

Organization and Function of Ventrolateral Medullary Afferents to the Nucleus of the Solitary Tract Complex

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I. Introduction

The ventrolateral medulla (VLM) has received considerable attention as an important integrating area for control of the circulation (1). Neurons in the VLM have been shown to be involved in the maintenance of vasomotor tone (2–4), in baroreceptor and chemoreceptor reflex mechanisms (5–11), in mediating the cerebral ischemic response and somatosympathetic reflexes (12–17), and in the control of the secretion of vasopressin (3,18–21). Most of these physiological functions have been thought to involve the catecholaminergic neurons within the VLM (3,18,22–28).

It has also been suggested that the VLM influences the gain of the baroreceptor reflex at the level of the nucleus of the solitary tract (NTS; Ref. 29), the primary site of termination of cardiovascular afferent fibers (30–33). In support of this suggestion, lesions of the caudal VLM that included the region of the A₁-noradrenergic cell group have been shown to alter baroreceptor reflex excitability (28). In addition, chemical and electrical stimulation of the VLM has been shown to elicit an increase in heart rate and in arterial pressure, during which vagal activity to the heart is inhibited (3) and sympathetic vasomotor

tone is insensitive to baroreceptor inhibition (9). Furthermore, several anatomical studies have demonstrated direct projections from the VLM region to the cardiovascular areas of the NTS (34–39). However, these anatomical data have been obtained in autoradiographic or horseradish peroxidase studies, in which the injection sites were not limited to the anatomical structures studied (35, 36, 38).

Additionally, although retrograde tract-tracer injections that overlap most of the NTS complex give a good indication of where the neurons that project to the NTS are located within the rostrocaudal extent of the VLM, they provide little information regarding the subnuclei in the NTS that receive the VLM projections (35, 36, 38–40). Retrograde tract-tracer studies must also be interpreted with caution, as fibers of passage in the injection area may have contributed to the retrograde labeling of neurons observed in the VLM. Autoradiographic studies have provided evidence for neurons within the rostral VLM that project to the ventrolateral and intermediate subnuclei of the NTS (36) and neurons within the caudal VLM that project primarily to the medial and commissural subnuclei of the NTS (35, 40). In a recent study using the selective anterograde tract-tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L), it was demonstrated that neurons within the rostral VLM project to the commissural and ventrolateral subnucleus of the NTS (34). Although there appears to be a discrepancy in the projections to the NTS with the use of the anterogradely transported tract tracers, it may be due to the location of the injection sites within the rostrocaudal extent of the VLM or to the fact that some neurons in the VLM do not take up and transport the tracers (41).

Injections of catecholamines into the NTS have been shown to elicit changes in arterial pressure and heart rate (42–44) and to alter baroreceptor reflex excitability (43, 45, 46). Therefore, the possibility exists that catecholaminergic neurons within the VLM may influence the function of NTS neurons. In support of this suggestion, the NTS has been shown to be densely innervated by catecholaminergic fibers (47–52). This catecholaminergic innervation of the NTS has been suggested to arise from neurons belonging to the A₁ and C1 catecholaminergic cell groups within the VLM (39). However, no attempt has been made to identify the subnuclei of the NTS that receive catecholaminergic inputs from VLM neurons and to determine whether a topographical organization exists within the NTS complex of projections from the A₁-noradrenergic and C1 adrenergic neurons in the VLM.

The NTS complex has also been shown to contain catecholaminergic neurons within its medial subdivision (47, 50, 51). These catecholaminergic neurons are also thought to play a role in cardiovascular regulation (42, 53). It has been suggested that catecholaminergic inputs onto the A₂-noradrenergic neurons within the NTS may be responsible for eliciting changes in blood pressure and heart rate observed after microinjections of catecholamines into the NTS (42). This suggestion is based on the observation that some catecholaminergic neurons

within the NTS receive catecholaminergic input (54), although the source of this catecholaminergic input is not known.

This chapter presents a mapping of the termination sites in the NTS complex of neurons located at different rostrocaudal levels within the VLM using the anterograde tract tracer PHA-L (55). PHA-L has the advantages of allowing the identification of the precise location of cell bodies that have incorporated the tracer for transport and permitting a clear identification of the resulting labeled axons with their presumptive terminal boutons at the light microscope level (55, 56). PHA-L tract tracing was also combined in these studies with immunohistochemistry for the catecholaminergic biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), and phenylethanolamine-*N*-methyltransferase (PNMT) to investigate the contribution of catecholaminergic neurons in the VLM to the innervation of the NTS and that of non-catecholaminergic VLM neurons to the catecholaminergic cell groups of the NTS. In addition, electrophysiological (57) and functional data are summarized, showing the effect of activation of VLM neurons on the aortic baroreceptor reflex.

II. Anatomical Organization of Afferent Projections to the NTS Complex from Neurons Within the VLM

The organization of afferent projections to the NTS complex from neurons located throughout the rostrocaudal extent of the VLM was investigated in the male Wistar rat. PHA-L injections were made within the caudal VLM that overlapped the A₁-noradrenergic neurons, within the rostral VLM that overlapped the C1 adrenergic neurons, and within the area between both the A₁- and C1 cells groups, which is known to contain a mixture of both A₁-noradrenergic and C1 adrenergic neurons (Fig. 1). This latter area will be referred to as the intermediate VLM in this chapter.

PHA-L injections within the caudal VLM (Fig. 2) resulted in a dense innervation of the commissural and medial subnuclei of the NTS caudal to area postrema (Fig. 3) bilaterally, but with an ipsilateral predominance. At the level of area postrema, the commissural and medial subnuclei of the NTS had very few PHA-L-labeled fibers. A moderate number of labeled fibers and presumptive terminal boutons were observed throughout the ventral and to a smaller extent the ventrolateral subnuclei of the NTS. The region of the lateral subnucleus of the NTS lying lateral and dorsal to the solitary tract received very few projections from caudal VLM neurons. At more rostral levels of the NTS (Fig. 2), fibers were also observed to be scattered medial to the solitary tract in the region in and around the central and medial subnuclei of the NTS. A few fibers and presumptive terminal boutons containing both PHA-L and TH and/or DBH immunoreactivity were observed in the ventral aspects of the commissural

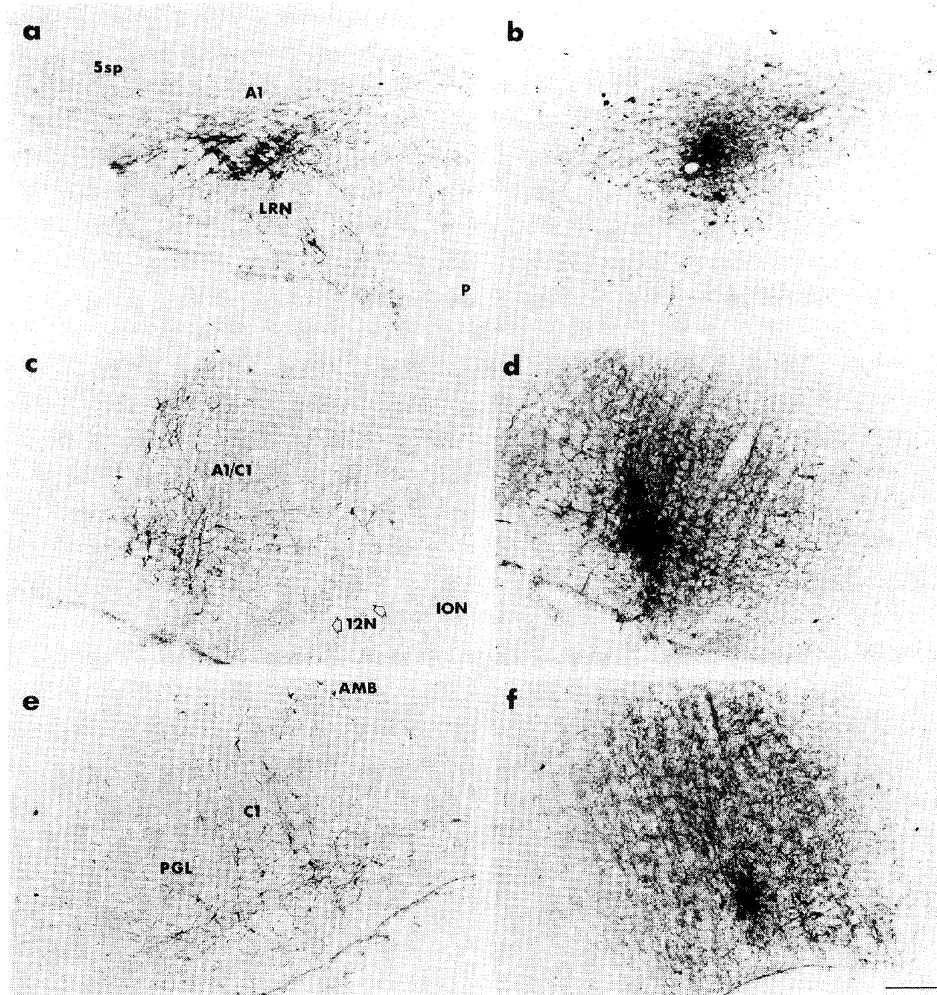


Figure 1 Bright-field photomicrographs of transverse sections of the medulla through the region of the VLM showing TH immunoreactive cells and PHA-L injection sites in the caudal (a) and intermediate (c) VLM and PNMT immunoreactive neurons (e) and a PHA-L injection (f) in the rostral VLM (f). Sections a, c, and e are adjacent to those shown in sections b, d, and f, respectively. Note that the PHA-L injection sites overlap the A₁-noradrenergic cell group (a and b), the A₁-/C1 catecholaminergic cell group (c and d) and the C1 adrenergic cell group (e and f). The calibration mark in f represents 200 μ m and applies to all photomicrographs. Abbreviations: A₁, A₁ noradrenergic cell group; C1, C1 adrenergic cell group; AMB, nucleus ambiguus; ION, inferior olivary nucleus; LRN, lateral reticular nucleus; P, pyramidal tract, PGL, paragigantocellularis lateralis nucleus; 5sp, spinal trigeminal nucleus; 12N, hypoglossal nerve.

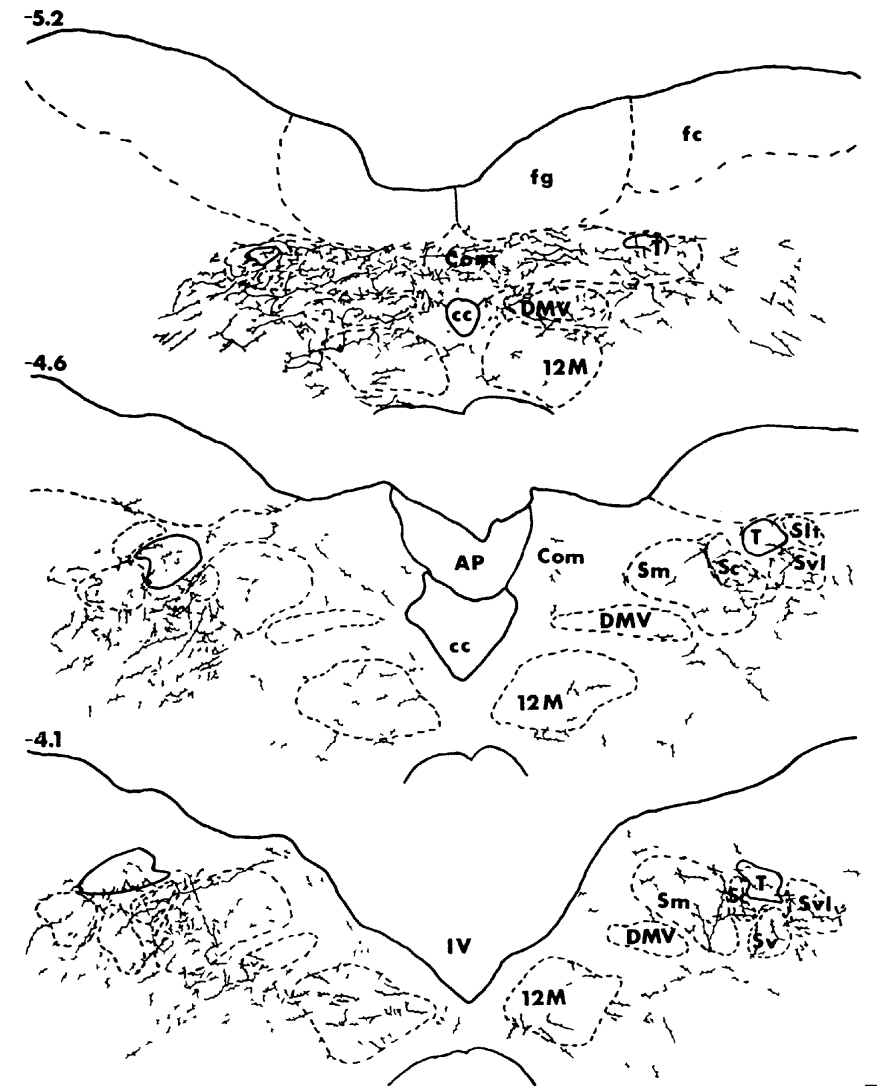


Figure 2 Series of camera lucida drawings of the rat dorsomedial medulla showing the distribution of labeled fibers and presumptive terminal boutons in the NTS complex resulting from a PHA-L injection into the caudal VLM. Calibration line represents 0.2 mm. Abbreviations: AP, area postrema; cc, central canal; Com, commissural subnucleus of nucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; fc, cuneate fasciculus; fg, gracilis fasciculus; Sm, medial subnucleus of the solitary tract; Slt, lateral subnucleus of nucleus of the solitary tract; Sv, ventral subnucleus of nucleus of the solitary tract; Svl, ventrolateral subnucleus of nucleus of the solitary tract; T, tractus solitarius; 12 M, hypoglossal nucleus; IV, fourth ventricle.

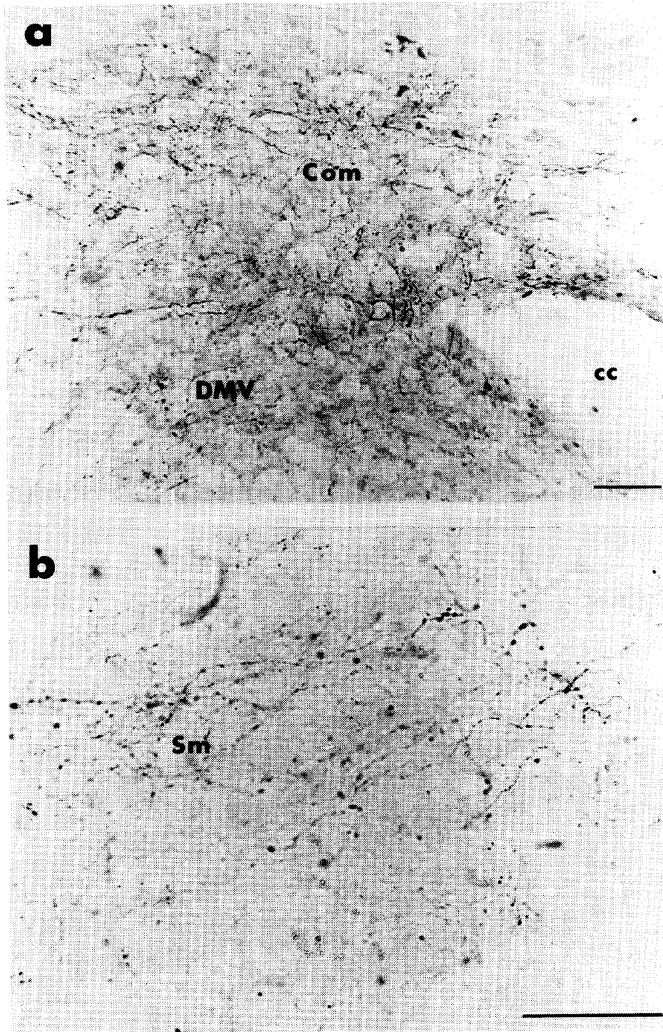


Figure 3 Bright-field photomicrographs of representative transverse sections through the NTS complex of the rat showing PHA-L labeled fibers and terminal boutons within the commissural subnucleus (Com, a) and the medial subnucleus of the nucleus (Sm; b) of the solitary tract resulting from a PHA-L injection site in the caudal VLM. The calibration marks represent 50 μm . Abbreviations: cc, central canal; Com, commissural subnucleus of nucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; sm, medial subnucleus of nucleus of the solitary tract.

nucleus (Fig. 4a and b). No other subnucleus in the NTS complex was found to contain fibers labeled for both PHA-L and TH and/or DBH.

PHA-L injections within the intermediate region of the VLM resulted in a different projection pattern in the NTS complex than that arising from the caudal VLM (Fig. 5). The commissural nucleus received few projections from

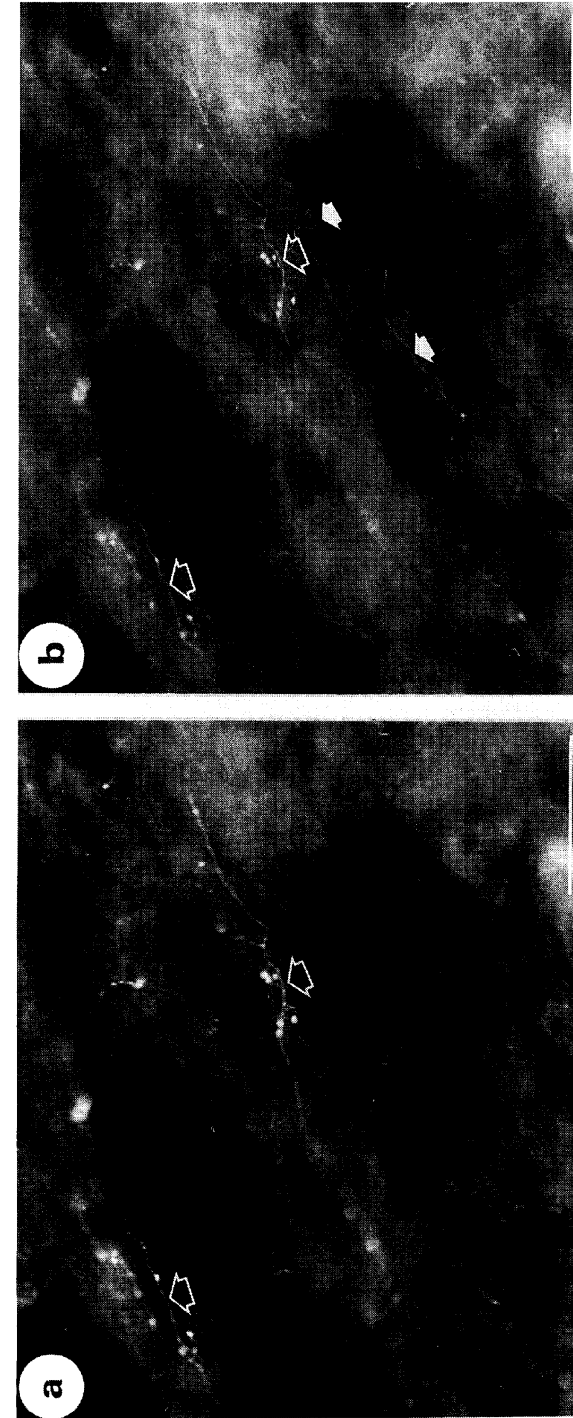


Figure 4 Photomicrographs of Texas red (a) and fluorescein (b) labeled PHA-L fiber and terminal labeling in the commissural subnucleus of the nucleus of the solitary tract (A), which also contained tyrosine hydroxylase immunoreactivity (TH, B). Note that PHA-L-labeled fibers and terminal boutons (a, open arrows) were also labeled for TH (b, open arrows). Solid arrows in b point to TH-labeled fibers not immunoreactive to PHA-L (compare to a). Photomicrographs a and b were taken from the same section but under different fluorescent illuminations. Calibration mark in all photomicrographs represent 50 μm .

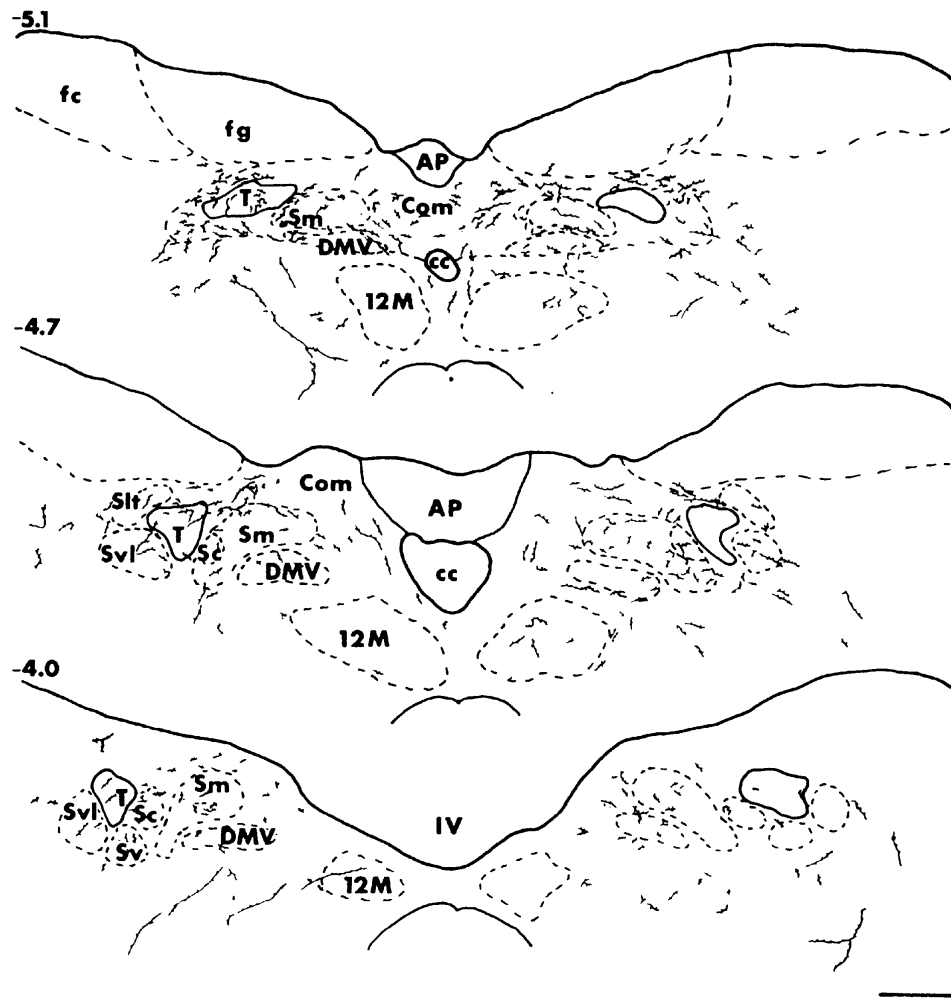


Figure 5 Series of camera lucida drawings of the rat dorsomedial medulla showing the distribution of labeled fibers and presumptive terminal boutons in the NTS complex resulting from a PHA-L injection into the intermediate VLM. Calibration line represents 0.2 mm. Abbreviations: AP, area postrema; cc, central canal; Com, commissural subnucleus of nucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; fc, cuneate fasciculus; fg, gracilis fasciculus; Sm, medial subnucleus of the solitary tract; Slt, lateral subnucleus of nucleus of the solitary tract; Sv, ventral subnucleus of nucleus of the solitary tract; Svl, ventrolateral subnucleus of nucleus of the solitary tract; T, tractus solitarius; 12 M, hypoglossal nucleus; IV, fourth ventricle.

VLM neurons in the intermediate area bilaterally. In addition, a few scattered fibers were observed around the perimeter of the medial subnucleus and encircling the central subnucleus of the NTS. Compared to projections from the caudal VLM, a denser innervation was observed to the lateral, ventral, and ventrolateral subnuclei of the caudal NTS. A small number of fibers and pre-

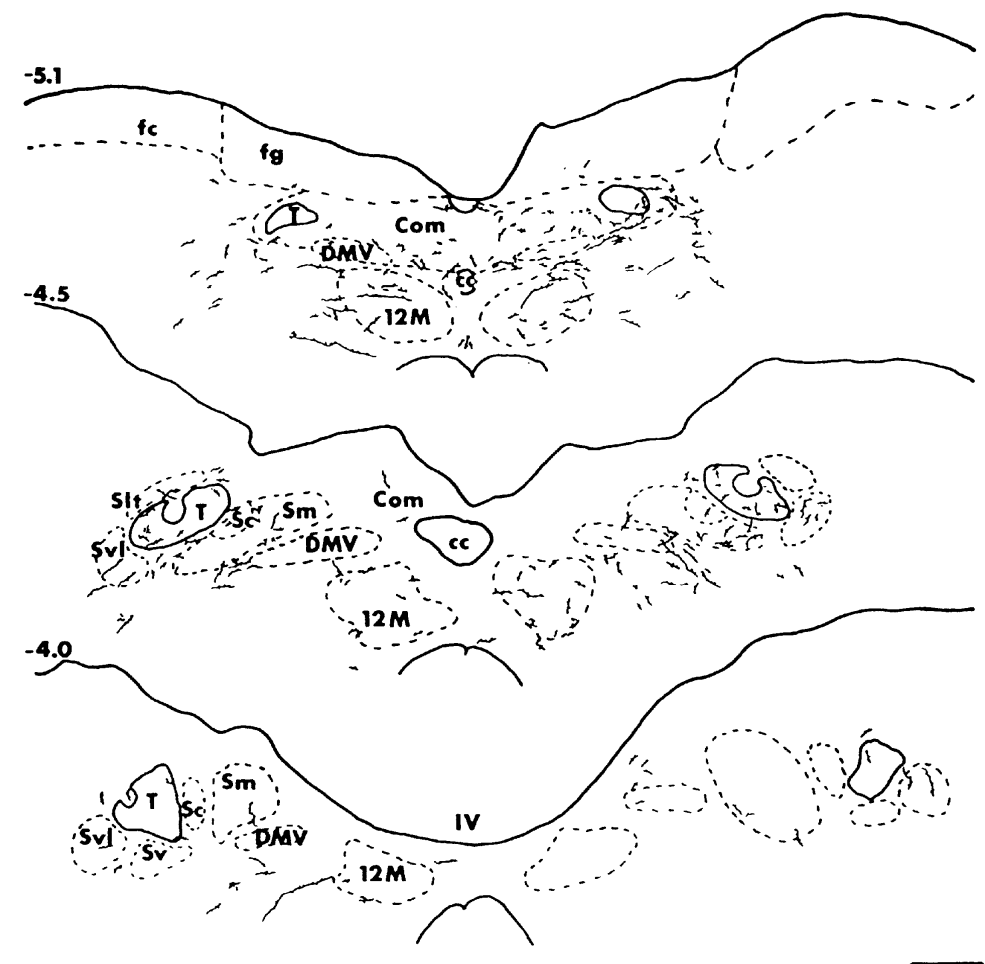


Figure 6 Series of camera lucida drawings of the rat dorsomedial medulla showing the distribution of labeled fibers and presumptive terminal boutons in the NTS complex resulting from a PHA-L injection into the rostral VLM. Calibration line represents 0.2 mm. AP, area postrema; cc, central canal; Com, commissural subnucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; fc, cuneate fasciculus; fg, gracilis fasciculus; Sm, medial subnucleus of the solitary tract; Slt, lateral subnucleus of nucleus of the solitary tract; Sv, ventral subnucleus of nucleus of the solitary tract; Svl, ventrolateral subnucleus of nucleus of the solitary tract; T, tractus solitarius; 12 M, hypoglossal nucleus; IV, fourth ventricle.

sumptive terminal boutons in the commissural and medial subnuclei of the NTS were double labeled for PHA-L and TH and/or DBH.

PHA-L injections within the rostral VLM (Fig. 6) resulted in the least number of PHA-L labeled fibers with terminal boutons in the NTS complex compared to that after PHA-L injections in either the caudal or rostral VLM.

Few labeled fibers with terminal boutons were observed in the caudal portions of the NTS complex, along the ventral border of the commissural nucleus. In addition, few fibers with terminal boutons were scattered lateral and medial to the solitary tract at approximately the level of the obex. The lateral, ventral, and ventrolateral subnuclei of the NTS were also sparsely innervated by rostral VLM neurons. Rostral to the obex, labeled fibers were rarely observed within the NTS complex. None of the PHA-L injections that overlapped the C1 adrenergic cell group resulted in PHA-L and PNMT double-labeled fibers in the NTS complex.

III. VLM Innervation of the Catecholaminergic Neurons Within the NTS Complex

In the present study, PHA-L injections overlapping either the caudal or intermediate VLM resulted in labeled fibers with presumptive terminal boutons within both the commissural and medial subnuclei of the NTS complex. Previous anatomical studies have demonstrated that the commissural and medial subnuclei of the NTS contain noradrenergic neurons of the A₂ cell group (47, 50, 51). In the present study, a few PHA-L labeled fibers with terminal boutons were observed to intermingle with TH immunoreactive neurons within the NTS at the level of the commissural subnucleus following injections of PHA-L into either the caudal or intermediate VLM (Fig. 7). At the level of the area postrema, very few PHA-L-labeled fibers with terminals were observed to intermingle with the A₂-neurons. The majority of PHA-L-labeled fibers were observed to terminate within the NTS region just lateral and dorsal to the A₂-noradrenergic cell group. PHA-L-labeled fibers with presumptive terminals were also observed rostrally within the NTS complex at the level of the C2 adrenergic neurons, although, very few of these PHA-L-labeled terminals were seen to be in close proximity to the C2 neurons. No fibers or presumptive terminal boutons immunoreactive to both PHA-L or TH and/or DBH were observed in close apposition to catecholaminergic neurons in the NTS complex.

IV. Electrophysiological Identification of Neurons Within the NTS Complex Receiving Inputs from VLM and Aortic Baroreceptors

The anatomical data presented above have provided evidence that neurons within the caudal and intermediate VLM have the greatest projection to subnuclei within the NTS complex that have previously been shown to receive inputs from baroreceptor afferent fibers carried in the aortic depressor nerve (ADN) in the rat (30-33). This observation suggests the possibility that the VLM may alter aortic baroreceptor reflex excitability at the level of the NTS.

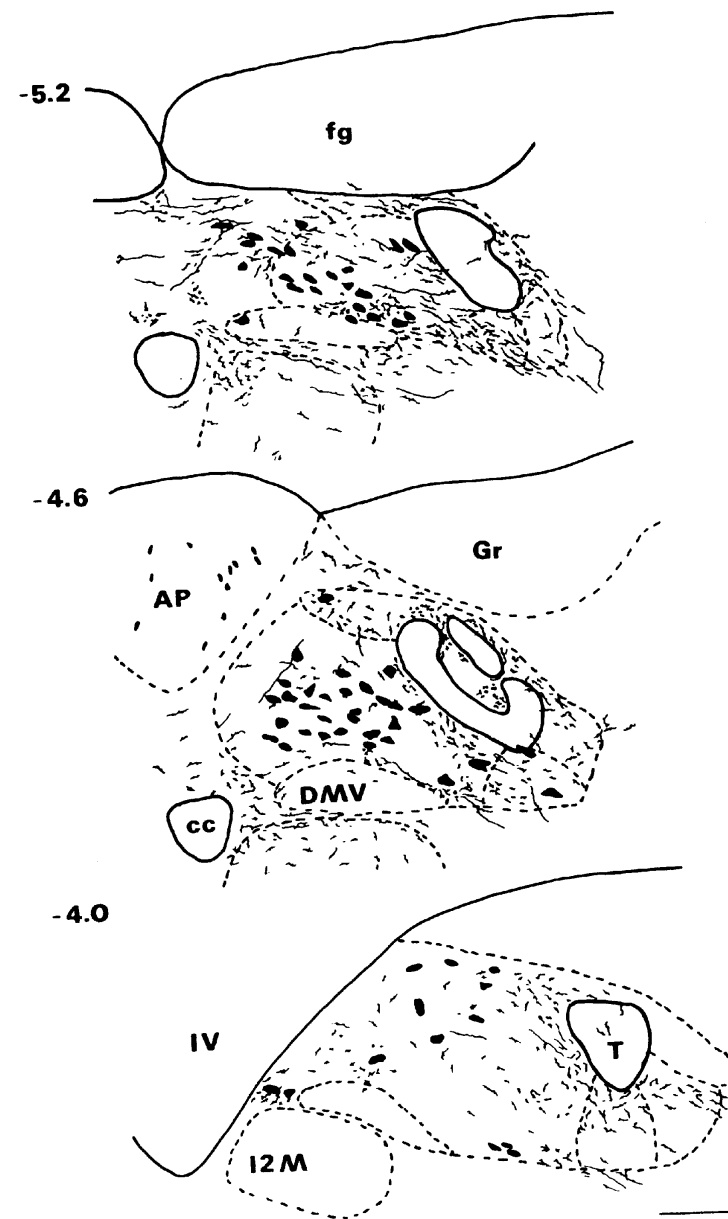


Figure 7 Series of projection drawings of the rat dorsomedial medulla showing the distribution of labeled fibers and presumptive terminal boutons resulting from a PHA-L injection that extended between the caudal and intermediate VLM in relation to the catecholaminergic neurons in the NTS complex. Calibration line represents 0.2 mm. Abbreviations: AP, area postrema; cc, central canal; DMV, dorsal motor nucleus of the vagus; fg, gracilis fasciculus; T, tractus solitarius; 12 M, hypoglossal nucleus; IV, fourth ventricle.

To test this possibility, electrophysiological experiments were done in alpha-chloralose-anesthetized male Wistar rats to determine the effect of VLM stimulation on the response of single units in NTS to ADN stimulation (57).

Of 178 single units recorded extracellularly from histologically verified sites in the NTS region, 28% (49/178) were identified as responding orthodromically to ADN stimulation and 37% (65/178) as responding orthodromi-

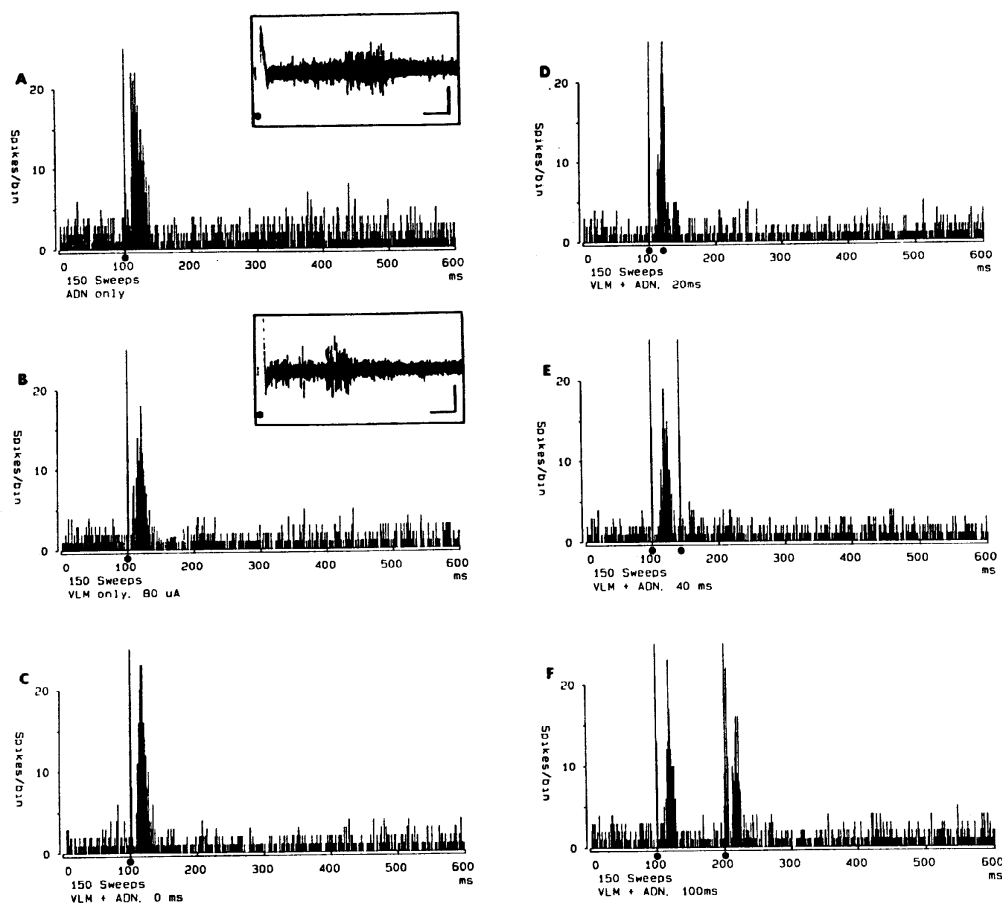


Figure 8 Peristimulus time histograms (1 ms/bin) showing the effect of applying conditioning stimuli to VLM on the firing frequency of NTS single units responding to stimulation of ADN (panels C through F). Note that the single unit increased its firing frequency during both ADN (panel A) and VLM (panel B) stimulation alone. Insets are five superimposed oscilloscope tracings of orthodromic responses of the unit to ADN (panel A) and VLM (panel B) stimulation. Calibrations marks represent 5 ms and 50 μ V. Note that a conditioning stimulus applied to VLM 20 to 40 ms prior to ADN stimulation (panels D through E) reduces the response of the unit to ADN stimulation. The response to ADN stimulation begins to return to control levels approximately 100 ms after the application of the VLM stimulus. (From Ref. 57.)

cally to VLM stimulation. Twenty-nine units within the NTS responding to both ADN (mean latency, 23.9 ± 4.5 ms) and VLM (mean latency, 21.5 ± 4.4 ms) stimulation were further tested for their response to ADN stimulation in the presence of a conditioning stimulus to the VLM. Of the 29 units, 13 (45%) showed a decrease in the ADN response of approximately 60% when a conditioning stimulus to the VLM preceded the ADN test stimulus by approximately 20 to 40 ms (Fig. 8). The response began to recover by approximately 100 ms.

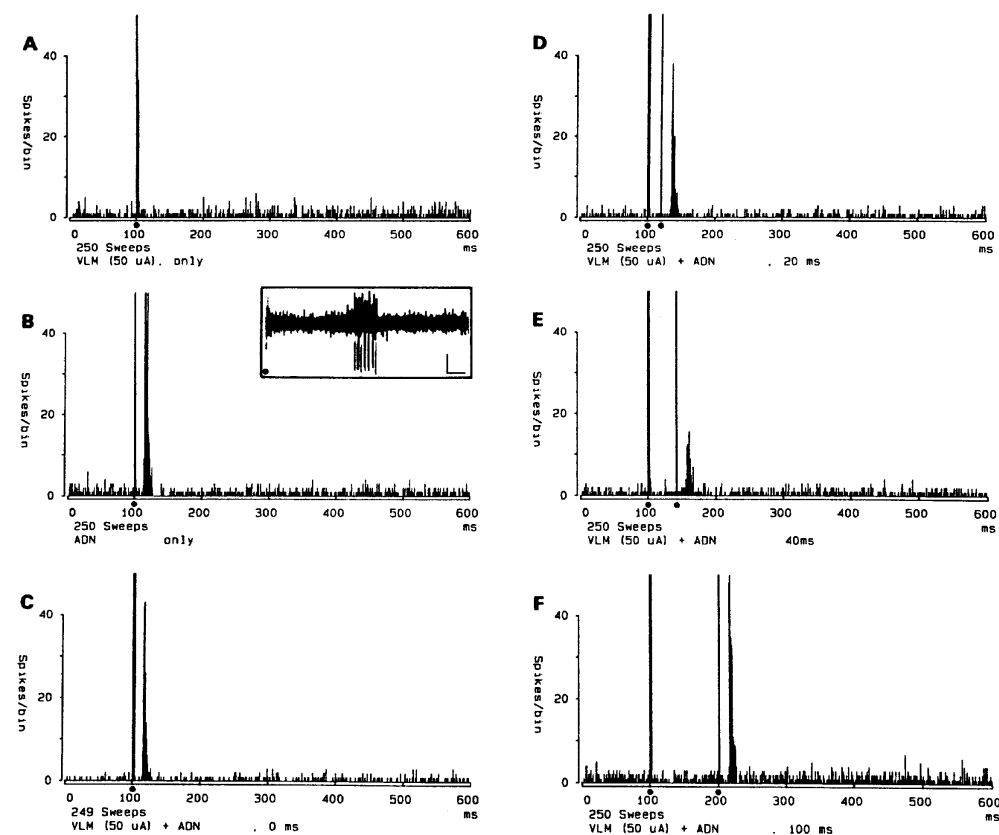


Figure 9 Peristimulus time histograms (1 ms/bin) showing the effect of conditioning stimuli to VLM on the firing frequency of an NTS single unit responding to stimulation of ADN. Note that VLM stimulation alone did not elicit a change in the firing frequency of the unit in panel A, whereas ADN stimulation excited the unit in panel B. The unit's response to ADN stimulation is reduced to less than half the original response when the conditioning stimulus applied to VLM precedes the ADN stimulation by approximately 40 ms (panel E). The response to ADN stimulation returns to control levels when the test stimulus to ADN is approximately 100 ms after the conditioning stimulus to VLM (panel F). The inset in panel B represents five superimposed oscilloscope tracings of the orthodromic response of the unit to ADN stimulation. Calibration is 100 ms and 50 μ V. Stimulus was delivered at the dot. (From Ref. 57.)

