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Immunohistochemical Localization of Neuropeptides and Neurotransmitters in the Nucleus Solitarius

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Abstract

The nucleus tractus solitarii (NTS), which receives visceral afferent information from the cardiovascular, respiratory, gastrointestinal and taste systems, contains multiple neurotrasmitters and neuropeptides throughout its rostral to caudal extent. The neurotransmitters and neuropeptides immunoreactivity is located predominately in varicose fibers and small puncta throughout the neuropil. In addition, immunoreactive NTS neurons for a variety of neurotransmitters and neuropeptides are present in subnuclear regions.

The neuroactive substances localized immunohistochemically in the NTS include acetylcholine, the neuropeptides, substance P, methionine- and leucine-enkephalin, β-endorphin, cholecystokinin, neurotensin, galanin, calcitonin gene-related peptide, somatostatin, FMRMamide, neuropeptide Y, angiotensin II, vasoactive intestinal polypeptide, vasopressin, oxytocin, thyrotropin-releasing hormone, luteinizing hormone-releasing hormone, atrial natriuretic peptide, the catecholamines, dopamine, norepinephrine, epinephrine, serotonin, histamine and the amino acids, GABA and glutamate. The pattern of innervation for each neurotransmitter and neuropeptide is not homogeneously distributed throughout the NTS. Each substance has a unique pattern within the NTS as each subnuclear region contains different immunohistochemical staining patterns and densities of fibers.

At the ultrastructural level both neurotransmitters and neuropeptides are present in synaptic terminals that are in contact with different parts of the neuronal membranes. Typically, the labeled terminals contain both small, clear vesicles and large, dense core vesicles with the exception of synaptic terminals containing acetylcholine, GABA and glutamate which do not typically have the large, dense core vesicles. The most frequent post-synaptic target are dendrites and spinous processes. Less frequently, synaptic contacts are present on the cell soma. **Chem. Senses 21: 367–376, 1996.**

The nucleus tractus solitarii (NTS) in the cat is a cylindrically-shaped nucleus located in the dorsomedial medulla extending from the level of the gracile and cuneate nuclei in the caudal medulla to the facial motor nucleus rostrally. Caudal to the obex, the NTS from each side fuses into a midline structure (Loewy and Burton, 1978). The NTS receives visceral afferent fibers from the cardiovascular, respiratory, gastrointestinal and gustatory systems traveling in cranial nerves VII, IX and X in a viscerotopically organized manner (Torvik, 1956; Cottle, 1964; Beckstead

and Norgren, 1979). The NTS in different animals has been divided into subnuclear divisions based mainly upon cytoarchitectonics (Loewy and Burton, 1978; Kalia and Mesulam, 1980; Kalia and Fuxe, 1985; Maley *et al.*, 1987; Whitehead, 1990). In the cat the commissural, intermediate, medial, interstitial, parvicellular, dorsal, dorsolateral, ventral and ventrolateral nuclei have been described (Loewy and Burton, 1978; Kalia and Mesulam, 1980). Most subdivisions do not extend the length of the NTS. For example, the commissural subdivision is present only in the portion caudal to the obex, while the parvicellular and interstitial are present only in the middle third of the nucleus. Some subdivisions, such as the medial, ventral and ventrolateral are present throughout the length of the nucleus. Afferent fibers are viscerotopically organized so that afferent fibers from different visceral systems terminate in specific subnuclear regions (Kalia and Mesulam, 1980). The majority of visceral afferent fibers from the cardiovascular, respiratory and gastrointestinal systems terminate in the caudal two-thirds of the nucleus, while gustatory afferent fibers are localized to its rostral third. In addition to its peripheral afferent innervation, the NTS receives a rich innervation from central nervous system nuclei. In the present report the distribution of neurotransmitters and neuropeptides will be discussed at three distinct levels of the cat NTS. First, a level caudal to the obex which contains the commissural, medial, ventral, dorsal and ventrolateral subdivisions. Secondly, an intermediate area at the level of the area postrema containing the ventral, medial, parvicellular, dorsolateral, dorsal, interstitial and ventrolateral subdivisions. Thirdly, a level at the near rostral extent of the nucleus containing medial, ventral, dorsolateral and ventrolateral subdivisions.

The diverse innervation of the NTS is reflected by the localization of numerous neurotransmitters and neuropeptides within its nuclear subdivisions. The list of neurotransmitters and neuropeptides in terminals and cell bodies reported in the NTS include acetylcholine, the excitatory amino acids, glutamate and aspartate, the inhibitory amino acid, GABA, the biogenic amines, dopamine, noradrenaline, adrenaline, serotonin, histamine, and the neuropeptides including substance P, enkephalin, galanin, cholecystokinin, vasopressin, oxytocin, neurotensin, somatostatin, calcitonin gene-related peptide, neuropeptide Y, α-melanocyte stimulating hormone, β-endorphin, vasoactive intestinal polypeptide, corticotropin-releasing factor, thyrotropin releasing hormone, bombesin, atrial natriuretic peptide and angiotensin II. As would be expected, each neurotransmitter or neuropeptide has its own unique pattern of distribution within the NTS.

While it is still not clear if each of the neuropeptides acts as a neurotransmitter, it is evident that they play major roles in synaptic transmission and circuitry of the NTS. The NTS contains numerous neuropeptides; some of the neuropeptides are present in large quantities, while others are represented by only a scattering of fibers throughout the nucleus. Substance P, enkephalin, galanin, calcitonin gene-related peptide,. cholecystokinin, neurotensin and neuropeptide Y, are present throughout the NTS in large quantities. The remaining neuropeptides, including endorphin, vasopressin, oxytocin, neurotensin, somatostatin, α-melanocyte stimulating hormone, β-endorphin, vasoactive intestinal polypeptide, corticotrophin-releasing factor, thyrotropin-releasing hormone, bombesin, atrial natriuretic peptide and angiotensin II are present in limited quantities, as scattered individual fibers and occasional cell bodies.

Substance P is present throughout the entire rostral to caudal extent of the NTS in both fibers and cell bodies (Maley and Elde, 1982b; Maley *et al.*, 1983, 1987). At levels of the nucleus caudal to the obex substance P immunoreactive fibers can be seen crossing from one side to the other in the commissural subdivision. At the level of the area postrema it is located in the medial, intermediate, dorsolateral and interstitial subdivisions, while far fewer substance P fibers are present in the ventrolateral and parvicellular regions (Maley and Elde, 1982b). In the parvicellular subdivision a noticeable band of substance P immunoreactive fibers surround a central core lacking any substantial substance P immunoreactivity. The same regions that contain substance P fibers also possess scattered substance P containing neurons (Maley and Elde, 1982b). At rostral levels the substance P is greatly reduced although a few immunoreactive fibers are present in the medial and ventral subdivisions. The majority of substance P is thought to be of vagal origin, although some undoubtedly arise from intrinsic substance P immunoreactive neurons (Brimijoin *et al.*, 1980; Katz and Karten, 1980; Helke *et al.*, 1984).

Enkephalin immunoreactivity occupies the same regions as substance P, however; the amount of enkephalin immunoreactivity is noticeably less (Maley and Elde, 1982b; Maley *et al.*, 1983; Mantyh and Hunt, 1984; Maley *et al.*, 1987; Maley and Panneton, 1988). Caudal to the level of the obex enkephalin immunoreactivity is most prominent in the commissural and intermediate subdivisions. Rostral to the obex at the level of the area postrema enkephalin immunoreactivity is located mainly in the medial, ventral and interstitial subdivisions. At rostral levels of the nucleus enkephalin immunoreactive fibers are present in the medial

and ventral subdivisions. Enkephalin immunoreactive neurons are present in the medial and commissural subdivisions, and they are presumed to be responsible for the majority of enkephalin immunoreactivity in the NTS (Maley *et al.*, 1983; Mantyh and Hunt, 1984; Maley *et al.*, 1987).

Galanin immunoreactive fibers are present throughout the rostral to caudal extent of the NTS. Caudal to the obex galanin immunoreactivity is located in the commissural, dorsal and medial subdivisions. In the middle third of the NTS galanin immunoreactivity is in the dorsal, dorsolateral, medial, parvicellular, interstitial and ventral subdivisions, while the ventrolateral subdivision has very few fibers (Melander *et al.*, 1986; Michener *et al.*, 1990; Maley *et al.*, 1993). Just rostral to the level of the area postrema level galanin immunoreactivity is lost in the medial, interstitial and central portions of the parvicellular subdivisions, while the ventral and dorsolateral subdivisions retains its immunoreactivity. At rostral levels galanin immunoreactivity is present in moderate levels in the medial, ventral and ventrolateral subdivisions, and most prominently in the dorsolateral subdivision as a thin band of immunoreactive staining. Galanin positive neurons are present in the medial, commissural, parvicellular and dorsolateral subdivisions (Herbert and Saper, 1990). Interestingly, the dorsal motor nucleus of the vagus nerve contains large amounts of galanin immunoreactivity from its caudal to rostral extent. It is not clear as to origin of the galanin fibers in the nucleus; however, some is probably of intrinsic origin.

Calcitonin gene-related peptide (CGRP) has one of the densest network of fibers in the NTS (Kruger *et al.*, 1988; Batten *et al.*, 1989; Maley, 1995). At levels caudal to the obex, CGRP immunoreactivity is in the dorsal subdivision and commissural subdivision crossing the midline to the contralateral nucleus. Less immunoreactivity is in the ventral medial and ventrolateral subdivisions. Rostral to the obex, the parvicellular, medial and interstitial subdivisions have the greatest density of immunoreactive. Slightly less immunoreactivity is present in the dorsolateral, dorsal and ventral subdivisions At rostral levels, the CGRP immunoreactivity persists in the pavicellular, medial, ventral, dorsal and ventrolateral subdivisions. At the most rostral level CGRP immunoreactivity is still present in the medial subdivision. At least in the cat, all the calcitonin gene-related peptide immunoreactive fibers are extrinsically derived since no cell bodies positive for this neuropeptide have been found (Rranco-Cereceda *et al.*, 1987). CGRP immunoreactive cell bodies have been localized in the NTS of the rat (Morishima *et al.*, 1985).

Cholecystokinin (CCK) is present in many of the same subdivisions as many of the neuropeptides in the NTS. At levels of the NTS caudal to the obex, a prominent band of CCK immunoreactivity is present in the commissural subdivision, while the medial and dorsal subdivisions have very little. The NTS at the level of the area postrema contain prominent CCK immunostaining in the interstitial, dorsolateral, medial and parvicellular subdivisions. Less CCK immunoreactivity is in the intermediate and ventrolateral subdivisions (Newton and Maley, 1985; Ladenheim *et al.*, 1988; Howes *et al.*, 1989). In the rostral third of the nucleus a thin rim of CCK immunoreactivity is present in the medial and ventrolateral subdivisions, immediately surrounding the solitary tract. CCK immunoreactive neurons are present in the medial, parvicellular and commissural subdivisions, and undoubtedly contribute to the immunoreactivity within the NTS. Some CCK immunoreactive neurons project to other regions of the central nervous system (Herbert and Saper, 1990; Wang *et al.*, 1992).

Neuropeptide Y within the interstitial, medial and commissural subdivisions of the NTS has a dense plexus (Loren *et al.*, 1979; Massari *et al.*, 1990). In the parvicellular, dorsolateral and intermediate subdivisions neuropeptide Y immunoreactivity is sparse. The ventrolateral subdivision typically does not contain neuropeptide Y. Neuropeptide Y immunoreactive cell bodies are present in the medial and commissural subdivisions (Hokfelt *et al.*, 1983; Pickel *et al.*, 1989a).

Neurotensin immunoreactivity at caudal levels of the NTS is located principally in the medial and commissural subdivisions. At intermediate levels it is in the intermediate, dorsolateral and medial subdivisions, while the ventrolateral and parvicellular subdivisions contain very little neurotensin immunoreactivity (De León *et al.*, 1991). In the rostral third of the nucleus, neurotensin immunostaining is situated as a thin rim surrounding the solitary tract in the medial and ventrolateral subdivisions. However, neurotensin immunoreactivity is less in the NTS when compared to the previously mentioned neuropeptides, although it has the same pattern within NTS subdivisions. Intrinsic neurotensin immunoreactive neurons have been reported in the NTS (Triepel *et al.*, 1984; Wang *et al.*, 1992). It is assumed that they contribute intrinsic fibers, as well as sending projections to other central nervous system nuclei.

The remaining neuropeptides which include endorphin, dynorphin, vassopressin, oxytocin, somatostatin, α-melanocyte stimulating hormone, vasoactive intestinal polypeptide, thyrotropin-releasing hormone, bombesin, atrial natriuretic

peptide and angiotensin II are present as scattered fibers in various NTS subdivisions.

Since it has not been possible to generate antibodies directly to the acetylcholine molecule, many studies describing its localization have utilized antibodies to its synthetic enzyme, choline acetyltransferase, to describe the distribution of acetylcholine (Kimura *et al.*, 1981; Maley *et al.*, 1988; Ruggiero *et al.*, 1990). Acetylcholine immunoreactivity is contained in thin, varicose fibers located within the NTS (Kimura *et al.*, 1981; Ruggiero *et al.*, 1990). At caudal levels of the NTS, fibers have been described crossing the midline in the commissural subdivision (Ruggiero *et al.*, 1990). In the NTS at the level of the area postrema the greatest amount of acetylcholine is present in the central subdivision of the rat with fewer immunoreactive fibers in the medial, intermediate, dorsolateral and ventrolateral subdivisions (Kimura *et al.*, 1981; Ruggiero *et al.*, 1990). At the rostral third of the nucleus the medial subdivision contains acetylcholine fibers. In the intermediate and medial subdivisions scattered cholinergic neurons are present (Kimura *et al.*, 1981; Armstrong *et al.*, 1988; Ruggiero *et al.*, 1990). Receptor autoradiography for both muscarinic (using [^{3}H]quinuclidinyl benzilate) and nicotonic (using [^{3}H]nicotine or [^{125}I]α-bungarotoxin) cholinergic binding sites indicates that both receptor subtypes are present in the NTS. Muscarinic binding sites are located in more caudal regions, including the commissural, intermediate, interstitial medial, parvicellular, ventrolateral, ventral and dorsal subdivisions (Maley and Seybold, 1993). Nicotinic cholinergic binding sites, identified using [^{3}H]nicotine, are predominately located at more rostral levels in the medial, ventrolateral and ventral subdivisions (Maley and Seybold, 1993). In the caudal two-thirds of the nucleus nicotinic cholinergic binding sites are located only in the interstitial subdivision (Maley and Seybold, 1993). Interestingly, [^{125}I]α-bungarotoxin, which is a second ligand used to identify nicotinic cholinergic binding sites, is absent at the rostral third of the nucleus, but are present in the caudal two thirds in many of the same subdivisions as muscarinic cholinergic binding sites (Maley and Seybold, 1993). The segregation of nicotine cholinergics as identified with [^{3}H]nicotine in rostral regions suggests that nicotine cholinergics play a role in gustatory sensations. The source of cholinergics to the NTS is most probably from extrinsic sources, such as the adjacent dorsal motor nucleus of the vagus nerve, since only a few cholinergic neurons have been reported in the NTS.

Both the excitatory amino acid, glutamate and the inhibitory amino acid, GABA, are present throughout the NTS (Dietrich *et al.*, 1982; Maley and Newton, 1985; Lasiter and Kachele, 1988). GABA and glutamate immunoreactivity are present in puncta, as well as in neurons throughout the length of the NTS (Dietrich *et al.*, 1982; Maley and Newton, 1985). The parvicellular subdivision contained the fewest GABA and glutamate immunoreactive varicosities; however, it had the greatest number of GABA immunoreactive neurons. Within many of the subdivisions GABA and glutamate immunoreactive puncta formed what appears to be pericellular arborizations around both GABA immunoreactive neurons and non-immunoreactive neurons (Maley and Newton, 1985). GABA immunoreactivity in the NTS is in part from intrinsic GABA containing neurons in the NTS (Maley and Newton, 1985). Much of the glutamate immunoreactivity is visceral afferents to the nucleus, although the presence of glutamate immunoreactive neurons in the nucleus suggests that they are also a possible source (Sved and Backes, 1992).

The NTS is a part of the A2 catecholaminergic cell group which contains a mixture of dopamine, noradrenalin, and adrenalin in cell bodies and fibers. Most studies which have examined the localization in the NTS of the catecholamines, dopamine, noradrenalin and adrenalin have used the synthetic enzymes, tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine-N-methyl transferase, respectively, as their marker (Kalia *et al.*, 1985a,b). Dopamine containing fibers are found throughout the nucleus, but predominately in the caudal two-thirds of the NTS. In particular, the dorsolateral and dorsal subdivisions contain the greatest amounts, while the intermediate, medial, ventral and ventrolateral regions have slightly less dopamine (Kalia *et al.*, 1985a). At rostral levels dopamine containing fibers are reduced in all subdivisions. Noradrenalin immunoreactive fibers are more extensive than dopamine in the NTS and also extend from the caudal extent to the rostral pole of the nucleus. Adrenaline containing fibers in the NTS is less than the other two catecholamines and is scattered throughout the entire nucleus. Catecholamine containing neurons in the NTS are predominately noradenaline, although there are dopamine-containing neurons scattered among these cells.

Serotonin immunoreactivity appears as varicose fibers spread throughout the rostral to caudal extent of the NTS (Maley and Elde, 1982b). In the commissural subdivision of the caudal NTS serotonin immunoreactive fibers cross from one side of the nucleus to the other (Maley and Elde, 1982b). At more rostral levels serotonin immunoreactive fibers are present throughout the nucleus with the exception

of the ventrolateral subdivision which has very little (Maley and Elde, 1982b). In the case of serotonin the neurons responsible for this innervation are located in the raphe system outside the NTS (Schaffar *et al.*, 1988; Thor and Helke, 1989; Li *et al.*, 1992). At rostral levels serotonin immunoreactivity is present in the medial and ventral subdivisions.

It is still not clear if all of the neurochemicals with the possible exception of acetylcholine, the catecholamines and amino acids localized within the NTS are, indeed, true neurotransmitters. The use of electron microscopy to localize neuropeptides within presynaptic terminals have attempted to answer in part if these neurochemicals are parts of synaptic circuits within the NTS.

The morphological appearance of neuropeptide-containing terminals in the NTS is remarkably similar (Voom and Buijs, 1983; Jaeger *et al.*, 1984, Maley, 1985; Morishima *et al.*, 1985; Kawai and Takagi, 1989; Rinaman *et al.*, 1989; Fodor *et al.*, 1990; Velley *et al.*, 1991). The majority of peptide containing terminals contain two distinct populations of synaptic vesicles. The first group of synaptic vesicles, which is the more numerous of the two, are small, clear vesicles that range in size from 40 to 60 nm. These small synaptic vesicles are the ones typically associated with the active zone of the synaptic junction (Jaeger *et al.*, 1984; Maley, 1985). The second category of synaptic vesicles are the large, granular vesicles (70–150 nm in diameter) that are usually not directly associated with the active zone of the terminal (Jaeger *et al.*, 1984). Typically, the large, granular vesicles are present some distance from the synaptic junction and are considered to be responsible for the storage/transportation of the neuropeptide in the axon terminal. Peptide containing terminals are usually associated with some type of synaptic specialization, whether an asymmetrical or symmetrical junction. Within the NTS the neuropeptides are typically associated more frequently with the asymmetrical terminal, although in the case of the enkephalins many of those terminals are of the symmetrical variety. The significance of symmetrical versus asymmetrical specializations is not totally understood at present; however, it has been proposed that symmetrical membrane specializations are associated with inhibitory neurotransmission while asymmetrical specializations are indicative of excitatory synaptic interactions (Anderson *et al.*, 1963; Eccles, 1964). However, a number of investigators do not support this view. In the NTS, substance P is considered to be excitatory and its synaptic junctions are typically asymmetrical, while enkephalin, which is inhibitory in the NTS, is present in synaptic terminals that have symmetrical synaptic junctions (Jaeger *et al.*, 1984; Maley, 1985). However, some substance P immunolabeled terminals do have symmetrical junctions and a some enkephalin containing terminals have asymmetrical junctions (Jaeger *et al.*, 1984; Maley, 1985). This would seem to suggest that there is no relationship between the form of the synaptic specialization and its function. However, many of the neuropeptides have been reported to be co-localized with classical neurotransmitters which may be either inhibitory or excitatory (Pickel *et al.*, 1990), thus making the association of symmetry of the junction with excitation and inhibition even more unclear (Magoul *et al.*, 1986).

Although the morphology of peptide-containing terminals is remarkably similar, there are noticeable differences in the distribution of different neuropeptide containing terminals along the neuronal membrane (Jaeger *et al.*, 1984). Substance P-containing terminals contact small, distal dendrites and spinous processes, and very seldom are in contact with the large, proximal dendrites and never the cell somata of NTS neurons (Maley, 1985). In contrast, enkephalin immunolabeled terminals are located on the cell somata and proximal dendrites, with far fewer synaptic contacts on distal dendrites and spinous processes (Maley, 1985). Additionally, enkephalin terminals are presynaptic to other axonal terminals, while substance P-labeled terminals have never been found presynaptic to other axonal terminals. These studies suggest that there is a segregation of synaptic terminals along the neuronal membrane of NTS neurons. This also may be indicative of the ability of a neuropeptide to excite or inhibit post-synaptic neurons. It has been reported that more proximally placed synaptic terminals have potentially a greater influence than those located along more peripheral portions of a neuron's membrane (Rall, 1962, 1967).

GABA-containing terminals in the NTS contain almost exclusively a population of small, clear vesicles (30–50 nm in diameter) and, typically, they have a symmetrical junctional complex at the synaptic junction (Lipski *et al.*, 1990). As suggested by light microscopic studies, GABA-containing terminals are found along the neuronal membrane of NTS neurons. However, a slightly greater number of the GABA terminals are presynaptic to proximal regions of the neuron (cell somata, large, proximal dendrites, intermediate dendrites) than more distal regions of the dendritic tree and spinous processes. Glutamanergic terminals, which are remarkably similar to GABAergic terminals, contain a single population of synaptic vesicles that are small, round and clear (40–60 nm in diameter). It has been estimated that

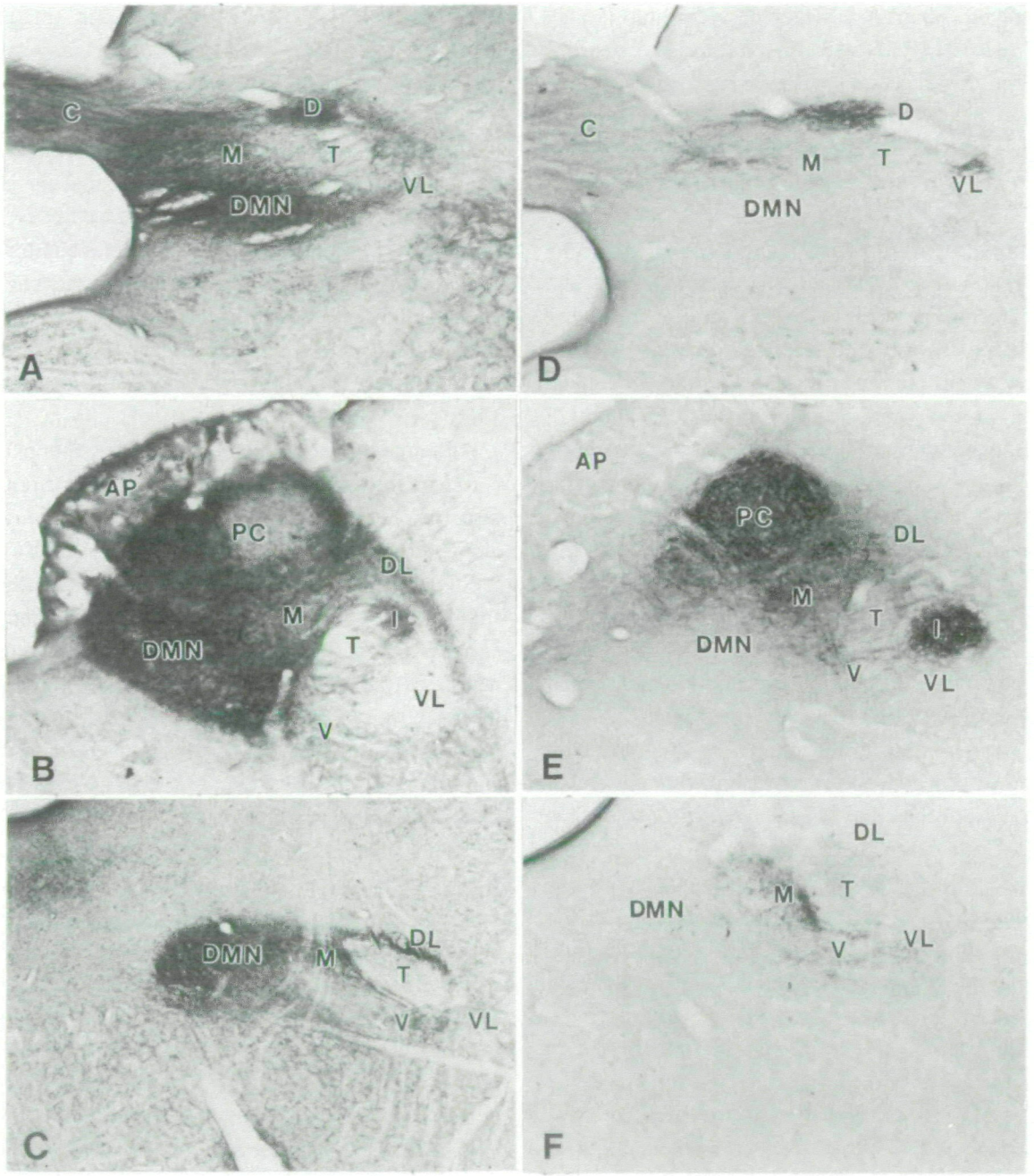

Figure 1 **(A)** Photomicrograph of galanin immunoreactivity in the caudal NTS of the cat. The commissural (C), dorsal (D), medial (M) and ventrolateral (VL) subdivisions contain galanin immunoreactive fibers. The dorsal motor nucleus of the vagus nerve (DMN) contains intense staining for galanin immunoreactivity Final magnification ×44. **(B)** Photomicrograph of galanin immunoreactivity in the cat NTS at the level of the area postrema (AP). The majority of galanin immunoreactivity is located in the medial (M), dorsolateral (DL), interstitial (I) subdivisions and dorsal motor nucleus of the vagus nerve (DMN). The parvicellular (PC) and ventral (V) subdivisions contain less immunoreactivity, while the ventrolateral subdivision (VL) has no discernible galanin immunoreactivity. The solitary tract (T) is indicated. Final magnification ×44. **(C)** Photomicrograph of galanin immunuoreactivity in the rostral NTS. Galanin immunoreactivity is reduced to thin bands in the medial (M), ventral (V), ventrolateral (VL) and dorsolateral (DL) subdivisions which surround the solitary tract (T). The dorsal motor nucleus of the vagus nerve (DMN) still contains intense immunostaining for galanin. Final magnification ×52. **(D)** Photomicrograph of calcitonin gene related peptide (CGRP) in the caudal NTS of the cat. CGRP immunoreactivity is localized at a dense band in the dorsal (D) subdivision which at even more caudal sections continues into the commissural (C) subdivision. The ventrolateral (VL) and the medial (M) subdvsions contain a small amount of CGRP immunoreactivity. The solitary tract (T) and dorsal motor nucleus of the vagus nerve (DMN) contain no CGRP immunoreactivity. Final magnification ×40. **(E)** Photomicrograph of calcitonin gene related peptide in the cat NTS at the level of the area postrema (AP). The majority of CGRP immunoreactivity is located in the parvicellular (PC) and interstitial (I) subdivisions. The medial (M), dorsolateral (DL) and ventral (V) subdivisions have considerably less immunoreactivity, while the ventrolateral subdivision (VL) has almost none. The dorsal motor nucleus of the vagus nerve (DMN) has no immunoreactivity. The solitary tract (T) is indicated. Final magnification ×44. **(F)** Photomicrograph of CGRP immunoreactivity in the rostral NTS of the cat. CGRP immunoreactivity is restricted to the medial (M) subdivision, while the ventral (V), ventrolateral (VL) and dorsolateral (DL) subdivisions lack any CGRP immunoreactivity. The solitary tract (T) and dorsal motor nucleus of the vagus nerve (DMN) are indicated. Final magnification ×40.

they comprise 40% of the synaptic population in the NTS (Saha *et al.*, 1995). They have both symmetrical and asymmetrical junctional complexes, and are in synaptic contact with all portions of the neuronal membrane (unpublished observations). Acetylcholine immunoreactive terminals are characterized by a single population of round, clear synaptic vesicles (50–60 nm; Maley *et al.*, 1988).

Serotonin-containing terminals contain numerous large, granular vesicles (70–120 nm in diameter) and a smaller population of small, clear synaptic vesicles, 40–60 nm in diameter. Typically, serotonin synaptic terminals make synaptic contacts on distal dendrites (Maley and Elde, 1982a; Maley *et al.*, 1990; Voss *et al.*, 1990). In addition to presynaptic terminals in the NTS serotonin immunoreactivity is contained in non-synaptic varicosities containing large granular vesicles. Dopamine, noradrenaline and adrenaline are characterized by a population of small, clear vesicles (40–60 nm in diameter) and a somewhat smaller group of large, granular vesicles (80–150 nm in diameter) (Chiba and Doba, 1975; Pickel *et al.*, 1976 1989b; Maley *et al.*, 1990). At the synaptic junction, which can be asymmetrical or symmetrical, only small, clear vesicles are found (Maley *et al.*, 1990). Catecholamine terminals contact predominately dendritic processes and spinous processes, although axosomatic synapses involving at least dopamine and noradrenaline have been reported.

The morphological appearance of synaptic terminals containing the neurotransmitters/neuropeptides is for the most part remarkably similar, i.e. most have two populations of vesicle types, a group of small, clear vesicles and a second group consisting of large, granular vesicles, and have either asymmetrical or symmetrical junctions associated with them. Typically, the amino acid containing terminals (GABA and glutamate) and acetylcholine labeled terminals have only the small, clear vesicles, although on occasional a few large, granular vesicles can be found in these terminals. It is not surprising that the morphological appearances of the many classes of neurotransmitters/neuropeptides are similar to one another, since it has been reported that synaptic terminals contain multiple neurotransmitters. What does appear to be distinctive of these chemically-labeled terminals is their distribution along the neuron's membrane. With regard to the neuropeptides which have been most extensively investigated, they have unique patterns of distribution along the membrane. It is probable that as the remaining neurotransmitter/neuromodulators are studied in the NTS similar types of patterns will emerge.

The localization of receptors in the NTS demonstrates that many of the neuropeptides and neurotransmitters have some role in the synaptic circuits in the nucleus. For the majority of receptors that have been studied in the NTS there is a good correlation between its presence and that of the neuropeptide or neurotransmitter. For the neuropeptides, substance P, CGRP and galanin the overlap of their receptor binding sites and immunohistochemical localization of the neuropeptide is in very good agreement. However, these light microscopic studies do not provide definitive evidence that there is a perfect registry between the presynaptic neuropeptide and its receptor binding site. In the case of the endogenous opiates (enkephalin, endorphin and dynorphin) it is clear that the opiate receptor subtypes reported to be associated with each of the opioid peptides do not correspond to the immunohistochemical localization of these three neuropeptides in the NTS. Clearly, the NTS contains far more enkephalin immunoreactivity than dynorphin and endorphin, suggesting that these opioid peptides or their precursor molecules interact at multiple opiate receptor types. It is also becoming apparent that the mu opioid receptor is present at non-synaptic sites, in addition to the active zone of the synaptic junction, suggesting that opioid peptides or their precursors are exerting their influence on target cells in almost a paracrine fashion. Obviously, additional investigations are needed to delineate the interactions of neuropeptides with their reported binding sites in the NTS.

REFERENCES

Anderson, P., Eccles J.C. and Voorhoeve P.E. (1963) Inhibitory synapses on somas of Purkinje cells in the cerebellum. *Nature*, **199,** 655–656.

Armstrong, D.M., Rotler, A., Hersh, L.B. and Pickel, V.M. (1988) Localization of choline acetyltransferase in perikarya and dendrites within the nuclei of the solitary tracts. *J. Neurosci. Res.*, **20,** 279–290.

Batten, T.F.C., Lo, V.K.F., Maqbool, A. and McWilliam, P.N. (1989) Distribution of calcitonin gene-related peptide-like immunoreactivity in the medulla oblongata of the cat, in relation to choline acetyltransferaseimmunoreactive motoneurones and substance P-immunoreactive fibres. *J. Chem. Neurol.*,. **2,** 163–176.

Beckstead, R.M. and Norgren, R. (1979) An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal and vagal nerves in the monkey. *J. Comp. Neurol.*, **184,** 455–472.

Brimijoin, S., Lundberg, J.M., Brodin, E., Hokfelt, T. and Nilsson,

G. (1980) Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. *Brain Res.*, **191,** 443–457.

Chiba, T. and Doba, N. (1975) The synaptic structure of catacholaminergic axon varicosities in the dorso–medial portion of the nucleus tractus solitarius of the cat: possible roles in the regulation of cardiovascular reflexes. *Brain Res.*, **84,** 31–46.

Cottle, M.K. (1964) Degeneration studies of primary afferents of IXth and Xth cranial nerves in the cat. *J. Comp. Neurol.*, **122,** 329–345.

De León, M., Coveñas, R., Narváez, J.A., Tramu, G., Aguirre, J.A., and González-Barón, S. (1991) Distribution of neurotensin-like immunoreactive cell bodies and fibers in the brainstem of the adult male cat. *Peptides*, **12,** 1201–1209.

Dietrich, W.D., Lowry, O.H. and Loewy, A.D. (1982) The distribution of glutamate, GABA and aspartate in the nucleus tractus solitarius of the cat. *Brain Res.*, **237,** 254–260.

Eccles, J.C. (1964) *The Physiology of Synapses*. Springer-Verlag, Berlin.

Fodor, M., Csiffáry, A., Kiss, P. and Palkovits, M. (1990) Dynorphin A-containing neural elements in the nucleus of the solitary tract of the rat. Light and electron microscopic immunohistochemistry. *Brain Res.*, **522,** 251–258.

Helke, C.J., Shults, C.W., Chase, T.N. and O'Donohue, T.L. (1984) Autoradiographic localization of substance P receptors in rat medulla: effect of vagotomy and nodose ganglionectomy. *Neuroscience*, **12**, 215–223.

Herbert, H. and Saper, C.B. (1990) Cholecystokinin-, galanin-, and corticotropin-releasing factor-like immunoreactive projections from the nucleus of the solitary tract to the parabrachial nucleus in the rat. *J.Comp.Neurol.*, **293,** 581–598.

Hokfelt, T., Lundberg, J.M., Tatemoto, K., Mutt, V., Terenius, L., Polak, J., Bloom, S., Sasek, C., Elde, R. and Goldstein, M. (1983) Neuropeptide Y (NPY)- and FMRFamide neuropeptide-like immunoreactivities in catecholamine neurons of the rat medulla oblongata. *Acta Physiol. Scand.*, **117,** 315–318.

Howes, K.A., Newton, B.W. and Maley, B.E. (I 989) Cholecystokinin octapeptide immunoreactivity in the nucleus tractus solitarius of the cat. *Peptides*, **10,** 73–78.

Jaeger, C.B., Ruggiero, D.A., Albert, V.R., Park, D.H., Joh, T.H. and Reis, D.J. (1984) Aromatic L-amino acid decarboxylase in the rat brain: immunocytochemical localization in neruons of the brain stem. *Neuroscience*, **11,** 691–713.

Kalia, M., Fuxe, K. and Goldstein, M. (1985a) Rat medulla oblongata. II. Dopaminergic, noradrenergic (Al and A2) and adrenergic neurons, nerve fibers, and presumptive terminal processes. *J. Comp. Neurol.*, **233,** 308–332.

Kalia, M., Woodward, D.J., Smith W.K. and Fuxe, K. (1985b) Rat medulla oblongata. IV. Topographical distribution of catecholaminergic neurons with quantitative three-dimensional computer reconstruction. *J. Comp. Neurol.*, **233,** 350–364.

Kalia, M. and Fuxe, K. (1985) Rat medulla oblongata. I. cytoarchitectonic considerations. *J. Comp. Neurol.*, **233,** 285–307.

Kalia, M. and Mesulam, M.M. (1980) Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. *J. Comp. Neurol.*, **193,** 467–508.

Katz, D.M. and Karten, H.J. (1980) Substance P in the vagal sensory ganglia: localization in cell bodies and pericellular arborizations. *J. Comp. Neurol.*, **193,** 549–564.

Kawai, Y. and Takagi, H. (1989) Parvicellular adrenergic neurons receive substance P-ergic inputs in the nucleus of the tractus solitarius of the rat. *Brain Res.*, **479,** 344–348.

Kimura, H., McGeer, P.L., Peng, J.H. and McGeer, E.G. (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. *J. Comp. Neurol.*, **200,** 151–201.

Kruger, W., Mantyh, P.W., Stemini, C., Brecha, N.C. and Mantyh, C.R. (1988) Calcitonin gene-related peptide (CGRP) in the rat central nervous system: patterns of immunoreactivity and receptor binding sites. *Brain Res.*, **463,** 223–244.

Ladenheim, E.E., Speth, R.C. and Ritter, R.C. (1988) Reduction of CCK-8 binding in the nucleus of the solitary tract in unilaterally nodosectomized rats. *Brain Res.*, **474,** 125–129.

Lasiter, P.S. and Kachele, D.L. (1988) Organization of GABA and GABA-transaminase containing neurons in the gustatory zone of the nucleus of the solitary tract. *Brain Res. Bull.*, **21,** 623–636.

Li, Y.-Q., Zeng, S.-L., Rao, Z-R. and Shi, J-W. (1992) Serotonin-, substance P- and tyrosine hydroxylase-like immunoreactive neurons projecting from the midbrain periaqueductal gray to the nucleus tractus solitarii in the rat. *Neurosci. Lett.*, **134,** 175–179.

Lipski, J., Waldvogel, H.J., Pilowsky, P. and Jiang, C. (1990) GABA-immunoreactive boutons make synapses with inspiratory neurons of the dorsal respiratory group. *Brain Res.*, **529,** 309–314.

Loewy, A.D. and Burton, H. (1978) Nuclei of the solitary tract: efferent projections to the lower brainstem and spinal cord of the cat. *J. Comp. Neurol.*, **181,** 421–450.

Loren, I., Alumets, J., Hakanson, R. and Sundler, F. (1979) Immunoreactive pancreatic polypeptide (PP) occurs in the central and peripheral nervous system: preliminary immunocytochemical observations. *Brain Res.*, **200,** 179–186.

Magoul, R., Onteniente, B., Oblin, A. and Calas, A. (1986) Inter- and intracellular relationship of substance P-containing neurons with serotonin and GABA in the dorsal raphe nucleus:

combination of autoradiographic and immunocytochemical techniques. *J. Histochem. Cytochem.*, **34,** 735–742.

Maley, B.E. (1995) Calcitonin gene related peptide in the nucleus solitarius of the cat. Submitted

Maley, B., Mullett, T. and Elde, R. (1983) The nucleus tractus solitarii of the cat: a comparison of Golgi impregnated neurons with methionine enkephalin- and substance P-immunoreactive neurons. *J. Comp. Neurol.*, **217,** 405–417.

Maley, B. and Elde, R. (1982a) The ultrastructural localization of serotonin immunoreactivity within the nucleus of the solitary tract. *J. Neurosci.*, **2,** 1499–1506.

Maley, B. and Elde, R. (1982b) Immunohistochemical localization of putative neurotransmitters within the feline nucleus tractus solitarius. *Neuroscience*, **7,** 2469–2490.

Maley, B.E. (1985) The ultrastructural localization of enkephalin and substance P immunoreactivities in the nucleus tractus solitarii of the cat. *J. Comp. Neurol.*, **233,** 490–496.

Maley, B.E , Newton, B.W., Howes, K.A., Herman, L.M., Oloff, C.M., Smith, K.C., and Elde, R.P. (1987) Immunohistochemical localization of substance P and enkephalin in the nucleus tractus solitarii of the Rhesus monkey, *Macaca mulatta*. *J. Comp. Neurol.*, **260,** 483–490.

Maley, B.E., Frick, M.L., Levey, A.I., Wainer, B.H. and Elde, R.P. (1988) Immunohistochemistry of choline acetyltransferase in the guinea pig brain. *Neurosci. Lett.*, **84,** 137–142.

Maley, B.E., Engle, M.G., Humphreys, S., Vascik, D.A., Howes, K.A., Newton, B.W. and Elde, R.P. (1990) Monoamine synaptic structure and localization in the central nervous system. *J. Elect. Micros. Tech.*, **15,** 20–33.

Maley, B.E., Colson, C., McGillis, J.P. and Hyde, J.F. (1993) Galanin immunoreactivity and binding sites in the nucleus tractus solitarii of the cat. *Soc. Neurosci. Abst.*, **19,** 1148

Maley, B.E. and Newton, B.W. (1985) Immunohistochemistry of γ-arninobutyric acid in the cat nucleus tractus solitarius. *Brain Res.*, **330,** 364–368.

Maley, B.E. and Panneton, W.M. (1988) Enkephalin-immunoreactive neurons in the nucleus tractus solitarius project to the parabrachial nucleus of the cat. *Brain Res.*, **442**, 340–344.

Maley, B.E. and Seybold, V.S. (1993) Distribution of [^{3}H]quinuclidinyl benzilate, [^{3}H]nicotine, and [^{125}I]α-bungarotoxin binding sites in the nucleus tractus solitarii of the cat. *J. Comp. Neurol.*, **327,** 194–204.

Mantyh, P.W. and Hunt, S.P. (1984) Neuropeptides are present in projection neurones at all levels in visceral and taste pathways: from periphery to sensory cortex. *Brain Res.*, **299,** 297–311.

Massari, V.J., Homby, P.J., Friedman, E.K., Milner, T.A., Gillis, R.A. and Gatti, P.J. (1990) Distribution of neuropeptide Y-like immunoreactive perikarya and processes in the medulla of the cat. *Neurosci. Lett.*, **115,** 37–42.

Melander, T., Hokfelt, T. and Rokaeus, A. (1986) Distribution of galanin-like immunoreactivity in the rat central nervous system. *J. Comp. Neurol.*, **248,** 475–517.

Michener, S.R., Aimone, L.D., Yaksh, T.L. and Go, V.L.W. (1990) Distribution of galanin-like immunoreactivity in the pig, rat and human central nervous system. *Peptides*, **11,** 1217–1223.

Morishima, Y., Takagi, H., Akai, F., Tohyama, M., Emson, P.C., Hillyard, C., Girgis, S. and MacIntyre, I. (1985) Light and electron microscopic studies of calcitonin gene-related peptide-like immunoreactive neurons and axon terminals of the nucleus of the tractus solitarius of the rat. *Brain Res.*, **344,** 191–195.

Newton, B.W. and Maley, B.E. (1985) Cholecystokinin-octapeptide like immunoreactivity in the area postrema of the rat and cat. *Regul. Pept.*, **13,** 31–40.

Pickel, V.M., Joh, T.H. and Reis, D.J. (1976) Monoamine-synthesizing enzymes in central dopaminergic, noradrenergic and serotoninergic neurons: immunocytochemical localization by light and electron microscopy. *J. Histochem. Cytochem.*, **24,** 792–806.

Pickel, V.M., Chan, J. and Massari, V.J. (1989a) Neuropeptide Y-like immunoreactivity in neurons of the solitary tract nuclei: vesicular localization and synaptic input from GABAergic terminals. *Brain Res.*, **476,** 265–278.

Pickel, V.M., Chan, J. and Milner, T.A. (1989b) Ultrastructural basis for interactions between central opioids and catecholamines. II. Nuclei of the solitary tracts. *J. Neurosci.*, **9,** 2519–2535.

Pickel, V.M., Chan, J. and Milner, T.A. (1990) Tyrosine hydroxylase and enkephalin in nuclei of the solitary tracts: co-existence and convergent synaptic input to catecholamine neurons. *Prog. Clin. Biol. Res.*, **328,** 267–270.

Rall, W. (1962) Theory of physiological properties of dendrites. *Ann. NY Acad. Sci.*, **96,** 1071–1092.

Rall, W. (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.*, **30,** 1138–1168.

Rinaman, L., Miselis, R.R. and Kreider, M.S. (1989) Ultrastructural localization of thyrotropin-releasing hormone immunoreactivity in the dorsal vagal complex in rat. *Neurosci. Lett.*, **104,** 7–12.

Rranco-Cereceda, A., Henke, H., Lundberg, J.M., Petermann, J.F., Hokfelt, T. and Fischer, J.A. (1987) Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man:distribution and release by capsaicin. *Peptides*, **8,** 399–410.

Ruggiero, D.A., Giuliano, R., Anwar, M., Stometta, R. and Reis, D.J. (1990) Anatomical substrates of cholinergic-autonomic regulation in the rat. *J. Comp. Neurol.*, **292,** 1–53.

Saha, S., Batten, T.F.C. and McWilliam, P.N. (1995) Glutamate, γ-aminobutyric acid and tachykinin-immunoreactive synapses in the cat nucleus tractus solitarii. *J. Neurocytol.*, **24,** 55–74.

Schaffar, N., Kessler, J.P., Bosler, O. and Jean, A. (1988) Central serotonergic projections to the nucleus tractus solitarii- evidence from a double labeling study in the rat. *Neuroscience*, **26,** 951–958.

Sved, A.F. and Backes, M.G. (1992) Neuroanatonical evidence that vagal afferent nerves do not possess a high affinity uptake system for glutamate. *J. Auton. Nerv. Syst.*, **38,** 219–230.

Thor, K.B. and Helke, C.J. (1989) Serotonin and substance P colocalization in medullary projections to the nucleus tractus solitarius: dual-color immunohistochemistry combined with retrograde tracing. *J. Chem. Neurol.*, **2,** 139–148.

Torvik, A. (1956) Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. An experimental study in the rat. *J. Comp. Neurol.*, **106,** 51–132.

Triepel, J., Mader, J., Weindi, A., Heinrich, D., Forssmann, W.G., and Metz, J. (1984) Distribution of NT-IR perikarya in the brain of the guinea pig with special reference to cardiovascular centers in the medulla oblongata. *Histochemistry*, **81,** 509–516.

Velley, L., Milner, T.A., Chan, J., Morrison, S.F. and Pickel, V.M. (1991) Relationship of Met-enkephalin-like immunoreactivity to vagal afferents and motor dendrites in the nucleus of the solitary tract: a light and electron microscopic dual labeling study. *Brain Res.*, **550,** 298–312.

Voorn, P. and Buijs, R.M. (1983) An immuno-electromicroscopical study comparing vasopressin, oxytocin, substance P and enkephalin-containing nerve terminals in the nucleus of the solitary tract of the rat. *Brain Res.*, **270,** 169–173.

Voss, M.D., De Castro, D., Lipski, J., Pilowsky, P.M. and Jiang, C. (1990) Serotonin immunoreactive boutons form close oppositions with respiratory neurons of the dorsal respiratory group in the cat. *J. Comp. Neurol.*, **295,** 208–218.

Wang, Z., Rao, Z. and Shi, J. (1992) Tyrosine hydroxylase-, neurotensin-, or cholecystokinin-containing neurons in the nucleus tractus solitarii send projection fibers to the nucleus accumbens in the rat. *Brain Res.*, **578,** 347–350.

Whitehead, M.C. (1990) Subdivisions and neuron types of the nucleus of the solitary tract that project to the parabrachial nucleus in the hamster. *J. Comp. Neurol.*, **301,** 554–574.