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CENTRAL ORGANIZATION OF THE CARDIAC VAGUS

by

G. Steven Geis

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

April

1979

Dedicated to my folks --
for teaching me the real basics.

BIOGRAPHY

G. Steven Geis, the son of Mr. and Mrs. Arthur J. Geis, was born on October 31, 1951, in Chicago, Illinois. He attended parochial elementary schools in the Archdiocese of Chicago and completed his secondary education in 1969 at Dundee Community High School, Carpentersville, Illinois.

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CHAPTER I

INTRODUCTION

The central organization of the cardiac vagus has not been clearly elucidated. Most studies were carried out to identify medullary nuclei containing cardiac vagal preganglionic somata. However, quantitative analyses of the morphology and distribution of the cell bodies ~~have~~ not been performed. Comparisons of somata with axons passing in the left and right cervical vagi have not been reported and the possibility of medullary decussation of cardiac vagal preganglionic axons has not been investigated. Such anatomical properties may have physiological significance.

Differential parasympathetic cardiac control has been described. Activation of discrete peripheral parasympathetic nerves produces specific cardiac responses. Right vagal stimulation predominately affects heart rate while left vagal activation induces changes in myocardial impulse conduction (44). Distinct areas of cardiac muscle are controlled by specific cardiac vagal branches (177).

Possibly, a central neuroanatomic correlate to the functional organization of the peripheral vagus may exist. Cardiac vagal preganglionic somata may be cardiotoxically organized. Cell bodies giving rise to axons regulating a specific myocardial function may be arranged into distinct groups. Such "functional" populations of somata may demonstrate distinctive morphological characteristics. Furthermore, various cardiovascular reflexes may differentially regulate the different populations of somata.

The location of cardiac vagal preganglionic somata has not been determined. Different investigators used various techniques and identified the cell bodies in either the dorsal motor nucleus of the vagus (DMN) or the nucleus ambiguus (NA). Due to the conflicting results, there is little evidence for central cardiotoxic parasympathetic organization.

The present study was performed for investigating the central anatomical and physiological correlates to peripheral vagal organization. In the initial studies, the horseradish peroxidase (HRP) technique was used for comparing the morphology of somata with axons passing in the left and right vagi. HRP labeled somata were arranged in distinct groups. Therefore, studies were

carried out for determining the functional significance of the organization. Medullary nuclei containing cardiac vagal preganglionic somata were electrically stimulated while recording various cardiac parameters. Finally, experiments were designed for localizing ascending spinal pathways involved in modulating the different populations of cardiac vagal preganglionic somata.

CHAPTER II

LITERATURE REVIEW

A. Anatomy of the Vagus

1. Cervical Vagus, Aortic Depressor Nerve and Cardiac Vagal Branches

The vagus demonstrates a complex structure as would be expected from the wide variety of visceral responses it controls. Primarily as a result of the studies by Agostoni et al. (5), DuBois and Foley (67), DuBois and Foley (68) and Evans and Murray (71) the anatomical and functional characteristics of the cervical vagus and vagal branches have been determined. Intracranial, supranodose and infranodose vagotomies were performed. After cut axons degenerated, the cervical vagus was stimulated while monitoring various levels were then histologically treated and the characteristics of degenerating and nondegenerating fibers were determined.

Control cats without chronic vagotomy demonstrated hypotension, bradycardia as well as esophageal, duodenal and stomach contractions during stimulation of the distal end of the cut cervical vagus. Responses were not elicited from all animals with chronic vagotomy. Thus, division of the vagus at all three levels produced degeneration of efferent fibers.

The data indicated that the cells of origin of vagal efferent fibers are located within the central nervous system (5,67,71,79).

Histological studies indicated that the cervical vagus is composed of about 30,000 fibers. 16% are myelinated while the remainder are non-myelinated. Diameters of myelinated fibers range from 1-18 μm . The changes in the cervical vagi after chronic supranodose or intracranial vagotomy provided evidence of the anatomical characteristics of vagal efferent fibers.

After vagotomy, both myelinated and non-myelinated fibers demonstrated Wallerian degeneration. Since about 6000 fibers degenerated only 20% of the total fibers in the cervical vagus are efferent. About 40% of the myelinated and 15% of the non-myelinated fibers degenerated. Nearly all the fibers above 12 μm in diameter and about 40-50% of the fibers between 8-12 μm were affected by the vagotomy.

Forty-50% of the small myelinated fibers (below 6 μm in diameter) disappeared after supranodose or intracranial vagotomy. Therefore, these axons are motor and have their cell bodies in the central nervous system. The majority (80%) of myelinated fibers of medium size remained after supranodose division of the vagus. Therefore, these neurons are afferents and have their cell bodies in the nodose or jugular ganglia.

Several studies demonstrated the existence of aberrant neurons. After infranodose vagotomy, several hundred nondegenerated non-myelinated fibers remained in the cervical vagus caudal to the division. In most cases, visceral responses were not elicited upon electrical stimulation of these fibers. Yet, in some instances stimulation of the axons produced a pressor response. It is conceivable that the fibers might arise from aberrant ganglia scattered throughout the cervical sympathetic nerve (75) and run caudally in the vagus. Sympathetic fibers running in the cervical vagus have been described (122,123).

The aortic depressor nerve is closely adhered to the cervical vagal trunk, yet has a separate sheath and can be dissected free of the vagus. The nerve contains two relatively discrete groups of myelinated fibers. The distribution of fiber diameters is bimodal with peaks at 4 μm and 10 μm . There have been no reports of degenerating fibers in the aortic depressor nerve after supranodose vagotomy. Therefore, all the fibers are afferents. In some animals the aortic depressor nerve is included within the vagal trunk and is not enclosed in a separate sheath. However, the aortic depressor fibers form a characteristically discrete group of myelinated fibers of about 10 μm in diameter. Such fiber groups are not usually found in the vagus nerve when the aortic depressor nerve exists as a separate sheathed bundle.

Comparisons have been made of fiber populations of normal cardiac branches with those after chronic supranodose or intracranial vagotomy. In this manner the characteristics of afferent and efferent cardiac fibers have been determined. A normal cardiac vagal branch is composed of about 2500 fibers. Approximately 500 fibers degenerate after chronic supranodose vagotomy. Thus, 80% of the fibers are afferents while 20% are efferents.

The cardiac vagal branches contain about 500-700 myelinated fibers with diameters ranging from 1-12 μ m. There is no apparent reduction in the number of these fibers after supranodose vagotomy. Thus, most myelinated fibers are afferents. However, of the considerable number of individual cardiac branches examined after supranodose or intracranial vagotomy sections taken from the finest branches and stained by the Weigert method showed scattered myelin droplets. These data suggest that a small number of myelinated fibers are efferents.

As the vagus passes through the thorax, it gradually loses most of the myelinated fibers. Practically all the small and medium sized myelinated fibers are distributed to the esophageal, bronchial and cardiac branches of the vagus. This was shown by the fact that only about 400 myelinated fibers in both the anterior and posterior abdominal vagal branches receive fibers from both cervical vagi.

2. Nodose and Jugular Ganglia

The cell bodies of vagal afferent fibers are located in the nodose ganglion (inferior or plexiform ganglion) and the jugular ganglion (superior ganglion). The nodose ganglion lies immediately posterior to the carotid sinus and inferior to the jugular ganglion. The nodose ganglion of the cat is composed of about 30,000 neurons (122,74,75) organized into two size groups. Most of the somata are 35-40 μm in diameter while a few have diameters of 20-30 μm (156). The cell bodies are predominately unipolar (32) but may demonstrate fenestrated (32,74,75) fusiform or bipolar morphology.

The axons demonstrate a convoluted course around one pole of the cell body and then divide into peripheral and central branches. The central branch is about one-third the diameter of the distal branch. Afferent fibers originating from ganglion cells are organized in the center of the ganglion while motor fibers of central origin pass along the perimeter (32).

The nodose ganglion of the cat contains chromaffin cells (114) which receive synapses from nerve terminals containing granular vesicles. However, the chromaffin cells demonstrate no efferent synapses. The cells are considered chemoreceptor-like cells of the carotid bodies, yet their function has not been elucidated.

The nodose ganglion provides afferent fibers to the

main trunk of the vagus while the jugular contributes 73% of its fibers to the auricular nerve.

3. Origin of Preganglionic Neurons

The location of the cell bodies of vagal preganglionic fibers has not been determined. The dorsal motor nucleus of the vagus demonstrates chromatolytic changes after vagotomy (93). However, physiological evidence suggests the cell bodies are in the nucleus ambiguus. Stimulation of the nucleus ambiguus produces bradycardia while activation of the dorsal motor nucleus produces no effect. Localizing the cell bodies of cardiac vagal preganglionic fibers is one of the purposes of the present study. A further review of the literature related to the topic will be carried out in Chapter V.

B. Anatomy and Physiology of Cardiac Innervation

1. Atrial Innervation

Since the classical studies of Gesell (86) and Wiggers (205), there has been general agreement that vagal stimulation depresses atrial contractility. Williams et al. (207) measured atrial force with strain gauge arches. Right atrial force decreased by 41% and left atrial force decreased by 19% during right vagal stimulation in intact paced canine hearts. Stimulation of the left vagus produced smaller decreases in contractile force in both atria. Infusion of acetylcholine in heart-lung preparations or isolated atria produced negative inotropic effects (12,104). Responses to

stimulation of either vagus or acetylcholine were abolished by atropine. Thus, the negative inotropic effects of vagal stimulation are mediated by the muscarinic actions of the parasympathetic nervous system on the atrial tissue.

2. Ventricular Innervation

Most of the early histological (53,162,163) and physiological (36,66) studies of cardiac innervation indicated no direct parasympathetic innervation of the ventricles. The notable exception was Nonidez (162) who identified sparse parasympathetic terminals in the ventricles of dogs and cats using the silver impregnation technique. The development of stains for cholinesterase activity, electron microscopic techniques and cardiac denervation procedures provided further evidence of ventricular cardiac parasympathetic innervation.

Cholinesterase stains produce light staining for adrenergic terminals and dark staining for cholinergic terminals. Carbonell (35) and James (117) used the technique for studying cardiac innervation. Definite concentrations of dark staining terminals were observed in the ventricles. These results were repeated and confirmed by a number of investigators (46,103,104). Cholinesterase activity in the ventricles was as high as one-third of that found in the atria. Tchong (196) demonstrated the existence of intramural ganglia at the base and apex of the ventricles. Since the association of nerve fibers with ganglia in an

organ is considered characteristic of the parasympathetic nervous system, the ganglia were considered to be post-ganglionic parasympathetic fibers. In addition, since the ganglion cells remain after extrinsic cardiac denervation (104, 105), they are considered to be parasympathetic. Electron microscopic techniques have confirmed the persistence of intrinsic neural elements in the ventricular myocardium after cardiac denervation (159,160).

Physiological studies have confirmed the anatomic data suggesting ventricular parasympathetic innervation. Negative effects on ventricular automaticity and rate during vagal stimulation have been reported (70). Complete AV block has been described consequent to vagal activation. However, until recent years most studies suggested that vagal stimulation produces no significant ventricular inotropic effects (11).

Yet most of the studies used contractile force as a measure of ventricular contractility (36,61). Contractility is an intrinsic myocardial property independent of preload, afterload and heart rate. Contractile force depends on these parameters as well as contractility (73,148,150, 185). Preload, afterload and heart rate were not controlled during most studies investigating vagal control of ventricular inotropism (12,36,61).

Eliakim et al. (70) produced a decrease in ventricular contractile force during vagal stimulation in dogs with complete heart block. Negative isovolumic responses on the left ventricle were demonstrated by DeGeest (53,58). Stimulation of either vagus produced decreased left ventricular systolic pressure when heart rate, coronary perfusion pressure and end-diastolic volume were kept constant. The reduction ranged from 7-34% depending on the stimulation frequency and the responses were abolished by atropine. Daggett (55) also demonstrated a negative inotropic effect on the left ventricle by vagal stimulation in a right heart bypass preparation in which aortic pressure, cardiac output and heart rate were held constant. Vagal stimulation during synchronized atrioventricular pacing produced decreases in left ventricular pressure and the maximal rate of rise of left ventricular, right ventricular contractile force and duration of systole. The investigators demonstrated that the changes in atrial contractility, ventricular activation, coronary blood flow or effectiveness of mitral valve closure did not contribute to the observed negative ventricular inotropic response. Negative inotropic effects of vagal stimulation on ventricular myocardium has been confirmed by several groups in the dog (99,144,166) and baboon (175).

The role of the parasympathetic nervous system in the regulation of tonic ventricular contractility has been

demonstrated. DeGeest (58) showed that electrical stimulation of the cut distal vagus with parameters sufficient to reduce heart rate to the spontaneous rate demonstrated by intact animals also produced a negative inotropic effect. In addition, increases in contractile force in the paced ventricle of dogs after bilateral vagotomy or atropine have been reported.

3. Coronary Circulation

Considerable controversy exists with regard to parasympathetic control of the coronary vessels. Vasoconstriction (7) vasodilation (15) or no response (24) to vagal stimulation have been reported. Several cardiac functions change during parasympathetic activation and may secondarily affect coronary flow. Thus, the direct affect of the vagus on the coronary vessels may be masked. The factors include heart rate, arterial pressure, extravascular myocardial compression and ventricular contractility. These parameters must be controlled to determine unequivocally the response of the coronary vessels to parasympathetic stimulation.

Katz and Jochim (126) controlled perfusion pressure, heart rate and extravascular compression by studying the coronary vessels of fibrillating hearts. Vagal stimulation produced increases in coronary flow which were abolished by atropine. Berne (15) observed coronary flow increases and coronary resistance decreases during cervical vagal

stimulation in paced, unpaced and fibrillating hearts with constant coronary perfusion pressure. Fibrillating hearts demonstrated increases in coronary flow as well as coronary sinus pO_2 during parasympathetic activation. The data suggest the vasodilation is a direct vascular effect rather than an indirect response resulting from increases in myocardial oxygen requirements. Further evidence of a vasodilator response to the vagus has been provided by Berne (14). Direct infusion of acetylcholine into the coronary arteries produced an increase in coronary flow.

4. Vagal Influences on Rhythm and Conduction.

The mechanisms through which the vagus affects SA node cells and consequent cardiac rhythmicity have been elucidated through the use of intracellular microelectrodes. Acetylcholine or vagal stimulation produces a reduction in the slope of spontaneous diastolic depolarization (phase 4 depolarization) an elevation of the maximum diastolic potential, decreased action potential duration and pacemaker shift (106,109). The net affect of these responses is a decrease in SA node firing rate and a subsequent heart rate decrease.

At the AV node. acetylcholine or vagal stimulation inhibits transmission resulting in partial or even complete atrio-ventricular dissociation (50,107). Decremental conduction is produced by a reduction in the rate of rise of

the action potential as well as the action potential amplitude (50,167).

Early investigators suggested that the vagus is incapable of affecting the specialized conduction system distal to the AV node (27,106). However, Bailey (9) used intracellular recording techniques for studying the cells of the bundle of His and the right bundle branch of the dog heart. The slope of diastolic depolarization decreased upon administration of acetylcholine.

5. Peripheral Mechanisms of Vagally Induced Changes in Cardiac Function

The concept of cardiac depression by vagal activation is at best an oversimplification of parasympathetic cardiac control. An intimate interaction of the parasympathetic nerves with the effector organ and the sympathetic nerves ultimately determines the cardiac responses to vagal activation. The phenomena of paradoxical tachycardia, the Burn-Rand Hypothesis and accentuated antagonism demonstrate this interaction.

a. Paradoxical Tachycardia

Levy et al. (141,142) showed that repetitive stimulation of the vagus could synchronize cardiac rate over a narrow range of stimulating frequencies. Raising the frequency of nerve stimulation above the synchronizing range produced a paradoxical increase in heart rate. The

mechanism of the phenomenon has not been clearly elucidated. However, it is independent of adrenergic mechanisms since it persists after β -blockade or stellatectomy. A single vagal stimulus may produce an excitation or inhibition of cardiac pacemaker activity depending on the time of arrival of the impulse at the SA node (25). Thus, vagal control of cardiac rhythm depends on the temporal relation between nerve impulses and the cardiac cycle. The phenomenon may be significant to "normal" cardiac parasympathetic control since efferent vagal fibers demonstrate groups of impulses with each cardiac cycle (119,125). The periodic impulse grouping may be the result of baroreceptor activation with each pressure pulse (112,146).

b. Burn-Rand Hypothesis

Vagal stimulation has been reported to produce excitatory and inhibitory effects on cardiac function. Negative and positive atrial (86) and ventricular responses to parasympathetic activation have been reported (11,70). These observations led to the development of the Burn-Rand Hypothesis (28,29,30) of sympathetic and parasympathetic interaction. According to this theory, acetylcholine released from postganglionic nerve terminals plays a role in norepinephrine release from adrenergic nerve fibers.

Abundant evidence has shown that cholinergic stimulation releases norepinephrine from cardiac depots. Hoffman

(109) noted an excitatory effect of acetylcholine on atropinized hearts. Acetylcholine produced positive inotropic and chronotropic effects and the appearance of an epinephrine-like substance in the perfusate (6,182). The positive effect of acetylcholine was markedly attenuated by pharmacological (31,38,48,60) or surgical (31,60) cardiac sympathectomy. Thus, with depletion of catecholamine stores, the cardiac stimulating effects of parasympathetic agents are abolished. Electroneurographic studies have verified the activation of postganglionic adrenergic fibers by cholinergic stimuli (31,195). In addition, Lassberg (134) reported an increase in cardiac norepinephrine concentration after acetylcholine administration. These data suggest parasympathetic stimuli may induce norepinephrine synthesis.

However, several studies indicate evidence against the Burn-Rand Hypothesis. Vagal stimulation still elicits a cardio-stimulatory effect after depletion of cardiac catecholamines stores by reserpine (202) or sympathectomy (38).

Buccino et al. (26) proposed the existence of two or three specific cholinergic receptors to explain the phenomenon. Type I receptors respond to small concentrations of acetylcholine. Activation of the receptors produces a negative inotropic effect which is blocked by atropine. Type II receptors respond to large concentrations of

acetylcholine and produce a positive inotropic effect. These receptors are not blocked by atropine or hexamethonium. Buccino concluded that Type I receptors are closely associated with vagal nerve endings while Type II receptors are spatially separate from the terminals.

However, relatively high concentrations of acetylcholine are necessary to activate the Type II sites. Thus, the action of acetylcholine may represent a nonspecific effect on the cell membrane rather than on a discrete receptor. Furthermore, the positive inotropic action of acetylcholine could not be elicited in the intact feline heart depleted of catecholamines by previous extrinsic denervation or reserprinization. These data suggest that the positive inotropic effects in the intact heart are mainly catecholamine dependent (60). Thus, although it seems possible that under special circumstances two mechanisms may exist by which acetylcholine can produce a positive inotropic effect, the significance of the catecholamine independent mechanism seems to be much less than the catecholamine-dependent mechanism.

c. Accentuated Antagonism

The chronotropic responses to activation of both divisions of the autonomic nervous system are not algebraically additive (139). Instead, accentuated antagonism occurs. The absolute reduction in heart rate produced by

cholinergic interventions is accentuated by high cardiac sympathetic tone (147,184,186). Grodner et al. (92) demonstrated a similar interaction in regard to the chronotropic effects of the two autonomic neurotransmitters acting directly on receptor sites.

When various combinations of norepinephrine and acetylcholine were added to an isolated atrial preparation, the bradycardia influence of acetylcholine predominated over the excitatory effect of norepinephrine. The negative inotropic effects of cholinergic interventions appear to be substantially exaggerated in the presence of high cardiac sympathetic tone (60,109,144). Levy et al. (144) demonstrated that accentuated antagonism occurs in the setting of autonomic nerve stimulation. They noted vagal stimulation produced a more intense negative inotropic effect when pre-existing tone was elevated by stellate stimulation. These results suggest that vagal effects on the heart are mediated, in part, by antagonizing the positive inotropic influence of the prevailing sympathetic activity. The findings of Dempsey and Cooper (60) indicating an attenuation of the inotropic response to norepinephrine in the presence of acetylcholine lend support to this possibility.

6. Central and Reflex Mechanisms of Vagally Induced Changes in Cardiac Function

a. Central Control

Accentuated antagonism also occurs through central neural mechanisms. Under certain circumstances simultaneous activation of sympathetic nerve fibers may exaggerate cardiovascular responses to parasympathetic intervention while under others it suppresses the responses.

Direct or reflex stimulation of the anterior hypothalamus induces cardiovascular responses characteristic of parasympathetic stimulation (83,84,85). Yet, direct or reflex activation of the posterior hypothalamic centers results in the enhancement of responses to sympathetic intervention and attenuation in those to parasympathetic interventions. This phenomenon has been termed "reciporical inhibition." States of preexisting elevated sympathetic tone were accompanied by enhanced sympathetic but decreased parasympathetic reactivity. The reverse changes occurred in states of preexisting increases in parasympathetic activity. Gebber (80,81,130) noted modulation of baroreceptor responsiveness by suprabulbar stimulation. Electrical activation of the lateral and posterior aspects of the hypothalamus blocked the bradycardia evoked by carotid sinus nerve stimulation in the high spinal preparation. On the other hand, stimulation of forebrain sites in the area preoptica, the septal area, and anterior hypothalamus greatly facilitated the parasympathetic component of the baroreceptor reflex (102,130). Thus a central autonomic interaction

may be responsible for attenuation of parasympathetically mediated bradycardia of the baroreceptor reflex in states of elevated sympathetic tone such as during exercise.

b. Reflex Control

Parasympathetic nerve fibers in the vagosympathetic trunk form a portion of the efferent limb of a number of cardiovascular reflexes. The relative importance of adrenergic and parasympathetic divisions of the autonomic nervous system in the negative chronotropic response to baroreceptor activation has been controversial (13,63,19). In conscious resting humans (69) and dogs (89,100) the predominant mechanism is through parasympathetic activation. However, in the presence of pentobarbital in the dog, withdrawal of tonic sympathetic activity mediates the reflex bradycardia (13,62).

Bristow et al. (22) evaluated the sensitivity of the baroreceptor reflex to pharmacologically induced blood pressure elevations. Since the reflex bradycardia observed with activation of the reflex is unaffected by β -blockade and abolished by atropine (170), the technique is used for evaluating parasympathetic control of the heart. Reflex bradycardia is decreased in elderly patients (91), patients with essential hypertension (22) and abolished during exercise (170). The parasympathetic component of the baroreflex in conscious human subjects is decreased by a number of heart

diseases (91) and in dogs with cardiac hypertrophy of failure (101). These results indicate a prominent defect in cardiac control by the parasympathetic system during heart disease.

The parasympathetic nervous system also plays a role in the reflex negative ventricular inotropic response to baroreceptor activation. Pressure increases in the isolated carotid sinus produce depression of peak systolic pressure in the innervated isovolumic contracting left ventricle. The response is decreased somewhat by adrenergic blockade (143) and completely abolished by atropine (199).

Vagally mediated bradycardia occurs in response to carotid chemoreceptor activation (45,56,57). The vagus also, in part, mediates the negative inotropic response of the atrium (65) and ventricles (59,65) during carotid body activation. However, the reflex inotropic responses to carotid and aortic body differ. DeGeest et al. (59) studied the responses of the paced innervated, isovolumic left ventricle of dogs. Hypoxia of the isolated carotid sinus produced a negative inotropic effect on left ventricular performance which was vagally mediated. Stimulation of aortic chemoreceptors produced a positive inotropic effect mediated by the sympathetic nerves.

Rhythmic frequency of impulse traffic in the parasympathetic fibers to the heart occur at a frequency similar

to the respiratory frequency (113). Furthermore, it has been shown that heart rate and contractility oscillations coincident with the respiratory cycle are, in part, mediated by the vagus (90,140).

Cardio-cardiac reflexes involving the vagus have been described (177). The vagus mediates the heart rate response to pressure increases on the right (10) and left sides of the heart. The reflex response to distension of the atria is attenuated by vagotomy (10) and unaffected by β -blockade (111).

CHAPTER III

MATERIALS AND METHODS

A. General Animal Preparation

The experiments were performed on 83 fed, anesthetized cats of either sex (2.3-4.4 kg) for horseradish peroxidase (HRP) injections and physiological studies. Femoral arterial and venous catheters were inserted in all cats used for physiological studies and 3 cats used for the HRP injections. Blood pressure was monitored by a P23Db pressure transducer while heart rates were determined by a cardiometer triggered by the pulse wave. Tracings were displayed on an oscillograph (Grass model 7).

Depending on the procedure, the heart was electrically paced and the medulla or peripheral nerves were electrically activated. Bipolar stainless steel electrodes were used for cardiac pacing while concentric bipolar electrodes (David Kopf, 100 μ m od) and bipolar insect pin (Type 000) electrodes were used for stimulating the medulla and peripheral nerves, respectively. Stimuli were delivered from a stimulus isolation unit (Grass model SIU5 or SIU 4678) connected to a stimulator (Grass S44, S48 or SD5). Current was monitored with a constant current unit (Grass 1A)

during medullary stimulations. Stimulation parameters were routinely recorded by a differential amplifier (Tektronix type 3A1 or 3A72) and displayed on an oscilloscope (Tektronix type 561A or 565).

Data were analyzed according to the statistical tests described by Gilbert (88). Variance (F) and probability (P) values less than 0.05 were considered statistically significant.

B. HRP Labeling of Cardiac Vagal Somata

Fifteen cats were anesthetized with pentobarbital (38 mg/kg, ip), intubated and placed on positive pressure ventilation (Puritan-Zep model AR). A thoracotomy was performed and the pericardium was incised. A 25% solution of horseradish peroxidase (HRP, HPOFF-Worthington Biochemical Corp., Freehold, NJ) in 0.9% saline was injected subepicardially with a 10 μ l Hamilton syringe using a 27 gauge needle. Injection sites included the SA node region, the borders of the left and right atrial appendages and bases of the aorta and pulmonary artery. The volume of solution injected at each site was approximately 2 μ l.

Two test groups were used for labeling somata with axons passing in the left and right cervical vagi. A left vagotomy was performed on 6 cats while the right vagus was sectioned in another 6 cats prior to HRP injection. A control group included 3 animals in which peripheral cardiac

parasympathectomies were performed prior to HRP injection. The controls served to determine the number of somata labeled due to HRP uptake by vagal fibers innervating non-myocardial tissue. Heart rate and blood pressure were monitored and the left cervical vagus was sectioned in these animals. Heart rate responses were then noted during electrical stimulation of the intact right vagus (10 v, 1 msec, 50 Hz) before and after cutting the ipsilateral cardiac vagal branches. The absence of bradycardia during vagal stimulation after cardiac vagal branch section indicated cardiac parasympathectomy.

Forty-eight hours after HRP injection each animal was reanesthetized, thoracotomized and the descending aorta was clamped. The rostral portion of the animal was perfused with 0.9% saline and fixed with 0.4% paraformaldehyde and 2.4% glutaraldehyde in 0.1 M phosphate buffer (pH=7.4). The brain stem was removed, marked for orientation, stored in fixative for 3 hours and washed with buffer. The tissue was successively infiltrated with 10% and 20% sucrose for 4 hour periods and stored overnight in 30% sucrose. Frozen 40 μ m serial sections were cut. Transverse sections were taken from the brain stems of 3 cats with right vagotomy, 3 cats with left vagotomy and all animals with cardiac parasympathectomy. Sagittal sections were taken from the tissues of the other cats. Sections were incubated in

3,3'-diaminobenzidine and 0.01% hydrogen peroxide in 0.1 M phosphate buffer (pH=7.4), dehydrated and mounted for examination.

Labeled somata were identified by the appearance of distinct cell margins, nuclei and golden-brown horseradish peroxidase granules in the cytoplasm. The total number of labeled cell bodies in test and control cats were compared by analysis of variance and the Scheffé test.

Labeled somata were found in 3 zones of the medulla in test animals (see results). An analysis of variance and the Scheffé test were used to compare the total number of cell bodies in each zone in each test group. The t-test for unpaired data was then used to compare the number of somata in corresponding medullary zones for the 2 test groups.

The distribution of somata along the longitudinal neuraxis was determined from transverse sections. For each animal, the total number of cell bodies in 400 μ m serial segments was calculated by summing the number of labeled somata in 10 successive serial sections using the obex as the reference point. The average number of cell bodies in the different segments was determined by pooling the data from all animals studied. The average number of cell bodies in the different segments was compared by analysis of variance for each test group. The number of somata in corresponding segments of the 2 test groups were then

compared by the t-test for unpaired data. The longitudinal distribution of somata in each of the 3 medullary zones were determined and compared in a similar manner.

Morphological characteristics were determined for somata demonstrating distinct nucleoli. The long and short axes were measured by an eyepiece micrometer. In each test group, the average dimensions of cell bodies in the different medullary areas were determined by pooling the data from individual animals. The dimensions of cell bodies in the different medullary areas were compared by analysis of variance and the Scheffé test in each group. Somata dimensions in corresponding medullary regions in the 2 groups were compared by the t-test for unpaired data.

C. Physiological Studies

1. Preliminary Surgical Procedure

Sixty-eight cats were preanesthetized with chloroform followed by alpha-chloralose (40-50 mg/kg, iv). A tracheotomy was performed. The aortic depressor and carotid sinus nerves were sectioned bilaterally. The animals were paralyzed with decamethonium bromide (C_{10} , 0.03 mg/kg, iv) and artificially ventilated (Puritan-Zep model AR). Endexpiratory %CO₂ was monitored (Beckman model LBl) and maintained at 4.0-4.5% throughout the experiment. Rectal temperature was monitored and maintained at 37 C by a heating pad.

Chemical cardiac sympathectomy was then performed. Heart rate responses to isoproterenol (5.0 $\mu\text{g}/\text{kg}$, iv, Elkins-Sinn, Inc., Cherry Hill, N.J.) were noted before and after β -blockade with propranolol (0.1 mg/kg, Ayerst Laboratories, Inc., New York, N.Y.). The absence of tachycardia in response to isoproterenol after propranolol administration indicated cardiac sympathectomy.

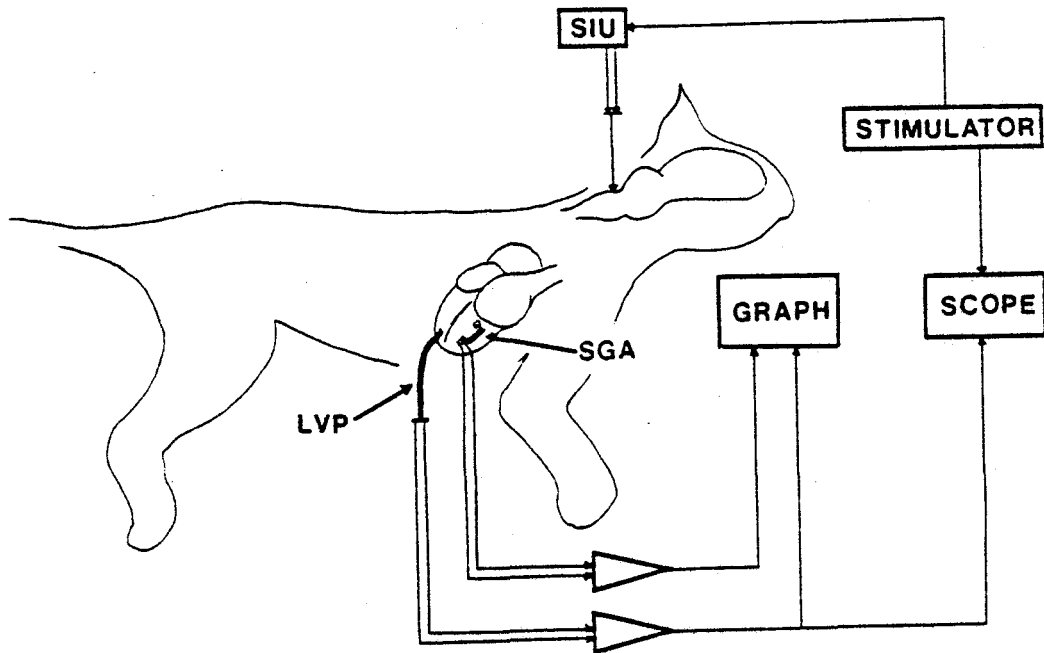
In some experiments, hearts were paced. Pacing rates were adjusted to the spontaneous heart rate demonstrated by the cat after β -blockade.

At the end of each experiment, the animal was perfused with 0.9% saline and fixed with 10% formalin. The brain stem or spinal cord was removed, depending on the procedure, and 10 μm serial sections were cut. Brain stems and spinal cords were stained with cresyl violet (175) and Kluver-Barrera (131), respectively.

2. Medullary Stimulations

The effect of electrical stimulation of the dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus (NA) on heart rate and contractility was investigated in 20 cats. Figure 1 illustrates the animal preparation used for the experiments. Each animal was secured in a Horsley-Clarke stereotaxic device. An occipital craniotomy was performed and the medulla was exposed by rostral displacement of the cerebellum with cotton swabs. The exposed tissue was kept moist with warm mineral oil throughout the experiment.

ANIMAL PREPARATION FOR MEDULLARY
STIMULATIONS



Diagrammatic representation of the animal preparation for medullary stimulation studies. SIU = stimulus isolation unit, SGA = strain gauge arch, LVC = left ventricular catheter, RVC = right ventricular catheter.

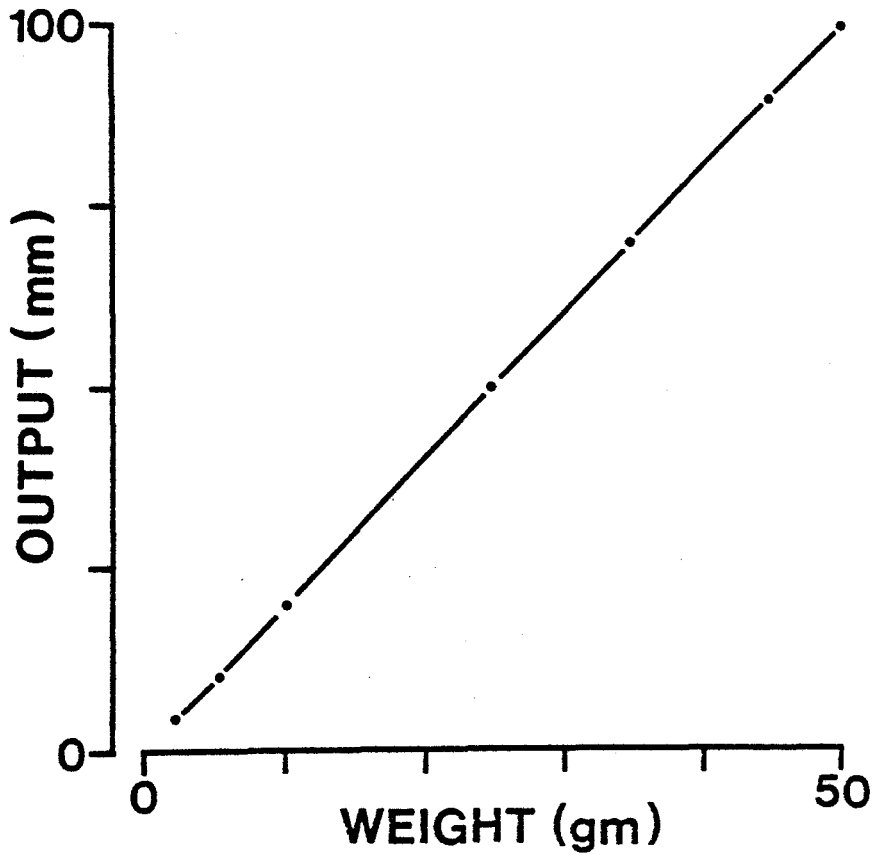
Ribs 2-5 were removed on the right side and the pericardium was incised. Pacing electrodes were inserted into the right ventricular myocardium. A Walton-Brodie strain gauge arch was sutured to the right ventricle and a catheter was inserted into the left ventricular chamber by cardiac puncture. The rate of rise of the rising limb of the left ventricular pressure curve (dp/dt) and the output from the strain gauge were used as indices of myocardial contractility.

Strain gauge output was displayed on an oscillograph (Grass model 7). Left ventricular pressure was monitored by a P23Db pressure transducer, amplified (Honeywell model 130-2C) and displayed on an oscilloscope (Grass model 365). Permanent records of left ventricular pressure were obtained by photographing the oscilloscope display (Grass Camera model C4N).

Each strain gauge arch was tested for linearity. Weights were hung from a gauge foot while monitoring outputs. Oscillograph pen displacement was plotted as a function of weight. Figure 2 shows a plot for a single gauge. All gauges were linear within a range of 2-50 gm.

The dynamic accuracies of the ventricular recording systems were determined. "Pop" tests were performed according to the methods described by Gabe (78) and Fry (76) using different sizes of polyethylene tubing connected to

STRAIN GAUGE CALIBRATION



Oscillograph pen deflection (ordinate) is plotted as a function of the weight (abscissa) hung from the foot of a strain gauge arch. Each dot indicates the oscillograph output for a specific weight.

needles of various gauges. A system composed of a 15 gauge needle connected to a 90 mm length of PE 205 tubing (0.062 id, 0.082 id) was chosen for recording. Such a system demonstrated a natural frequency of 102 Hz and damping factor of 0.4.

In 10 of the animals, a catheter was inserted into the right ventricular chamber by cardiac puncture. Right ventricular pressure was monitored by a P23Db pressure transducer and displayed on the oscillograph. Left ventricular pressures were displayed on the oscillograph in addition to the oscilloscope in these experiments. Pre-amplifier gains were adjusted for accurate monitoring of end-diastolic pressures.

After surgical instrumentation, the DMN and NA were stimulated on either the left or right side of the brain stem. The tip of an electrode was stereotaxically (193) inserted into each nucleus at 3 different medullary planes including the obex level, 1.5 mm rostral to the obex and 1.5 mm caudal to the obex. At each site, a stimulus train of 20 sec duration (0.1-0.15 mA, 1 msec, 50 Hz) was delivered. Stimuli were applied with and without cardiac pacing at each site. The sequence of DMN and NA stimulation was random. Each site was stimulated 3 times. The ipsilateral vagus was then sectioned and each medullary site was again stimulated 3 times with and without cardiac pacing.

The remaining cervical vagus was subsequently cut in the 10 animals with left and right ventricular catheters. Cardiovascular parameters were noted during injections of phenylephrine (30 $\mu\text{g}/\text{kg}$, iv, Winthrop Laboratories, New York, N.Y.) and histamine (2.5 $\mu\text{g}/\text{kg}$, iv, Lilly Laboratories, Indianapolis, Ind.).

Each animal was perfused and fixed. The brain stem was removed and histologically prepared for confirmation of stimulation sites.

Data were organized into groups corresponding to the nucleus stimulated. Thus, NA and DMN groups were formed. For each group comparisons were made between prevagotomy controls and responses, pre- and postvagotomy controls, pre- and postvagotomy responses and postvagotomy controls and responses using the paired t-test.

For each data group, parameters during pacing were compared with corresponding parameters with and without pacing using the paired t-test. Pre- and postvagotomy controls and pre- and postvagotomy responses were compared in this manner. Control and response parameters observed during pacing were then compared using the paired t-test. The test was made between prevagotomy controls and responses and postvagotomy controls and responses.

Cardiovascular responses to each drug were compared with controls by the paired t-test.

3. Spinal Lesion Studies

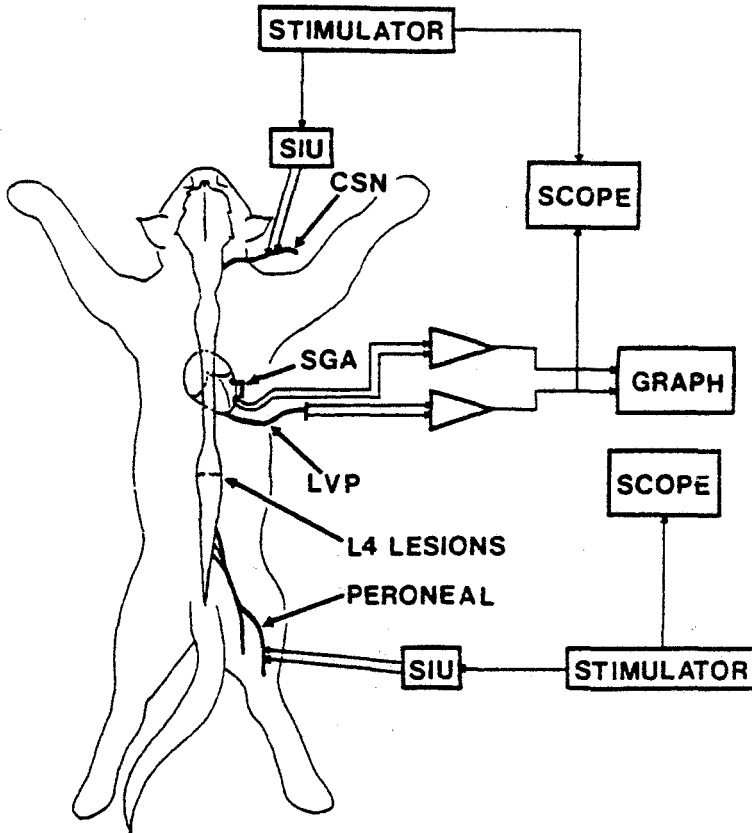
a. Localization of Ascending Pathways

Two series of experiments were performed on 28 cats for localizing spinal pathways affecting the cardiac vagus. The animal preparation used for these studies is illustrated in Figure 3. Each animal was secured with a head holder. A laminectomy was performed at L4 and the dura was incised. The right peroneal nerve was isolated and cut. The central ends of the peroneal and right carotid sinus nerves were placed on stimulating electrodes. The exposed nerves and spinal cord were bathed with mineral oil throughout the experiment.

Heart rate and blood pressure were monitored during individual and simultaneous stimulation of the nerves. The nerves were stimulated with 20 sec trains. Stimulus parameters were 10 v, 1 msec, 50 Hz and 4 v, 1 msec, 50 Hz for the peroneal and carotid sinus nerves, respectively. Lesions were then placed in the spinal cord at L4 and the stimuli were repeated.

Lesions were made in the dorsolateral sulcus region (DLS) with a scalpel. In 10 cats, the stimuli were repeated after making a right DLS lesion and then after bilateral lesions. Stimuli were repeated only after making bilateral lesions in the remaining animals.

ANIMAL PREPARATION FOR SPINAL LESIONS



Diagrammatic representation of the animal preparation used for spinal lesion studies. SIU = stimulus isolation unit, CSN = carotid sinus nerve, SGA = strain gauge arch.

In another 10 cats, myocardial contractility was monitored according to the methods described under "Medullary Stimulations" and the hearts were paced. Cardiovascular responses were monitored during individual and simultaneous stimulation of the right carotid sinus and peroneal nerves. The stimulations were repeated after making a lesion in the right DLS and then after making bilateral DLS lesions.

b. Medullary Projections of Spinal Pathways

A final series of experiments were carried out in 20 cats to determine whether ascending pathways project bilaterally or unilaterally to medullary cardiac vagal nuclei. The experimental preparation for the series was similar to that described in "Localization of Ascending Pathways". However, a left cervical vagotomy was performed prior to nerve stimulation and making lesions in this series.

In 10 animals heart rate and blood pressure were monitored. Contractility was recorded in another 10 animals and the hearts were paced.

Cardiovascular responses were monitored during individual and simultaneous stimulation of the carotid sinus and peroneal nerves. The stimuli were repeated before and after placing a lesion in the right DLS at L4.

Each animal used for "Spinal Lesion Studies" was perfused and fixed at the end of the experiment. Spinal

segment L4 was removed and the lesion sites were histologically verified.

Pre- and postlesion control parameters were compared by analysis of variance. Analysis of variance and the Scheffé test were used for comparing pre- and postlesion responses to nerve stimulation.

CHAPTER IV

RESULTS

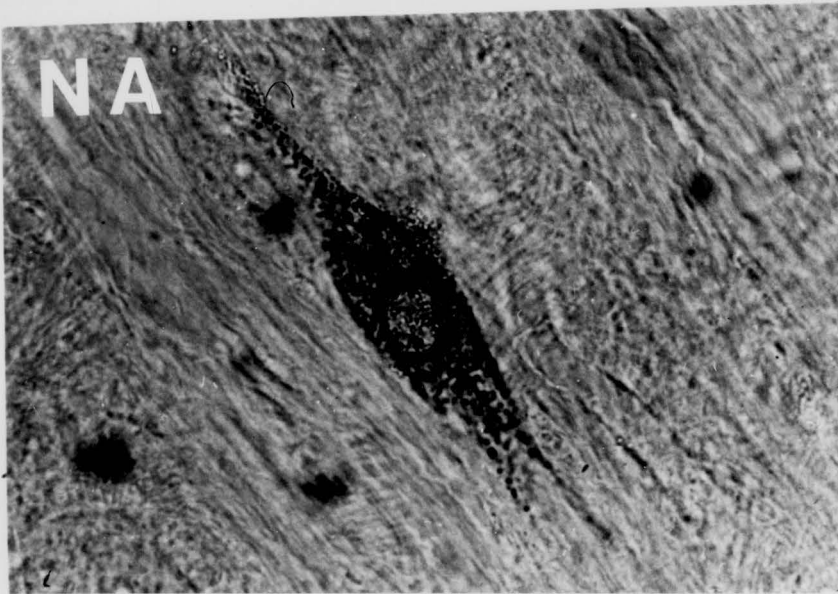
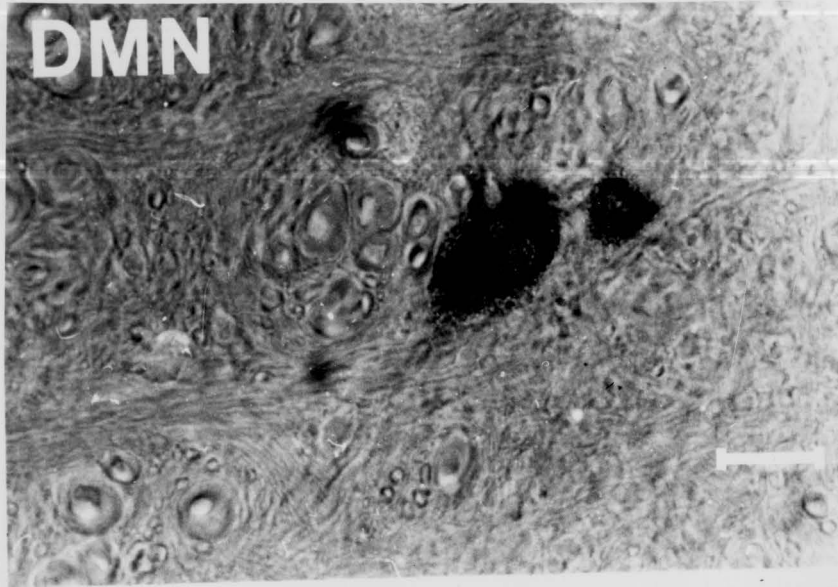
A. HRP Labeled Cardiac Vagal Somata

Labeled somata demonstrated distinct cell margins, nuclei and golden-brown horseradish peroxidase granules in the cytoplasm (Fig. 4). Large, flat-brown endogenous diaminobenzidine staining particles were present bilaterally in the region of the ala cinerea. Care was taken to avoid identifying aggregates of these particles as HRP labeled cardiac vagal preganglionic somata.

The 3 control cats with left cervical vagotomy demonstrated significant heart rate (HR) and mean arterial blood pressure (MABP) decreases during right cervical vagal stimulation ($P < 0.05$). The responses were abolished by cutting the right cardiac vagal branches (Fig. 5). These data indicated peripheral cardiac denervation.

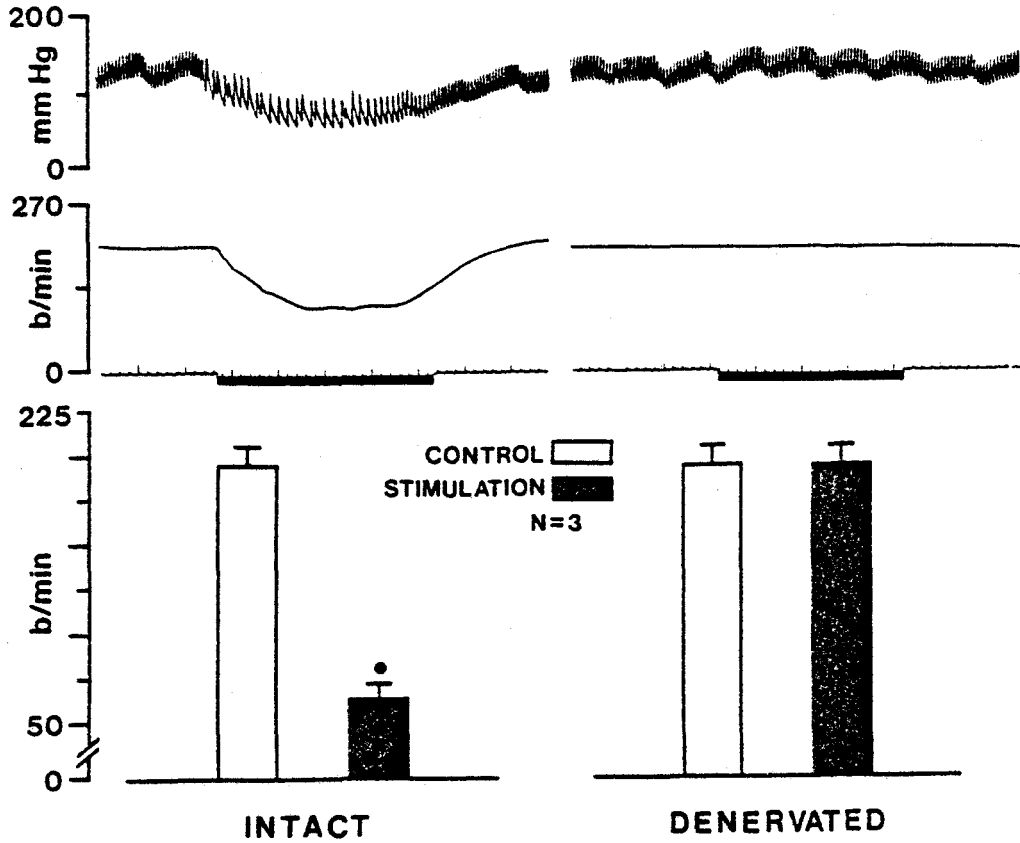
The population sizes of labeled cell bodies in the test and control groups were significantly different ($F < 0.05$). Cats with an intact left and right vagus demonstrated 366.1 ± 14.2 and 354.5 ± 17.3 labeled somata, respectively, while 24.8 ± 6.2 cell bodies were identified in the control group. The sizes of the populations in the 2 test groups were not significantly different ($P > 0.05$) while the number of somata in the control group was significantly less than

HRP LABELED SOMATA



HRP labeled somata identified in the DMN and NA. The cell bodies were identified in a transverse section from a cat with an intact right vagus. Calibration bar indicates 10 μ m.

HEART RATE RESPONSES TO RIGHT
CERVICAL VAGAL STIMULATION



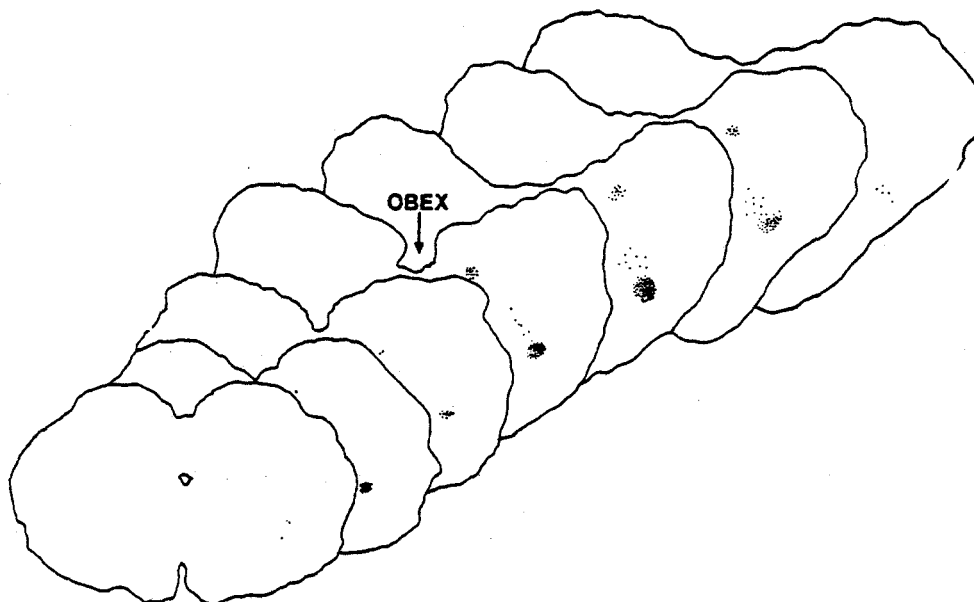
Average heart rate responses to right cervical vagal stimulation in control cats before and after cutting the right cardiac vagal branches. Solid circle indicates significant difference from control. Brackets indicate S.E. Top tracings are from a single cat. The tracings are from top to bottom: blood pressure, heart rate and time marker. Time marker indicates 1 and 5 sec intervals. Downward deflection of time marker indicates stimulus duration.

the number of cell bodies in animals with either the left or right vagus intact ($P < 0.05$). Therefore, only about 6.8% of the labeled somata in test cats were associated with non-myocardial tissue or with axons traveling to the heart by nerves other than the cardiac vagal branches.

Labeled somata of test cats were ipsilateral to the intact vagus in the DMN, the NA and an intermediate zone (IZ) between the DMN and NA (Fig. 6). The number of labeled somata in the DMN, NA and IZ were significantly different ($F < 0.05$) in each test group (Fig. 7). The NA had significantly more somata than either the DMN or IZ ($P < 0.05$) while the DMN demonstrated a larger cell body population than the IZ ($P < 0.05$). The number of labeled somata in corresponding medullary areas in the 2 test groups were not significantly different ($P > 0.05$).

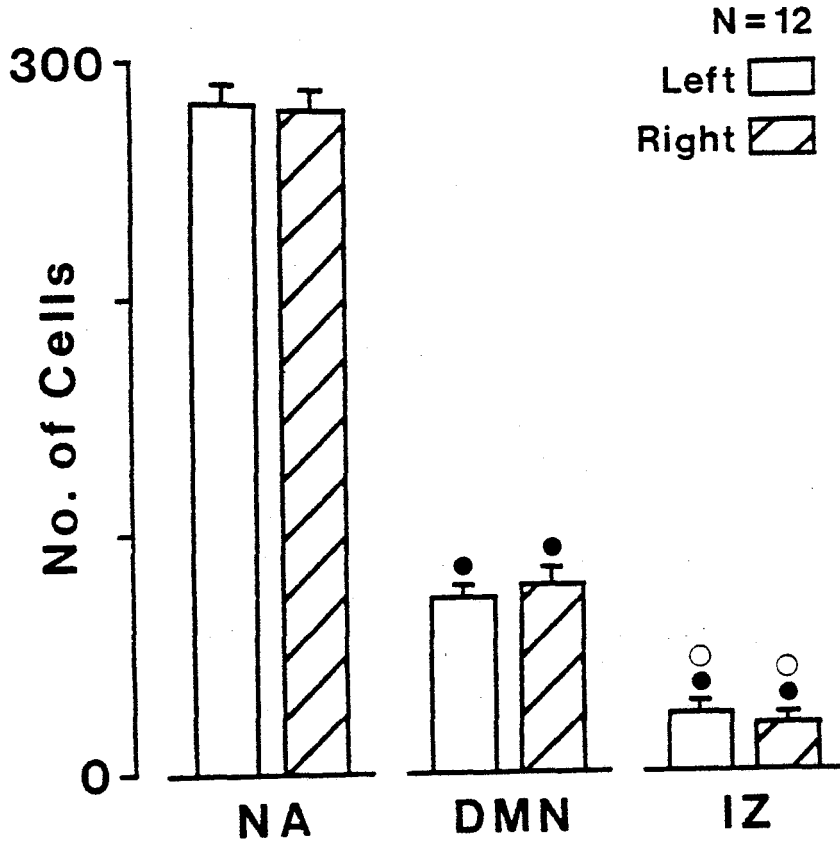
The longitudinal distribution of labeled somata was determined from transverse serial sections. The distribution was described as nonuniform if an analysis of variance indicated a significant difference ($F < 0.05$) in the number of cell bodies in the 400 μm serial segments. The distribution was described as uniform if no difference was shown ($F > 0.05$). For cats with either an intact left or intact right vagus, the longitudinal distribution of the total number of labeled somata was nonuniform ($F < 0.05$). There were no differences in the number of somata in corresponding segments in the 2

MEDULLARY DISTRIBUTION OF
HRP LABELED SOMATA



Reconstruction of the medulla of a single cat with an intact right vagus. Each section represents a brain stem segment 800 μ m in thickness. Each dot indicates the location of a labeled soma.

LOCATIONS OF HRP LABELED SOMATA

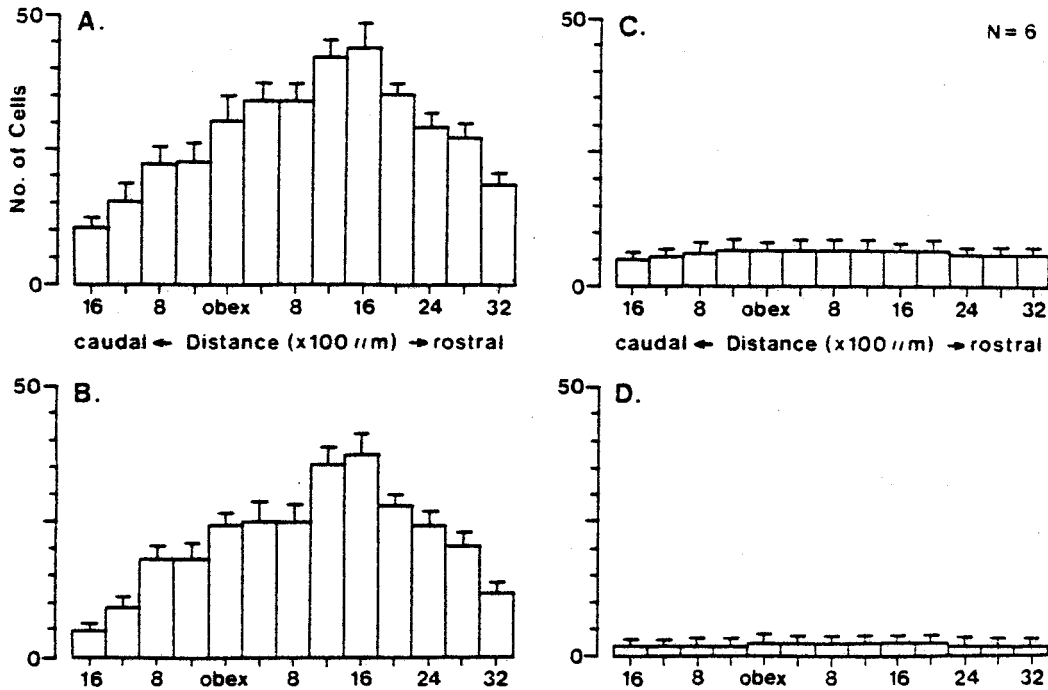


The number of labeled somata in the NA, DMN and IZ in cats with an intact left or intact right vagus. Solid circles indicate a significant difference from the number of labeled somata in the NA of the corresponding test group. Open circles indicate a significant difference from the number of labeled somata in the DMN of the corresponding test group.

groups ($P > 0.05$). Therefore, the data for the test animals were pooled (Fig. 8A). The NA cell body distribution was nonuniform ($F < 0.05$) in each test group (Fig. 8B). Both test groups demonstrated uniform distributions of somata ($F > 0.05$) in the DMN (Fig. 8C) and IZ (Fig. 8D). There were no differences ($P > 0.05$) in the number of somata in corresponding segments of the test groups in the NA, DMN, or IZ. Therefore, the data for the test groups were pooled.

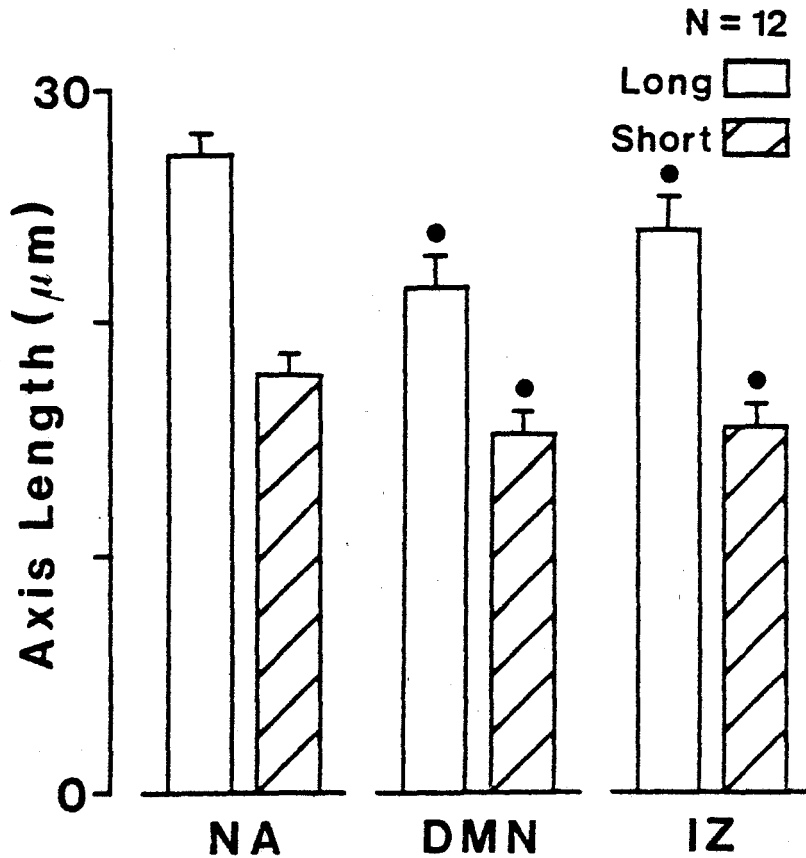
Distinct nucleoli were apparent in 326.6 ± 10.4 and 324.0 ± 12.3 labeled somata in cats with an intact left and intact right vagus, respectively. The dimensions of cell bodies cut in transverse and sagittal planes were not significantly different ($P > 0.05$). Therefore, the data obtained from transverse and sagittal histological sections were pooled. Data from the test groups were pooled since the dimensions of somata in these groups were not significantly different ($P > 0.05$). The dimensions of somata found in the DMN, NA, and IZ (Fig. 9) were significantly different ($F < 0.05$). The long and short axes of cell bodies in the NA were greater ($P < 0.05$) than corresponding dimensions of somata in other regions (Figs. 4 & 9). There were no significant differences ($P > 0.05$) in the dimensions of somata in the DMN and IZ (Fig. 9).

LONGITUDINAL DISTRIBUTION OF
HRP LABELED SOMATA



Longitudinal distribution of labeled somata. (A) distribution of all labeled somata. (B), (C) and (D) demonstrate the distributions of labeled somata in the NA, DMN and IZ, respectively. Each bar indicates the number of labeled somata in a 400 μm segment of the medulla. Brackets indicate S.E.

DIMENSIONS OF HRP LABELED SOMATA



Long and short axis dimensions of somata labeled in the NA, DMN and IZ. Solid circles indicate significant difference from corresponding dimension of NA somata.

B. Responses to Medullary Stimulations

Strain gauge outputs were converted to grams from calibration curves similar to the one illustrated in Figure 2. The conversions provided quantitative values for the outputs which are necessary for statistical comparisons. The values are not interpreted as absolute measures of cardiac force. Strain gauge outputs will be referred to as strain gauge forces (SGF) in the remaining discussions.

The medullary stimulation experiments were performed for investigating the possibility that DMN and NA somata control different cardiac functions. As indicated in the "HRP Labeled Cardiac Vagal Somata" section, the DMN and NA contained most of the HRP labeled cell bodies. The IZ was excluded from the study due to the paucity of somata in the region. The DMN and NA were stimulated while monitoring heart rate and ventricular contractility. Stimuli were repeated after vagotomy to ensure responses were vagally mediated.

However, dP/dt and SGF are measures of contractile force which depend on preload and afterload as well as myocardial contractility (97). To determine the effect of HR on the parameters, the responses were monitored with and without cardiac pacing. Left (LVEDP) and right ventricular end-diastolic pressures (RVEDP) were monitored as indices

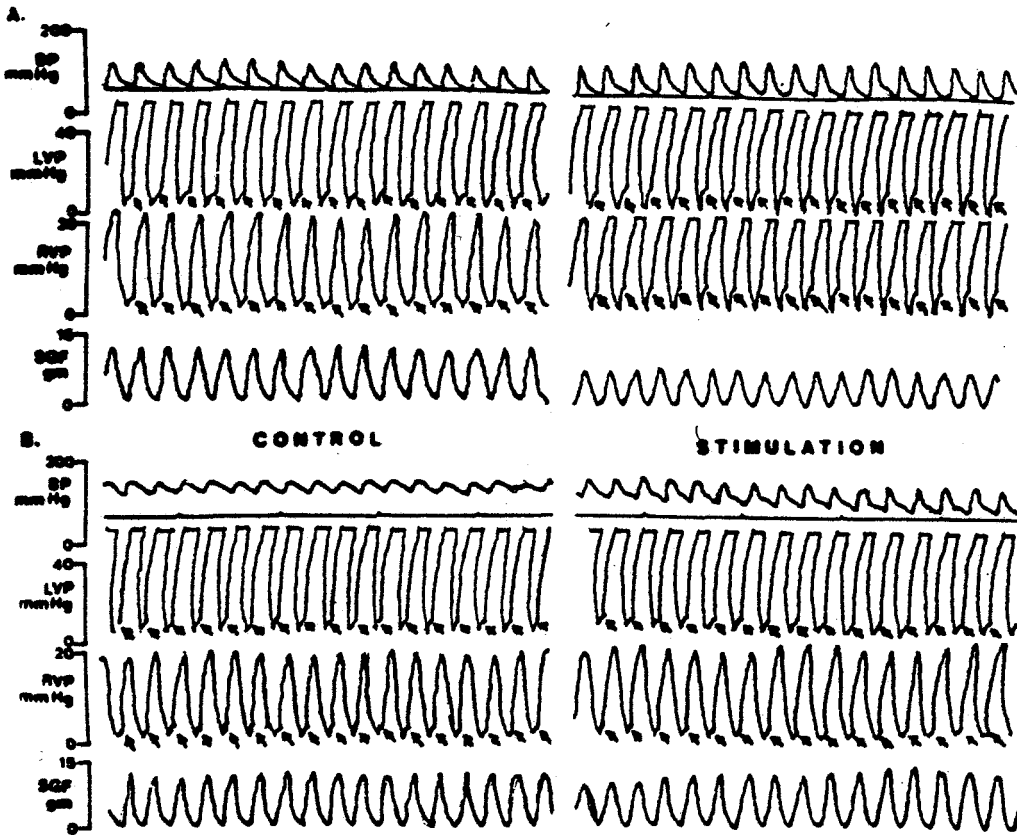
of preload. The effects of changes in afterload were determined by monitoring the responses to pressor and depressor agents.

Stimuli were applied to both the DMN and NA on different sides of the medulla and compared. For each nucleus the responses to left and right side stimulation were not different ($P > 0.05$). Therefore, the data from the 2 sides were pooled.

Stimuli were applied at 3 points along the longitudinal extent of each nucleus. The procedure was performed to exclude the possibility that different groups of somata along the longitudinal axis of a given nucleus affect different cardiac functions. Analysis of variance of the responses to DMN stimulation at the 3 points indicated no significant differences ($F > 0.05$). The responses to NA stimulation at the 3 points were not different ($F > 0.05$). Therefore, the data corresponding to the 3 points of each nucleus were pooled.

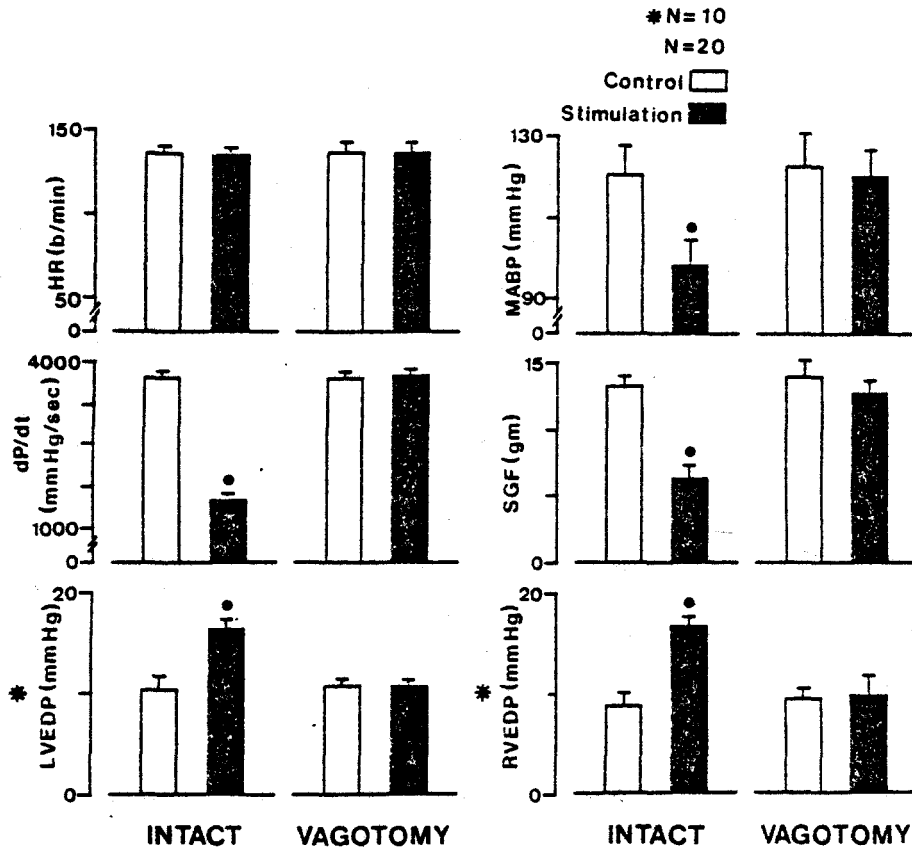
Figure 10 shows the effects of vagotomy on control parameters and the responses to DMN stimulation in a single cat. Figure 11 summarizes the data obtained from the cats without cardiac pacing. dP/dt , SGF and MABP decreased significantly ($P < 0.05$) during DMN stimulation. The stimulus did not affect HR ($P > 0.05$) but produced significant increases in LVEDP and RVEDP ($P < 0.05$). Ipsilateral vagotomy did not

CARDIOVASCULAR RESPONSES IN A SINGLE CAT
TO DMN STIMULATION



Responses of a single cat to DMN stimulation are shown before and after vagotomy. Time marker indicates 1 and 5 sec intervals. Downward deflection of the time marker indicates stimulus duration. Left ventricular pressure curves on the left of each panel are controls while pressure curves on the right of each panel were taken from cardiac cycles during the last 5 sec of stimulations. Arrows indicate end-diastolic pressures.

AVERAGE CARDIOVASCULAR CHANGES
DURING DMN STIMULATION



Average cardiovascular responses to DMN stimulation without cardiac pacing before and after vagotomy. Solid circles indicate significant difference from control. Brackets indicate S.E.

affect control parameters ($P > 0.05$) but abolished the responses to DMN stimulation. After vagotomy, parameters did not change significantly ($P > 0.05$) from controls during DMN stimulation.

HR and MABP with and without cardiac pacing were similar (Table I). Before and after vagotomy control parameters and the responses to DMN stimulation were not different ($P > 0.05$) whether the hearts were paced or unpaced.

The effects of cardiac pacing and vagotomy on control parameters and the responses to NA stimulation for a single cat are illustrated in Figure 12. Figure 13 and Table II summarize the data. Without cardiac pacing, intact animals demonstrated significant decreases ($P < 0.05$) in MABP and HR but significant increases ($P < 0.05$) in dP/dt , SGF, LVEDP and RVEDP. Cardiac pacing did not affect control parameters ($P > 0.05$) but abolished the responses to stimulation. Parameters did not change significantly ($P > 0.05$) during NA stimulation when the hearts were paced. Vagotomy had no effect on control parameters with or without pacing ($P > 0.05$). NA stimulation produced no significant changes ($P > 0.05$) in parameters after vagotomy.

The electrode tracts in each of the 3 medullary planes penetrated are illustrated in Figure 14 for each of the experiments. Since cresyl violet stains cell bodies (157), various nuclei are apparent. The electrode tip was

TABLE I
CARDIOVASCULAR RESPONSES TO DMN STIMULATION

| Parameter | N | | INTACT | | VAGOTOMY | |
|----------------------|----|---|-------------|-------------|-------------|-------------|
| | | | UNPACED | PACED | UNPACED | PACED |
| MABP (mm Hg) | 20 | C | 120.6+ 8.7* | 118.5+ 9.4 | 124.6+ 8.5 | 122.9+ 7.4 |
| | | S | 102.8+ 7.5 | 98.7+ 8.4 | 120.4+ 6.7 | 118.4+ 6.5 |
| HR (b/min) | 20 | C | 136.8+ 4.4 | 133.6+ 5.2 | 139.3+ 6.1 | 135.3+ 3.9 |
| | | S | 136.6+ 3.7 | 134.0+ 4.7 | 139.5+ 5.3 | 135.9+ 3.6 |
| dP/dt (mm Hg/sec) | 20 | C | 3725.8+91.5 | 3744.7+85.3 | 3754.4+92.5 | 3737.4+79.8 |
| | | S | 1488.4+89.5 | 1450.2+94.6 | 3762.8+87.2 | 3748.8+87.4 |
| SGF (gm) | 20 | C | 13.4+ 0.8 | 14.3+ 0.4 | 13.9+ 1.2 | 14.0+ 0.7 |
| | | S | 6.4+ 1.1 | 8.1+ 0.9 | 12.9+ 0.9 | 13.3+ 1.0 |
| LVEDP (mm Hg) | 20 | C | 10.6+ 0.7 | 11.4+ 0.6 | 10.9+ 0.5 | 9.8+ 0.4 |
| | | S | 16.5+ 1.1 | 15.3+ 0.9 | 10.4+ 0.8 | 10.3+ 0.7 |
| RVEDP (mm Hg) | 10 | C | 8.7+ 1.5 | 11.2+ 0.8 | 9.4+ 1.1 | 8.9+ 1.2 |
| | | S | 16.7+ 0.9 | 9.4+ 1.3 | 9.7+ 1.9 | 9.3+ 0.8 |

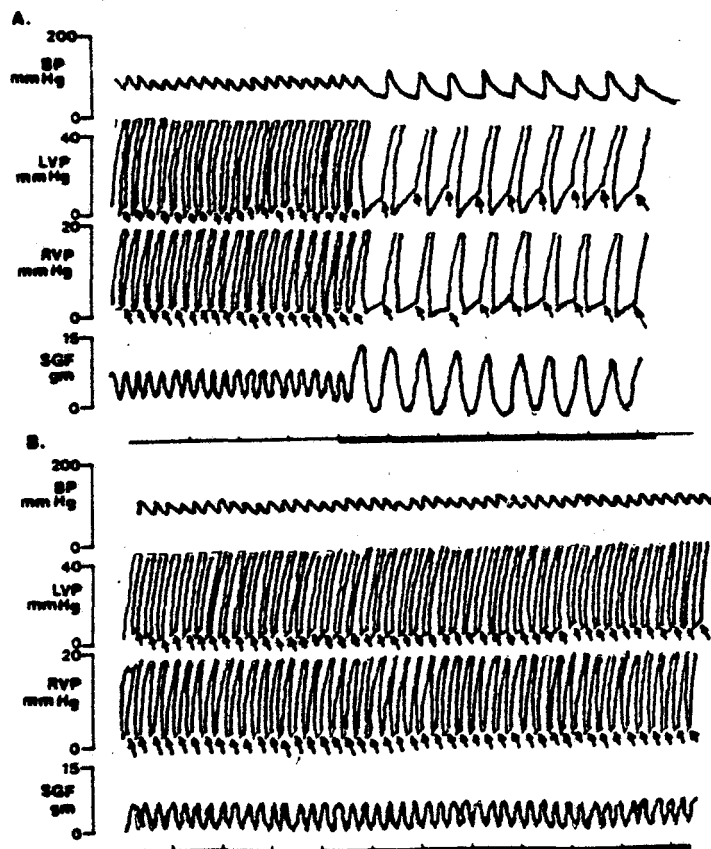
C = control

* = S.E.

S = stimulation

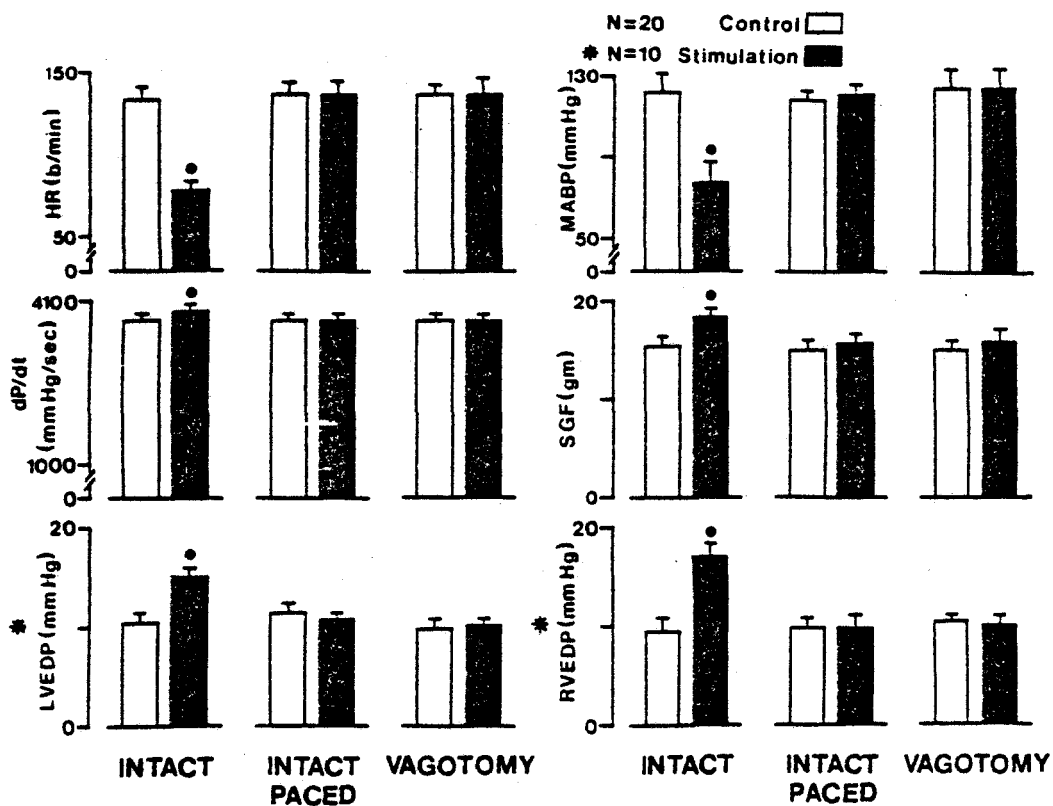
SGF = strain gauge force

CARDIOVASCULAR RESPONSES IN A SINGLE CAT
TO NA STIMULATION



Responses of a single cat to NA stimulation are shown with and without cardiac pacing and after vagotomy without pacing. Time marker indicates 1 and 5 sec intervals. Downward deflection of the time marker indicates stimulus duration. Left ventricular pressure curves on the left of each panel are controls while pressure curves on the right of each panel were taken from cardiac cycles during the last 5 sec of stimulations. Arrows indicate end-diastolic pressures.

AVERAGE CARDIOVASCULAR CHANGES
DURING NA STIMULATION



Average cardiovascular responses to NA stimulation are shown for intact cats without cardiac pacing, intact cats with pacing and after vagotomy without pacing. Solid circle indicates significant difference from control. Brackets indicate S.E.

TABLE II
CARDIOVASCULAR RESPONSES TO NA STIMULATION

| Parameter | N | | INTACT | | VAGOTOMY | |
|----------------------|----|---|-------------|-------------|-------------|-------------|
| | | | UNPACED | PACED | UNPACED | PACED |
| MABP (mm Hg) | 20 | C | 123.4+ 8.6* | 120.2+ 2.2 | 125.6+ 9.3 | 124.3+ 7.4 |
| | | S | 78.6+10.3 | 121.6+ 3.6 | 125.9+ 8.7 | 123.9+ 6.5 |
| HR (b/min) | 20 | C | 135.3+ 5.6 | 138.8+ 6.0 | 139.2+ 4.7 | 138.2+ 7.2 |
| | | S | 54.3+ 4.2 | 138.3+ 6.7 | 136.4+ 7.3 | 138.9+ 6.4 |
| dP/dt (mm Hg/sec) | 20 | C | 3752.6+78.5 | 3734.8+68.5 | 3769.3+82.5 | 3742.5+75.8 |
| | | S | 3975.6+83.7 | 3743.5+72.5 | 3775.4+70.6 | 3756.7+69.4 |
| SGF (gm) | 20 | C | 15.6+ 1.2 | 15.2+ 0.9 | 14.9+ 1.5 | 15.0+ 1.7 |
| | | S | 16.5+ 1.7 | 15.9+ 1.1 | 15.7+ 1.8 | 15.9+ 0.7 |
| LVEDP (mm Hg) | 20 | C | 10.4+ 0.8 | 10.8+ 0.7 | 9.9+ 0.8 | 10.7+ 0.7 |
| | | S | 15.2+ 0.5 | 10.7+ 0.5 | 10.1+ 0.4 | 10.3+ 0.5 |
| RVEDP (mm Hg) | 10 | C | 9.5+ 1.7 | 9.9+ 0.8 | 10.5+ 0.9 | 9.7+ 1.1 |
| | | S | 17.2+ 1.2 | 9.8+ 1.1 | 9.9+ 0.8 | 10.1+ 0.7 |

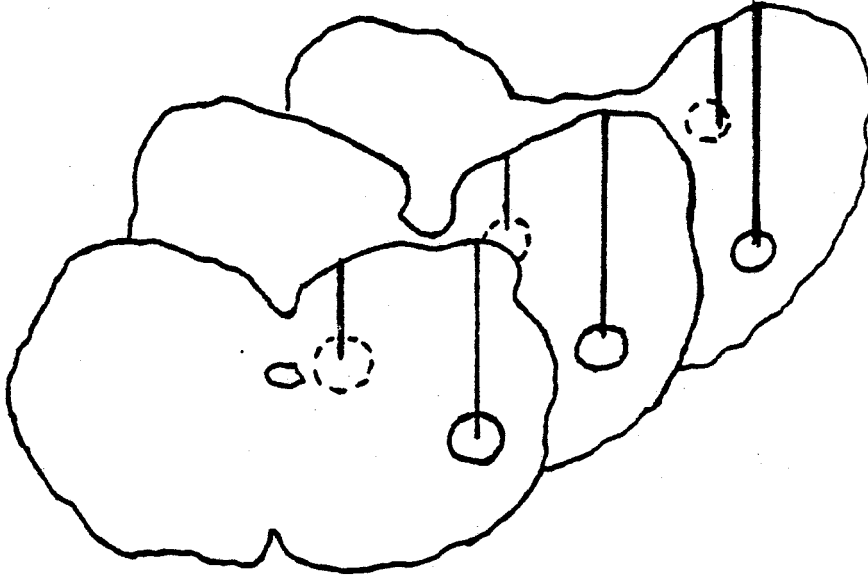
C = control

* = S.E.

S = stimulation

SGF = strain gauge force

MEDULLARY ELECTRODE TRACTS



The 3 planes of electrode penetration are shown for one animal studied. Dotted lines encircle the DMN while solid lines encircle the NA. The locations of the nuclei were evident since cresyl violet stains cell bodies (157). Black bars indicate the limits of the electrode tracts.

located in either the DMN or NA.

In bilaterally vagotomized cats, phenylephrine and histamine produced significant MABP increases ($P < 0.05$) and decreases ($P < 0.05$), respectively (Table III). The other cardiovascular parameters did not change significantly ($P > 0.05$) after administration of either drug.

C. Localization of Ascending Pathways

Spinal ascending pathways affecting the cardiac vagus were localized by noting cardiac responses to peripheral nerve stimulation before and after making spinal lesions. Lesion sites were above the point of nerve entry into the cord. Figure 15 illustrates the HR and MABP responses of a single cat to nerve stimulation while Table IV summarizes the data obtained from all animals. Intact animals demonstrated no significant changes in HR during peroneal nerve stimulation ($P > 0.05$) and significant decreases ($P < 0.05$) in HR during carotid sinus nerve stimulation. Stimulation of the peroneal nerve during carotid sinus nerve stimulation significantly attenuated ($P < 0.05$) the bradycardia observed during individual carotid sinus nerve stimulation. These data indicate the existence of an ascending pathway which modulates vagal control of heart rate. Peroneal activity and subsequent pathway activity apparently cannot regulate spontaneous vagal control of HR. However, the pathway inhibits vagal inhibition of HR produced by carotid sinus nerve stimulation.

TABLE III

CARDIOVASCULAR RESPONSES TO PHENYLEPHRINE AND HISTAMINE

| | | PHENYLEPHRINE | HISTAMINE |
|----------------------|---|---------------|-------------|
| Parameter | | | |
| MABP (mm Hg) | C | 120.4+ 6.7* | 125.4+ 8.5 |
| | R | 165.3+ 8.3 | 95.3+ 7.4 |
| HR (b/min) | C | 133.4+ 6.5 | 134.5+ 7.8 |
| | R | 130.5+ 5.3 | 133.6+ 8.3 |
| dP/dt (mm Hg/sec) | C | 4013.7+70.4 | 4054.8+79.5 |
| | R | 4123.4+67.3 | 4002.6+80.2 |
| SGF (gm) | C | 14.3+ 1.2 | 13.7+ 1.5 |
| | R | 15.6+ 0.9 | 15.6+ 1.7 |
| LVEDP (mm Hg) | C | 9.3+ 1.1 | 9.5+ 1.2 |
| | R | 9.7+ 0.9 | 9.6+ 1.4 |
| RVEDP (mm Hg) | C | 10.1+00.8 | 10.2+ 0.7 |
| | R | 10.7+ 1.0 | 10.6+ 0.9 |

C = control

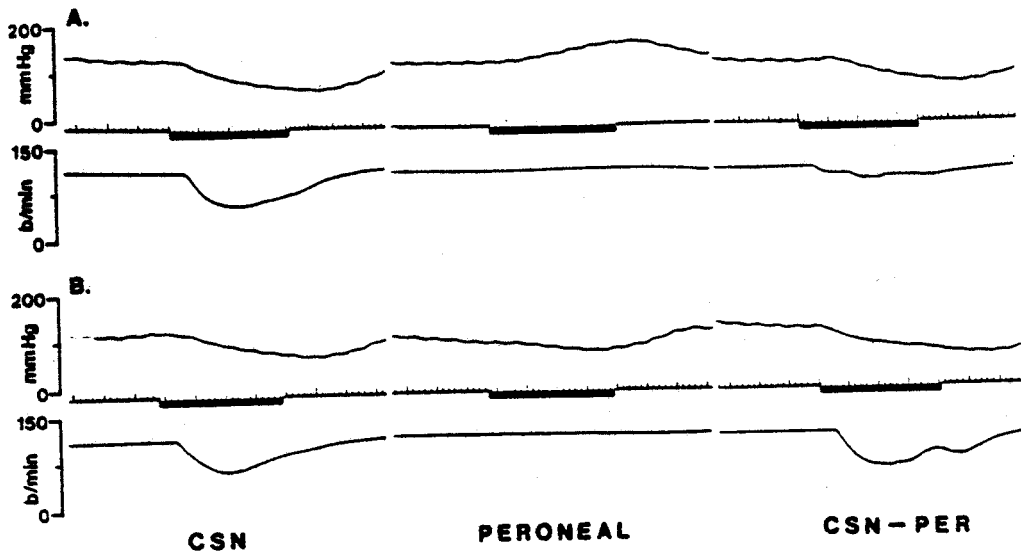
* = S.E.

N = 10

S = stimulation

SGF = strain gauge force

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
 IN A SINGLE CAT BEFORE AND AFTER MAKING BILATERAL
 SPINAL LESIONS



Blood pressure and heart rates are shown during individual carotid sinus (CSN) and peroneal nerve stimulation and simultaneous stimulation of both nerves (CSN-PER). Responses are shown before (A) and after (B) placing bilateral lesions in the DLS at L4. Time marker indicates 1 and 5 sec intervals. Downward deflection of time marker indicates stimulus duration.

TABLE IV
HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER BILATERAL SPINAL LESIONS

| | | CSN | | PERONEAL | |
|-----------------|---|------------|---------------------|-----------|---------------------|
| Parameter | | INTACT | BILATERAL LESION | INTACT | BILATERAL LESION |
| MABP (mm Hg) | C | 132.6+2.0* | 123.3+5.0 | 133.1+2.0 | 124.3+5.3 |
| | S | 80.8+3.5 | 73.0+4.3 | 166.8+6.3 | 110.7+6.6 |
| HR (b/min) | C | 138.4+7.8 | 130.3+6.0 | 132.2+7.6 | 131.2+6.3 |
| | S | 75.9+7.2 | 61.2+6.9 | 137.0+7.5 | 129.4+6.0 |

C = control

S = stimulation

* = S.E.

N = 18

TABLE IV (Continued)

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER BILATERAL SPINAL LESIONS

| | | CSN & PERONEAL | |
|-----------------|---|----------------|---------------------|
| | | INTACT | BILATERAL LESION |
| Parameter | | | |
| MABP (mm Hg) | C | 131.5+1.9* | 122.3+4.9 |
| | S | 127.6+7.4 | 73.8+5.1 |
| HR (b/min) | C | 131.2+7.8 | 130.4+6.3 |
| | S | 103.5+7.4 | 63.8+6.5 |

C = control

S = stimulation

* = S.E.

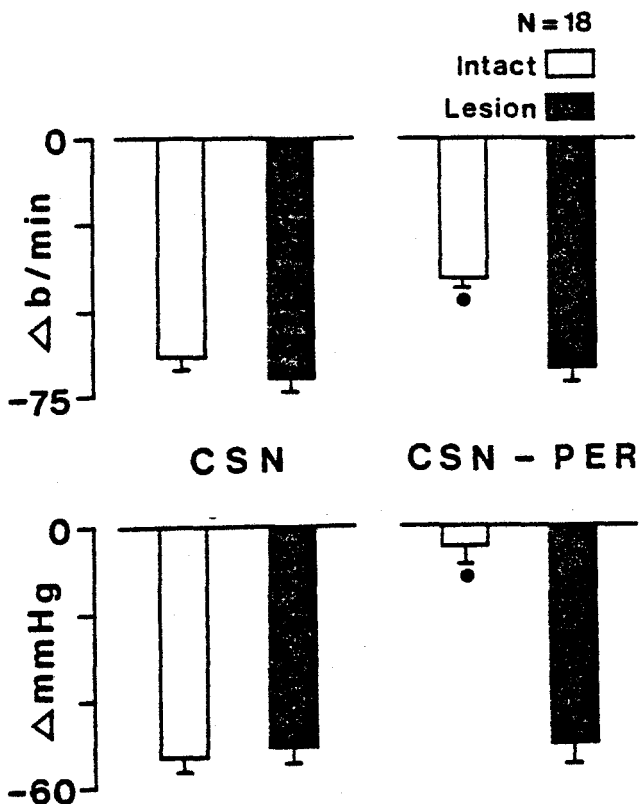
N = 18

Thus, comparisons of cardiac responses to carotid sinus nerve stimulation with responses to simultaneous carotid sinus and peroneal nerve stimulation before and after making spinal lesions were used for localizing ascending pathways involved in cardiac vagal control.

An analysis of variance showed no significant differences ($F > 0.05$) in control parameters associated with carotid sinus nerve stimulation and simultaneous carotid sinus and peroneal nerve stimulation (Table IV). Therefore, data were expressed as the changes in HR and MABP during nerve stimulation (Fig. 16).

Carotid sinus nerve stimulation produced HR and MABP decreases which were significantly attenuated ($P < 0.05$) by simultaneous peroneal nerve stimulation (Figs. 15 & 16). After making bilateral lesions in the dorsolateral sulcus region (DLS) at L4, carotid sinus nerve stimulation produced decreases in HR and MABP which were not significantly different ($P > 0.05$) from corresponding prelesion responses. However, HR and MABP decreases during simultaneous carotid sinus and peroneal nerve stimulation were significantly greater ($P < 0.05$) than corresponding prelesion responses. The postlesion changes in HR and MABP during simultaneous nerve stimulation were not significantly different ($P > 0.05$) from the changes produced by carotid sinus nerve stimulation before or after making the lesions.

HEART RATE AND BLOOD PRESSURE CHANGES DURING NERVE STIMULATION BEFORE AND AFTER MAKING BILATERAL SPINAL LESIONS



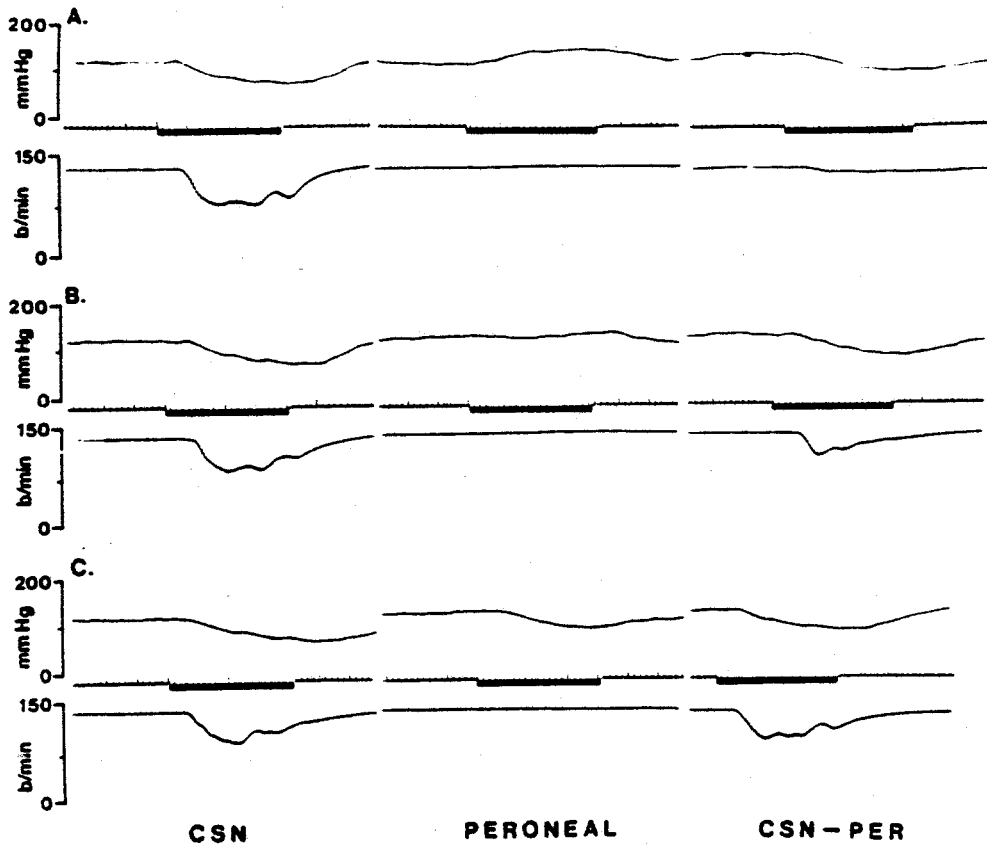
Heart rate (top) and blood pressure (bottom) changes during carotid sinus nerve (CSN) stimulation and simultaneous stimulation of the carotid sinus and peroneal nerves (CSN-PER). Responses are shown before and after making bilateral lesions in the DLS at L4. Closed circles indicate a significant difference from the other responses. Brackets indicate S.E.

Investigations were performed to determine whether the pathway ascends ipsilateral, contralateral or ipsilateral and contralateral. HR and MABP responses to nerve stimulations were monitored before and after making a right DLS lesion and then after making bilateral lesions. The responses of a single cat and a summary of all the data are shown in Figure 17 and Table V, respectively. Since unilateral and bilateral lesions did not affect control parameters ($F > 0.05$), the data were plotted as changes in HR and MABP in response to nerve stimulation (Fig. 18).

Before making the lesion, carotid sinus nerve stimulation produced HR and MABP decreases which were significantly attenuated ($P < 0.05$) by simultaneous peroneal nerve stimulation. A unilateral lesion placed in the right DLS did not alter the responses to carotid sinus nerve stimulation ($P > 0.05$). After the lesion, peroneal nerve stimulation still significantly attenuated the responses to carotid sinus nerve stimulation ($P < 0.05$). However, postlesion HR and MABP decreases during simultaneous nerve stimulation were significantly greater ($P < 0.05$) than corresponding decreases before the lesion.

Subsequent bilateral lesions of the DLS completely abolished the attenuation of the bradycardia during carotid sinus nerve stimulation by simultaneous peroneal nerve stimulation. Responses to carotid sinus nerve stimulation after

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
 IN A SINGLE CAT BEFORE AND AFTER MAKING UNILATERAL
 AND THEN BILATERAL SPINAL LESIONS



Blood pressure and heart rates are shown during individual carotid sinus (CSN) and peroneal nerve stimulation and simultaneous stimulation of both nerves (CSN-PER). Responses are shown before (A) and after (B) placing a right unilateral lesion and then after placing bilateral lesions (C) in the DLS at L4. Time marker indicates 1 and 5 sec intervals. Downward deflection of time marker indicates stimulus duration.

TABLE V

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | CSN | | |
|-----------------|---|-------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP (mm Hg) | C | 131.2+ 2.7* | 128.2+ 4.1 | 128.8+7.8 |
| | S | 80.1+ 5.1 | 77.9+ 5.7 | 70.3+5.6 |
| HR (b/min) | C | 135.5+11.7 | 139.1+12.5 | 133.7+9.7 |
| | S | 83.7+10.5 | 87.5+10.3 | 85.7+9.7 |

C = control

S = stimulation

* = S.E.

N = 10

TABLE V (Continued)

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | PERONEAL | | |
|-----------------|---|-------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP (mm Hg) | C | 130.6+ 2.8* | 127.0+ 4.6 | 118.9+ 8.1 |
| | S | 155.1+ 9.9 | 135.2+13.1 | 99.7+10.0 |
| HR (b/min) | C | 130.8+11.0 | 135.5+11.9 | 133.4+ 8.1 |
| | S | 36.4+10.7 | 132.7+11.5 | 131.2+ 7.5 |

C = control

S = stimulation

* = S.E.

N = 10

TABLE V (Continued)

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | CSN-PERONEAL | | |
|-----------|---|--------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP | C | 129.0+ 2.5* | 133.7+ 5.5 | 124.9+7.8 |
| (mm Hg) | S | 116.6+10.7 | 114.5+ 9.3 | 69.3+6.0 |
| HR | C | 130.4+11.1 | 133.1+11.8 | 131.9+8.3 |
| (b/min) | S | 118.3+10.6 | 113.1+11.9 | 83.5+8.8 |

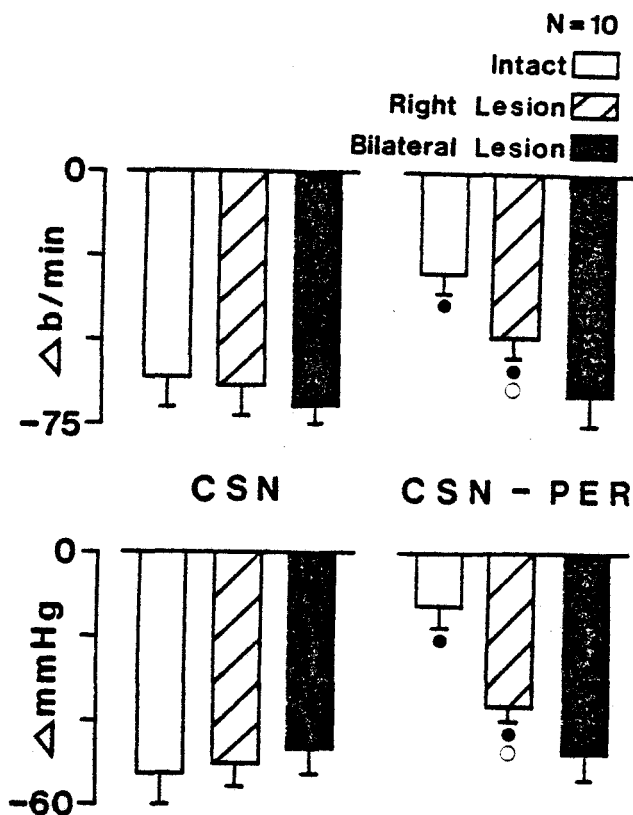
C = control

S = stimulation

* = S.E.

N = 10

HEART RATE AND BLOOD PRESSURE CHANGES DURING NERVE
STIMULATION BEFORE AND AFTER MAKING UNILATERAL
AND THEN BILATERAL SPINAL LESIONS



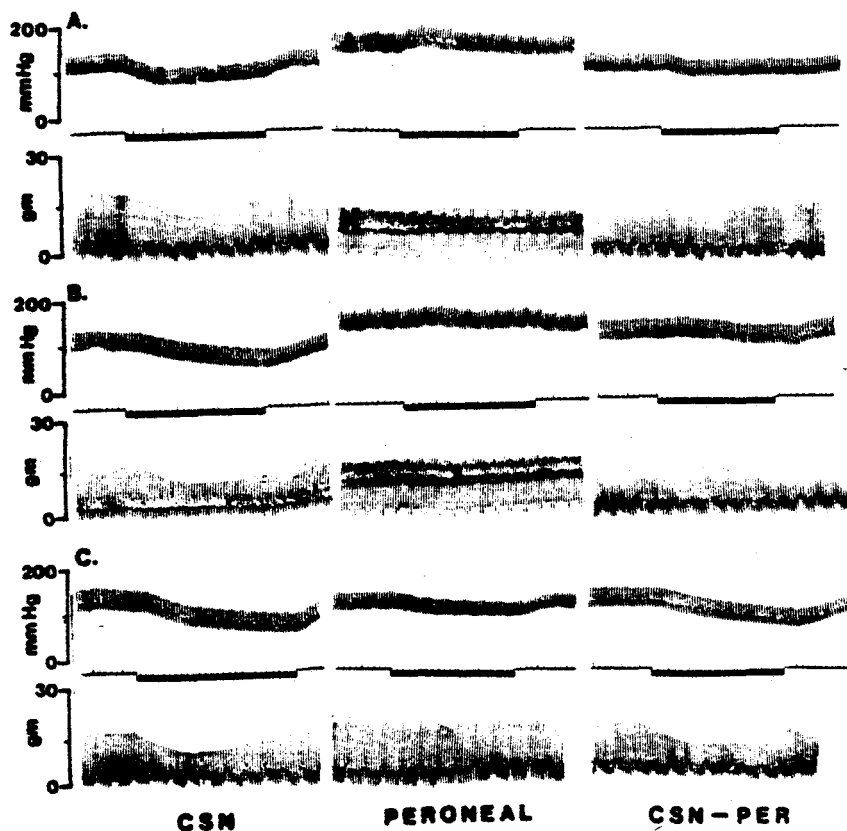
Heart rate (top) and blood pressure (bottom) changes during carotid sinus nerve (CSN) stimulation and simultaneous stimulation of the carotid sinus and peroneal nerves (CSN-PER). Responses are shown before and after making a right DLS and then after making bilateral DLS lesions at L4. Closed circles indicate significant difference from responses without circles. Open circles indicate significant difference from all other responses. Brackets indicate S.E.

making bilateral lesions were not significantly different ($P > 0.05$) from responses before or after making a right DLS lesion. After bilateral lesions, the responses to simultaneous carotid sinus and peroneal nerve stimulation were not significantly different from the responses to carotid sinus nerve stimulation before or after unilateral spinal lesions and after bilateral lesions ($P > 0.05$).

D. Medullary Projections of Spinal Pathways

The locations of ascending spinal pathways modulating vagal control of cardiac contractility were determined. Hearts were paced throughout the experiments and MABP, dP/dt and SGF were monitored. Cardiovascular responses were noted before and after making spinal lesions during carotid sinus nerve stimulation, peroneal nerve stimulation and simultaneous stimulation of both nerves. Figure 19 shows the responses of a single cat and Table VI summarizes the data from all animals studied. Intact cats did not demonstrate significant changes in dP/dt and SGF during peroneal nerve stimulation ($P > 0.05$). The parameters decreased significantly during carotid sinus nerve stimulation ($P < 0.05$). The responses during simultaneous stimulation of both nerves were significantly less ($P < 0.05$) than the responses to carotid sinus nerve stimulation. The data indicate that peroneal nerve stimulation activates a spinal pathway which opposes vagal inhibition of ventricular

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION IN A SINGLE
 CAT BEFORE AND AFTER MAKING UNILATERAL AND
 THEN BILATERAL SPINAL LESIONS



Responses are shown during individual carotid sinus (CSN) and peroneal nerve stimulation and simultaneous stimulation of both nerves (CSN-PER). Responses are shown before (A) and after (B) placing a right unilateral lesion and then after placing bilateral lesions (C) in the DLS at L4. Time marker indicates 1 and 5 sec intervals. Downward deflection of time marker indicates stimulus duration. Left ventricular pressure curves on the left of each panel are controls while pressure curves on the right of each panel were taken from a cardiac cycle during the last 5 sec of stimulation.

TABLE VI

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | CSN | | |
|----------------------|---|-------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP (mm Hg) | C | 118.5+ 7.2* | 123.4+ 9.3 | 125.4+ 7.4 |
| | S | 105.3+ 6.4 | 110.4+ 8.6 | 100.3+ 6.3 |
| HR (b/min) | C | 142.7+ 3.8 | 142.6+ 3.5 | 141.9+ 4.1 |
| | S | 142.1+ 4.2 | 143.1+ 3.0 | 142.0+ 3.7 |
| dP/dt (mm Hg/sec) | C | 4267.8+80.5 | 4285.8+90.8 | 4278.2+93.4 |
| | S | 2560.7+85.7 | 2657.2+83.7 | 2524.1+87.4 |
| SGF (gm) | C | 15.3+ 0.7 | 16.2+ 1.7 | 14.9+ 1.1 |
| | S | 9.2+ 1.6 | 8.9+ 0.9 | 9.4+ 1.8 |

C = control

SGF = strain gauge force

S = stimulation

* = S.E.

N = 10

TABLE VI (Continued)

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | PERONEAL | | |
|----------------------|---|-------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP (mm Hg) | C | 122.5+ 8.2* | 118.9+ 6.2 | 121.6+ 8.1 |
| | S | 143.2+ 6.7 | 129.4+ 7.8 | 106.4+ 7.1 |
| HR (b/min) | C | 142.7+ 3.8 | 142.0+ 2.1 | 143.1+ 3.2 |
| | S | 142.5+ 4.0 | 142.1+ 2.0 | 143.4+ 3.2 |
| dP/dt (mm Hg/sec) | C | 4259.3+79.8 | 4270.6+80.5 | 4284.7+98.6 |
| | S | 4328.4+90.4 | 4234.7+78.6 | 4187.9+75.8 |
| SGF (gm) | C | 15.3+ 1.5 | 15.9+ 0.9 | 14.8+ 1.7 |
| | S | 14.3+ 0.8 | 15.6+ 1.4 | 15.9+ 1.3 |

C = control

SGF = strain gauge force

S = stimulation

* = S.E.

N = 10

TABLE VI (Continued)

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL AND THEN BILATERAL SPINAL LESIONS

| | | CSN-PERONEAL | | |
|----------------------|---|--------------|-----------------|---------------------|
| | | INTACT | RIGHT LESION | BILATERAL LESION |
| Parameter | | | | |
| MABP (mm Hg) | C | 118.6+ 7.2* | 115.3+ 8.3 | 117.6+ 7.3 |
| | S | 115.7+ 6.2 | 107.3+ 6.2 | 108.4+ 6.3 |
| HR (b/min) | C | 142.6+ 4.3 | 142.4+ 2.9 | 142.2+ 3.0 |
| | S | 141.9+ 3.7 | 142.2+ 2.8 | 142.7+ 2.8 |
| dP/dt (mm Hg/sec) | C | 4260.7+81.4 | 4274.3+84.4 | 4282.5+76.8 |
| | S | 4250.6+79.1 | 3218.5+91.4 | 2483.9+82.3 |
| SGF (gm) | C | 14.9+ 1.3 | 15.4+ 1.7 | 15.7+ 1.2 |
| | S | 14.7+ 0.9 | 12.3+ 1.2 | 9.5+ 1.4 |

C = control

SGF = strain gauge force

S = stimulation

* = S.E.

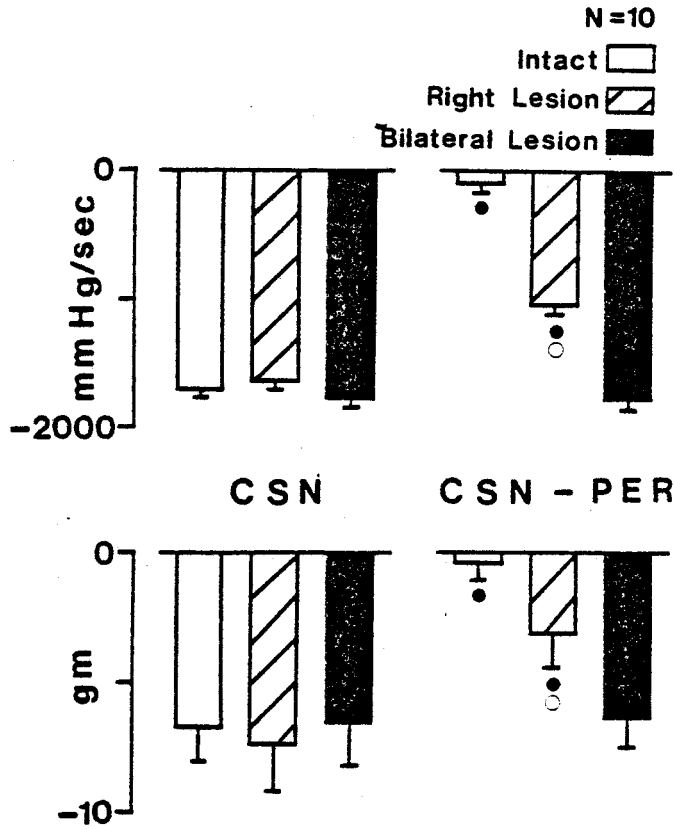
N = 10

contractility consequent to carotid sinus nerve stimulation. Peroneal activation of the pathway does not affect spontaneous vagal inotropic control. Therefore, comparisons of cardiac responses to carotid sinus nerve stimulation with responses to simultaneous carotid sinus and peroneal nerve stimulation before and after making spinal lesions were used for localizing the pathway.

Control parameters of intact animals associated with the various nerve stimulations (Table VI) were not significantly different ($F > 0.05$). Control parameters did not change significantly ($F > 0.05$) after unilateral and bilateral spinal lesions (Table VI). Therefore, the responses to nerve stimulations were expressed as changes from controls and are plotted in figure 20.

In intact cats, simultaneous carotid sinus and peroneal nerve stimulation produced dP/dt and SGF decreases which were significantly less ($P < 0.05$) than the decreases produced by carotid sinus nerve stimulation. After placing a lesion in the right DLS, simultaneous nerve stimulation produced decreases in dP/dt and SGF which were significantly greater ($P < 0.05$) than the decreases produced by simultaneous nerve stimulation before the lesion. However, after unilateral lesion, the responses to stimulation of both nerves was significantly less than ($P < 0.05$) the responses to carotid sinus nerve stimulation. After bilateral DLS lesions, the

CARDIOVASCULAR CHANGES DURING NERVE STIMULATION BEFORE AND AFTER MAKING UNILATERAL AND THEN BILATERAL SPINAL LESIONS



dP/dt (top) and SGF (bottom) changes during carotid sinus nerve (CSN) stimulation and simultaneous stimulation of the carotid sinus and peroneal nerves (CSN-PER). Responses are shown before and after making a right DLS lesion and then after making bilateral DLS lesions at L4. Closed circles indicate significant difference from responses without circles. Open circles indicate significant difference from all other responses. Brackets indicate S.E.

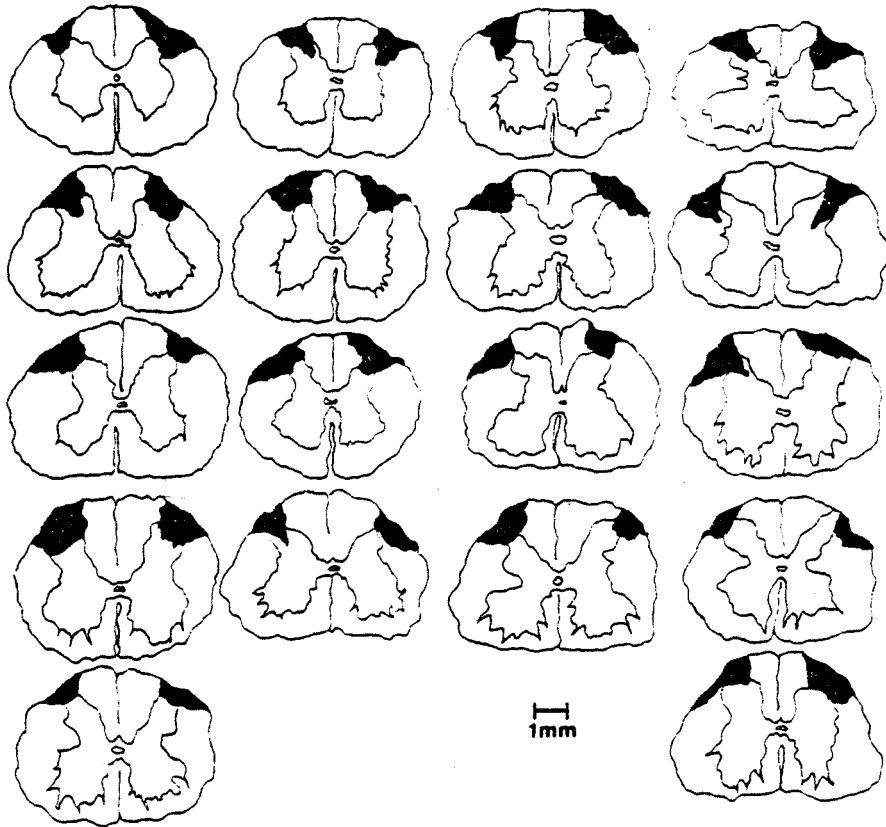
responses to stimulation of both nerves was not different ($P > 0.05$) from the response to carotid sinus nerve stimulation. The responses to carotid sinus nerve stimulation before and after unilateral lesion and after bilateral lesions were not different ($F > 0.05$).

Spinal lesions for localizing pathways involved in vagal modulation of HR and contractility were similar (Figs. 21 & 22). The lesions were about 1.0 mm in depth in the DLS region of the spinal cord. The lesions extended slightly into the posterior columns and dorsolateral funiculus (DLF).

In the final experiments a left vagotomy was performed in each cat. Cardiovascular responses were monitored during right carotid sinus nerve stimulation, right peroneal nerve stimulation and simultaneous stimulation of both nerves before and after making a right DLS lesion. In one series of cats, HR and MABP were monitored. Hearts were paced and MABP, dP/dt and SGF were recorded in another group of animals.

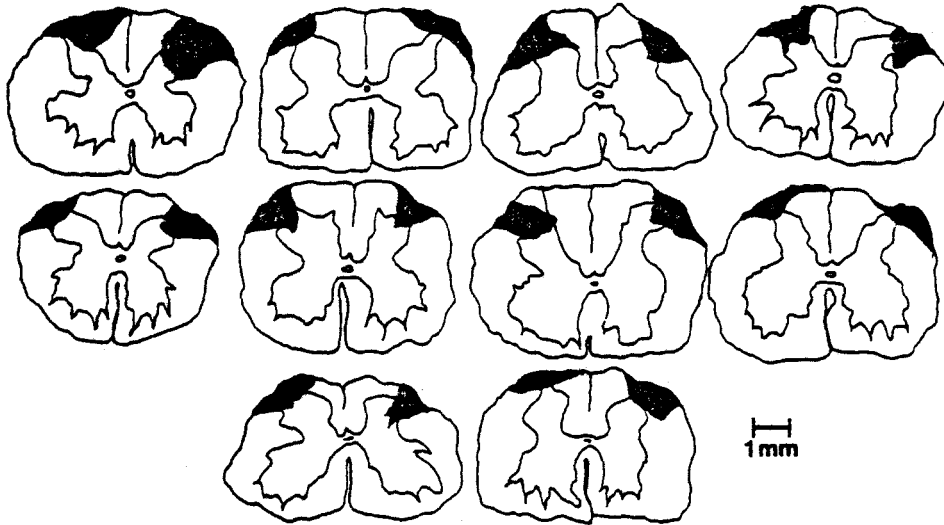
HR and MABP from a single cat are shown in figure 23 while the summarized data is shown in Table VII. The changes in HR and MABP during nerve stimulation are plotted in figure 24. Control parameters associated with the different nerve stimulations were not significantly different ($P > 0.05$) before or after the lesion (Table VII).

LESION SITES FOR HEART RATE AND
BLOOD PRESSURE STUDIES



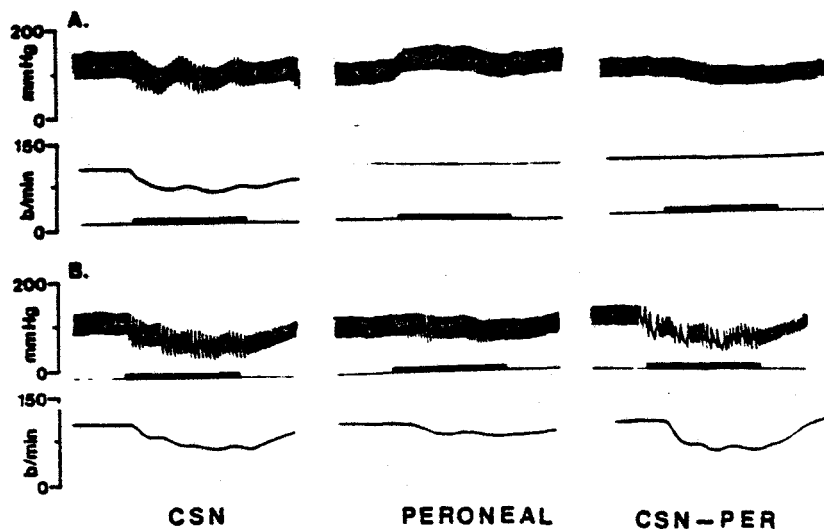
Cross sections of L4 spinal cords from animals used for heart rate and blood pressure studies. Blackened areas indicate lesion sites.

LESION SITES FOR CONTRACTILITY STUDIES



Cross sections of L4 spinal cords from animals used for contractility studies. Blackened areas indicate lesion sites.

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE
STIMULATION IN A SINGLE CAT BEFORE AND AFTER
MAKING UNILATERAL SPINAL LESIONS



Blood pressure and heart rates are shown during individual carotid sinus (CSN) and peroneal nerve stimulation and simultaneous stimulation of both nerves (CSN-PER). Responses are shown before (A) and after (B) placing a right unilateral lesion in the DLS at L4. Time marker indicates 1 and 5 sec intervals. Upward deflection of the time marker indicates stimulus duration.

TABLE VII

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL SPINAL LESIONS

| | | CSN | | PERONEAL | |
|-----------------|---|------------|-----------------|-----------|-----------------|
| | | INTACT | RIGHT LESION | INTACT | RIGHT LESION |
| Parameter | | | | | |
| MABP (mm Hg) | C | 125.6+8.2* | 124.7+6.3 | 120.5+6.2 | 129.4+8.3 |
| | S | 111.2+7.3 | 110.3+5.2 | 152.4+7.3 | 137.4+6.7 |
| HR (b/min) | C | 141.4+6.7 | 145.7+7.0 | 140.4+7.3 | 139.6+8.3 |
| | S | 98.0+7.3 | 104.9+8.3 | 143.7+6.2 | 138.4+6.2 |

C = control

S = stimulation

* = S.E.

N = 10

TABLE VII (Continued)

HEART RATE AND BLOOD PRESSURE RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL SPINAL LESIONS

| CSN & PERONEAL | | | |
|-----------------|---|------------|-----------------|
| | | INTACT | RIGHT LESION |
| Parameter | | | |
| MABP (mm Hg) | C | 130.5+6.7* | 124.3+8.7 |
| | S | 127.2+4.7 | 111.0+7.2 |
| HR (b/min) | C | 143.7+8.5 | 140.6+7.2 |
| | S | 122.0+7.9 | 98.1+6.4 |

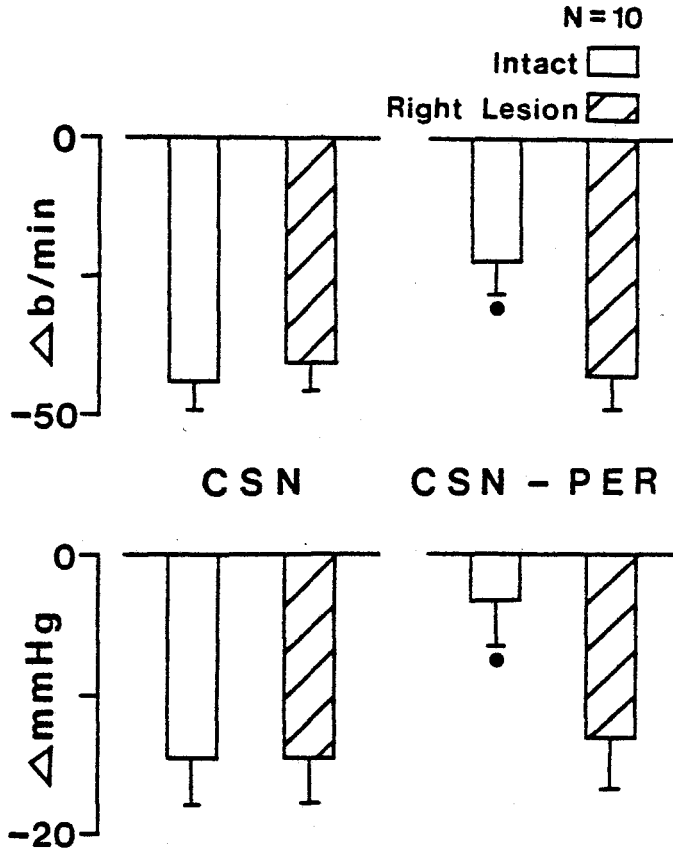
C = control

S = stimulation

* = S.E.

N = 10

HEART RATE AND BLOOD PRESSURE CHANGES DURING NERVE
STIMULATION BEFORE AND AFTER MAKING A
UNILATERAL SPINAL LESION

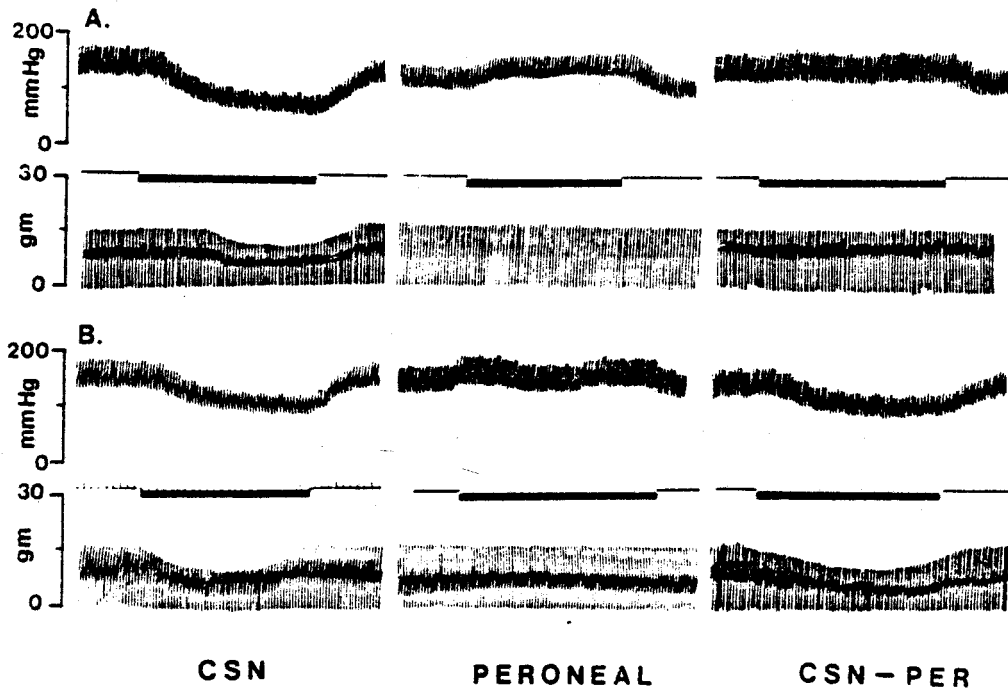


Heart rate (top) and blood pressure (bottom) changes during carotid sinus nerve (CSN) stimulation and simultaneous stimulation of the carotid sinus and peroneal nerves (CSN-PER). Responses are shown before and after making a right DLS lesion at L4. Closed circles indicate significant difference from other responses. Brackets indicate S.E.

Carotid sinus nerve stimulation produced HR and MABP decreases which were significantly attenuated ($P < 0.05$) by simultaneous peroneal nerve stimulation. A right DLS lesion did not affect the responses to carotid sinus nerve stimulation ($P > 0.05$) but abolished the effect of simultaneous peroneal nerve stimulation. After the lesion, the changes in HR and MABP during simultaneous nerve stimulation were not significantly different from the changes produced by carotid sinus nerve stimulation before or after the lesion ($P > 0.05$). Responses to carotid sinus nerve stimulation were not affected by the lesion ($P > 0.05$).

A right DLS lesion had similar effects on dP/dt and SGF. The responses of one cat are illustrated in figure 25 and the summarized data is shown in Table VIII. Data are plotted in figure 26 as changes in parameters from controls during nerve stimulation. Control values (Table VIII) did not change significantly after the lesions ($F > 0.05$). During cardiac pacing, carotid sinus stimulation produced dP/dt and SGF decreases which were significantly attenuated ($P < 0.05$) by simultaneous peroneal nerve stimulation. After making a right DLS lesion, the responses to simultaneous nerve stimulation were not significantly different from the responses to carotid sinus nerve stimulation before or after lesions ($P > 0.05$). Pre- and postlesion responses to carotid sinus nerve stimulation were not significantly different ($P > 0.05$).

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION IN A SINGLE
CAT BEFORE AND AFTER MAKING UNILATERAL SPINAL LESIONS



Responses are shown during individual carotid sinus (CSN) and peroneal nerve stimulation and simultaneous stimulation of both nerves (CSN-PER). Responses are shown before (A) and after (B) placing a right unilateral lesion in the DLS at L4. Time marker indicates 1 and 5 sec intervals. Downward deflection of the time marker indicates stimulus duration. Left ventricular pressure curves on the left of each panel are control while pressure curves on the right of each panel were taken from a cardiac cycle during the last 5 sec of stimulation.

TABLE VIII
 CARDIOVASCULAR RESPONSES TO NERVE STIMULATION
 BEFORE AND AFTER UNILATERAL SPINAL LESIONS

| | | CSN | | PERONEAL | |
|----------------------|---|-------------|-----------------|-------------|-----------------|
| Parameter | | INTACT | RIGHT LESION | INTACT | RIGHT LESION |
| MABP (mm Hg) | C | 132.4+ 7.5* | 135.1+ 6.5 | 130.3+ 8.2 | 133.4+ 7.2 |
| | S | 114.3+ 6.4 | 112.3+ 7.2 | 148.4+ 7.3 | 141.6+ 6.2 |
| HR (b/min) | C | 135.4+ 4.4 | 135.4+ 4.5 | 135.3+ 4.1 | 135.5+ 3.9 |
| | S | 135.2+ 4.2 | 135.1+ 4.7 | 135.3+ 4.2 | 135.6+ 4.0 |
| dP/dt (mm Hg/sec) | C | 3837.3+81.2 | 3858.6+79.3 | 3830.3+69.3 | 3841.4+70.2 |
| | S | 1937.4+72.3 | 1968.4+78.7 | 3921.4+75.4 | 3682.5+81.3 |
| SGF (gm) | C | 16.2+ 1.7 | 15.9+ 1.4 | 16.7+ 1.2 | 16.8+ 0.9 |
| | S | 9.7+ 0.8 | 9.1+ 1.7 | 17.5+ 1.3 | 17.2+ 1.7 |

C = control

SGF = strain gauge force

S = stimulation

* = S.E.

N = 10

TABLE VIII (Continued)

CARDIOVASCULAR RESPONSES TO NERVE STIMULATION
BEFORE AND AFTER UNILATERAL SPINAL LESIONS

| | | CSN & PERONEAL | |
|----------------------|---|---------------------|---------------------|
| | | INTACT | RIGHT LESION |
| Parameter | | | |
| MABP (mm Hg) | C | 129.4+ 6.3* | 132.4+ 6.9 |
| | S | 129.5+ <u>6.8</u> | 110.3+ <u>7.2</u> |
| HR (b/min) | C | 135.7+ 3.8 | 135.6+ 3.7 |
| | S | 135.2+ <u>4.1</u> | 135.6+ <u>3.4</u> |
| dP/dt (mm Hg/sec) | C | 3861.4+78.3 | 3848.4+82.7 |
| | S | 3845.6+ <u>80.1</u> | 1946.4+ <u>74.3</u> |
| SGF (gm) | C | 17.0+ 1.7 | 16.0+ 1.2 |
| | S | 16.7+ <u>0.9</u> | 10.0+ <u>1.6</u> |

C = control

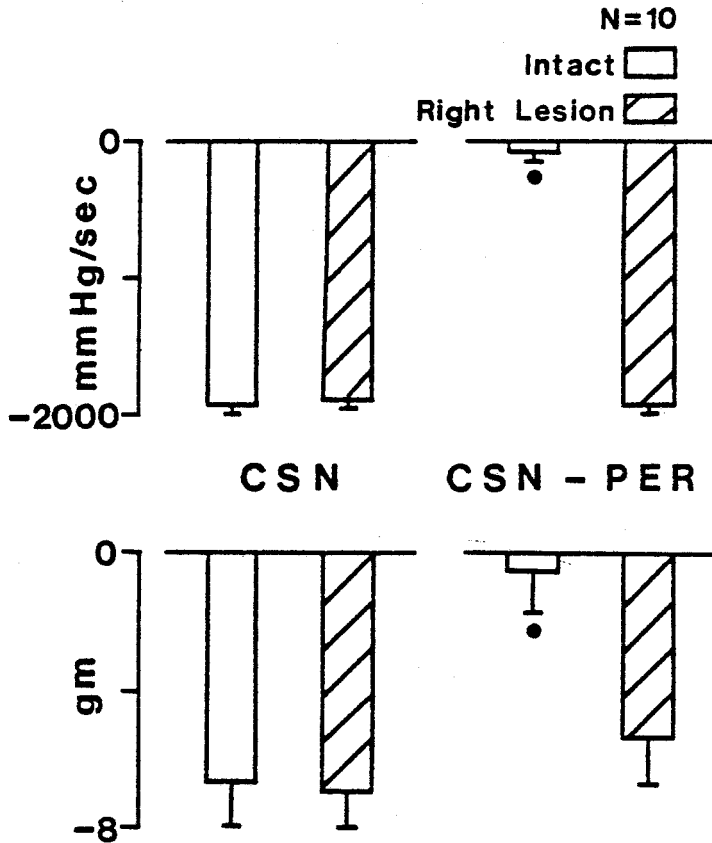
SGF = strain gauge force

S = stimulation

* = S.E.

N = 10

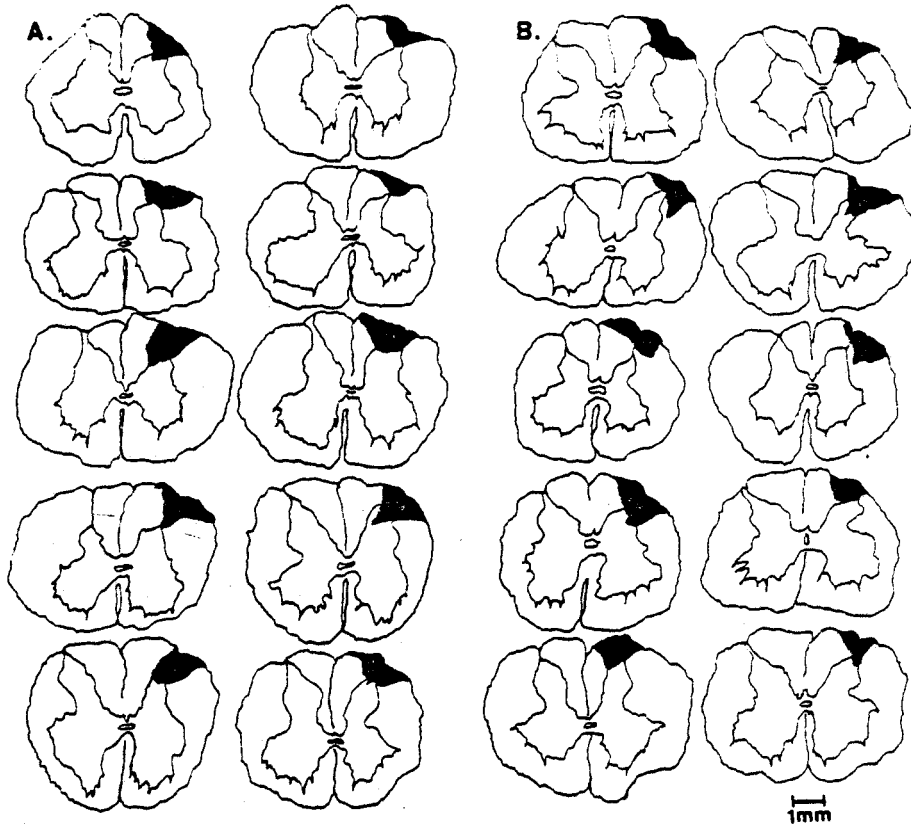
CARDIOVASCULAR CHANGES DURING NERVE STIMULATION BEFORE
AND AFTER MAKING UNILATERAL SPINAL LESIONS



dP/dt (top) and SGF (bottom) changes during carotid sinus nerve (CSN) stimulation and simultaneous stimulation of the carotid sinus and peroneal nerves (CSN-PER). Responses are shown before and after making a right DLS lesion at L4. Closed circles indicate significant difference from other responses. Brackets indicate S.E.

The sites of the lesions placed in cats with left vagotomy are illustrated in figure 27. The lesions for HR and MABP studies (Fig. 27A) and contractility studies (Fig. 27B) were similar. Lesions extended about 1.0 mm in depth in the region of the DLS.

LESION SITES FOR MEDULLARY
PROJECTION STUDIES



Cross sections of L4 spinal cords from animals used for determining the medullary projections of ascending pathways. (A) indicates spinal cords from cats in which heart rate and blood pressure alone were monitored. (B) shows spinal cords from cats in which dP/dt and SGF were monitored. Blackened areas indicate lesion sites.

CHAPTER V

DISCUSSION

The number, distribution and sizes of horseradish peroxidase labeled somata with axons passing in the left and right cervical vagi are similar. The cell bodies are located in 3 regions ipsilateral to the vagus carrying their axons: the nucleus ambiguus (NA), the dorsal motor nucleus of the vagus (DMN) and an intermediate zone (IZ) between the NA and DMN (Fig. 6). The NA contain the maximum number of somata while successively fewer cell bodies are located in the DMN and IZ (Fig. 7). NA cell bodies are heterogeneously distributed along the longitudinal neuraxis and are maximally concentrated in the brain stem segment extending 1.0 to 1.8 mm rostral to the obex (Fig. 8). Somata of the DMN and IZ are homogeneously and sparsely distributed along the neuraxis (Fig. 8). The long and short axes of NA somata are larger than corresponding dimensions of cell bodies in the DMN or IZ (Figs. 4 & 9). The dimensions of somata in the DMN and IZ are similar.

Anatomical studies of cat embryology indicate the DMN, IZ and NA are the expected locations of parasympathetic preganglionic cell bodies. Windle (207) studied the

anatomical development of the cat fetus. Brain stems of embryos were sectioned and stained with cresyl violet or silver impregnation. During early gestation, vagal preganglionic cell bodies form a compact nucleus immediately below the neural tube. A number of the somata migrate ventrolaterally during later developmental stages. The migrating neurons form the NA while immobile somata become the DMN. Cell bodies of the IZ may be aberrant neurons which do not complete migration.

Anatomical studies suggest parasympathetic preganglionic somata develop a viscerotopic organization. Vagal branches to different viscera were sectioned in the rabbit (87) and monkey (154). The locations of the resulting chromatolytic neurons were compared. Cell bodies innervating a given organ were arranged in relatively distinct groups. The studies are limited since the entire brain stems were not thoroughly examined. However, the possibility of viscerotropic organization may be significant to the present study. HRP labeled somata of the DMN, NA and IZ may innervate different organs. HRP may have been taken up by fibers passing near the myocardium to innervate other viscera or by axon terminals in organs adjacent the heart (trachea, esophagus, lung, pulmonary vasculature) resulting from HRP spread from injection sites.

Reports by LaVail and LaVail (135), Kuypers et al. (133) and Nauta (161) indicate HRP remains well localized

at injection sites. Furthermore, the enzyme is taken up by axon terminals and not by fibers en passage. In addition, to ensure the labeled somata are cardiac vagal preganglionic neurons, control studies were performed.

Hearts were parasympathectomized in control cats prior to HRP injection. The intrapericardial techniques for cardiac denervation described by Geis et al. (82) and Kaye et al. (127) were not used since adventitial stripping could produce cut axons in the myocardium. HRP is readily taken up by cut fibers (3,4,96,188,192). Instead, a left cervical vagotomy was performed and the right caudal and cranial cardiac vagal branches were sectioned at a distance of about 1.0 cm from the heart. The absence of a HR response to right cervical vagal stimulation after cutting the cardiac vagal branches indicated cardiac parasympathectomy (Fig. 5). McAllen and Spyer (151) showed the procedure produces cardiac denervation without altering the innervation of other viscera in the cat. However, abolishing the HR response to cervical vagal stimulation does not indicate complete cardiac parasympathectomy. Fibers modulating cardiac inotropy and dromotropy may pass in branches other than the caudal and cranial cardiac vagal branches (8,177). Attempts were not made to record cardiac inotropic responses in the control cats since the necessary techniques for the measurements would introduce the variable of myocardial tissue trauma in the controls.

However, control animals demonstrated 24.8 ± 6.2 labeled cell bodies compared with 366.1 ± 14.2 and 354.5 ± 17.3 labeled somata in cats with an intact left and right vagus, respectively. Therefore, approximately 6.8% of the labeled somata of test animals were the result of HRP uptake by fibers innervating nonmyocardial tissue or by cardiac parasympathetic axons traveling in nerves other than the right caudal and cranial cardiac vagal branches.

Fibers of medullary origin producing positive chronotropic (124), inotropic (173) and dromotropic (172) effects pass with the vagus to the heart. Weiss and Priola (203) localized the cell bodies of origin in the NA in the dog. Since such fibers have not been described in the cat, all HRP labeled somata in the NA are considered to be cardiac parasympathetic preganglionic neurons.

The results of the present investigation conflict with electrophysiological, physiological and anatomical studies which localized cardiac vagal preganglionic somata in either the DMN (42,43,87,153,156,190,198,199) or NA (17,151,195,197,201). Different investigators studied various animal species. Therefore, the results may reflect intrinsic species variations (93).

However, experiments on cats have produced conflicting results. McAllen and Spyer (151), Urabe and Tsubakawa (201) and Porter (171) explored cat brain stems with recording electrodes while stimulating cardiac vagal branches.

Evoked discharges were elicited from NA somata, exclusively. Szentagothai (195) observed Wallerian degeneration in the cardiac vagal branches after NA ablation but not after DMN destruction. However, Calaresu and Cottle (33) reported degenerating axons in these branches after making DMN lesions. The different results can be explained by the longitudinal distributions of cardiac vagal preganglionic somata in the DMN, NA and IZ.

Somata of the DMN and IZ were uniformly and sparsely distributed along the medullary longitudinal axis while cell bodies of the NA are nonuniformly distributed (Fig. 8). A maximum of 2 somata were labeled in the DMN or IZ of any transverse brain stem section compared with 14 labeled somata in the NA. Therefore, the probability of electrophysiologically localizing a somata in the NA is much greater than in the DMN or IZ. Furthermore, producing orthograde degeneration of DMN or IZ axons by brain stem ablation would require complete destruction of the regions. Such lesions have proven difficult to produce (17,33,129).

Recently, Todo (198) and Todo et al. (199) used the HRP technique to identify cardiac vagal preganglionic somata. Cell bodies were found exclusively in the DMN and the nucleus tractus solitarius (NTS). The conflicting results between these studies and the present investigation may be due to injection site differences, variations in the labeling material or improper identification of the diaminobenzidine stained

granules.

In his first report, Todo et al. (199) injected HRP in the regions of the SA and AV nodes exclusively, while the injections of his later report (198) were similar to the present study. Labeled somata were identified in the DMN and NTS of both studies. Thus, injection site differences do not explain the conflicting results.

HRP labeling of somata depends on the HRP enzymatic activity and the distance from the axon terminal to the cell body. Adams (2) reported better labeling with HPOFF: Worthington Biochemical Corp. (Freehold, NJ) than with HRP Type VI: Sigma Chemical Co. (St. Louis, MO). HPOFF had 3.2 times the unit activity per mg than Type VI. The data reported by Adams suggests better labeling by the HRP with greater enzymatic activity. The data of Sherlock and Raisman (192) suggest faster retrograde transport of the HRP with greater enzymatic activity. The reported transport rate of Type VI HRP is 84 mm per day (137).

Windle (207) provided evidence that axons arising from the NA somata are longer than fibers originating from DMN cell bodies. Silver impregnation techniques were used to stain fibers and observe their pathways. Axons from DMN somata project in a ventrolateral direction from the medulla. The length of NA fibers may be up to 10 mm longer than those of the DMN. Axons of NA somata course dorsomedially to a genu in the region of the DMN. The fibers then turn back and pass

ventrolaterally with DMN axons to exit the medulla. The estimated length of the vagus from the DMN to the myocardium is 165 mm.

Todo injected cats with Type II HRP and sacrificed the animals after 48 hours. Type II HRP has 30% the enzymatic activity of Type VI HRP and 18% the activity of HPOFF HRP. Type II would travel less than 168 mm during the survival period. Thus, the survival time may not have been sufficient for HRP to reach NA somata.

Furthermore, Todo may have been identifying aggregates of endogenous diaminobenzidine staining particles as cardiac vagal preganglionic somata. Such particles have been described in the region of the ala cinerea which includes the DMN and NTS (128,208). The presence of these granules was confirmed in the present study. Dark brown particles distributed bilaterally throughout the DMN and NTS were apparent. The particles were distinctly different in quality from the golden-brown granules appearing in the cytoplasm of HRP labeled somata. Todo did not acknowledge the presence of such granules in his reports nor did he describe qualitative differences between exogenous and endogenous staining material.

Calaresu and Pearce (34) and Gunn and Sevelius (93) monitored heart rate responses to stimulation of a variety of brain stem sites. Short latency bradycardia was produced during NA stimulation, exclusively. The authors concluded

that cardiac vagal preganglionic somata were located in the NA. However, changes in heart rate alone do not necessarily reflect cardiac innervation. Parasympathetic nerve fibers also control cardiac inotropic and dromotropic activity (177). A central organization of cardiac vagal preganglionic somata according to physiological function is an alternative explanation of the results. DMN somata could affect cardiac inotropism while NA cell bodies control cardiac rhythmicity.

Differential organization of the autonomic nervous system has been well described. Different regions of the nervous system control specific myocardial functions. Stimulation of the right hypothalamus (168), medullary vasomotor center (37), stellate ganglion and ventral roots (178,179) produce more rapid heart rates than stimulation of corresponding structures on the left side. Wurster (209) indicated that the right descending pressor pathway of the cat regulates tonic heart rate control while the left pathway has minimal effects on cardiac rhythmicity. Right vagal stimulation produces primarily negative chronotropic responses while left vagal stimulation affects AV nodal conduction (44,98). Selective stimulation of different cardiac sympathetic and parasympathetic nerves as well as thoracic roots produces inotropic responses in specific myocardial regions (164,165,177).

The anatomical data of the present investigation indicates no neuroanatomic correlate with the myocardial

effects of left and right vagal stimulation. The number, distribution (Figs. 7 & 8) and sizes (Fig. 9) of the labeled somata were not significantly different on the two sides of the medulla.

Malone (149) compared cell body sizes of brain stem somata considered to be vagal preganglionic neurons. Sizes of the vagal cell bodies were correlated with the types of muscle innervated. Apparently, large cell bodies innervate skeletal muscle while medium and small somata innervate cardiac and smooth muscle, respectively. Thus, a correlation between size and function of neurons exists.

The differences in the sizes of cardiac vagal pre-ganglionic somata in the DMN and NA and the distinct separation of the 2 cell body groups could be the central neuro-anatomical substrate for a differential organization of the cardiac vagus. The physiological responses of the heart to stimulation of the two nuclei confirmed this hypothesis.

The DMN cell bodies control myocardial contractility while NA somata regulate rhythmicity. Stimulation of the DMN produced dP/dt and SGF decreases but no HR changes (Fig. 11 and Table I). Contractility is an intrinsic myocardial property (73,148,150,185). dP/dt and SGF are measures of contractile force dependant on contractility, preload and afterload. LVEDP and RVEDP increased during DMN stimulation (Fig. 11 & Table I). Preload increases could not produce contractile force decreases unless the heart functions in

the descending limb of the Frank-Starling curve (185,188, 194). The dP/dt values shown in figure 11 and Table I compare well with normal values reported for cats (72). Aortic pressure increases or decreases did not affect dP/dt and SGF in bilaterally vagotomized cats. Ipsilateral vagotomy abolished the responses to DMN stimulation. Therefore, the decreases in contractile force produced by DMN activation are the result of vagally mediated decreases in ventricular contractility.

Stimulation of the NA produced HR decreases and increases in dP/dt , SGF, LVEDP and RVEDP (Fig. 13 & Table II). The alterations in contractile force could be secondary responses to vagally mediated decreases in heart rate. HR decreases increase diastolic filling times and end-diastolic pressure which ultimately produces a contractile force increase (97). Cardiac pacing abolished all responses to NA stimulation. Therefore, the changes in dP/dt , SGF, LVEDP and RVEDP are passive mechanical alterations produced by bradycardia. Since all responses were abolished by ipsilateral vagotomy, the cardiac changes during DMN stimulation were neurally mediated by the vagus.

However, the responses to stimulating a nucleus at one point may not reflect cardiac control by all somata at one nucleus. The DMN and NA may demonstrate cardiotoxic organization. Conceivably, DMN somata at the obex level control ventricular contractility while more rostral cell bodies

regulate heart rate. NA somata at the obex may regulate HR while somata of the rostral NA may affect contractility. Each nucleus was stimulated at the obex level, 1.0 mm rostral and 1.0 mm caudal to the obex. There were no significant differences ($F > 0.05$) in the responses to stimulating at the 3 points, for each nucleus. Thus, the responses shown in figures 11 and 13 characterize myocardial control by all somata of the DMN and NA, respectively.

The stimulating electrodes may have been activating axons or interneurons of a cardiac vagal reflex directly or as the result of current spread. This possibility is excluded for three reasons. First, histological data indicate the electrode tips were located in the DMN or NA (Fig. 14). Second, the electrical currents used for stimulation (0.1-0.15 mA) were within the accepted limits for activating central neural structures with minimal spread (205). Finally, all responses were abolished by vagotomy ipsilateral to the stimulating electrode.

Medullary reflex pathways affecting the cardiac vagus were bilateral. The bradycardia consequent to unilateral carotid sinus nerve stimulation is abolished by bilateral vagotomy (174). Carotid sinus chemoreceptor pathways project bilaterally as do aortic baroreceptor and aortic chemoreceptor pathways (132). The nucleus tractus solitarius (NTS) lies immediately dorsolateral to the DMN and contains the second order neurons of the baroreceptor and chemoreceptor

reflexes (41,49,57,155,158). Calaresu and Pearce (134) produced bradycardia by unilateral electrical stimulation of the NTS. The response was abolished by bilateral vagotomy. Ipsilateral vagotomy abolished the responses to DMN and NA stimulation in the present experiments (Figs. 11 & 13 and Tables I & II). Therefore, electrical currents were activating vagal preganglionic neurons and not afferent components of cardiac vagal reflexes.

Physiological and electrophysiological evidence for the existence of an ascending spinal pathway controlling the cardiac vagus has been described. Quest and Gebber (174) stimulated the sciatic nerves bilaterally while monitoring HR and aortic blood pressure in cats treated with propranolol. Bilateral sciatic nerve stimulation produced tachycardia and inhibited the bradycardia produced by carotid sinus nerve stimulation or physiological activation of the baroreceptors. Iriuchijima and Kumada (112,113) recorded the activity from single units or slips of the cardiac vagal branches in dogs. Stimulation of the peroneal, saphenous or brachial nerves inhibited spontaneous activity. Evoked potentials produced by carotid sinus nerve stimulation were inhibited by simultaneous stimulation of a peripheral nerve. The somatosympathetic reflex is a phenomenon in which somata afferent nerves modulate sympathetic preganglionic activity. The reports by Quest and Gebber and Iriuchijima and Kumada indicate that existence of a somatoparasymphathetic reflex.

The lesion studies of the present investigation localized a spinal pathway of the somatoparasymphathetic reflex. The peroneal nerve was stimulated while monitoring cardiovascular parameters in β -blocked cats before and after placing spinal lesions above the junction of the peroneal nerve and the cord. The peroneal nerve enters the cord at segments L7 through S4 (52). Classical ascending crossed sensory pathways decussate within 3 segments above the point of entry of the afferent fibers into the cord (23,200). The lesions of the present study were placed at L4.

The spinal pathway of the somatoparasymphathetic reflex is located bilaterally in the dorsolateral sulcus region of the spinal cord at L4. Right peroneal nerve stimulation significantly inhibited the negative inotropic and chronotropic responses to right carotid sinus nerve stimulation (Figs. 16,18,20 & Tables IV,V & VI). After making a right DLS lesion, the peroneal nerve still significantly ($P < 0.05$) inhibited the effects of carotid sinus nerve stimulation. However, the inhibition after the lesion was significantly less than before the lesion. These data indicate that the lesion interrupted a portion of the pathway ascending to inhibit the cardiac vagus. After placing a second lesion in the left DLS the responses to stimulating both nerves was not different ($P > 0.05$) from the responses to carotid sinus nerve stimulation. Therefore, the second lesion interrupted the remaining fibers of the pathway.

The pathway does not demonstrate medullary decussation. To demonstrate this characteristic, cats were β -blocked and the left vagus was sectioned. Thus, the right vagus was the only efferent nerve capable of modulating cardiac function. Previous studies showed that right peroneal nerve stimulation activates the pathway on both sides of the cord (Figs. 12,18 & 20 and Tables IV,V & VI). It is reasoned that if medullary decussation takes place, peroneal stimulation would continue to modulate the cardiac vagus after the right limb of the pathway has been sectioned. However, a right DLS lesion abolished the inhibition of the negative chronotropic and inotropic responses to right carotid sinus nerve stimulation by right peroneal nerve stimulation (Figs. 24 & 26 and Tables VII & VIII). Therefore, the intact left limb of the pathway did not affect the right cardiac vagus.

The HRP and medullary stimulation studies localized preganglionic parasympathetic somata regulating HR and contractility in the DMN and NA, respectively. DLS lesions interrupted both HR and chronotropic responses to peroneal nerve stimulation. These data indicate the pathways projecting to the NA and DMN are a relatively discrete bundle of fibers.

The data provided by Quest and Gebber (174) and Iriuchijima and Kumada (112,113) suggest that pathway regulates tonic vagal control of the heart. Peripheral nerve stimulation produced tachycardia and inhibition of

spontaneous activity of cardiac vagal branches. The data in Tables V and VI conflict with these results. Peroneal nerve stimulation did not produce significant HR, dP/dt or SGF changes and interruption of the pathway did not affect control parameters. The conflicting results may be due to the different animal preparations used.

Quest and Gebber stimulated the sciatic nerves while the peroneal nerve was activated in the present study. Both nerves contain Groups I, II, III and IV afferent fibers (121). However, the sciatic nerve is composed of more fibers than the peroneal. Furthermore, Quest and Gebber stimulated bilaterally while unilateral activation was used in the present study. Therefore, spatial summation may be necessary for altering spontaneous cardiac vagal preganglionic activity. However, Quest and Gebber showed the data from a single animal in their report and provided no index of the average responses of all animals studied. In the present study, some cats demonstrated tachycardia during peroneal nerve stimulation. Yet, pooled data from all cats indicated no significant difference from control ($P > 0.05$) during peroneal nerve stimulation.

The conflicting results between this study and the work of Iriuchijima and Kumada may be due to species variations or inappropriate interpretation of the electrophysiological data. Nerve activity does not necessarily reflect the response of the innervated organ. Levy (142) showed

heart rate responses to vagal stimulation depend on the time during the cardiac cycle at which the nerve impulses reach the SA node. The response of organs to nerve stimulation depend on temporal and spatial summation (200). Thus, the discharge of the vagus during peripheral nerve stimulation may not produce sufficient temporal and spatial summation in the heart to induce functional alterations.

The locations of the pathways compare well with the location of ascending spinal pathways involved in blood pressure regulation (48,121,180,181). Chung and Wurster (41) used a similar animal preparation to localize the ascending pressor pathway. The blood pressure data shown on Table V compares well with their results. Unilateral stimulation of the peroneal nerve produced a pressor response which was significantly attenuated by making a right DLS lesion. The response to peroneal nerve stimulation was converted to a slight depressor response after making bilateral DLS lesions. The fibers of the ascending pressor pathway and the pathway involved in cardiac vagal control may be identical.

The pathway controlling the cardiac vagus may be identical to the tract of Lissaur which ascends in the spinal gray at the apex of the dorsal horn (23,200). The lesion sites (Figs. 21,22 & 27) were too extensive to determine whether the ascending pathways for cardiac vagal control are confined to the spinal gray or are diffusely organized in the white matter of the posterior columns and dorsolateral

funiculus.

Since the lesions extended into the dorsolateral funiculus and posterior columns, the pathway may be a component of classical posterior columns and dorsospinocerebellar pathways (23,200).

The close approximation of the pathway with the dorsospinocerebellar pathway suggests the possibility that the pathway courses to the cerebellum to regulate neuronal populations involved in vagal cardiac control. Stimulation of the fastigial nucleus produces tachycardia due to vagal inhibition (64). The pathway may exert vagal inhibition by activation of neurons of the fastigial nucleus.

Speculations regarding baroreceptor function and differential control of the heart can be made from the results of the present investigation. Several studies have demonstrated a progressive decrease in baroreceptor reflex sensitivity with increasing exercise levels. The slope of the regression line relating heart rate to blood pressure decreases as moderate exercise increases (16,21,169). The reflex bradycardia in resting conscious dogs to methoxamine induced hypertension is abolished during severe exercise (152).

Studies carried out by Gebber (80,81) suggest activated central suprobulbar centers depress the reflex with exercise. However, the somatoparasympathetic reflex may regulate baroreceptor activation of vagal preganglionic

neurons as well. Conceivably, impulses elicited from exercising limbs and muscles could reflexly activate the ascending spinal pathways and inhibit the baroreceptor induced activation of cardiac vagal neurons.

In addition, the data provides evidence for a central cardiotoxic organization of the cardiac vagus. NA somata regulate cardiac rhythmicity while cell bodies of the DMN affect contractility. Thus, the anatomy and physiology exists for specific activation of "chronotropic" neurons or "inotropic" neurons by different reflexes or different central neural centers.

CHAPTER VI

CONCLUSIONS

The characteristics of cardiac vagal central organization are illustrated in figure 28 and are described as follows:

1. The anatomy of cardiac vagal preganglionic somata giving rise to axons passing in the left and right vagi are similar.

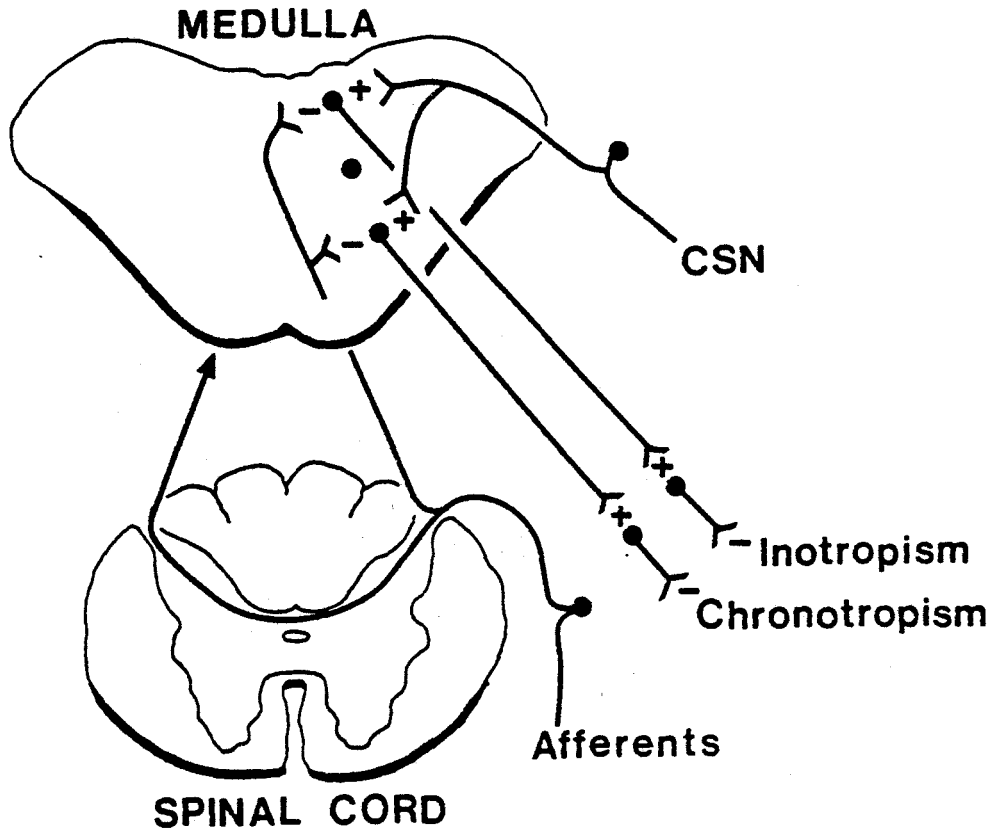
2. The cell bodies are located in 3 medullary regions, ipsilateral to the vagus carrying their axons: the dorsal motor nucleus of the vagus (DMN), the nucleus ambiguus (NA) and an intermediate zone (IZ) between the DMN and NA.

3. The NA contain the maximum number of cardiac vagal preganglionic cell bodies while successively fewer somata are located in the DMN and IZ.

4. NA somata demonstrate a nonuniform distribution along the longitudinal neuraxis while DMN and IZ cell bodies are uniformly distributed.

5. The long and short axes of NA somata are larger than corresponding dimensions of cell bodies in the DMN or IZ. The dimensions of somata in the DMN and IZ are similar.

SCHEMATIC DRAWING OF CARDIAC VAGAL PREGANGLIONIC
SOMATA AND CARDIAC VAGAL SPINAL PATHWAYS



See text for details.

6. DMN somata have the capacity to control ventricular contractility while NA somata are capable of controlling heart rate.

7. Right carotid sinus nerve stimulation produces vagally mediated negative inotropic and chronotropic responses. Responses to right carotid sinus nerve stimulation are inhibited by simultaneous right peroneal nerve stimulation.

8. The spinal pathway mediating the responses to right peroneal nerve stimulation is located bilaterally in the dorsolateral sulcus (DLS) region of the cord at L4. The pathway decussates below L4.

9. The 2 limbs of the pathway ascend to inhibit somata of the ipsilateral NA and DMN. Medullary decussation of the pathway does not occur.

10. The spinal pathway does not control tonic heart rate and ventricular contractility.

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The final copies have been examined by the director of the dissertation and the signature which appears below certifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 23, 1979
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