Exp Brain Res (1995) 105:241–253

RESEARCH ARTICLE

Robert D. Sweazey

Distribution of aspartate and glutamate in the nucleus of the solitary tract of the lamb

Received: 22 September 1994 / Accepted: 23 March 1995

Abstract Excitatory amino acids have been implicated in several nucleus of the solitary tract (NST)-mediated functions. The distribution of the excitatory amino acids aspartate and glutamate has been described in both cat and rat. However, the distribution of these amino acids has not been described for the lamb, a species frequently used in the investigation of NST-mediated behaviors. Thus, this study was designed to investigate the distribution of aspartate-like (ASP) and glutamate-like (GLU) immunoreactivity in the lamb NST using pre- and post-embedding immunohistochemistry. Both ASP- and GLU-immunoreactive cells and puncta were observed throughout the rostral to caudal extent of the lamb NST. The most intense ASP- and GLU-immunoreactive cell and puncta staining was found ventromedial, ventral and ventrolateral to the solitary tract at intermediate and caudal levels of the lamb NST. The relative numbers of both cells and puncta stained were lower at rostral levels of the NST corresponding to the gustatory NST. The intense ASP and GLU immunoreactivity observed in areas of the lamb intermediate and caudal NST that are involved in respiration, deglutition and cardiovascular functions suggests excitatory amino acids play an important role in NST neural processing that underlies these behaviors in lamb.

Key words Amino acid neurotransmitters · Immunocytochemistry · Nucleus tractus solitarii · Sheep

Introduction

Excitatory amino acids are distributed throughout the vertebrate central nervous system and serve as important transmitters in neural pathways that underlie numerous diverse behaviors. Anatomical and neurochemical studies conducted in cat and rat have demonstrated that excitatory amino acids and their receptors are widespread throughout the nucleus of the solitary tract (NST) (Dietrich et al. 1982; Drewe et al. 1990; Meeley et al. 1989; Philippu 1988; Simon et al. 1985). The NST is the primary central nervous system relay for sensory information transmitted via the facial, glossopharyngeal and vagus nerves (Hamilton and Norgren 1984; Kalia and Mesulam 1980; Kalia and Sullivan 1982). The NST has also been implicated as an important component in the neural pathways that underlie gustatory, cardiovascular, respiratory and alimentary tract functions such as swallowing.

Anatomical and neurochemical studies conducted by several laboratories have provided various lines of evidence for a role of amino acids in NST synaptic transmission. For example, evidence exists for a glutamate high-affinity uptake system in the NST, while removal of vagal afferent cell bodies in the nodose ganglion reduces glutamate levels in the NST and stimulation of vagal afferent fibers results in a release of excitatory amino acids (Allchin et al. 1994; Granata and Reis 1983; Perrone 1981; Simon et al. 1985; Talman et al. 1980).

Physiological studies conducted in vivo and in vitro have indicated that excitatory amino acids are important neurotransmitters in the rostral gustatory portion of the NST (King and Bradley 1993, 1994; Wang et al. 1993), as well as in more caudally located regions of the NST involved in swallowing, respiration, cardiovascular control and gastrointestinal functions (Dampney 1994; Karius et al. 1994; Kessler et al. 1990; Priddy et al. 1992; Tell and Jean 1990, 1993; Van Giersbergen et al. 1992). Previous studies have described the distribution of excitatory amino acids in the NST of the rat and cat, but equivalent information for lambs is currently lacking. Lambs have been used extensively for investigation of the physiology and development of taste, deglutition, upper airway reflexes, cardiovascular function and respiration, functions in which the NST plays an important role (Amri et al. 1990, 1991; Burrows et al. 1986; Car and Jean 1971; Grogaard et al. 1982; Harding et al. 1978;

R. D. Sweazey
Department of Anatomy, Indiana University School of Medicine, 2101Coliseum Boulevard East, Ft. Wayne, IN 46805–1499, USA; Tel: (219) 481–6739, Fax: (219) 481–6408

Jean 1972a,b; Kovar et al. 1979; Mistretta and Bradley 1983a,b; Praud et al. 1992; Ross et al. 1990; Sweazey and Bradley 1988, 1989; Webb et al. 1994). A description of the distribution of the excitatory amino acids aspartate and glutamate in the lamb NST would contribute information important for understanding the significance of these neurotransmitters in the lamb NST. Therefore, the goal of this study was to describe the distribution of aspartate and glutamate in the lamb NST using immunohistochemistry on thick and semi-thin tissue sections.

Material and methods

The following procedures were approved by the university's Committee on Use and Care of Animals and are in compliance with NIH guidelines for the care and use of laboratory animals.

Experiments were performed using ten Suffolk lambs aged 30–60 days, weighing 9–16 kg. An overdose of sodium pentobarbital was given by jugular puncture and the animals perfused through the carotid arteries. Blood was flushed from the vascular system using 1.5 l of phosphate-buffered saline (PBS, pH 7.4), followed by 1 l of a solution of 3% paraformaldehyde and 0.2–0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3–7.4). After perfusion, the brainstem was removed from the skull and stored overnight in 0.1 M phosphate buffer at 4°C.

Using tissue from eight animals, five serial 60-μm transverse sections of the lamb brainstem were collected every 1 mm throughout the rostral-caudal extent of the NST. Following collection of the fifth 60-μm section, a 150-μm section was taken for subsequent post-embedding immunocytochemistry as noted below. All tissue sections were cut using a Vibratome (Pelco). The free-floating 60-μm sections were processed for the visualization of aspartate or glutamate according to the protocol of Helfert et al. (1989). Briefly, sections were first pre-incubated in a blocking solution containing 5% normal goat serum in PBS containing Triton-X 100 (Sigma). Sections were then transferred into a solution containing one of the following affinity-purified, polyclonal rabbit antisera diluted in blocking serum: glutamate 1:1500–1:2000; aspartate 1:2000 (Montero and Wenthold 1989). The tissue sections were then incubated overnight at 4°C with gentle agitation.

Following incubation in the primary antisera, the tissue was processed according to the avidin-biotin method of Hsu et al. (1981) using the Vectastain ABC kit (Vector Labs), and the peroxidase activity was visualized by exposing the sections to 0.05% diaminobenzidine and 0.001% hydrogen peroxide in PBS for 5–10 min (Helfert et al. 1989). Tissue was then rinsed in distilled water, mounted on chrom-alum-subbed slides and coverslipped with Krystallon mounting medium (Harleco) or Permount. In addition to the transverse sections, two animals had five 60-μm serial horizontal sections taken every 1 mm throughout the dorsal-ventral extent of the NST and processed in a manner similar to that used for transverse sections. No difference in the pattern of immunoreactive cells and puncta was observed between the transverse and horizontal orientations.

Although immunoreactive cell bodies are easily demonstrated using immunohistochemistry on 60-μm sections, identification of immunoreactive puncta, indicative of axon terminals, is less than ideal. Therefore, post-embedding immunohistochemistry in semi-thin sections (1–2 μm) was used to investigate the distribution of immunoreactive puncta in the lamb NST. This technique has been shown to be superior to thick section immunohistochemistry for the identification of immunoreactive puncta (Helfert et al. 1989).

With only slight modification, the 150-μm-thick sections were processed for post-embedding immunocytochemistry according the technique of Helfert et al. (1989). Briefly, the sections were post-fixed in 0.1% OsO_4 in 0.1 M phosphate buffer for 2 h, dehydrated through a graded series of ethanol followed by acetone, and flat-embedded in Epon resin. The embedded sections were then trimmed to include only the region around the NST and thin 1- to 2-μm sections through the NST were cut on an ultramicrotome and mounted on glass slides. To increase penetration of the antiserum, sections were etched in a solution of sodium ethoxide/ethanol and osmium was bleached from the sections using 1% sodium *m*-periodate followed by distilled water rinses. Sections were then placed in 5% normal goat serum in PBS, followed by incubation in aspartate or glutamate antiserum diluted in blocking serum (1:200–1:500) in a humidified chamber at 4°C for 48 h. The tissue sections were then processed as noted above for thick sections, except that the Vectastain reagents were prepared at twice the concentration recommended by the manufacturer (Helfert et al. 1989).

Controls for both thick and semi-thin sections included incubating some sections in the blocking serum minus the primary antisera (n=10) or incubating in the different primary antisera preadsorbed with the appropriate amino acid (n=1). These procedures resulted in the absence of immunoreactive labeling. Incubation of the tissue with the primary antisera preadsorbed with the inappropriate amino acid produced little decrease in the observed level of immunoreactivity (n=1).

The tissue was examined and photographed using a Leitz Diaplan Microscope equipped with both bright-field and differential interference contrast optics. The relative densities of cell bodies, fibers and puncta showing aspartate-like (ASP) and glutamate-like (GLU) immunoreactivity were mapped onto standard drawings of the lamb NST using a camera lucida. Although strict quantitative measures of immunoreactivity are not possible using the ABC technique, the densities of immunoreactivity were subjectively evaluated separately for each animal and rated on a scale of light (Fig. 3c) to intense (Figs. 6b,c). The subjective evaluations were then combined across subjects to produce the distribution maps shown in Figs. 2 and 5. For clarity, the NST was divided into caudal, intermediate and rostral levels. A representative section from each level of the lamb NST is shown in Fig. 1. The caudal NST included all sections caudal to 0.5 mm anterior to the obex (Fig. 1a). Intermediate levels of the NST were from 0.5 mm to 3.5 mm rostral to the obex (Fig. 1b), and the rostral NST included all of the NST rostral to 3.5 mm (Fig. 1c).

Results

Both ASP- and GLU-immunoreactive cell bodies, fibers and puncta were located throughout the rostral-caudal extent of the NST (Figs. 2, 5). However, the highest concentrations of ASP and GLU immunoreactivity were observed in the caudal two-thirds of the NST that encompasses the caudal and intermediate levels. At these levels ASP and GLU immunoreactivity was particularly pronounced in regions ventrolateral, ventral and ventromedial to the solitary tract (ST).

ASP-immunoreactive cells in the NST

The density of ASP immunoreactivity was the lightest at rostral levels of the NST. At the most rostral NST locations examined, fewer ASP-immunoreactive cells were present than at more caudal levels of the nucleus (Fig. 2). This was particularly true for areas of the NST ventrolateral to the ST. At rostral levels of the NST, intense ASP immunoreactivity was restricted to a relatively small region of the nucleus located just lateral to the ST. The remaining ASP-immunoreactive cells were located either ventral or medial to the ST, and the numbers of immunoreactive cells present were lower than was

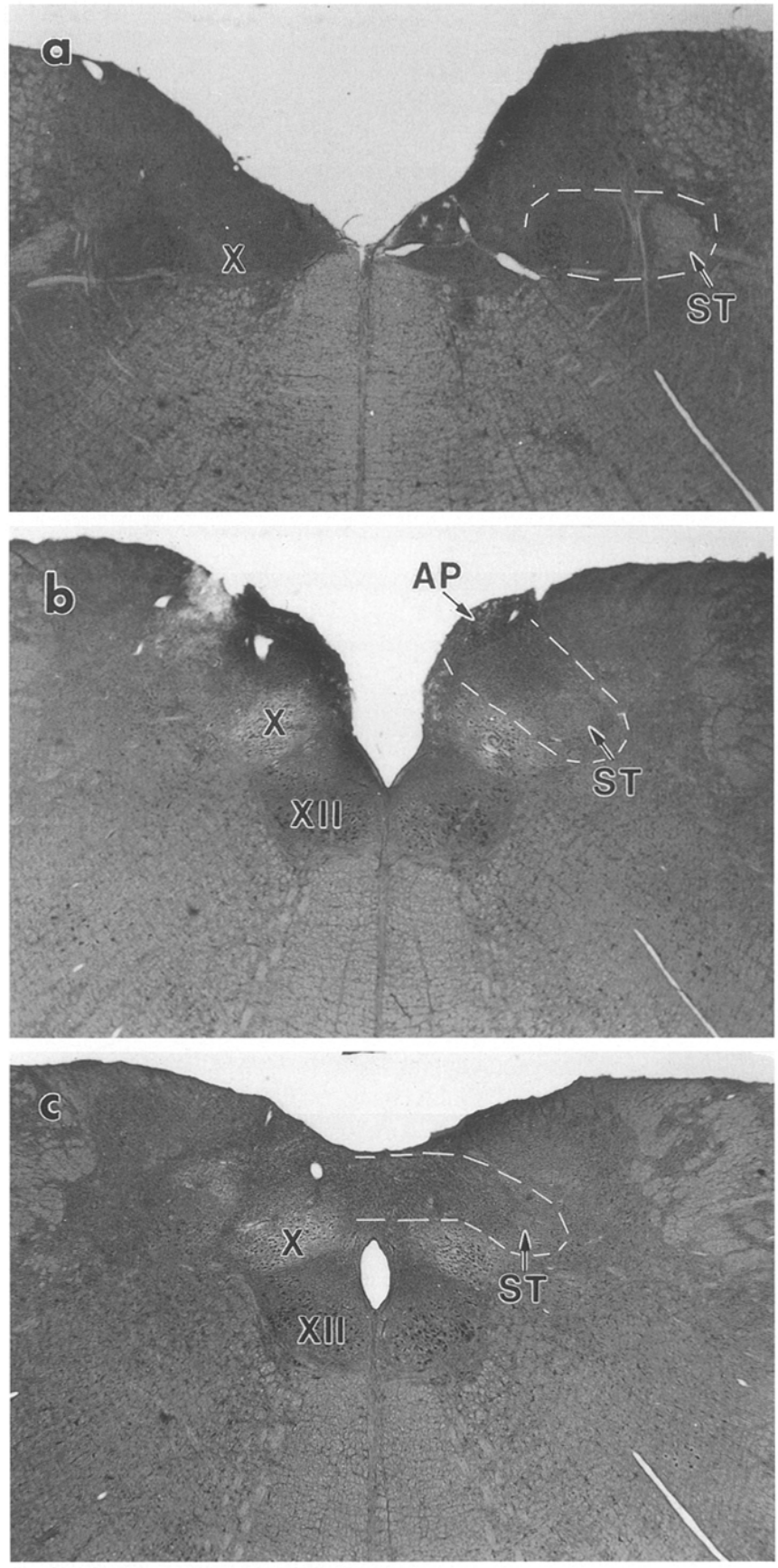


Fig. 1a–c Photomicrographs of representative sections from the three different levels of the lamb NST as defined in Material and methods: **a** rostral NST; **b** intermediate NST; **c** caudal NST. The aproximate boundaries of the NST are indicated by the *dashed lines*. *AP* area postrema, *ST* solitary tract, *X* dorsal motor nucleus of vagus, *XII* hypoglossal nucleus

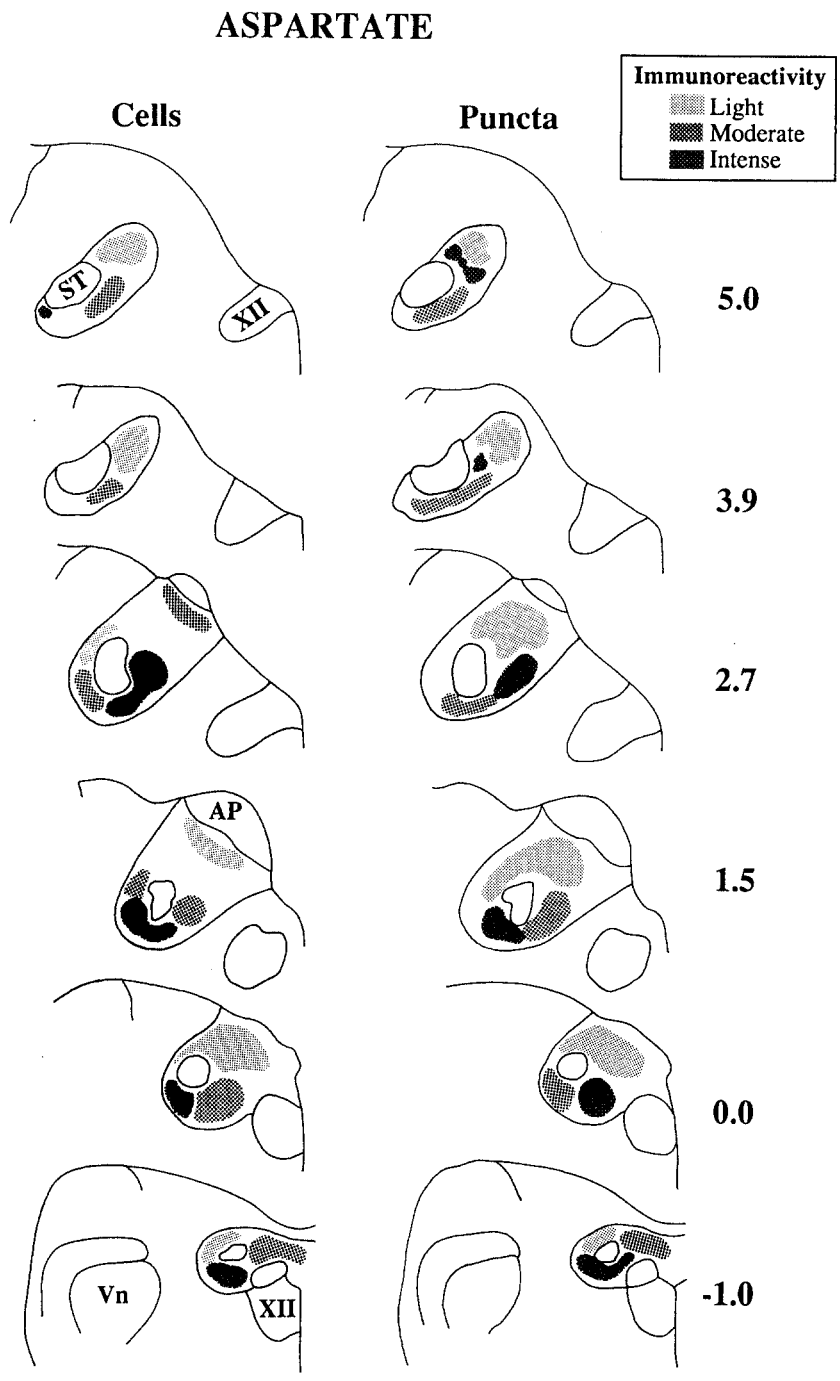
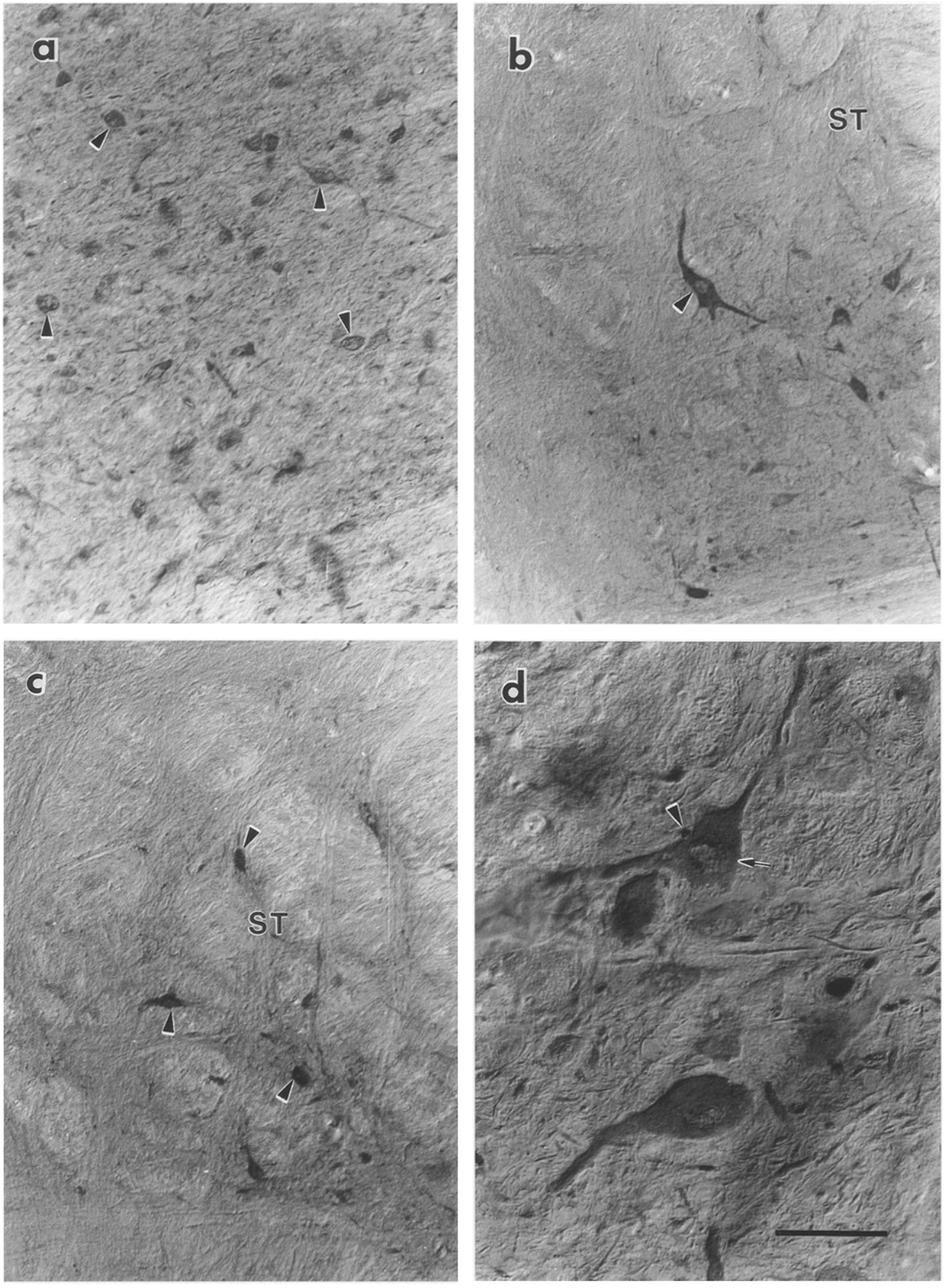


Fig. 2 Distribution of ASP immunoreactivity in the lamb NST. The *left column* of schematic drawings shows the distribution of immunoreactive cells and the *right column* shows the distribution of immunoreactive puncta. The *numbers* adjacent to the right column indicate the distance (in mm) of each section from the obex. The intensity of ASP-immunoreactive staining is coded according to the key at *upper right*. Areas with *no shading* contained only an occasional immunoreactive cell or punctum or no immunoreactivity at all. *AP* Area postrema, *ST* solitary tract, *Vn* trigeminal nucleus, *XII* hypoglossal nucleus

observed at levels of the NST caudal to the obex (Fig. 2).

At intermediate levels of the NST the largest concentrations of ASP-immunoreactive cells were located ventrolateral, ventral and ventromedial to the ST (Fig. 2). ASP-immunoreactive cells in the ventromedial and medial NST were small to medium in size and were generally round or fusiform in shape (Fig. 3a). Cells located ventrolateral and ventral to the ST were medium to large in size and multipolar or fusiform in shape, and the dendrites of some ASP cells extended into the ST and/or subjacent reticular formation (Fig. 3b). The density of ASP immunoreactivity was moderate to intense in NST

Fig. 3a–d Photomicrographs of ASP immunoreactivity in different regions of the lamb NST. **a** ASP-immunoreactive cells (*arrowheads*) located ventromedial to the solitary tract (*ST*) in the intermediate NST. Most immunoreactive neurons in this region of the NST were small to medium in size and had ovoid to elongated somata. **b** Immunoreactive neurons located ventrolateral to the ST in the intermediate NST. This photomicrograph shows an example of the large multipolar immunoreactive neurons (*arrowhead*) that were frequently observed in this region of the NST after incubation in antiserum to aspartate. **c** Immunoreactive neurons (*arrowheads*) interspersed among the fiber bundles that make up the lamb ST at intermediate levels of the NST. **d** Immunoreactive neurons and puncta ventral to the ST in the caudal NST. An immunoreactive punctum (*arrowhead*) can be seen adjacent to an immunoreactive cell body (*arrow*). *Scale bars*: **a** 100 μm; **b,c** 175 μm; **d** 43 μm


a
b
ST
c
ST
d

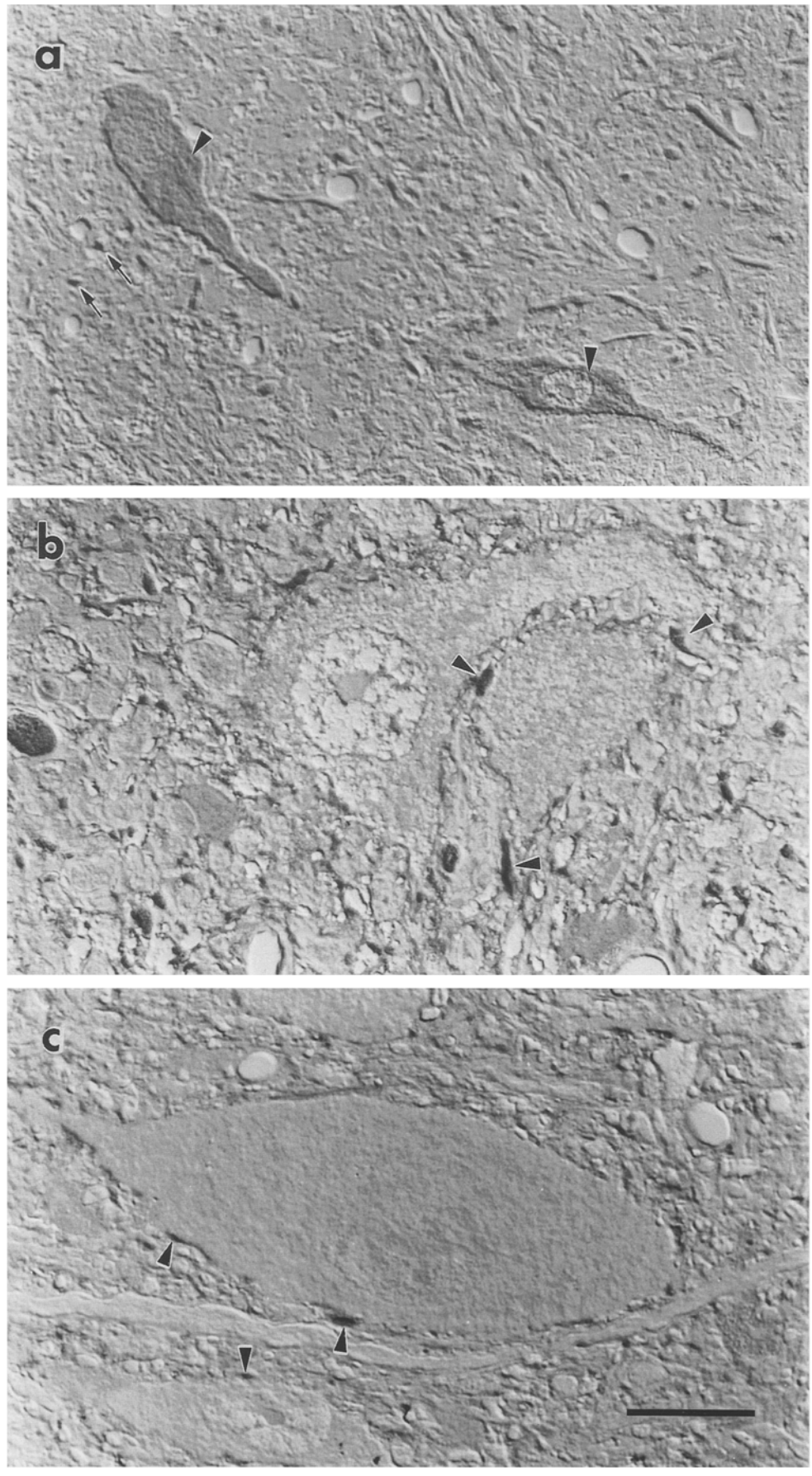


Fig. 4a–c Immunohistochemistry in semi-thin sections of the lamb NST. **a** ASP-immunoreactive cell bodies (*arrowheads*), fibers and puncta (*arrows*) in a region of the intermediate NST ventrolateral to the solitary tract. **b** ASP-immunoreactive puncta (*arrowheads*) adjacent to non-immunoreactive cell processes in the intermediate NST. These puncta were located in the cellular region between the bundles of fibers that make up the lamb ST (interstitial subnucleus). **c** GLU-immunoreactive puncta (*arrowheads*) adjacent to non-immunoreactive soma. These puncta were located dorsolateral to the solitary tract in the rostral NST. *Scale bars*: **a** 35 μm; **b,c** 15 μm

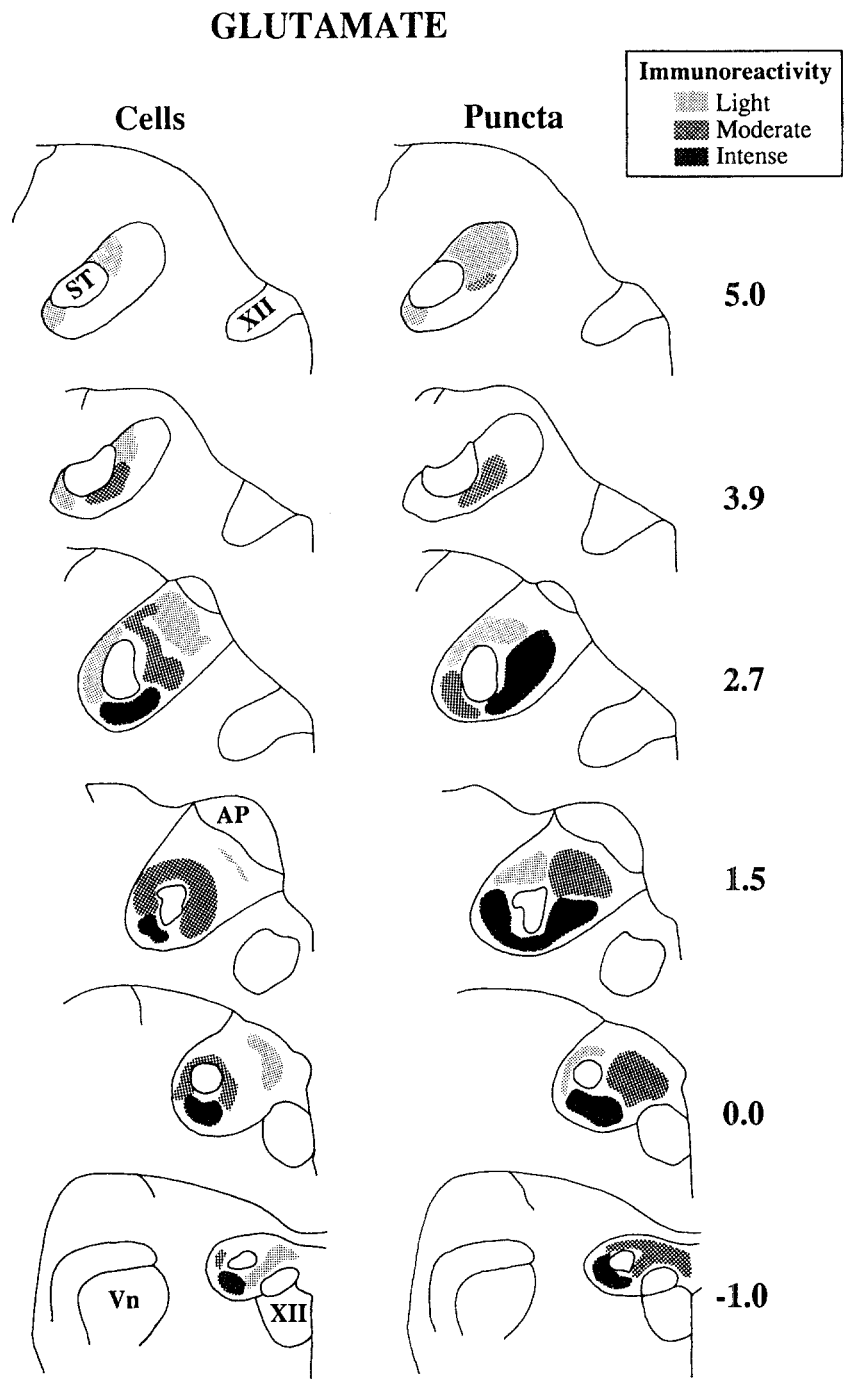
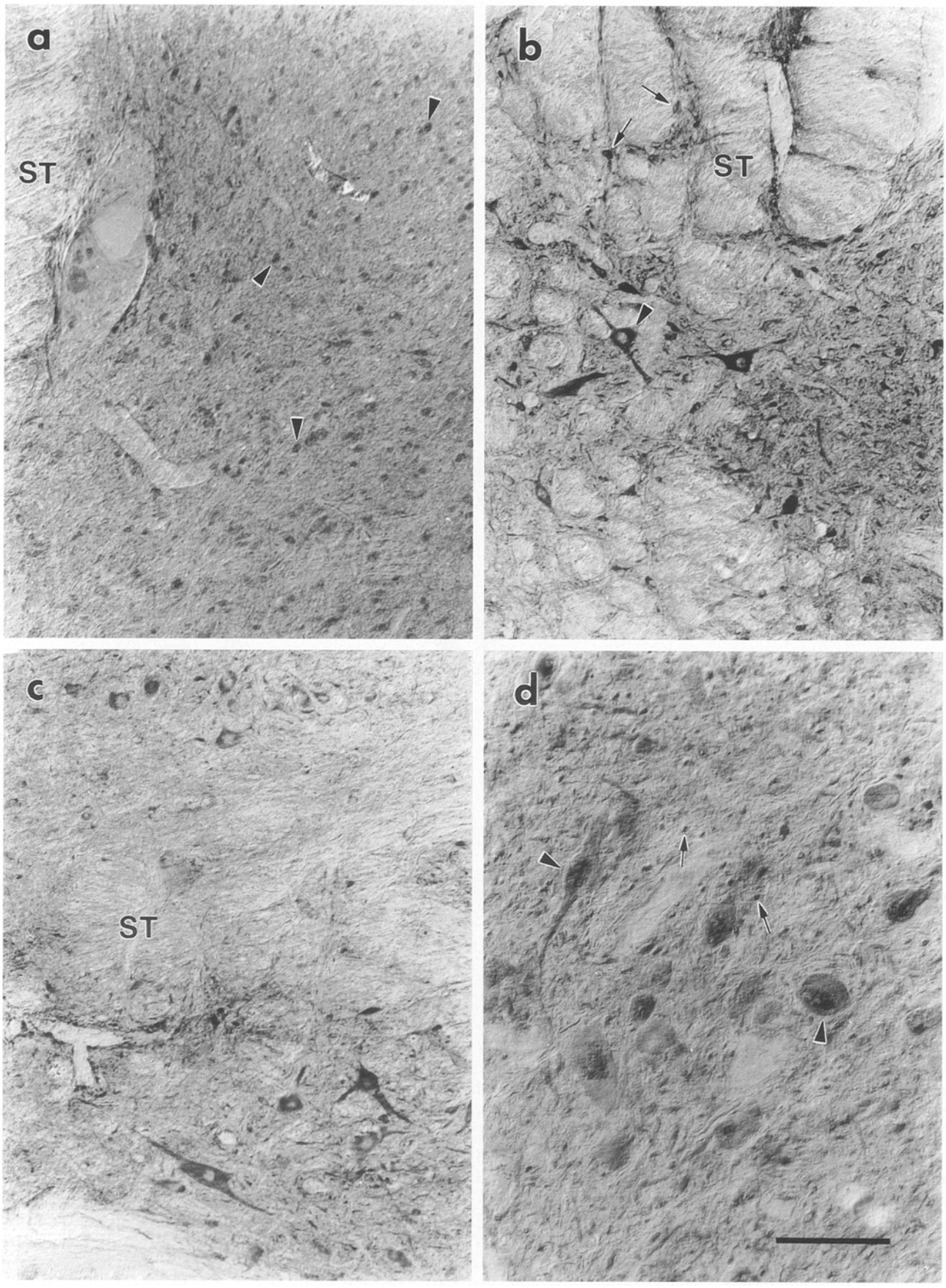


Fig. 5 Distribution of GLU immunoreactivity in the lamb NST. The *left column* of schematic drawings shows the distribution of immunoreactive cells and the *right column* shows the distribution of immunoreactive puncta. The *numbers* adjacent to the right column indicate the distance (in mm) of each section from the obex. The intensity of GLU-immunoreactive staining is coded according to the key at *upper right*. *AP* Area postrema, *ST* solitary tract, *Vn* trigeminal nucleus, *XII* hypoglossal nucleus

regions lateral and dorsal to the ST, while only a few ASP-immunoreactive cells were observed adjacent to the area postrema (Fig. 2). In contrast to more rostral and caudal levels of the NST, there were relatively few ASP-immunoreactive cells in NST regions medial to the ST except, as noted previously, ventromedial to the ST. In addition to the distribution of ASP cells noted above and in Fig. 2, small to moderate numbers of ASP-immunoreactive cells were observed in the spaces between the bundles of fibers that make up the lamb ST (Fig. 3c), an area corresponding to the interstitial subnucleus described in cat and rat (Kalia and Mesulam 1980; Kalia and Sullivan 1982).

At caudal levels of the NST moderate numbers of immunoreactive cells were observed medial and dorsomedial to the ST and in the commissural nucleus at levels of the NST caudal to and near the obex (Fig. 2). In contrast, the density of ASP immunoreactivity was greater ventral and ventrolateral to the ST at this level of the NST with larger numbers of ASP-immunoreactive cells present (Fig. 2). These ASP-immunoreactive cells were generally large to medium in size and were either fusiform or multipolar in shape. Relatively few ASP-immunoreactive cells were observed in the regions of the NST dorsolateral to the ST at levels of the NST caudal to the obex (Fig. 2).


a
ST
b
ST
c
ST
d

ASP-immunoreactive puncta in the NST

ASP-immunoreactive puncta were seen adjacent to both immunoreactive and non-immunoreactive somata throughout the NST (Figs. 4a,b). ASP-immunoreactive puncta tended to be more widely distributed than ASP-immunoreactive cells. However, like the distribution of ASP-immunoreactive cells, there tended to be more intense immunoreactive staining at intermediate and caudal levels of the NST (Fig. 2).

The density of ASP-immunoreactive puncta was generally moderate to light at the most rostral levels of the NST (Fig. 2). An exception was an area immediately adjacent and medial to the ST, where the intense level of immunoreactive puncta staining was equivalent to that observed more caudally in the NST (Fig. 2). In contrast to more caudal levels of the NST, the intensity of puncta staining in ventrolateral and lateral regions of the rostral NST was relatively light. Like all other levels of the NST, the most medial regions of the rostral NST exhibited only light ASP-immunoreactive puncta staining.

At intermediate levels of the NST, moderate to intense ASP-immunoreactive puncta labeling was observed lateral, ventrolateral and ventral to the ST. In particular, between 1.0 and 2.0 mm rostral to the obex, large numbers of immunoreactive puncta were observed ventrolateral to the ST in a region corresponding to a major termination site of the lamb superior laryngeal nerve (SLN) (Sweazey and Bradley 1986). The intensity of ASP-immunoreactive puncta staining was relatively light dorsolateral and medial to the ST, although the region ventromedial to the ST exhibited moderate to intense ASP immunoreactivity throughout the extent of the intermediate NST (Fig. 2).

At the most caudal levels of the NST, the distribution of immunoreactive puncta was similar to that of ASP-immunoreactive cells. The greatest concentrations of immunoreactive puncta were observed ventrolateral, ventral and ventromedial to the ST (Fig. 2). Moderate quantities of immunoreactive puncta were observed in the commissural subnucleus caudal to the obex. In contrast, only moderate to light ASP immunoreactivity was observed dorsolateral and medial to the ST at the level of the obex.

Fig. 6a–d Photomicrographs of GLU immunoreactivity in different regions of the lamb NST. **a** GLU-immunoreactive neurons in the intermediate NST. Large numbers of immunoreactive cell bodies (*arrowheads*) and a few immunoreactive processes can be seen distributed throughout the NST medial to the ST. **b** Photomicrograph of the intermediate NST showing intense GLU immunoreactivity. Large numbers of GLU-immunoreactive somata (*arrowhead*) and numerous processes are present ventral to the ST. GLU-immunoreactive staining is also quite intense among the bundles of the ST, where numerous small immunoreactive cell bodies can be observed (*arrows*). **c** Photomicrograph showing GLU-immunoreactive cells and processes in NST areas adjacent to the ST in the caudal NST. **d** Photomicrograph showing the moderate numbers of GLU-immunoreactive cells (*arrows*) and puncta (*arrowheads*) present ventromedial to the ST in the rostral NST. Like ASP-immunoreactive cells in this region of the NST, most GLU-immunoreactive somata were round to elongate in shape. *Bars*: **a–c** 175 μm, **d** 42 μm

GLU-immunoreactive cells in the NST

The relative numbers of GLU-immunoreactive cells were generally similar to ASP throughout the NST, although some differences were observed in their distribution (Figs. 2, 5). GLU-immunoreactive cells were most numerous at intermediate levels of the lamb NST. Moderate to large numbers of immunoreactive cells were located ventromedial, ventrolateral and ventral to the ST (Figs. 5, 6a,b). GLU-immunoreactive cell bodies in ventromedial areas of the intermediate NST were generally ovoid in shape and small to medium in size, a finding similar to that observed for ASP (Fig. 6a). GLU-immunoreactive cells in the ventral and ventrolateral areas of the NST were medium to large, elongated or multipolar (Fig. 6b). Smaller numbers of GLU-immunoreactive cells were located dorsolateral and in regions of the NST adjacent to the area postrema (Fig. 5). In addition, in a manner similar to that seen following incubation of sections in aspartate antiserum, numerous GLU-immunoreactive cells were located between the bundles of fibers that constitute the ST (Fig. 6b).

At NST levels equal or caudal to the obex, the largest numbers of GLU-immunoreactive cells were found ventrolateral and ventral to the ST (Figs. 5, 6c). Although direct comparisons are not possible, the overall numbers and distribution of immunoreactive cells and fibers in the caudal NST ventrolateral and ventral to the ST appeared similar to those previously described for aspartate. Furthermore, most GLU-immunoreactive cells in this area of the caudal NST were medium to large fusiform or multipolar neurons, a finding similar to that observed at intermediate NST levels. These shapes of GLU-immunoreactive cells in the ventrolateral, ventral and ventromedial areas of the NST were also similar to those of neurons observed following incubation of the caudal NST tissue with antiserum to aspartate. In contrast, fewer GLU-immunoreactive cells were found in the commissural subnucleus and regions medial to the ST at the level of the obex, and their distribution was less widespread than that of ASP immunoreactivity (Figs. 2, 5).

GLU-immunoreactive cells at the most rostral levels of the NST were small to medium in size and had an ovoid or elongated soma (Fig. 6d). These immunoreactive cells were generally located in the area of the NST immediately adjacent to the ST, with the greatest numbers located ventromedial and ventral to the ST (Fig. 5). In a manner similar to that described for ASP-immunoreactive cells, the quantities of GLU-immunoreactive soma in the most medial portions of the rostral NST were relatively small compared to more caudal regions of the NST.

GLU-immunoreactive puncta in the NST

GLU-immunoreactive puncta were observed at all levels of the NST and were located on the soma and dendrites of immunoreactive and non-immunoreactive cells

(Fig. 4c). A comparison of Figs. 2 and 5 shows that although GLU-immunoreactive cells in the NST were slightly less frequent and a little less widely distributed than ASP-immunoreactive cells, the density of GLU puncta labeling was greater than that observed for aspartate. Large numbers of GLU-immunoreactive puncta were observed at both intermediate and caudal levels of the NST. At NST levels caudal to or near the level of the obex, moderate to intense levels of GLU-immunoreactive puncta were observed in all areas of the NST. GLU-immunoreactive puncta were especially numerous in areas of the NST ventral and ventrolateral to the ST (Figs. 5, 6c).

GLU-immunoreactive staining of puncta was intense over a wide area of the intermediate NST (Fig. 5). Large numbers of GLU-immunoreactive puncta were observed lateral, ventrolateral, ventral, ventromedial and medial to the ST (Figs. 5, 6b). The intensity of immunoreactive puncta was only light to moderate in regions of the NST dorsal and dorsomedial to the ST and very scattered in the most medial areas of the intermediate NST (Fig. 5).

The relative density of GLU-immunoreactive puncta staining was much less at the most rostral levels of the NST examined. Generally, light GLU puncta labeling was located in the medial or dorsolateral regions of the nucleus at this level. An exception was in the ventromedial region of the rostral NST, where there were moderate levels of GLU-immunoreactive puncta labeling (Fig. 5).

Discussion

Both ASP and GLU immunoreactivity were widely distributed throughout the lamb NST. The most concentrated ASP and GLU immunoreactivity was located at intermediate levels of the NST between 1 and 3 mm rostral to the obex. At this level there were numerous immunoreactive cells and puncta, particularly in NST areas ventromedial, ventral and ventrolateral of the ST. These areas of the lamb intermediate NST are involved in the control of respiratory- and swallowing-related functions (Car and Jean 1971; Jean 1984; Sweazey and Bradley 1988, 1989), and several studies conducted in cats and rats have shown a role for excitatory amino acids in both respiration and swallowing (Champagnet et al. 1980; Hashim and Bieger 1989; Henry and Sessle 1985; Karius et al. 1994; Kessler et al. 1990). Although this study provides no direct information as to the functional role of ASP- and GLU-immunoreactive cells in the ventral and ventrolateral NST, the morphology of the immunoreactive cells in the present study exhibited some similarity to physiologically identified, intracellularly labeled neurons in the cat ventral and ventrolateral NST that receive SLN inputs and are involved in respiration (Bellingham and Lipski 1992; Berger et al. 1984). Like the cat NST neurons, the immunoreactive cells in ventrolateral regions of the lamb NST were frequently multipolar or fusiform in shape, had medium-sized to large somata and extended dendritic processes into the adjacent ST (see Figs. 3b,d, 6b,c).

The intense levels of puncta staining observed in the lamb intermediate NST in the present study suggest that, as in the cat and rat, excitatory amino acids may be involved in the afferent control of respiratory- and swallowing-related reflexes in sheep. The ventrolateral, ventral and ventromedial areas of the intermediate NST receive the largest projection of the lamb SLN, a branch of the vagus important for the elicitation of respiratory- and swallowing-related reflexes, and many terminations of the cervical vagus (Sweazey and Bradley 1986). Several lines of evidence suggest a role for excitatory amino acids in the transmission of afferent information between the vagus and NST. Stimulation of the vagus nerve results in the release of both glutamate and aspartate in the intermediate NST, and removal of the cell bodies of vagal sensory neurons by nodose ganglionectomy produces a decrease in the levels of excitatory amino acids (Allchin et al. 1994; Granata and Reis 1983; Granata et al. 1984). Furthermore, injections of tritiated D-aspartate into this area of the rat NST result in labeling of primary vagal sensory neurons in the nodose ganglion, suggesting the presence of an uptake mechanism in these neurons (Schaffar et al. 1990). Although the current study does not provide direct evidence for vagal origin of puncta labeling, the intense immunoreactive puncta staining in regions of the NST that receive dense terminations of the SLN (Sweazey and Bradley 1986) suggests that aspartate and glutamate may play an important role in the transmission of afferent information between the lamb SLN fibers and NST neurons. Physiological investigations in other species support this hypothesis (Henry and Sessle 1985; Karius et al. 1993, 1994).

ASP and GLU immunoreactivity, particularly puncta labeling, was also moderate to intense in the lamb caudal NST. This region of the NST has been shown to be an important relay in cardiovascular and gastrointestinal neural pathways (Dampney 1994; Davison 1987; Van Giersbergen et al. 1992). Numerous investigations have provided evidence for the role of excitatory amino acids in the control of various NST-mediated cardiovascular functions, including the baroreceptor reflex (Kubo and Kihara 1991; Le Galloudec et al. 1989; Talman et al. 1980). Extracellular, intracellular, or whole-cell patch recordings in brain slice preparations containing the caudal NST or from dissociated caudal NST neurons have suggested a role for excitatory amino acids in afferent synaptic transmission onto caudal NST neurons. Evidence provided by these studies suggests that both ionotrophic and metabotrophic glutamate receptor mechanisms are involved in synaptic transmission in the caudal NST (Andresen and Yang 1994; Drewe et al. 1988, 1990; Glaum and Miller 1992, 1993). Neurons located in the caudal NST also serve as a medullary substrate involved in antinociception in the rat, and glutamate appears to be one of the chemical messengers involved in NST media-

tion of this effect (Lewis et al. 1987; Randich et al. 1988). The intense ASP- and GLU-immunoreactive puncta staining observed in caudal NST in the present study suggests that excitatory amino acids probably play a significant role in synaptic functioning in the lamb NST in a manner similar to that described in other species.

Both ASP- and GLU-immunoreactive staining were less dense in the lamb rostral NST than at more caudal locations. This contrasts with previous findings in rats. Analysis of glutamate uptake in rats revealed an increase in activity in this parameter from caudal to rostral NST (Simon et al. 1985). The high level of glutamate uptake in the rat rostral NST is suggestive of higher concentrations of excitatory amino acids at this level of the rat NST than in the lamb. The differences observed between the present study and findings in the rat may reflect species differences. While investigating the distribution of adrenergic neurons in the sheep brain, Tillet (1988) found that sheep lacked both the C1 and C3 cell groups that have been described in the rat.

The rostral NST is the primary relay for gustatory afferent fibers carried in the facial and glossopharyngeal nerves, and this region of the NST is often referred to as the gustatory zone of the NST (Hamilton and Norgren 1984; Whitehead 1994). Currently, little is known about the role of excitatory amino acids in rostral NST function. What information is available comes from whole-cell patch recordings in rat brainstem slices. Applications of ionotropic *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists to the bathing medium reduced or abolished the amplitude of the EPSP evoked by ST stimulation (Wang et al. 1993). These data indicate that, like caudal and intermediate NST neurons, the excitatory post-synaptic potentials evoked by ST stimulation in the rostral NST neurons are mediated by both ionotropic NMDA and non-NMDA glutamate receptors (Bradley 1994; King and Bradley 1993, 1994; Wang et al. 1993). Furthermore, rostral NST neurons also respond to metabotrophic glutamate receptor agonists and antagonists (R. Bradley, personal communication), implying that these rostral neurons contain the same extensive excitatory amino acid receptor sites and many of the response properties as caudal NST neurons. Although these in vitro studies provide evidence for the action of excitatory amino acids on rostral NST neurons, there is currently no information available as to what effect these compounds have on gustatory processing in the intact animal.

In summary, both ASP- and GLU-immunoreactive cells and puncta are observed throughout the lamb NST. The most intense immunoreactivity for both aspartate and glutamate was observed in the ventrolateral, ventral and ventromedial regions of the intermediate and caudal lamb NST. At the most rostral levels of the NST examined, the density of ASP and GLU immunoreactivity was less than that observed at more caudal locations. The intense ASP and GLU immunoreactivity seen in regions of the NST that are involved in respiration, deglutition and cardiovascular functions suggests that, as in other species, excitatory amino acids play an important role in the neural processing that underlies these behaviors in the lamb.

Acknowledgements I would like to thank Dr. R.A. Altschuler and Dr. R.J. Wenthold for providing the antisera used in this study. I would also like to thank J.A. Cook and T.M. Todoran for their technical assistance. This research was supported by National Institute of Deafness and Other Communication Disorders Grant DC00735.

References

Allchin RE, Batten TFC, McWilliam PN, Vaughan PFT (1994) Electrical stimulation of the vagus increases extracellular glutamate recovered from the nucleus tractus solitarii of the cat by in vivo microdialysis. Exp Physiol 79:265–268

Amri M, Car A, Roman C (1990) Axonal branching of medullary swallowing neurons projecting on the trigeminal and hypoglossal motor nuclei: demonstration by electrophysiological and fluorescent double labeling techniques. Exp Brain Res 81:384–390

Amri M, Lamkadem M, Car A (1991) Effects of lingual nerve and chewing cortex stimulation upon activity of the swallowing neurons located in the region of the hypoglossal motor nucleus. Brain Res 548:149–155

Andresen MC, Yang M (1994) Excitatory amino acid receptors and afferent synaptic transmission in the nucleus tractus solitarius. In: Barraco IRA (ed) Nucleus of the solitary tract. CRC Press, Boca Raton, pp 187–192

Bellingham MC, Lipski J (1992) Morphology and electrophysiology of superior laryngeal nerve afferents and postsynaptic neurons in the medulla oblongata of the rat. Neuroscience 48:205–216

Berger AJ, Averill DB, Cameron WE (1984) Morphology of inspiratory neurons located in the ventrolateral nucleus of the tractus solitarius of the cat. J Comp Neurol 224:60–70

Bradley RM (1994) Neurobiology of the gustatory zone of nucleus tractus solitarius. In: Barraco IRA (ed) Nucleus of the solitary tract. CRC Press, Boca Raton, pp 51–62

Burrows FA, Norton JB, Fewel J (1986) Cardiovascular and respiratory effects of ketamine in the neonatal lamb. Can Anaesthesiol Soc J 33:10–15

Car A, Jean A (1971) Potentiels evoques dans le rhombencephale du mouton par la stimulation du nerf larynge superieur. Contribution a l'etude de la localisation du centre deglutiteur. J Physiol (Paris) 63:715–730

Champagnat J, Denavit-Saubie M, Velluti JC (1980) Excitability of bulbar respiratory neurones: a study using microiontophoretic applications of depolarizing agents. Brain Res 191: 359–377

Dampney RAL (1994) Functional organization of central pathways regulating the cardiovascular system. Physiol Rev 74: 323–364

Davison JS (1987) The central organization of gastrointestinal reflexes. In: Szurszewski JH (ed) Cellular physiology and clinical studies of gastrointestinal smooth muscle. Excerpta Medica, Amsterdam, pp 187–207

Dietrich WD, Lowry OH, Loewy AD (1982) The distribution of glutamate, GABA and aspartate in the nucleus tractus solitarius of the cat. Brain Res 237:254–260

Drewe JA, Childs GV, Kunze DL (1988) Synaptic transmission between dissociated adult mammalian neurons and attached synaptic boutons. Science 241:1810–1813

Drewe JA, Miles R, Kunze DL (1990) Excitatory amino acid receptors of guinea pig medial nucleus tractus solitarius neurons. Am J Physiol 259:H1389–H1395

Glaum SR, Miller RJ (1992) Metabotropic glutamate receptors mediate excitatory transmission in the nucleus of the solitary tract. J Neurosci 12:2251–2258

Glaum SR, Miller RJ (1993) Activation of metabotropic glutamate receptors produces reciprocal regulation of ionotropic glutamate and GABA responses in the nucleus of the tractus solitarius of the rat. J Neurosci 13:1636–1641

Granata AR, Reis DJ (1983) Release of (3H)L-glutamine acid (L-Glu) and (3H)D-aspartic acid (D-Asp) in the area of nucleus tractus solitarius in vivo produced by stimulation of the vagus nerve. Brain Res 259:77–93

Granata AR, Sved AF, Reis DJ (1984) In vivo release by vagal stimulation of L-[3H] glutamic acid in the nucleus tractus solitarius preloaded with L-[3H] glutamine. Brain Res Bull 12:5–9

Grogaard J, Lindstrom DP, Stahlman MT, Marchal F, Sundell H (1982) The cardiovascular response to laryngeal water administration in young lambs. J Dev Physiol 4:353–370

Hamilton RB, Norgren R (1984) Central projections of gustatory nerves in the rat. J Comp Neurol 222:560–577

Harding R, Johnson P, McClelland ME (1978) Liquid-sensitive laryngeal receptors in the developing sheep, cat and monkey. J Physiol (Lond) 277:409–422

Hashim MA, Bieger D (1989) Excitatory amino acid receptor-mediated activation of solitarial deglutitive loci. Neuropharmacology 28:913–921

Helfert RH, Bonneau JM, Wenthold RJ, Altschuler RA (1989) GABA and glycine immunoreactivity in the guinea pig superior olivary complex. Brain Res 501:269–286

Henry JL, Sessle BJ (1985) Effects of glutamate, substance P and endosin-related peptide on solitary tract neurones involved in respiration and respiratory reflexes. Neuroscience 14:863–873

Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Jean A (1972a) Localisation et activite des neurones deglutiteurs bulbaires. J Physiol (Paris) 64:227–268

Jean A (1972b) Effet de lesions localisees du bulbe rachidien sur le staade oesophaagien de la deglutition. J Physiol (Paris) 64:507–516

Jean A (1984) Brainstem organization of the swallowing network. Brain Behav Evol 25:109–116

Kalia M, Mesulam MM (1980) Brain stem projections of sensory and motor components of the vagus complex in the cat. I. The cervical vagus and nodose ganglion. J Comp Neurol 193:435–465

Kalia M, Sullivan JM (1982) Brainstem projections of sensory and motor components of the vagus nerve in the rat. J Comp Neurol 211:248–264

Karius DR, Ling L, Speck DF (1993) Excitatory amino acid neurotransmission in superior laryngeal nerve-evoked inspiratory termination. J Appl Physiol 74:1840–1847

Karius DR, Ling L, Speck DF (1994) Nucleus tractus solitarius and excitatory amino acids in afferent-evoked inspiratory termination. J Appl Physiol 76:1293–1301

Kessler JP, Cherkaoui N, Catalin D, Jean A (1990) Swallowing responses induced by microinjection of glutamate and glutamate agonists into the nucleus tractus solitarius of ketamine-anesthetized rats. Exp Brain Res 83:151–158

King MS, Bradley RM (1993) Biophysical properties of morphologically characterized neurons in the rostral nucleus tractus solitarius (rNTS). Neurosci Abstr 19:1430

King MS, Bradley RM (1994) Relationship between structure and function of neurons in the rat rostral nucleus tractus solitarii. J Comp Neurol 344:50–64

Kovar I, Selstam U, Catterton WZ, Stahlman MT, Sundell HW (1979) Laryngeal chemoreflex in newborn lambs: respiratory and swallowing response to salts, acids, and sugars. Pediatr Res 13:1144–1149

Kubo T, Kihara M (1991) Unilateral blockade of excitatory amino acid receptors in the nucleus tractus solitarii produces an inhibition of baroreflexes in rats. Naunyn Schmiedebergs Arch Pharmacol 343:317–322

Le Galloudec E, Merahi N, Laguzzi R (1989) Cardiovascular changes induced by the local application of glutamate-related drugs in the rat nucleus tractus solitarii. Brain Res 503:322–325

Lewis JW, Baldrighi G, Akil H (1987) A possible interface between autonomic function and pain control: opioid analgesia and the nucleus tractus solitarius. Brain Res 424:65–70

Meeley MP, Underwood MD, Talman WT, Reis DJ (1989) Content and in vitro release of endogenous amino acids in the area of the nucleus of the solitary tract of the rat. J Neurochem 53:1807–1817

Mistretta CM, Bradley RM (1983a) Developmental changes in taste responses from glossopharyngeal nerve in sheep and comparisons with chorda tympani responses. Develop Brain Res 11:107–117

Mistretta CM, Bradley RM (1983b) Neural basis of developing salt taste sensation: response changes in fetal, postnatal, and adult sheep. J Comp Neurol 215:199–210

Montero VM, Wenthold RJ (1989) Quantitative immunogold analysis reveals high glutamate levels in retinal and cortical synaptic terminals in the lateral geniculate nucleus of the macaque. Neuroscience 31:639–647

Perrone MH (1981) Biochemical evidence that L-glutamate is a neurotransmitter of primary vagal afferent nerve fibers. Brain Res 230:283–293

Philippu A (1988) Regulation of blood pressure by central neurotransmitters and neuropeptides. Rev Physiol Biochem Pharmacol 111:3–115

Praud J-P, Canet E, Bureau MA (1992) Chemoreceptor and vagal influences on thyroarytenoid muscle activity in awake lambs during hypoxia. J Appl Physiol 72:962–969

Priddy M, Drewe JA, Kunze DL (1992) L-Glutamate inhibition of an inward potassium current in neonatal neurons from the nucleus of the solitary tract. Neurosci Lett 136:131–135

Randich A, Roose MG, Gebhart GF (1988) Characterization of antinociception produced by glutamate microinjection in the nucleus tractus solitarius and the nucleus reticularis ventralis. J Neurosci 8:4675–4684

Ross MG, Sherman DJ, Ervin MG, Day L, Humme J (1990) Fetal swallowing: response to systemic hypotension. Am J Physiol 258:R130–R134

Schaffar N, Pio J, Jean A (1990) Selective retrograde labeling of primary vagal afferent cell-bodies after injection of [^{3}H]D-aspartate into the rat nucleus tractus solitarii. Neurosci Lett 114:253–258

Simon JR, DiMicco SK, Aprison MH (1985) Neurochemical studies of the nucleus of the solitary tract, dorsal motor nucleus of the vagus and the hypoglossal nucleus in rat: topographical distribution of glutamate uptake, GABA uptake and glutamic acid decarboxylase activity. Brain Res Bull 14:49–53

Sweazey RD, Bradley RM (1986) Central connections of the lingual-tonsillar branch of the glossopharyngeal nerve and the superior laryngeal nerve in lamb. J Comp Neurol 245:471–482

Sweazey RD, Bradley RM (1988) Response of lamb nucleus of the solitary tract neurons to chemical stimulation of the epiglottis. Brain Res 439:195–210

Sweazey RD, Bradley RM (1989) Responses of neurons in the lamb nucleus tractus solitarius to stimulation of the caudal oral cavity and epiglottis with different stimulus modalities. Brain Res 480:133–150

Talman WT, Perrone MH, Reis DJ (1980) Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers. Science 209:813–815

Tell F, Jean A (1990) Rhythmic bursting patterns induced in neurons of the rat nucleus tractus solitarii, in vitro, in response to *N*-methyl-D-aspartate. Brain Res 533:152–156

Tell F, Jean A (1993) Ionic basis for endogenous rhythmic patterns induced by activation of *N*-methyl-D-aspartate receptors in neurons of the rat nucleus tractus solitarii. J Neurophysiol 70:2379–2390

Tillet Y (1988) Adrenergic neurons in sheep brain demonstrated by immunohistochemistry with antibodies to phenylethanolamine *N*-methyltransferase (PNMT) and dopamine-—hydroxylase (DBH): absence of the C_1 cell group in the sheep brain. Neurosci Lett 95:107–112

Van Giersbergen PLM, Palkovits M, de Jong W (1992) Involvement of neurotransmitters in the nucleus tractus solitarii in cardiovascular regulation. Physiol Rev 72:789–824

Wang L, King MS, Bradley RM (1993) In vitro patch clamp analysis of postsynaptic potentials mediated by excitatory and inhibitory amino acids on neurons in the gustatory zone of the nucleus tractus solitarius. Chem Senses 18:578

Webb B, Hutchison AA, Davenport PW (1994) Vagally mediated volume-dependent modulation of inspiratory duration in the neonatal lamb. J Appl Physiol 76:397–402

Whitehead MC (1994) Functional connections of the rostral nucleus of the solitary tract in viscerosensory integration of ingestion reflexes. In: Barraco IRA (ed) Nucleus of the solitary tract. CRC Press, Boca Raton, pp 105–118