 1976) on the face, in combination with breathholding, is as effective as immersion in producing a response. The magnitude of the cardiovascular response to face immersion varies inversely with water temperature and is insignificant above a "critical temperature" of about 30^{o}C (Hong et al. 1967; Paulev 1968; Kobayasi and Ogawa 1973). In several studies of diving species, no effect of water temperature on the intensity or time

course of bradycardia during head immersion was found (Andersen 1963a; Butler and Jones 1968; Dykes 1974; Drummond 1980). Dykes (1974) argued that, since air temperature in the seal's natural environment may vary from above to below water temperature, it is "necessary that the dive reflex of the seal be insensitive to temperature" and concluded that thermal stimuli play no important role in the initiation of the diving response. Florin-Christensen et al. (1986) found that, with electrocoagulation of the receptor areas for the diving response in ducks, the response to head immersion was eliminated in 36^{o}C water but a normal response was produced in 15^{o}C water. This suggests that temperature may play a role in eliciting the diving response. An effective system of thermoafferent signaling of the response in diving species might include enhanced sensitivity of receptor function over a wide range of temperature and an all-or-nothing type of response. The above findings are consistent with such a system. Thus, it seems premature to rule out thermoreception as a mechanism for initiating the diving response.

A functional organization of trigeminal afferent projections has been proposed since the last century, from clinical observations of sensory dissociations following central nervous system lesions. Hun (1897) reported a loss of thermal and pain sensibility, but retained ability to detect light touch, following posterior inferior cerebellar artery occlusion, which destroys Sp5C and possibly Sp5I. Gerard (1923) proposed that trigeminal pain and temperature afferents terminate in Sp5C. The results of trigeminal tractotomy at the level of the obex, introduced by Sjoquist (1938) for the treatment of trigeminal

neuralgia, generally have supported this proposal (Kunc 1970). Many animal experiments confirm the presence of thermoreceptive and nociceptive neurons in Sp5C and mechanoreceptive neurons at all levels of the trigeminal sensory complex (e.g., Price et al. 1976; Dostrovsky and Hellon 1978; Dickenson et al. 1979; see Dallel et al. 1988 for review). It has generally been accepted, therefore, that Sp5C is the essential relay for thermal and nociceptive afferents from orofacial structures. Segregation of thermal and nociceptive inputs is suggested by some clinical results, but has not been clearly demonstrated (Darian-Smith 1984). The results of human and animal tractotomy, trigeminal nuclear lesion, recordings from rostral spinal trigeminal nuclei, and projections traced from tooth pulp, on the other hand, amply demonstrate that the representation of oral and paramedian facial nociception incl des trigeminal subdivisions rostral to the obex (see Broton and Rosenfeld 1985, Dallel et al. 1988 for reviews). This suggests that thermal afferents from oral and perioral facial areas also may project rostral to the obex. In a recent review of thermal sensibility, Darian-Smith (1984) states, "thermoreceptive neurons within the trigeminal complex are found only within nucleus caudalis." To my knowledge, no systematic search for such neurons in regions other than Sp5C, however, has so far been made. Thermoreceptive and nociceptive neurons in Sp5C are found primarily in layers I-II and V-VI of the medullary dorsal horn (Mosso and Kruger 1973; Price et al. 1976; Poulos and Molt 1977; Dostrovsky and Hellon 1978; Burton et al. 1979; Dickenson et al. 1979; Hu et al. 1981). Low-threshold mechanoreceptive

neurons (LTM) have been located in layers III-IV and V-VI (Mosso and Kruger 1973; Price et al. 1976; Hu et al. 1981; Amano et al. 1986). In a study of the functional organization of Sp5I, Hayashi et al. (1984) found LTM neurons uniformly distributed throughout the nucleus, whereas nociceptive neurons were limited to the lateral/marginal and medial/ventral borders.

The prominence of labeled terminals throughout the rostrocaudal extent of Sp5C and the concentration of labeling in layers I-II suggest the importance of thermal and nociceptive projections from the nose in both the muskrat and rat. An elaboration of thermoafferent terminations from the nose would be consistent with the concentration of thermoreceptors in this area (Kenshalo et al. 1971). Labeling of layers V-VI of Sp5C and the medial border of Sp5I and adjacent reticular formation in the muskrat also is consistent with thermal and nociceptive projections, and suggests the involvement of thermoreception in the elicitation of the diving response.

Primary afferent projections from the nose, soft palate, posterior pharynx and larynx to Pa5 in the muskrat suggest that this structure may participate in eliciting the diving response and related upper respiratory tract reflexes. The function of Pa5 is not known. Several findings point to nociceptive and thermoreceptive functions, however, which suggests that Pa5 also may be involved in thermoafferent signaling of the diving response. Pa5 has been described by numerous investigators as a rostral extension of Sp5C, specifically of layers I-II (Gobel and Hockfield 1977; Panneton and Burton 1981; Craig and Burton 1981; Cruz and Basbaum 1985; Shigenaga et al. 1986a, 1988). Pa5

is histologically similar to layer I and contains cells and receives fibers with Substance P-like immunoreactivity (SPLI) (Chan-Palay 1978b). SPLI is present in cells of the spinal cord and medullary dorsal horns, with highest concentrations in layers I-II, and in C and A delta fibers, and therefore has been implicated in pain pathways (Cuello and Kanazawa 1978; Emson 1979). These findings are also consistent with the possible involvement of Pa5 in thermoreceptive pathways. Afferent projections have been traced from peripheral nerves or tissue sites to both Pa5 and layers I-II of Sp5C in numerous studies (Panneton and Loewy 1980; Panneton and Burton 1981; Marfurt 1981; Shigenaga et al. 1986a,b; Menetrey and Basbaum 1987; Pfaller and Arvidsson 1988; Shigenaga et al. 1988). In some studies, labeling of Pa5 was continuous with, or appeared to be an extension of, labeling in layers I-II (Craig and Burton 1981; Shigenaga et al. 19186a,b; Menetrey and Basbaum 1987). In a study by Shigenaga et al. (1986a,b) in which the central projections of many trigeminal branches were traced in the cat, branches with projections to Pa5 (lingual, infraorbital, palatine, buccal, and corneal) included those that innervate areas with concentrations of thermoreceptors (Poulos and Benjamin 1968; Kenshalo et al. 1971; Dostrovsky and Hellon 1978). Converging efferent projections to central sites also have been traced from cells of both Pa5 and layer I, further suggesting a relationship between these structures, and the possible involvement of Pa5 in thermal and pain pathways (Craig and Burton 1981; Cechetto et al. 1985; Panneton and Burton 1985; Nasution and Shigenaga 1987; Menetrey and Basbaum 1987).

Recently, Shults and Light (1987) traced collaterals of a cooling-specific fiber, with a receptive field on the tip of the tongue, to Pa5 at Sp5I and Sp5O levels in the cat. They also recorded from nociceptive cells in Pa5 with oral and perioral receptive fields. Efferent projections to the medial and lateral parabrachial nuclei have been traced in anterograde studies following injections into a region of marginal zone cells responsive to cooling of oral and perioral receptive fields, including the nasal region (Burton et al. 1979). In retrograde studies, efferent projections were traced from both Pa5 and layer I cells following injections into the medial and lateral parabrachial nuclei (Loewy and Burton 1978; Cechetto et al. 1985; Panneton and Burton 1985; Nasution and Shigenaga 1987). It has been suggested that projections from Pa5 and layer I cells to the medial parabrachial nucleus participate in the convergence of gustatory and thermoreceptive or mechanoreceptive afferents from the tongue and may be involved in somatovisceral reflex behavior (Cechetto et al. 1985; Panneton and Burton 1985). It also has been suggested that projections from these cells to the lateral parabrachial nucleus mediate cardiopulmonary responses to orolingual thermoreceptive, nociceptive and chemoreceptive afferents. There is evidence that Pa5 conveys nociceptive signals from lingual, buccal and palatine nerves to the parabrachial nucleus and the thalamus, directly and indirectly, via the parabrachial nucleus (Nasution and Shigenaga 1987). A direct projection has been reported from Pa5 to nucleus submedius of the thalamus, which contains cells responding to noxious thermal stimulation of the tip of the snout and may be involved in pain and

thermal pathways (Panneton and Burton 1985; Craig and Burton 1981). Sites in the medial part of the ventroposterior medial thalamus (VPM) that respond to cooling of the tongue have been identified (Landgren 1959; Emmers 1966; Poulos and Benjamin 1968; Burton et al. 1979; Craig and Burton 1981). Burton et al. (1979) found retrograde labeling from this area to layer I cells, but did not distinguish the Pa5. Kilduff et al. (1983) cite an unpublished study by Mehler in which HRP injected into the VPM retrogradely labeled cells in both layer I and Pa5.

The possibility of a thermoafferent function of Pa5 has been most strongly suggested by Kilduff and colleagues, from measurements of local cerebral metabolic activity in hibernating ground squirrels, using the relative 2-deoxyglucose method (Kilduff et al. 1983, 1987). Pa5 was the only one of 95 structures examined to significantly increase its activity during hibernation. A progressive activation and deactivation of Pa5, on entrance to and arousal from hibernation, respectively, paralleled the progressive changes in body temperature during these transitional periods. Pa5 also was activated during forced hypothermia. The caudalmost zone of increased activity in Pa5 was continuous with layer I of Sp5C, suggesting a functional homology between these structures.

Finally, Pa5 may be related to a similar nucleus, the lateral descending trigeminal nucleus (LDTN), and the thermoafferent relay function of the LDTN therefore suggests a similar role for Pa5. The LDTN is a large nucleus present in pit vipers and some boid snakes with infrared receptors, to which these receptors exclusively project

(Molenaar 1974; Schroeder and Loop 1976). It is situated lateral to the main descending trigeminal nucleus and is bounded medially by fibers of the main descending trigeminal tract, and laterally by its lateral descending trigeminal tract. The location of LDTN in the brainstem, and its physical relationship to the main descending trigeminal tract and nucleus, is therefore similar to that of Pa5.

Thus the heaviest projections from the nose in muskrats and rats, the more highly developed projections in the muskrat in comparison with the rat, and the overlapping projections from structures injected in the muskrat, are strongly correlated with thermal and nociceptive terminations. This suggests that thermoafferent signaling may play an important role in the diving response.

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