

Blessing, Chapter 3

Anatomy of the Lower Brainstem, part 2

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Nucleus Ambiguus

Nucleus ambiguus (nA) motoneurons, regulating striated muscle in the pharynx, larynx, cervical region, and esophagus, are part of the ventrolateral branchial arch column of motoneurons, extending throughout the length of the medulla oblongata. Székely and Matesz (1993) consider the nA to be in a common rostro-caudal line with the accessory facial nucleus rather than with the facial nucleus itself. The myelinated axons of nA enter cranial nerves IX and X, looping dorsally before exiting the medulla laterally through the spinal tract of V (Fig. 3.9A) and terminating peripherally in neuromuscular junctions. The most rostral nA is sometimes called the retrofacial nucleus, and the most caudal portion is sometimes referred to as the retroambiguus nucleus, but the whole column of neurons is best referred to as the nucleus ambiguus. Neurons of the nA do not have collateral axons projecting to other CNS neurons, so use of the name ambiguus to include other nearby neurons (McKellar and Loewy, 1982) is inappropriate. Some neurons in the nA are parasympathetic preganglionic neurons projecting to final motoneurons that innervate the airways, the heart, and other thoracic and (to a limited extent) abdominal structures. These neurons are considered in a separate section in this chapter.

Figure 3.9 A, The nucleus ambiguus in the rat, defined by retrograde transport of cholera toxin-HRP to the brain after application to the cervical vagus in the rat. The tractus solitarius is labeled by anterograde transport from the nodose ganglion. **B**, Viscerotopic representation of the upper alimentary tract and the respiratory tract in the nA, as projected on the sagittal plane. LRN, lateral reticular nucleus; VII, facial nucleus. (A, B, modified from Bieger and Hopkins, 1987.)
Abbreviations listed on pages xiii-xiv.

Lawn (1966b,a) defined the rabbit nA using retrograde degeneration procedures. Most of his conclusions have been confirmed and extended by subsequent retrograde transport investigations in this species (Kitamura et al., 1993a), in cat and dog (Kalia and Mesulam, 1980a, 1980b; Bennett et al., 1981; Nomura and Mizuno, 1983a), and in monkey (Gwyn et al., 1985). Studies in the rat include a most informative one by Bieger and Hopkins (1987), involving careful retrograde transport studies with deposition of tracer in the various structures innervated by nA axons. The following account derives mainly from this paper and from Altschuler and colleagues (1991a) and Shapiro and Miselis (1985b).

Bieger and Hopkins (1987) indicate that the name ambiguus evidently reflects a nineteenth century controversy concerning whether the nucleus was actually connected with the vagus and glossopharyngeal nerves, presumably reflecting its ill-defined borders. The anatomy of the nA is summarized in Figure 3.9.

The nA in the rat consists of two major groups. Neurons in the first group, innervating striated branchial arch musculature, are subdivided into a compact formation (esophagus), a semicompact formation (pharyngeal constrictors and cricothyroid muscle), and a loose formation (intrinsic laryngeal musculature except cricothyroid). The semicompact formation extends rostrally in a position generally ventral to the compact formation. Neurons in the second group (parasympathetic preganglionic neurons) occur in the external formation of the nA and project to the heart, lungs, and airways (Nosaka et al., 1979; Sugimoto et al., 1979; Ciriello and Calaresu, 1982; Hopkins and Armour, 1982; Haxhiu et al., 1993) and to the cardia region of the stomach (Coil and Norgren, 1979; Kalia and Mesulam, 1980b; Leslie et al., 1982; Takayama et al., 1982; Gwyn et al., 1985; Shapiro and Miselis, 1985b; Norgren and Smith, 1988; Hudson, 1989).

In addition to acetylcholine, the special visceral motoneurons in VII, IX, and XII that innervate striated muscle (both somitic mesodermal and branchial arch muscle) contain calcitonin gene-related peptide (CGRP) or galanin, but the general visceral (parasympathetic) motoneurons do not contain these peptides (Kimura et al., 1981; Armstrong et al., 1983; Kawai et al., 1985; Takami et al., 1985; Batten et al., 1989; McWilliam et al., 1989; Moore, 1989; Tago et al., 1989; Unger and Lange, 1991; Lee et al., 1992; Merchenthaler et al., 1993). Pituitary adenylate cyclase-activating polypeptide is present in nA neurons (Légrádi et al., 1994), but it is not certain to which subpopulation the positive neurons belong.

Dorsal Motor Nucleus of the Vagus (Parasympathetic Efferents)

The term dorsal motor nucleus of the vagus (dmnX) is now part of neuroanatomical lore so that any change in terminology would be more confusing than helpful. Nevertheless, as noted by Székely and Matesz (1993), VII and IX also contain axons of (more rostral) dorsomedially situated parasympathetic preganglionic neurons; thus it would be preferable to rename the dorsal motor nucleus of the vagus the dorsal visceromotor column of the medulla.

The dmnX (may the name long be with us!) consists of a column of densely packed general visceral (parasympathetic) preganglionic motoneurons, present throughout the rostrocaudal extent of the medulla oblongata. The topographical anatomy reflects the manner in which the central grey masses are displaced laterally as the canal becomes the fourth ventricle. In the caudal part of the medulla the dmnX is ventral to the nTS. More rostrally it is medial to the nTS, directly beneath the floor of the fourth ventricle, and then it is displaced laterally in the medulla, ventral to the vestibular nuclei. Cajal (1909) noted that the dmnX contains some smaller neurons whose axons do not exit in cranial nerve X, presumably neurons that have "migrated in from neighboring parts of either the central grey or the nucleus of the solitary tract." These neurons have been studied in cat and monkey dmnX (McLean and Hopkins, 1981; McLean and Hopkins, 1985). The dmnX can be seen in the Nissl stain of the dorsomedial medulla of a rat shown in Figure 3.10. An early study of the rabbit dmnX (Getz and Sirnes, 1949) contains useful references to older literature as well as a challenging topographical analysis based on retrograde degeneration of dmnX neurons after sectioning of the various peripheral branches of the tenth nerve. References to early studies of the dmnX can also be found in Mitchell (1985).

Figure 3.10 Nissl stain of transverse section through rat dorsomedial medulla at the level of area postrema. Abbreviations listed on pages xiii-xiv.

Efferent projections of dmnX motoneurons

All the axonal projections are to peripheral targets. Many retrograde neuroanatomical analyses have now been completed (Getz and Sirnes, 1949; Coil and Norgren, 1979; Nosaka et al., 1979; Kalia and Mesulam, 1980a, 1980b; Dennison et al., 1981; Kalia and Sullivan, 1982; Takayama et al., 1982; Nomura and Mizuno, 1983a; Fox and Powley, 1985, 1992; Gwyn et al., 1985; Shapiro and Miselis, 1985b; Rinaman and Miselis, 1987; Norgren and Smith, 1988; Altschuler et al., 1989, 1991b; Hudson, 1989; Berthoud et al., 1990a; Izzo et al., 1992). A limited population of neurons in the dmnX project to ganglionic neurons located in or near major airways and lungs and, possibly, the heart (see below); the major projection is to viscera, particularly to the stomach. For the stomach, upper small intestine, and cecum, the vagal efferents project to neurons in the enteric nervous system. Presumably some dmnX neurons also project to parasympathetic final motoneurons whose axons innervate blood vessels supplying thoracic and abdominal viscera, but this matter has not been widely investigated.

The subdiaphragmatic projection has been carefully studied in the rat, a species in which more than 90% of dmnX neurons are labeled after injection of retrograde tracer into the stomach (Fox and Powley, 1985; Shapiro and Miselis, 1985b; Norgren and Smith, 1988). This may mean that individual axons innervate more than one abdominal viscus. Alternatively, axons destined for other organs may take up tracer as they traverse the stomach. The gastric innervation is from perikarya in the more medial columns of the dmnX, throughout its rostrocaudal extent. Neurons in the right and left medial columns innervate the dorsal and ventral aspects, respectively, of the stomach via axonal projections through the dorsal and ventral gastric nerves. There does not seem to be a major topographical organization in dmnX neurons innervating the different abdomen organs. Preganglionic neurons innervating the cecum are mainly confined to the lateral portion of the nucleus (Altschuler et al., 1991b).

The location of parasympathetic preganglionic neurons innervating the heart and lungs has been more difficult to establish. The peripheral branches of the relevant nerves are small, and physiological identification is usually required. Movement of thoracic structures with heart and lung activity makes the injection procedure more difficult. There is the possibility of injecting nerves that include aberrant esophageal branches. In the case of the heart, parasympathetic final motoneurons may be collected together in the epicardial region rather than being located in the atrial muscle or in the ventricular myocardium so that conventional agents injected into the heart may not transport to neurons in the medulla oblongata.

The detailed study in the cat by Kalia and Mesulam (1980b) reports many retrogradely dmnX neurons after injections of HRP into the larynx, the intrathoracic large airways, the lung, or the heart. For the larynx and the extrathoracic large airways, the neurons were in the more rostral part of the dmnX, but for the other organs the neurons were found at all rostrocaudal levels for distances as long as 11 mm. Almost as many dmnX neurons, in a similar extensive rostrocaudal extension, were labeled after

HRP injections into the stomach. The results prompted Kalia and Mesulam to argue against any topographic representation for the different organs in the dmnX. However, the marked contrast between the limited rostral dmnX distribution after injections into the extrathoracic trachea and the extensive rostrocaudal distribution after injections into the intrathoracic trachea is most unexpected. Now that we have a broader perspective, we may wonder whether the intrathoracically injected HRP spread to esophageal vagal axons destined for subdiaphragmatic targets, artifactually increasing the extent of labeling in the dmnX.

Hopkins and Ellenberger (1995), in a recent review, still consider that the cardiac innervation probably arises principally from the nA rather than the dmnX. A transneuronal viral tracing study (Standish et al., 1994) has revived the controversy by concluding that dmnX neurons contribute extensively to the vagal cardiac projection in the rat. However, in that viral study, so many dmnX neurons were labeled that, taken at face value, the results imply that the same dmnX preganglionic neuron must project to both heart and sub-diaphragmatic structures. The brainstem origin of the parasympathetic innervation of cardiac blood vessels should also be kept in mind. At present it is probably wise to maintain a sceptical view concerning the dmnX and the heart. It would be informative to have additional careful conventional retrograde studies in the rat to confirm the parasympathetic innervation of different thoracic structures in this species.

Haxhui and colleagues (1993) injected tracer into the tracheal wall in rats and noted retrogradely labeled neurons, relatively small, in the rostral dmnX and in the medial part of the rostral nTS. Retrogradely labeled neurons also occur in this region after application of tracer to the superior laryngeal nerve in rats (Hamilton and Norgren, 1984), cats (Kalia and Mesulam, 1980b), rabbits (Hanamori and Smith, 1989), muskrats (Panneton, 1991a), and hamsters (Hanamori and Smith, 1986). Although the superior laryngeal projection was not observed by Contreras and colleagues (1980), the neurons are probably situated in the same rostral dmnX-nTS region as the preganglionic neurons exiting in the lingual-tonsillar branch of IX, as demonstrated by Hamilton and Norgren (1984).

Neurotransmitter-related markers in dmnX neurons

The issue of transmitter-related substances other than acetylcholine (Armstrong et al., 1983; Jones and Beaudet, 1987; Kimura et al., 1981) in the dmnX is an important one, since the complex effects on gastric function produced by stimulating the efferent vagus (see Chapter 7) suggest the possibility of different classes of neurotransmitter agents in dmnX motoneurons. Some studies have detected markers for catecholamine synthesis in dmnX neurons with axons in cranial nerve X, but other double-labeling studies in rats and rabbits (Blessing et al., 1985, 1986) have suggested that catecholamine-synthesizing neurons in dmnX are displaced nTS cells belonging to the A2 or C2 catecholamine group. This issue is still controversial.

Small GABA-synthesizing neurons in the dmnX (Blessing et al., 1984a; Blessing, 1990) are presumably either displaced nTS cells or interneurons. In the human, many dmnX cells contain substance P, and axons from these cells appear to project in cranial nerve X (Halliday et al., 1988b, 1990). Other neurochemical markers present in dmnX neurons include neuropeptide Y and pituitary

adenylate cyclase-activating polypeptide (Blessing et al., 1986; Légrádi et al., 1994). The dmnX motoneurons do not contain CGRP or markers for nitric oxide-synthesis (Batten et al., 1989; Gai et al., 1995).

Direct inputs to dmnX neurons from peripheral afferents

Dendrites of many dmnX neurons enter the nTS, where there is a monosynaptic innervation of the dendrites by terminating vagal afferents, true vagovagal synaptic connections (Rinaman et al., 1989). Direct input from vagal afferents to dmnX perikarya can be also inferred from the most sensitive transganglionic HRP transport studies (Kalia and Sullivan, 1982; Shapiro and Miselis, 1985b). Since the contralateral motoneurons are not retrogradely labeled after application of tracer to one peripheral vagal trunk, the reaction product present in the contralateral dmnX must be transganglionically transported in terminals of afferent vagal fibers.

Parasympathetic Preganglionic Neurons With Axons Exiting in Cranial Nerves VII and IX

Parasympathetic preganglionic neurons in the pons and upper medulla regulate the function of exocrine glands (lacrimal, nasal, and oropharyngeal mucosal glands; salivary glands) in the head and upper cervical region. In addition, given the extensive vascular distribution of postganglionic parasympathetic neurons (Suzuki and Hardebo, 1993), the preganglionic cells must have a major role in the regulation of blood vessels in the head (see Chapter 5). The two roles are complementary given the rapidity with which the exocrine glands filter their vascular supply during the formation of their secretory products (tears, saliva, etc). It may be that individual preganglionic parasympathetic neurons project to ganglionic perikarya controlling both a gland and its supplying blood vessels. Historically, control of the salivary glands has dominated anatomical studies, and groups of pontine parasympathetic preganglionic neurons are often loosely referred to as "salivatory nuclei." Axons of pontine parasympathetic preganglionic neurons leave the brainstem in the intermediate division of the facial nerve, joining either facial (VII) or glossopharyngeal (IX) nerves (Fig. 3.7). Secretomotor fibers travel in the lesser petrosal nerve to the otic ganglion, and postganglionic fibers innervate the parotid gland. Other preganglionic fibers travel in the chorda tympani nerve, and then in the lingual nerve, before running along the ducts to the ganglionic cells situated in the sublingual or submandibular glands or along the ducts just before the glands. A third group of secretomotor fibers distribute in the greater petrosal nerve and reach the sphenopalatine (pterygopalatine) ganglion. Postganglionic fibers enter branches of the maxillary nerve and supply lacrimal, nasal, and palatine glands.

The location of the pontine preganglionic parasympathetic neurons has been determined by application of conventional retrograde tracing agents to the relevant peripheral ganglia and preganglionic nerves in rat, cat, rabbit, dog, and primate (Hiura, 1977; Satomi et al., 1979; Chibuzo and Cummings, 1980; Contreras et al., 1980; Matsuo et al., 1980; Mitchell and Templeton, 1981; Nicholson and Severin, 1981; Nomura et al., 1981; Perwaiz and Karim, 1982; Nomura and Mizuno, 1983b; Whitehead and Frank, 1983; Tramonte and Bauer, 1986; Hanamori and Smith, 1989; Spencer

et al., 1990; Nemoto et al., 1995). The general distribution is summarized in Figure 3.11A, adapted from the detailed study of Contreras and colleagues (1980). These authors question the existence of separate superior and inferior subnuclei. Neurons with axons traveling in the glossopharyngeal nerve and its branches have a brainstem distribution virtually indistinguishable from those with axons exiting in the facial nerve. More ventrally located neurons can be retrogradely labeled from the sphenopalatine ganglion and the greater superficial petrosal nerve.

Presence of Markers for Acetylcholine and Nitric Oxide Synthesis in Pontine Parasympathetic Preganglionic Neurons

Choline acetyltransferase (ChAT) is a specific marker for acetylcholine-synthesizing neurons. The distribution of these neurons in the rat pons has been described by Armstrong and colleagues (1983). However, the presence of ChAT in the accessory facial nucleus and in other neurons in the region has limited its use as a marker for parasympathetic preganglionic neurons with axons in VII and IX (see discussions in Zhu et al., 1996; Gai and Blessing, 1996b).

Markers for nitric oxide (NO) synthesis have been demonstrated in pontine regions known to contain parasympathetic neurons (Vincent and Kimura, 1992; Gai et al., 1995; Takemura et al., 1994; Kowall and Mueller, 1988; Dun et al., 1994), raising the possibility that preganglionic parasympathetic salivary neurons may contain markers for NO synthesis. Appropriate double-labeling studies have demonstrated markers for NO synthesis in nearly all submandibular salivary preganglionic brainstem neurons in rabbits, but the same procedure demonstrated very limited numbers of doublelabeled neurons in rats (Zhu et al., 1996). The distribution of NO-synthesizing neurons in corresponding regions of the human pons and upper medulla is likely to include the parasympathetic preganglionic "salivatory" neurons, as discussed in Chapter 8.

Parasympathetic Premotor Neurons

Cranial Outflow: Premotor neurons for the dmnX

The proximity of the dmnX to the nTS has made it difficult for retrograde transport studies to determine central inputs to dmnX neurons. Anterograde transport studies of dmnX afferents, after injection of tracer into brain regions hypothesized to project to the dmnX, are particularly valuable for confirmation of presumed CNS inputs established by retrograde transport studies.

Sawchenko (1983) considers that forebrain projections directly to the dmnX arise from the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamus, and the lateral hypothalamic area. Autoradiography studies by Schwaber and colleagues (1982) document the amygdaloid projection in rabbits. The studies of Ter Horst (1984) confirm projections to the dmnX from the lateral, dorsomedial, and paraventricular hypothalamic nuclei. Brainstem inputs arise from ventrolateral catecholamine cells (A1, C1, A5), but not from the parabrachial nuclei or Kölliker-Fuse nuclei. A projection from the locus coeruleus may contribute to the dopamine B-hydroxylase (DBH)-positive terminals in the dmnX (Swanson and Hartman, 1975; Ter Horst et al., 1991b), but a careful study combining *Phaseolus vulgaris* leucoagglutinin (PHA-L) anterograde tracing with DBH immunohistochemistry did not confirm this (Fritschy and Grzanna, 1990b). Probable inputs to the

dmnX from raphe pallidus and obscurus, and the parapyramidal region, contain thyrotropin release hormone (TRH) (Lynn et al., 1991), but they need to be checked by appropriate anterograde transport studies to make sure the termination is in the dmnX rather than the nTS.

Transneuronal herpes viral tracing studies involving branches of the vagus nerve or innervation targets have been used to assess central inputs to the dmnX (Loewy and Haxhiu, 1993; Loewy et al., 1994; Rinaman et al., 1993; Card et al., 1993). The studies need to be interpreted cautiously, because the virus may travel in either motor or sensory vagal axons, with virus-positive neurons appearing in nodose ganglion neurons with both Herpes simplex type I (Blessing et al., 1991) and pseudorabies strains. Some of the nTS labeling seen in the viral studies may thus reflect transport in afferents rather than retrograde transfer from dmnX neurons.

Cranial Outflow: Premotor neurons for parasympathetic preganglionic neurons in nA and lower pons

Transneuronal virus tracing studies have made an important contribution to the localization of the parasympathetic premotor neurons in the nA and the lower pons. The distribution of CNS neurons transneuronally labeled after injection of virus into the sphenopalatine ganglion or the submandibular gland in the rat has been described (Spencer et al., 1990; Jansen et al., 1992). These neurons occur in restricted regions of basal forebrain, including the bed nucleus of the stria terminalis (but not the amygdala), in the cerebral cortex near the amygdala, the lateral preoptic area, and the substantia innominata. In the hypothalamus, many virus-positive neurons occurred in the parvicellular paraventricular nuclei and in the lateral hypothalamic area. In the midbrain there was labeling in the periaqueductal grey and in the retrorubral fields. In the pons the lateral central parabrachial nucleus and the Kölliker-Fuse nuclei were labeled. In the medulla, the lateral nucleus tractus solitarius, raphe and parapyramidal cells, and spinal trigeminal nucleus contained virus. Occasional A1 catecholamine neurons were virus positive. The distribution of positive neurons after application of virus to the submandibular salivary gland is shown in Figure 3.11B. Anterograde tracing studies have confirmed a projection from the hypothalamic paraventricular nucleus to the parasympathetic cells projecting to the sphenopalatine ganglion (Hosoya et al., 1990). Monoamine and neuropeptide-containing boutons can be found in synaptic relationship with these preganglionic neurons (Nemoto et al., 1995).

Figure 3.11 A, Projection onto midline sagittal plane of parasympathetic preganglionic neurons, defined by retrograde transport of tracer after peripheral injections in rat. Filled squares, vagus nerve; filled circles, tympanic branch of glossopharyngeal nerve (relaying in otic ganglion to supply the parotid gland and hindbrain vasculature); filled triangles, greater superficial petrosal nerve (sphenopalatine ganglion) (supplying nasal and lacrimal glands as well as forebrain cerebral vasculature); open circles, lingual-tonsillar branch of glossopharyngeal nerve; open triangles, chorda tympani nerve (submandibular and sublingual salivary glands). (Modified from Contreras et al., 1980.) B, Premotor neurons projecting to parasympathetic preganglionic neurons, defined by retrograde transneuronal transport of live virus from the submandibular gland of the rat. The transverse section at the level of the facial nucleus (where preganglionic neurons were labeled) is

omitted from the diagram. (Modified from Jansen et al., 1992.) Abbreviations listed on pages xiii-xiv.

Sacral Outflow: Brainstem projections to preganglionic neurons innervating pelvic organs

This very important topic is dealt with only briefly, because, regrettably, this book does not review brainstem control of sexual and eliminative functions. The reader is referred to the excellent review by De Groat and Steers (1990). More recent literature dealing with brainstem neurons projecting directly to parasympathetic preganglionic neurons in the sacral spinal cord is well discussed in a transneuronal transport study with application of pseudorabies virus to the rat penis and clitoris and to the rat urinary bladder (Nadelhaft et al., 1992; Marson et al., 1993; Marson, 1995). Virus-positive neurons were found in raphe and parapyramidal nuclei, rostral ventrolateral medulla, A5 region, Barrington's nucleus, and paraventricular and preoptic nuclei of the hypothalamus.

It is useful to compare the brainstem neurons retrogradely labeled when a conventional tracer is applied to spinal regions containing sympathetic (see next section) or parasympathetic preganglionic neurons with those labeled after application of the tracer to the S3-S4 level of the spinal cord, where all neural inflow and outflow is to somatic motor and sensory neurons relevant to the function of the tail (Masson et al., 1991). There are dense projections from raphe and parapyramidal neurons to the S3-S4 cord level, just as there are dense projections from these neurons to sympathetic and parasympathetic preganglionic neurons. The tail is important in micturition, defecation, and in sexual activity, functions involving both somatic and autonomic preganglionic neurons. It may be that individual raphe and parapyramidal neurons project to both classes of cells, thereby coordinating their discharge.

Brainstem and Hypothalamic Neurons Projecting to the Spinal Cord

The origin, within the brain, of neurons projecting to the thoracic spinal cord was originally described by degeneration techniques following appropriately situated lesions (Brodal, 1957). The combination of lesioning and fluorescence histochemical procedures established bulbospinal projections of monoamine-synthesizing neurons (Dahlström and Fuxe, 1965). The HRP retrograde axonal transport method was first used to study the origins of descending axons by Kuypers and colleagues (1975), with other studies following (Hancock, 1976; Bjorklund and Skagerberg, 1979; Blessing and Chalmers, 1979; Hökfelt et al., 1979; Martin et al., 1979, 1981; Satoh, 1979; Tohyama et al., 1979a,b; Hosoya, 1980; Swanson and Kuypers, 1980; Blessing et al., 1981a; Miura et al., 1983; Schwanzel-Fukuda et al., 1984; Skagerberg and Lindvall, 1985; Skagerberg and Björklund, 1985; Holstege, 1987). The distribution of all CNS neurons retrogradely labeled after injection of HRP into the thoracic spinal cord is shown in Figure 3.12.

Figure 3.12 Retrogradely labeled neurons (open circles) in hypothalamus and brainstem after application of HRP to one half of the thoracic spinal cord of the cat (see injection site). (Hypothalamic

panels modified from Kuypers and Maisky, 1975; other panels modified from Tohyama et al., 1979a.)

Brainstem projections to the dorsal horn of the spinal cord

Brainstem catecholamine and 5-HT neurons have major projections to the dorsal horn of the spinal cord (see references in Tables 3.5 and 3.6, below). TH-immunoreactive neurons in the locus coeruleus, subcoeruleus, and A5 and A7 cell groups project to the dorsal horn. 5-HT neurons (as well as non-5-HT cells) within rostroventromedial medulla and caudal pons, including the nucleus raphe magnus and the parapyramidal area, also project to the dorsal horn.

Sympathetic premotor neurons

The first evidence for the location of bulbar sympathetic premotor neurons came when HRP injections were confined to the intermediolateral region of the upper thoracic spinal cord of the cat (Amendt et al., 1978, 1979). Retrogradely labeled brainstem neurons were observed in the nTS, in the raphe, and in the ventrolateral medulla from 3 to 7 mm rostral to the obex (Fig. 3.13A).

Figure 3.13 **A**, First demonstration of bulbospinal neurons underlying the vasomotor "glycine-sensitive area" in the cat rostral medulla. Filled circles indicate neurons retrogradely labeled after deposition of HRP in the intermediolateral region of the thoracic spinal cord. (Modified from Amendt et al., 1978, 1979.) **B**, Summary of brainstem distribution of presympathetic neurons in rat demonstrated by transneuronal transport of live virus from the adrenal medulla. (Modified from Strack et al., 1989b.) **C**, Presympathetic vasomotor neurons in rabbit brain, demonstrated by transneuronal labeling after injection of live virus into the renal nerve. (Modified from Ding et al., 1993.) Abbreviations listed on pages xiii-xiv.

The transneuronal viral tracing technique has provided most valuable information concerning the location of the sympathetic premotor neurons in rat and rabbit (Strack et al., 1989ab; Wesselingh et al., 1989; Strack and Loewy, 1990; Ding et al., 1993; Blessing et al., 1994; Jansen et al., 1995b). Taken together with information based on other procedures (see below), we can be fairly confident that sympathetic premotor neurons occur only in the hypothalamus (in the paraventricular nucleus and in the dorsomedial and lateral hypothalamic regions), in the A5 region of the caudal pons, and in the rostral ventrolateral medulla and the raphe-parapyramidal regions of the medulla oblongata. The distributions of sympathetic premotor neurons for rats and rabbits are summarized in Figure 3.13B,C.

Adrenal preganglionic neurons are distributed in the spinal cord from T3 to L1 (Haase et al., 1982; Jensen et al., 1992; Li et al., 1992a). Transneuronal tracing studies with live viruses indicate that premotor neurons with direct projections from the brain to adrenal sympathetic preganglionic cells are located in the same four brain regions that contain premotor neurons innervating nonadrenal sympathetic preganglionic neurons (Strack et al., 1989b; Li et al., 1992d).

Neurotransmitter-related antigens in these spinally projecting neurons have been examined in the transneuronal viral tracing experiments (see references above) and in conventional retrograde transport double-label studies (results from the latter studies include projections to spinal cord sites other than sympathetic preganglionic cells). In the rostral ventrolateral medulla, at least 50% of presympathetic neurons are catecholamine-synthesizing C1 neurons (Minson et al., 1990). The remaining 50% of neurons do not contain catecholamine-synthesizing enzymes, and at present little is known concerning the neurochemical identity of these neurons. Double-labeling studies in the rat suggest that sympathetic premotor neurons in raphe and parapyramidal regions are mostly 5-HT and/or substance P cells, possibly also containing glutamate and aspartate (Bowker et al., 1981, 1988; Charlton and Helke, 1987; Helke et al., 1989, 1986; Bowker and Abbott, 1990; Nicholas et al., 1992). Those in the A5 area are mainly noradrenaline synthesizing. Spinally projecting neurons in the paraventricular nucleus of the hypothalamus include dopamine, substance P, oxytocin, vasopressin, neurotensin, somatostatin, and enkephalin neurons (Skagerberg and Lindvall, 1985; Sawchenko and Swanson, 1982b; Cechetto and Saper, 1988).

Anterograde transport studies with deposition of tracer in brainstem regions containing spinally projecting neurons has helped establish the existence of direct projections to the sympathetic spinal regions. Ross and colleagues (1984a) injected tritiated proline into the ventrolateral medullary region containing C1 neurons and showed projections to intermediolateral and intermediomedial columns, with terminals close to sympathetic neurons retrogradely labeled from the adrenal gland (see also Dampney et al., 1987). This projection from the C1 region has been elegantly confirmed with an anterograde PHA-L study that includes ultrastructural demonstration of PHA-L-positive axosomatic and axodendritic synaptic inputs to identified adrenal preganglionic neurons (Zagon and Smith, 1993). Anterograde transport studies support the existence of a direct projection from the A5 region and from the hypothalamic paraventricular nuclei to spinal sympathetic preganglionic neurons (Saper et al., 1976a; Loewy et al., 1979; Luiten et al., 1985; Hosoya et al., 1991). In the hypothalamic projection study by Hosoya and colleagues (1991), sympathetic preganglionic neurons were identified by retrograde transport of cholera-toxin HRP from the superior cervical ganglion, so the relationships between PHA-L-positive fibers (processes of paraventricular neurons) and both the perikarya and the dendrites of sympathetic preganglionic neurons are beautifully demonstrated at the light microscopic level.

Studies of the thoracolumbar spinal distribution of different neurotransmitter related markers have also helped define the categories of brainstem neurons likely to function as premotor sympathetic cells. Work on the spinal distribution of monoamine nerve terminals remains particularly important (Dahlström and Fuxe, 1964, 1965; Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz, 1974; Hökfelt et al., 1974; Anderson et al., 1989; Ridet et al., 1992; Mouchet et al., 1992), because the absence of monoamine-synthesizing perikarya from the spinal cord implied an origin from relevant brainstem or hypothalamic neurons. Taken in conjunction with the brainstem localization of PNMT-synthesizing neurons, the distribution of PNMT-containing terminals in the rat spinal cord remains compelling evidence for a direct projection from the medullary PNMT-containing neurons to sympathetic preganglionic neurons, probably from C1 and C3 groups (Hökfelt et al., 1974; Ross et

al., 1984a; Ruggiero et al., 1985b; Kalia et al., 1985b; Tucker et al., 1987; Carlton et al., 1987, 1991; Minson et al., 1990). Supporting ultrastructural evidence for a monosynaptic connection is now available (Milner et al., 1988a; Bernstein-Goral and Bohn, 1989). Similar arguments have been advanced for neuropeptide Y, an additional neurotransmitter marker in C1 neurons (Blessing et al., 1987b; Llewellyn-Smith et al., 1990; Tseng et al., 1993). Close appositions between 5-HT boutons and sympathetic preganglionic cells (Jensen et al., 1995) is consistent with an input from B1-B3 groups in the raphe and parapyramidal area of the medulla oblongata. Innervation by boutons containing dopamine (Ridet et al., 1992; Mouchet et al., 1992) suggests an input from the paraventricular nucleus of the hypothalamus. Innervation of sympathetic preganglionic neurons by boutons containing other neurotransmitter markers such as substance P (Pilowsky et al., 1992) supports the idea of a projection from defined brainstem groups, but these boutons could arise from intrinsic spinal cord perikarya. Similarly, inputs containing markers for glutamate (Morrison et al., 1989; Minson et al., 1991; Llewellyn-Smith et al., 1992) and GABA could arise from various sources.

It is notable that, after transneuronal transport through sympathetic preganglionic cells, virus-positive neurons do not occur in the A1 noradrenaline group, in the nTS, in the parabrachial nucleus (including the Kölliker-Fuse subdivision), in the A7 group, in the locus coeruleus, or in the periaqueductal gray. In the forebrain, only hypothalamic regions, principally the paraventricular nuclei, are labeled. There is no evidence for any direct monosynaptic projection from the cerebral cortex to sympathetic preganglionic cells. Dissenting views, based on alternative sources of evidence, suggest that there are direct projections to spinal sympathetic preganglionic neurons from the nTS (Mtui et al., 1993), from the Kölliker-Fuse nucleus (Tucker and Saper, 1985), and possibly from the medial prefrontal cortex (Bacon and Smith, 1993).

The neuroanatomical studies do not indicate which classes of sympathetic premotor neurons are located in the different brainstem regions. As noted at the beginning of this chapter, the presence of a vascular supply to all tissues means that when a virus is transneuronally transported after application to a particular peripheral organ or tissue, the labeled sympathetic premotor neurons might be vasomotor in function, or they might be involved in control of the specific function of the organ. The relative numbers of virus-positive cells in the different regions may provide some clue to functional specificity of the various groups of premotor neurons. The premotor cells in the rostral ventrolateral medulla are thought to be principally vasomotor in function (see Chapter 5). When there is a predominance of positive neurons in other premotor regions, as observed, for example, in the paraventricular nucleus after injection of virus into the iris muscle (Blessing et al., 1994), there are some grounds for inferring specificity of function. Thus, in the iris muscle it may be that the premotor sympathetic pupillodilator neurons are located in the paraventricular nucleus of the hypothalamus.

Inputs to Sympathetic Premotor Neurons

As already discussed, the rostral ventrolateral medulla contains different populations of neurons, with their own specific connectivity and function. More detail concerning inputs to particular populations

of neurons is given in Chapters 4, 5, and 6. References to nuclei with inputs to the rostral ventrolateral medulla are included in those listed in Table 3.5 at the end of this chapter. Neurons with little or very sparse projections to the RVLM include the locus coeruleus cells (Fritschy and Grzanna, 1990b). A useful study of local interconnections in the rostral ventrolateral medulla region has been completed by Zagon (1995). A summary of probable inputs to the bulbospinal rostral ventrolateral medulla presympathetic cardiovascular neurons (see Chapter 5), with an example of evidence suggesting an input from the caudal ventrolateral medulla, is shown in Figure 3.14.

Trigeminal Nuclei and Paratrigeminal Islands

The anatomy of the principal sensory nucleus of V and the mesencephalic nucleus of V are not dealt with in this book. Projections to these nuclei, to spinal nuclei of the trigeminal nerve, and to the paratrigeminal islands are described in papers detailing anterograde tracing studies (Marfurt, 1981; Nomura et al., 1984, 1986; Mizuno and Nomura, 1986; Marfurt and Rajchert, 1991). The spinal nucleus of V and the paratrigeminal islands are the only extra-nTS regions anterogradely labeled after tracer injections into the sensory branches of lower cranial nerves. The paratrigeminal nuclei are diffuse collections (islands) of neurons located within the spinal trigeminal tract near the obex. The papers of Panneton and colleagues contain useful information and references concerning the paratrigeminal islands (Panneton and Burton, 1985; Panneton, 1991a). Major projections from the dorsal horn of the spinal cord, spinal V, and paratrigeminal islands to the nTS have been described (Nomura et al., 1984; Menetrey and Basbaum, 1987).

Figure 3.14 A, Summary of brain regions innervating presympathetic vasomotor neurons in the rostral ventrolateral medulla. (Modified from Dampney, 1994.) **B1**, Boutons containing *Phaseolus vulgaris* leucoagglutinin, anterogradely transported from the caudal ventrolateral medulla, surround a rostral ventrolateral medulla neuron trans-neuronally labeled after injection of live virus into the adrenal gland. The full distribution of *Phaseolus* in the rostral ventrolateral medulla is shown in **B2**, and the full distribution of virus-positive neurons in the same area is shown in **B3**. (Modified from Li et al., 1992d.) Abbreviations listed on pages xiii-xiv.