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**A STUDY OF THE DORSAL VAGAL NUCLEUS
IN RELATION TO VAGAL BRADYCARDIA**

by

Joeseeph Ponessa

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

January

1969

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BIOGRAPHY

Joseph T. Ponessa was born on September 10, 1941, in New York City. There he attended St. Francis Preparatory High School and St. Francis College, receiving the Bachelor of Science degree in Chemistry in 1962.

A course of study was then undertaken as a graduate teaching assistant at the University of North Dakota Medical School at Grand Forks, leading to the degree of Master of Science in Physiology. In September of 1964, he enrolled in the Physiology Department at Stritch School of Medicine, working under the direction of Dr. Clarence N. Peiss, and supported by an PHS Predoctoral Traineeship.

In November of 1965, he was married to the former Joan Doerschling, a graduate of this Department, and they now have two daughters.

PUBLICATIONS

1. PONESSA, J.T. and B. de Boer. The effect of reserpine and syrotingopine on pentobarbital depression. Arch. internat. pharmacodyn. 164: 67, 1966.
2. PONESSA, J.T. and C.N. Peiss. Role of the dorsal motor nucleus in vagal bradycardia. Fed. Proc. 26: 717, 1967 (abstract).

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CHAPTER I

INTRODUCTION: STATEMENT OF PROBLEM

According to most texts of neuroanatomy and physiology, the efferent vagal fibers which supply the heart arise from cell bodies located in the dorsal vagal nucleus (DVN) in the medulla oblongata. This concept however, has recently been questioned. Calaresu and associates (1965; 1965a and b) reported that discrete stimulation of DVN is often not effective in producing cardiac inhibition. Furthermore, electrical recording from this nucleus showed a relatively small number of cells with discharge patterns similar to those expected of vagal cardiac fibers, and study of degeneration in vagal cardiac branches following DVN lesions showed only a very small number of degenerating fibers. They noted the possibility that characteristic vagal cardiac effects might be mediated by a relatively small number of fibers, but made the alternative suggestion that DVN may indeed be secondary to another, principal nucleus involved in vagal cardiac inhibition. Gunn and associates (1965 and 1968) contended that in the cat (but not in the dog) DVN is not at all related to vagal cardiac fibers. Their proposal concurred with a report by Kerr (1967) who cauterized DVN in the cat and after an appropriate recovery period which would allow for degeneration,

showed that stimulation of the ipsilateral cervical vagus still was capable of producing bradycardia.

One basis for the general acceptance of the role of DVN as the origin of vagal cardiac fibers is the relative ease with which vagally mediated cardiac effects can be obtained by electrical stimulation of this area in the brain stem. However, a cursory examination of the anatomy in the region of DVN will show that nearby nuclei and tracts (notably the nucleus and tractus solitarius, NTS) might also account for reduction in heart rate, at least ultimately mediated by the vagus. Calaresu and Pearce (1965b) inferred that bradycardia evoked from stimulation in the region of DVN is actually accomplished through stimulation of NTS, and their stimulation studies led Gunn and associates (1965 and 1968) to conclude that, in the cat, the nucleus ambiguus (NA) is the origin of vagal cardio-motor fibers.

The studies described above, especially the discrete stimulations by Calaresu's group (1965b), are quite provocative and indeed seriously question the "textbook concepts" of the role of DVN. Nevertheless, the evidence given is by no means conclusive, and must certainly be evaluated with some consideration of the numerous studies over the past 150 years which support or disclaim the role of DVN.

The purpose of this study is to obtain additional information regarding the role of DVN in relation to vagal cardiac outflow, using an approach somewhat different than the simple stimulation, recording or degeneration studies that have

been conducted previously.

It was thought that if the bradycardia elicited by stimulation in the region of DVN is actually due to activation of the nearby NTS rather than the final common efferent pathway, functional studies might be useful in differentiating the response to stimulation in the region of DVN from the response to stimulation of the final common pathway. It seems not unreasonable to predict that, if one or more synapses are interposed between a stimulating electrode and the vagal preganglionic fiber, imposed changes in systemic blood pressure, as well as variation in stimulation parameters and technique, should be manifest at these synapses as facilitation, occlusion or fatigue. If, on the other hand, central synapses distal to the stimulating electrode are not present, response to stimulation in the region of DVN should be quantitatively and qualitatively similar to those elicited from the emergent vagal preganglionics.

It is with these basic considerations that the following experiments were undertaken.

CHAPTER II

REVIEW OF LITERATURE

General Anatomical and Embryological Considerations:

A. Vagal Nuclei; Topography and Cytology.

It is generally understood that the vagus nerve has components associated with three nuclei in the brain stem: the dorsal nucleus (DVN), which corresponds to the general visceral efferent column; the nucleus ambiguus (NA), which is part of the special visceral efferent column; and the nucleus and tractus solitarius (NTS), which is classified as part of the visceral sensory column.

The DVN is positioned in the dorsal medulla, just beneath the floor of the fourth ventricle, lateral and somewhat dorsal to the hypoglossal nucleus but medial to NTS. In the cat, DVN extends for a length of about 4.2 mm (Taber, 1961) for the most part rostral to the level of the obex. Histological examination of the brain stem reveals that the cell bodies of DVN are smaller than the somatic efferent cells of the hypoglossal nucleus, and also smaller than the cells of NA. Taber (1961) described three cell types in the cat DVN, of small, intermediate and medium size. The latter are dispersed throughout the nucleus while the intermediate sized cells are said to be more abundant in the middle region of the nucleus.

Kimmel (1940) observed two cell types in the adult rabbit DVN, both smaller than cells in NA.

Malone (1913), studying the brain stem of the monkey and lemur, has pointed out this size distinction, and classified two cell types in DVN; smaller cells at the rostral and caudal poles of this nucleus, and relatively large cells in its middle portion. Partly on the basis of Molhant's earlier (1912) statement that the large cells of NA supply striated muscle exclusively, Malone (1913) reasoned that the smallest DVN cells must supply fibers to smooth muscle while the larger DVN cells (which are smaller than the smallest cells in NA) give rise to vagal cardiac fibers. On this basis, Malone (1913) designated the "nucleus cardiacus nervi vagi" within DVN. It should be emphasized, however, that on the basis of evidence presented in his report, this nuclear specification is little more than speculation. Mitchell and Warwick (1955) furthermore were unable to confirm the existence of this circumscribed intermediate zone in the rhesus monkey.

Etemadi (1961) made quantitative measurements of these nuclei in human brains, and reported that there are about five times as many cells in DVN as in NA. He also measured cellular dimensions and calculated that the total cross sectional area of all cells in DVN is approximately 1 mm^2 , compared to a total cellular area of 0.6 mm^2 in NA. On the average then, a DVN cell in the human should be approximately one-third the diameter of a cell in NA.

The NTS is lateral to and approximately coextensive with DVN. Taber (1961) stated that the cat NTS is composed

primarily of small and medium sized cells, with a small number of large cells scattered throughout. The solitary nuclei on either side of the brain stem are contiguous with each other at the level of the obex just dorsal to the central canal; the connection is regularly described as the commissural nucleus of Cajal. Several investigators (Kimmel, Kimmel and Zarkin, 1961; Kerr, 1962; Cottle, 1964 and Rhoton, O'Leary and Ferguson, 1966) have described, on the basis of degeneration studies, afferent vagal as well as glossopharyngeal contributions to the commissural nucleus and contralateral solitary complex. Anderson and Berry (1956) reported similar connections on the basis of intramedullary electrical recordings after central vagal stimulation in the cat.

Mitchell and Warwick (1955) have described a small number of chromatolytic changes in DVN after section of the contralateral vagus and suggested that vagal fibers (presumably motor) decussate at least to a small extent.

B. Vagal Nuclei; Embryology.

Because of certain anatomical features of the vagal nuclei, in the context of their electrical stimulation, it is useful to consider briefly their embryological development.

Kimmel (1940) described the formation of the rabbit's hypoglossal and vagal nuclei from a primitive efferent cell column, whose medial cells give rise to the nucleus of the hypoglossal and whose lateral cells form the column which will become DVN. Mitchell and Warwick (1955), on the basis of degeneration studies, stated that the hypoglossal nucleus in the monkey shows no chromatolytic changes after vagisection at

various levels below the nodose ganglion and therefore makes no contribution to the vagus. However, chromatolytic changes were observed in cells located between DVN and the hypoglossal nucleus.

Of great pertinence to this study is the arrangement of medullary fibers originating from NA. Kimmel (1940), studying development of the rabbit, and Windle (1933) using the cat, have described the establishment of NA. The latter investigator showed that NA is composed of cells which have migrated from the region of the developing DVN column in a ventrolateral direction. Their axons emerge from the medulla together with DVN fibers, and this cell body migration thus results in a genu which is evident in the 11.5 mm embryo. The final position of NA is in and dorsolateral to the lateral longitudinal bundle of the reticular formation. Windle could not state positively that this genu persists in the adult cat, but felt that the presence of at least some remnants of it is a likely possibility. The presence of this genu in the adult is shown in a number of textbooks (Crosby, Humphrey and Lauer, 1961; Ranson and Clark, 1959).

It is known that visceral afferent fibers from the facial, glossopharyngeal and vagus nerves enter the solitary tract (Ranson and Clark, 1959). Kimmel (1941) described the embryology of these afferent connections in the rabbit, and pointed out that such connections are first established (in the 11 day embryo) with the longitudinal sensory bundle which subsequently develops into the solitary fasciculus and the spinal root of the trigeminal. He stated that a proportion of fibers from the IXth and Xth nerves enter the latter structure,

as well as other structures such as nucleus intercalatus. Indeed, he even suggested that "...all of the motor nuclei of the brain stem may be functionally related with the nucleus of the mesencephalic root of the trigeminal nerve through cell processes which course to motor nuclei...". Kimmel and associates later (1961) described the vagal and facial afferent contribution to the solitary tract in the guinea pig, as well as these nerves' connections with the spinal cord, spinal trigeminal nucleus, reticular formation and commissural nucleus. Wilson, Windle and Fitzgerald (1941) studied NTS development in the cat. In addition to their general agreement with Kimmel (1941) they also stated that a few spinal accessory fibers, as well as some trigeminal afferents contribute to the solitary tract, the latter perhaps mediating visceral afferent impulses. Wilson's group also mentioned an earlier study showing connections between the maxillary and mandibular branches of the trigeminal and rostral portions of NTS in the 25 day sheep. These fibers might possibly mediate taste, but this is uncertain. Wilson and associates (1941) also described a "fanning out" of NTS fibers towards the region of the medial motor cell column, and a decussation process in the formation of the commissural nucleus.

C. Vagal Nuclei; Functional Considerations.

Although certain cranial nuclei appear to be commonly derived, regularly aligned and functionally ordered so that common designations (e.g. "general somatic efferent column") have been widely used, there is at the same time the likelihood of and developmental framework for functional

mixing. Thus it is probably incorrect to classify even a single nucleus as having only one functional type of fiber. Functional relations will be considered briefly here.

Although most texts designate DVN as mainly supplying efferent fibers to thoracic and abdominal viscera, this basic concept has been seriously questioned as recently as 1952 when Szentagothai reported that partial or complete destruction of DVN in the cat failed to produce any degeneration in the cervical vagus. Cottle and Mitchell (1966) have recently stated that, unlike the situation in the central nervous system, the postoperative survival time for demonstration of degeneration in a peripheral nerve is quite critical. Although there have been similar reports prior to that of Szentagothai, his conclusion that DVN is a purely sensory nucleus finds no support in the modern literature.

Mitchell and Warwick (1955) studied degeneration in the brain stem of the monkey following extracranial vagi-section at various levels below the nodose ganglion and concluded that DVN is a mixed nucleus, having smaller, presumably secondary sensory cells along its lateral margin. In some animals these cells almost formed a separate column. Their statement that some of the smaller cells in or immediately lateral to DVN were sensory was based on the fact that these cells "...always...show no or only equivocal changes after section of the vagus nerves.", and is thus evidence by default. This position was contested by Cottle (1964) who performed Nauta studies in the cat after supra-nodose vagotomy or intracranial section of rootlets of the ninth and tenth nerves.

The findings of Rhoton, O'Leary and Ferguson (1966)

are also at variance with the conclusion of Mitchell and Warwick (1955). Rhoton's group studied the afferent connections of various cranial nerves in the monkey by means of supranodose vagotomy but observed no degenerated fibers going to DVN.

Kerr (1962) reported the same type of experiment in the cat and he also failed to find evidence of primary afferent vagal (or glossopharyngeal) connections to DVN. These findings suggest that most, or all reflexes involving DVN are mediated by means of a multisynaptic path; Szentagothai had earlier (1948) made this claim for all vegetative spinal and brain stem reflexes. Other findings of these workers (Kerr, Cottle and Rhoton's group) are substantially in agreement; they have shown that most of the glossopharyngeal and vagal afferents form the solitary tract, and Rhoton and associates state:

"It seems probable that the portion of the solitary tract which extends caudally from the entrance of IX and X mediates general visceral sensibility and forms the afferent limb for visceral reflexes, such as those involved in cardiorespiratory control."

Both Rhoton's group (1966) and Kerr (1962) concur regarding numerous other details pertaining to the afferent connections of the ninth and tenth nerves. Degenerating afferents have been traced to the dorsal horn at C1 and C2, by way of the spinal trigeminal tract. Afferents have also been found in the contralateral NTS via the commissural nucleus, and in the medial and lateral cuneate nuclei. Both studies showed that a relatively small number of fibers can

be traced to the reticular formation. Kerr (1962), in a single experiment in which degeneration was studied after section between the nodose and jugular ganglion, saw degeneration only in NTS and concluded that the nodose ganglion is of prime importance for visceral sensation.

The above discussion indicates that the tractus solitarius is mainly composed of visceral sensory fibers of the ninth and tenth nerves. The role of these nerves in cardiovascular reflexes and the proximity of NTS to DVN makes obvious the importance of NTS in the context of the stimulation experiments described below. There have been numerous studies to determine the contribution of other afferent nerves to NTS, and some of these will be considered here and also in the discussion. Torvik (1956) made a thorough study of afferent contributions to NTS and the trigeminal sensory nuclei in the rat, and provided an extensive review of earlier literature in this area. He, too, reported numerous sensory fibers from the mandibular and maxillary branches of the trigeminal ending in the solitary tract as well as a few going to the dorsal reticular formation. A small number of afferents from the facial, glossopharyngeal and vagus nerves were reported to terminate in the dorsal reticular formation, and afferent connections between the two last mentioned nerves and the spinal nucleus of the fifth nerve were confirmed. Degeneration of sensory components of the trigeminal have been observed in the dorsal horn at levels as low as C4. In addition, a small number of dorsal root fibers from C1 to C3 have been traced to NTS. Torvik (1956) concluded that it is not proper to distinguish sharp boundaries between peripheral visceral and

somatic structures since there probably exist transitional structures which serve both visceral and somatic afferent functions.

The role of NTS in cardiovascular reflexes is supported by a number of physiologic studies. Anderson and Berry (1956) recorded from the region of NTS in the cat after stimulation of the central end of the cut vagus and on the basis of latency measurements described three conduction velocities in NTS. The relative complexity of recorded electrical activity here and its temporal spread, especially in the caudal parts of NTS, led to the conclusion that secondary or higher order fibers descend in the tract along with primary sensory fibers. There were similar findings following afferent stimulation of the aortic depressor nerve. Koepchen and associates (1967), and Crill and Reis (1968) presented additional evidence supporting the role of NTS in cardiovascular reflexes. The latter workers proposed parallel afferent cardiovascular paths in NTS, one monosynaptic and the other polysynaptic. All of these authors suggested the possibility that reticular formation fibers are involved in cardiovascular control. Anderson and Berry (1956) listed numerous medullary structures that showed evoked responses after central vagal stimulation including NA, the contralateral DVN and the spinal tract and nucleus of the trigeminal; in the latter structure, they felt that the observed activity was representative of fiber terminations, rather than fibers simply passing through the trigeminal nucleus.

With regard to NA, this structure is commonly regarded as the origin of specialized motor fibers of the

ninth, tenth and eleventh nerves which supply some muscles of the pharynx and larynx. On the basis of degeneration and stimulation studies, Furstenberg and Magielski (1955) have even described a motor pattern in this nucleus, whereby specific regions of it are associated with specific muscle groups. However, Gunn and associates (1968) have proposed that, in the cat, NA is the principal source of preganglionic vagal fibers to the heart. Their work will be discussed in detail below.

D. The Peripheral Vagus; Fiber Population and Type.

There have been numerous histologic and physiologic studies to characterize the fiber population in the cervical vagus and to determine the type of vagal fiber(s) acting on the heart. It is generally agreed that the vagus nerve is largely (most investigators estimate about 75%) composed of afferent fibers (Foley and DuBois, 1937; Middleton, Middleton and Grundfest, 1950; Daly and Evans, 1953; Evans and Murray, 1954; Agostoni et al., 1957 and Hoffman and Kuntz, 1957). Furthermore, most investigators agree that the majority of vagal fibers are unmyelinated. These experiments have mainly involved chronic preparations (cats and rabbits) with transection above or below the nodose ganglion, subsequent histologic analysis and, in some cases, physiologic testing. Regarding myelin, it should be recalled, as Hoffman and Kuntz (1957) point out, that a myelinated fiber may become divested of its covering during the course of its travel, so that myelin may be lacking in more distal parts of that fiber.

Various authors have counted the number of fibers in

the vagus nerve. Foley and DuBois (1937) reported that they counted approximately 36,000 fibers just distal to the jugular ganglion in the cat. They claimed that 7-10,000 of these are motor fibers, while the majority of vagal fibers are sensory. Agostoni and associates (1957) reported a total of about 30,000 vagal fibers in the cat, of which approximately 6,000 were described as having motor functions. Individual variations in fiber counts, as well as the different values reported by various investigators, may be in part due to branching of nonmyelinated fibers in the vagus, as Heinbecker and O'Leary (1933) suggested. This factor is emphasized by Hoffman and Kuntz (1957) who have shown, in individual specimens, that the proportion of unmyelinated fibers in the cervical vagus is progressively increased at sites more distal to the nodose ganglion.

There have been numerous attempts to characterize those fibers which constitute the motor innervation of the heart. Heinbecker and associates conducted a number of studies to determine the nature of vagal cardiac efferents. In general, they stimulated the vagus nerve at various intensities, recording both cardiac effects and the propagated impulse from the vagus trunk at a site removed from the stimulation point in order to determine the fiber types participating in the response. In 1930, Heinbecker described six maxima in the neurogram from the cat vagus; the B2 peak was attributed to small diameter, thinly myelinated fibers, and the C wave was identified with unmyelinated fibers. The several peaks designated in the C group are of questionable meaning. In the same year, Bishop and Heinbecker (1930) in

a study of cold-blooded animals, as well as the rabbit, cat and dog, described four fiber types, designating them as A, B₁, B₂ and C, and inferred that the latter two groups mediated autonomic efferent activity. In addition, information on their relative thresholds suggested that the smaller B₂ and C fibers were less sensitive than the larger fibers by a factor of four. In a later study of the vagus in the frog and turtle, Heinbecker (1931) demonstrated a thin, myelinated fiber (B₃) which had a negative inotropic effect on the atria with no effect on heart rate. Stronger stimulation, which gave rise to the C component of the neurogram, was associated with bradycardia or cardiac arrest.

Experiments with cats and rabbits (Heinbecker and Bishop, 1935-6) under ether anesthesia showed that, unlike the turtle and frog, the B₂ component was associated with the largest part of the rate effect; an increase in stimulus intensity to activate C fibers enhanced the bradycardia response by no more than 10 per cent. In this situation it is difficult to assess the actual role of C fibers in relation to the smallest members of the B group.

In a histological study of the vagus and its smallest branches in the cat, rabbit and dog, Heinbecker and O'Leary (1933) showed that the cardiac branches of the vagus are largely composed of nonmyelinated fibers and small (less than 7 μ) myelinated fibers. Supranodose vagotomy 20 days prior to physiological testing and histological examination abolished vagal bradycardia and resulted in a "small but appreciable loss of myelinated fibers" in cardiac vagal branches. They found that a large number of nonmyelinated

fibers persist, but in relation to these, state that "The lack of definite areas of degeneration [within the cardiac branches of the vagus] does not permit a definite statement as to the number of fibers that are removed." Heinbecker and O'Leary (1933) did, however, observe degeneration of small myelinated and nonmyelinated fibers from a restricted area of the cervical vagus, which they regard as evidence consistent with the earlier proposal (Heinbecker, 1931) that both fiber types have efferent cardiac functions.

In their study of 1933, Heinbecker and O'Leary claimed that myelinated motor fibers to the bronchi and duodenum persist after supranodose vagotomy and suggested that cell bodies of these fibers must be located, without synapse, in the nodose ganglion. Numerous investigators have since disputed this claim (see, for example, Agostoni et al., 1957) and although Heinbecker and O'Leary (1933) stated that cervical sympathetics contribute few, if any, nonmyelinated fibers to the vagus, Middleton, Middleton and Grundfest (1950) suggested that these results might well be due to sympathetic fibers in the vagus.

Middleton, Middleton and Grundfest (1950) have confirmed the finding that vagal cardiac activity is largely mediated by B fibers in the cat. They also identified a small delta component between the A and B neurogram waves, and suggested that this might also have a slight chronotropic effect, while fibers of the C group are apparently without cardiac effect. Since prior supranodose vagotomy (which abolished the cardiac response to vagal stimulation) had no appreciable effect on the neurogram, it was proposed that

motor fibers were relatively small in number. These authors also pointed out the unusual individual variations sometimes observed; in a few cases, there was no cardiac effect in spite of a large B elevation, while in other animals, strong cardiac effects were seen with relatively weak vagal stimulation.

Hoffman and Kuntz (1957) stated that in the cat the vagal cardiac rami are composed of myelinated (smaller than 7 micra) and unmyelinated fibers (relatively few of these greater than 7 micra). Daly and Evans (1953) have done detailed anatomical and functional studies in the cat; they reported that although prior supranodose vagotomy does result in degeneration of small myelinated and nonmyelinated fibers in the vagus and its bronchial branches there is "...no obvious reduction in the number of myelinated fibers" in the cardiac branches. Vagal stimulation under these circumstances was, as expected, without cardiac effects. Evans and Murray (1957) conducted similar studies in the rabbit, and concluded that cardiac afferent and efferent activity was mediated by myelinated and nonmyelinated fibers. After supranodose vagotomy however, some cardiac branches showed no degeneration while in other branches all or nearly all of the myelinated fibers degenerated.

Agostoni and co-workers (1957) obtained more quantitative information in the cat. They stated that all large vagal fibers (greater than 12 micra) and about half of those less than 6 micra are efferent; these together were said to constitute 20 per cent of the fiber population in the vagus. A total of 3000 fibers was counted in the vagal cardiac branches on one side of the cat. On the basis of degenerative

section, the number of efferent vagal cardiac fibers was set at 500, and these were described as predominantly nonmyelinated. Approximately 600 myelinated fibers (of 12 micra or less) are present in these branches and almost all of these are afferent. Douglas and Ritchie (1962) have reviewed the literature dealing with C fibers and state that, in the vagus, most of these are afferents.

It is not intended to present here an extensive critical analysis of the above findings. However, the discrepancy in these various studies is unusual in light of current teaching (Patton, 1965) that autonomic preganglionic fibers are of the B type. There are several factors which may explain the diversity in description of vagal cardiac efferents. Foley and DuBois (1937) pointed out the difficulty in histologic examination of small, unmyelinated fibers, and such technical considerations may especially influence earlier studies, particularly when relatively small numbers or widely scattered C fibers are involved as in the cardiac branches. Furthermore, as Cottle and Mitchell (1966) pointed out, the time period in degenerative section is much more crucial for peripheral than for central nervous system fibers, and disappearance of myelinated and/or nonmyelinated fibers may have significantly influenced various findings. Axon branching, as well as peripheral loss of myelin, may account for such apparent discrepancies as the claim based mainly on histologic evidence that vagal cardiac fibers are mainly nonmyelinated (Agostoni et al., 1957) and the claim of Middleton's group (1950) that such efferents are of the B group.

In light of all the evidence presented here, it

seems reasonable to propose that both types of fiber might be involved in vagal cardiac control. The possibility of a relatively small number of fibers accounting for the large part of vagal activity remains open, and the methods of the studies mentioned above may have failed to recognize those few fibers of greatest importance.

Development Of Concepts Regarding DVN.

Since the Weber brothers (1845) first demonstrated the effect of the vagus nerves on the heart, there have been innumerable studies to further characterize the action of these nerves. In spite of these investigations, there remain at present many fundamental questions regarding the nature of peripheral vagal activity. Moreover, there is currently a lack of agreement on a most basic aspect of central vagal relationships - that is, the location in the central nervous system of cell bodies of vagal cardiac fibers.

McDowall (1938) and Mitchell and Warwick (1955) reviewed the literature regarding the development of present concepts of central vagal relationships. Except for studies of special pertinence or extraordinary historical interest, much of the material which they have reviewed will not be repeated here. Mitchell and Warwick's (1955) survey can be aptly summarized by pointing out that they not only cited many studies in favor of DVN as the origin of vagal cardiac fibers, but they referred to a number of workers who denied this role of DVN. Moreover, they cited several studies which implicate NA as the origin of these fibers. It is thus seen that the "recent" controversy regarding the roles of DVN and NA in relation to vagal cardiac supply has its origin in disputes dating back to the last century.

Stilling (1843) is said to have first described DVN in relation to the vagus, although he regarded it as a sensory nucleus. Deiters (1865) and later Dees (1889) stated that NA, as well as DVN, gives rise to vagal fibers. Laborde

(1888), according to Gunn and associates (1968), identified NA as the origin of vagal cardiac fibers in the cat and dog by means of needle puncture stimulations. Marinesco (1897) suggested that NA supplies fibers to striated muscle and DVN supplies non-striated muscle. The observations of Malone (1913-14) and his conclusion that medium sized cells in DVN supply the heart have been discussed above. His conclusions were largely based on earlier reports such as those of Marinesco (1897) and Molhant (1912) that NA supplied vagal fibers to skeletal muscle, and the statement of vanGehuchten and Molhant (1912) that DVN supplied fine vagal motor fibers to striated muscle of the esophagus and heart in the rabbit.

Among others, Kosaka (1909) and Yagita (1910) have been cited by Mitchell and Warwick (1955) as having claimed that NA gives rise to vagal cardiac preganglionics in the rabbit, dog, monkey and human. Mitchell and Warwick (1955) also listed the many earlier workers who claimed that DVN is primarily a sensory nucleus, but this will not be further discussed here as there is little modern support for this concept.

Miller and Bowman (1916) are among the first to have designated DVN as the origin of cardiac fibers on the basis of electrical stimulation of this structure. Using monopolar electrodes, they reported that in the acute spinal dog, a "minimum effective stimulus" elicited bradycardia from the entire extent of DVN. Ranson and Billingsly (1916) mentioned that, in the cat, cardioinhibition sometimes accompanied pressor responses elicited by electrical stimulation of the apex of the ala cinerea. However, it is not

clear whether the slowing was direct or reflex in nature.

In the last two decades there have been a number of anatomical studies designed to clarify the role of vagal nuclei. Perhaps most intriguing is the study of Getz and Sirnes (1949) who transected the vagus at one of four cervical or thoracic sites in seven rabbits. Subsequent comparison of the varying degrees of degeneration in DVN led them to designate specific regions of the nucleus as related to the vagal innervation of specific structures or regions. A portion of DVN just rostral to its most caudal quarter has been identified as the region giving rise to vagal cardiac fibers, and it corresponds closely to the area described by Malone (1913-14). Kitchell, Stromberg and Davis (1956) have also described regional localization in DVN, consistent for a variety of species, in which thoracic structures are represented in the caudal part of the nucleus. They did not provide specific information in their abstract with regard to the heart. Furstenberg and Magielski (1955) described a similar pattern in NA for laryngeal motor fibers in the monkey.

Mitchell and Warwick (1955), who studied the monkey, stated that although such localization may exist in DVN, there is a fair degree of overlap. Furthermore, they questioned the specification of a cardiac zone in DVN since they observed some degeneration in this general part of DVN even when the vagus was cut below the origin of cardiac branches. Removal of the cardiac plexus produced degeneration in many, but not all cells in this portion of DVN, while degenerative changes attributed to cardiac efferents were also seen in other parts of the nucleus.

Calaresu and Cottle (1965) made small lesions in DVN in 5 cats and noted modest degeneration in the cervical vagus while degeneration in the cardiac branches was slight and was seen in only 2 of these animals. Although they verified a direct connection between DVN and cardiac vagal branches, they concluded that either vagal cardiomotor functions are mediated over a relatively small number of fibers or that DVN plays a relatively minor role in such functions.

Kerr (1967) combined degeneration techniques and electrical stimulation, and described experiments in the cat in which DVN was selectively destroyed by cautery. Following a recovery period to ensure degeneration of fibers arising from this nucleus, it was found that this procedure failed to prevent bradycardia, as well as gastrointestinal and bronchiolar responses to stimulation of the ipsilateral vagus. On this basis, he claimed that DVN is not the source of visceral motor fibers. It may be of significance, however, that in the single record presented in his brief report, a relatively strong (6 volts, 1 msec, 10 cps) stimulation of the distal cut vagus ipsilateral to extensive destruction of DVN only reduced heart rate to about 70 beats per minute. Although Kerr (1967) described this as a normal response, it is our experience that the common Porter electrode will ordinarily produce vagal arrest at 2 to 3 volts unless the nerve is badly deteriorated. Furthermore, his conclusions are based on the presence of this questionable bradycardia, and he has not taken into account the report of Sperti, Midrio and Xamin (1962) who showed that, in the dog, the bulbar root of the accessory nerve contributes a modest bradycardia

component to the vagus. Stimulation of the vagus after prior intracranial section and degeneration of vagal rootlets was still capable of producing some slowing unless the bulbar accessory component had also been cut. Thus it appears possible that the bradycardia which Kerr (1967) demonstrated following degenerative destruction of DVN might actually be due to cardioinhibitory fibers of the eleventh nerve which course in the vagus.

Several recent studies have employed electrical stimulation of DVN and obtained the expected bradycardia; some of these, however, neglect to provide exact evidence of electrode placement (Sperti and Xamin, 1960; Buryak, 1964 and Varma, 1966). Buryak (1964) claimed that electrical stimulation of NA caused cardiac arrhythmias as well as altered coronary circulation. It is unclear whether his statement regarding the latter is based on direct flow measurements, changes in the T wave which he observed, or earlier reports in the literature.

Kovalev and Bondarev (1962/3) conducted discrete stimulation studies of numerous brain stem nuclei in thalamic and bulbar cats using 50 micra unipolar stimulating electrodes. Although they did not present the details of heart rate changes in relation to specific structures and stimulations, it was stated that bradycardia often accompanied evoked pressure changes. In 18 of 20 DVN stimulations, a depressor response resulted. One can only speculate as to the magnitude and importance of cardiac slowing in these responses, if indeed it did occur. Concomitant DVN bradycardia would seem the more likely possibility here, since failure of this nucleus

to elicit slowing would probably have been commented upon.

Chai, Mu and Brobeck (1965) used fairly precise stimulation techniques and reported that stimulation in or near DVN produced bradycardia and sometimes arrest. Their stimulations may have also included NTS, but this is difficult to evaluate. It is surprising that local heating of this region did not produce slowing. Local heating of regions in the lateral reticular formation was effective in this regard while electrical stimulation of these same points was ineffective.

Calaresu and Pearce (1965b) employed precise stimulation techniques using an electrode pair with tip diameters of 20 to 40 micra, spaced about 0.3 mm apart. They were unable to produce bradycardia (and usually failed to obtain evoked potentials in the ipsilateral vagus) after stimulation of DVN. Bradycardia was, however, obtained from stimulation of NTS and was mediated over both vagi. Two areas of NTS were designated effective in the production of bradycardia - one rostral and the other caudal to the obex. Excitation of the former region was accompanied by vagal activity conducted at about 10 to 60 meters per second, which is suggestive of afferent fiber activity. Responses evoked from the caudal parts of NTS were conducted in the vagus at a lower velocity (7 to 35 meters per second) and showed variable latencies suggesting the presence of synapses and possibly discharge in vagal efferent fibers.

In three animals in which NTS stimulation resulted in slowing, stimulation of DVN with extremely strong shocks (up to 30 volts, 2 msec and 200 cps) did not produce a re-

duction in heart rate. These authors suggested that earlier reports of bradycardia evoked by DVN stimulation may have been due to NTS stimulation. It was concluded that either DVN is not the site of origin of cardiomotor fibers or that such fibers are so few in number and/or so widely dispersed in DVN that their stimulation techniques were not adequate to produce bradycardia.

This position was supported by a related study (Calaresu and Pearce, 1965a) to be discussed below, in which unit activity in DVN was measured during reflex bradycardia.

Although it is commonly accepted that the sympathetic system may exert a beat-to-beat regulation on the heart, it does not necessarily follow that such a situation exists in the parasympathetic system. Thus negative evidence (failure to observe DVN activity related to the cardiac cycle) may not have special significance. Indeed, several authors (Green, 1959, Weidinger, Hetzel and Schaefer, 1962, Iriuchijima and Kumada, 1963 and Widdicombe, 1966) have recorded cardiac rhythms at various levels of the vagus from its central end. Calaresu and Pearce (1965a) cautioned that some studies of peripheral vagal branches have failed to evaluate the role of sympathetic fibers also present in these nerves. Green (1959) and Widdicombe (1966) suggested that the fibers firing with the cardiac cycle arise from (carotid) barosensory areas and travel an aberrant course away from the central nervous system. They may be involved in beat-to-beat regulation of the heart by going directly to it from these sensory areas. Humphrey (1966) suggested recently that the complex polysynaptic pathways by which NTS reflexes are probably mediated

tend to obliterate cardiac rhythms in medullary units which are responsive to carotid sinus nerve stimulation.

Gunn and associates (1968) stimulated vagal nuclei using a bipolar 26 gauge needle electrode in the cat (chloralose anesthesia) and dog (chloralose or pentobarbital anesthesia). In 28 cats, each with one or more electrode penetrations in DVN so that its entire length was explored, a 10 second train of stimulation (30 cps, 0.2 msec duration, 1 to 9 volts) in no instance produced bradycardia. In all of these cats, bradycardia was obtained from stimulation in either the NA area or NTS or both. It was stated that NTS stimulation "occasionally" resulted in bradycardia, and that this stimulus-bound slowing was accompanied by a pressor response of 25 mm Hg or more. Stimulation in the region of NA was also effective in producing slowing in the dog, which persisted after ipsilateral vagisection; latency of potentials in the contralateral vagus was about 1.4 msec greater than the latency in the ipsilateral side (about 6.25 msec). Since their stimulation experiments have also shown that bradycardia can be elicited in the dog from DVN, Gunn et al. (1968) concluded that there was an important species difference, with that this nucleus serving as an origin of vagal cardiac fibers in the dog but not in the cat. Regarding stimulation thresholds, they reported the lowest threshold in the NA region, while that for DVN (in the dog) appeared to be slightly higher. Latency for cervical vagal potentials after DVN stimulation ranged from 6.5 to 7.7 msec.

Another line of approach to questions regarding DVN-vagal relationships has been to record electrical activity

in this nucleus and related areas during reflex vagal activity or central vagal stimulation. There are a number of such studies dealing with fibers from barosensory areas which implicate NTS as an important relay point for cardiovascular reflexes (see, for example, the reports of Oberholzer, 1960; Hellner and vonBaumgarten, 1961; Porter, 1963; Humphrey, 1966 and Crill and Reis, 1968).

Anderson and Berry (1956) recorded electrical activity from small regions of the cat medulla after stimulation of the central end of the vagus below the level of the superior laryngeal nerve. They described the presence of evoked activity in the ipsilateral and contralateral DVN as well as in many other structures in the vicinity including the intercalated nucleus, medial longitudinal fasciculus, NTS and the commissural nucleus. From the relative complexity of NTS recordings (especially in its more caudal portions) and the observed latencies it was reasoned that secondary and higher order neurons were involved as well as primary afferents. Furthermore, they reported evoked activity in NA and in the reticular formation.

The work of Anderson and Berry (1956) has been criticized by various investigators on the grounds that their recording method lacked sufficient precision. Porter (1963) conducted a similar study of vagal nuclei using microelectrodes in the Nembutalized or decerebrate cat. He too designated NA as an origin of vagal preganglionics on the basis of the unvarying 2 msec latencies of recordings from this area (similar to those reported by Anderson and Berry in 1956 and ascribed to intramedullary vagal rootlets) and the ability of these

units to follow 300 cps stimulation of the central vagus. Vagal stimulation evoked activity in both DVN and NTS, and the description of NTS was much like that of Anderson and Berry (1956). Porter (1963) was reluctant to claim which of his unit recordings were from DVN, and which were from NTS; recordings in this region were never as stable as those from NA, nor were the latencies as consistent. Most latencies from the DVN-NTS region were in the range of 3 to 10 msec and were sometimes reduced if a stronger stimulus was employed. Most of these units failed to follow a driving frequency greater than 50 cps; others followed 100 cps stimulation and some units could be activated at 250 cps. Porter was unable to determine whether the latter two responses were from primary NTS afferents or antidromically stimulated DVN motor fibers.

It was also reported that vagal stimulation "of sufficient intensity" produced what Porter (1963) termed a "mass response" in NA and NTS. He suggested that this was due to propagated activity in a large number of fibers and/or cell bodies in the region of the recording electrode and was mediated by the fastest conducting fibers in the vagus. Stronger stimulation produced a second wave of activity with a 10 msec latency and a 5 msec duration, but a further increase in stimulus intensity did not produce any other responses. In evaluating this and similar studies, it is important to point out, as did Porter (1963), that because of the distance involved, activity in the slowest-conducting fibers may be expected to be attenuated and temporally dispersed and thus not appear as "mass potentials".

It is curious that Porter (1963) was unable to obtain

any evoked responses in the medulla following stimulation of the abdominal branches of the vagus even at intensities adequate to stimulate C fibers. Aside from the unlikely possibility of damage to the preparation, one might speculate that either none of the above-mentioned nuclei are involved with abdominal visceral afferents (or motor fibers) or that some aspect of his methodology was inadequate to his purpose. Perhaps the simple statistical considerations of stimulation of a relatively small group of fibers in an abdominal vagal branch while recording from a discrete brain stem locus prevented the observation of activity which was in fact evoked in the medulla. This would be especially true if, by virtue of vagal fiber branching, a peripheral fiber bundle was represented by a smaller number of fibers in the brain stem.

Calaresu and Pearce (1965a) studied electrical activity in the cervical vagus of the cat (anesthetized with chloralose). In 285 single and multifiber preparations they never observed spontaneous potentials related to the cardiac cycle, and in only seven instances did they observe increased activity related to reflex bradycardia. Again, this might reflect a wide range of temporal recruitment. Microelectrode exploration of DVN revealed that, of 49 penetrations, seven showed increased activity during reflex or drug induced bradycardia; however, in six of these cases, the increased activity did not appear until 0.3 to 2.4 seconds after the onset of slowing. The reduced rate usually outlasted this increased activity by 1 to 23 seconds, although maximum discharges approximated peak slowing. On the other hand, activity measured in single or multifiber preparations in the cervical

vagus preceded the onset of bradycardia. These findings were presented in support of the contention of Calaresu and Pearce (1965a and b) that either a very small number of vagal efferents are involved in rate regulation or that DVN is not the major origin of such fibers.

Although it is difficult to explain the time relationships in DVN during reflex bradycardia, it seems reasonable to state that Calaresu and Pearce (1965a) may have overly discounted their findings in DVN. Indeed, if all those units showing increased activity during bradycardia are functionally related to the heart, it would be somewhat surprising if as many as 14 % of the cells of the principal motor nucleus of the vagus are related to cardiac regulation. Of course, the meaningfulness of this figure is questionable since such a small population of DVN cells was tested.

In summary, evidence has been reviewed here to show that on the basis of early as well as modern studies, the role of DVN in relation to vagal cardiac innervation is still open to question. Degeneration studies have not been entirely satisfactory because of technical considerations, as well as complexities related to the admixture of sensory and motor nerves (as well as sympathetic fibers) in the vagus and its branches. Detailed investigations such as that of Getz and Sirnes (1949) have been provocative but of necessity used a small number of animals. It is especially difficult to evaluate the role of individual variations which may be of considerable importance; species differences may be involved and for this reason comparison of studies using different species must be done with regard for this possibility.

Stimulation studies have also failed to provide entirely satisfactory answers for a number of reasons, especially considering the variety of nuclei, tracts etc. which may be involved in bradycardia and which may be affected by stimulating electrodes in the region of DVN or NA. These considerations will be treated in more detail in the discussion section below.

Recording the electrical activity of vagal nuclei is similarly beset with a number of related difficulties, and the information gained from the studies discussed above appears less than satisfactory especially because of the equivocal nature of findings related to DVN. Thus we have attempted to study these nuclei using a somewhat different experimental approach, hoping to capitalize on changes in synaptic fields (subliminal fringe) produced for the most part by various maneuvers to change reflex vagal activity. If such synaptic areas were present "downstream" from the stimulating electrode, these should be manifest by a greater change in response to stimulation, in comparison to a response evoked by an electrode in the final common pathway.

CHAPTER III

METHODS

Animals; Surgical Preparation.

These experiments were performed on 73 cats of both sexes, and of weights ranging from 1.5 to 4 kg. Animals used for stereotaxic work were within the range of 2 to 2.8 kg.

Most animals were premedicated with intramuscular phencyclidine hydrochloride (Sernylan, Parke Davis), 2 mg/kg and, after a period of 20 to 30 minutes, anesthetized with intravenous alpha-chloralose, 40 to 60 mg/kg, dissolved in polyethylene glycol. The injected volume was usually between 1 and 2 ml. Injection of additional amounts of chloralose was rarely indicated. In a few experiments, anesthesia was accomplished with sodium pentobarbital (Nembutal, Abbott) in a dose of 30 to 35 mg/kg by the intraperitoneal or intrathoracic route. Premedication was not used in these animals, and one or more supplemental doses of 5 to 10 mg of pentobarbital were often required. Intravenous decamethonium, 0.1 mg/kg was used when necessary to abolish the skeletal motor activity which sometimes accompanied electrical stimulation of the brain stem. All animals receiving this drug were maintained with artificial respiration.

Surgical preparation included cannulation of the femoral artery and vein, care being taken to minimize trauma to the femoral nerve. The arterial cannula, consisting of polyethylene tubing fitted over a 21 or 22 gauge needle, was passed into the abdominal or thoracic aorta. Blood pressure was recorded using a Statham P 23 Dc transducer in conjunction with a Grass polygraph, model 7 or model 5. The use of an integrating cardiometer (McCook and Peiss, 1959) permitted a direct read-out of heart rate.

A midline incision was made in the neck, and the carotid arteries were freed from the surrounding fascia for a distance of 2 to 3 cm. The vagi were carefully isolated by blunt dissection. Loops of umbilical tape or silk suture material were placed around each vagus to facilitate access to them during the experiment. In some experiments the left vagus was sectioned at this time. The trachea was cannulated and in many animals, artificial ventilation was employed, using a positive pressure respiration pump (Harvard Apparatus). The pump operated at a fixed frequency of 23 strokes per minute; volume of the stroke and exhaust resistance were adjusted so that spontaneous respiration was just abolished and blood pressure remained relatively unchanged from the value observed prior to the initiation of artificial respiration. During the course of the experiment, the lungs were periodically hyperinflated to minimize the atelectasis which ordinarily develops during the course of positive pressure ventilation in the anesthetized subject.

Upon completion of the surgical procedures, the preparation was mounted in a standard cat stereotaxic device

(Kopf or Johnson), the scalp incised and the underlying muscle reflected back. An opening about 1 cm² was made in the caudal portion of the skull, involving the parietal and interparietal bones. Bone wax was routinely used to provide hemostasis and minimize or prevent aspiration of air into opened vascular channels in the bone. The dura was cut and reflected back, and electrodes were passed through the cerebellum into the medulla. In a few experiments the cerebellum was totally or partially removed by suction. In most instances, the electrodes were plunged deep into the substance of the medulla to regions more ventral than those subsequently studied, in order to establish electrode tracts and minimize artifacts due to displacement and/or compression of medullary tissue by the electrodes. The stereotaxic atlas of Snider and Niemer (1961) was used as a guide to electrode placement, and electrode loci was subsequently verified by histological examination.

The electrodes were made in this laboratory and were of the bipolar type. They were constructed of 22 gauge, 4 inch stainless steel hypodermic needles, with an inner core of 0.0142 inch diameter Nichrome V wire (Driver-Harris Company) insulated with formvar except at the tip, which extended approximately 0.5 mm beyond the end of the needle. The inner core of the electrode served as the cathode.

General Stimulation Protocol.

In most experiments, two banks of 3 electrodes each were placed in the right medulla, 1 to 2 mm and 4 to 5 mm lateral to midline, with the electrodes positioned at 11, 13 and 15 mm posterior to the standard interaural reference point. In descriptions to follow, stereotaxic coordinates will be referred to as "LR", "V" and "P" positions indicating respectively right lateral vertical and posterior loci in relation to the stereotaxic interaural zero point.

Unless otherwise noted, stimulation parameters were 20 cycles per second (cps), 0.2 milliseconds (msec.) duration and 2 - 7 volts. Stimulation pulses were monophasic and were delivered by a Grass stimulator (model 34 or SD5). Frequency and duration were regularly checked on a Tektronix 502 oscilloscope, and stimulation voltage was monitored throughout every experiment.

Virtually all stimulations were applied for a period of 5 seconds. However, in those instances in which extreme responses occurred, for example cardiac arrest, profound pressor reaction (rare) or profound skeletal motor activity (rare) stimulation was terminated prior to 5 seconds. During an exploratory type of procedure, or when stimulation had little or no apparent effect on blood pressure or heart rate, a recovery period of about 15 seconds was considered adequate. However, when it was desired to make a quantitative comparison of two stimulations, 1 - 2 minute periods were allowed to elapse between stimulations, at which time blood pressure and heart rate had returned to control levels. When the procedure produced what were considered to be extreme changes in

pressure and/or rate, relatively longer periods were allowed for recovery. To ascertain that such recovery periods were adequate and that quantitative comparisons were at least not affected by fatigue, consecutive stimulations producing a mild to moderate slowing were carried out in a number of animals. It was invariably found that several 5 second stimulations (with constant voltage) produced a bradycardia of consistent magnitude.

The terminal procedure in numerous experiments was to test whether or not the observed bradycardia might be attributed, in whole or in part, to sympathoinhibitory rather than vagal pathways. Thus, stimulation of active points was repeated when neither vagus was intact, sometimes with parameters much higher (eg 10 volts, 2 msec and 20 cps) than those used during the experiment. In no instance was slowing seen.

The animals were routinely sacrificed with an intravenous air embolism, and appropriate lesions were made in the medulla, using the stimulating electrodes and a Grass LM3 radiofrequency lesion maker. The electrode banks were subsequently removed from the medulla by bringing them up in the vertical plane only. Additional portions of the skull and the entire cerebellum were removed, affording good exposure of the medulla, and the electrodes were then brought down to the dorsal surface of the brain stem. Their position was thus macroscopically verified and recorded. The medulla was then carefully removed, fixed in ten percent formalin or Dietrich's solution and subsequently sectioned on the freezing microtome. Most sections were 18 to 30 micra in thickness, and were stained with thionin.

Exploration.

A preliminary study was conducted in which the right medulla was explored in an area from the approximate level of the obex to a point 4 or 5 mm rostral to it (electrodes at P 11, 13 and 15, obex at about P 15) and in vertical planes from the midline to a region 5 mm lateral to it. In all but two cases, exploration was at 1,2,3,4 or 5 mm lateral to the midline, and the single bank of three exploratory electrodes was advanced in the vertical plane in 1 mm steps.

Stimulus intensity was monitored for each stimulus, and maintained constant at 7 volts. Other details of stimulation are as described above.

Eleven animals are included in this series. All five planes were not explored in each animal; the planes at LR 4 and 5 represent four experiments, whereas LR 1 was explored in ten animals of this series.

Bilateral Carotid Occlusion.

Bilateral carotid occlusion (BCO) was applied for a period of 5 to 10 seconds, using bulldog clamps; care was taken to minimize distortion of the arteries as they were pulled from the neck for placement of the clamps. In various experiments, electrical stimulation periods of 5, 6, 10 or 15 seconds were used.

The consecutive "control" and "BCO" stimulations were generally done in the early part of the experiment, with 1 to 2 minute intervals usually allowed for recovery. The stimulation during BCO was not applied until the peak pressure response had occurred, generally 15 to 30 seconds after the start of BCO.

In many cases the procedure was repeated and it was observed that both control and BCO responses were reproducible or qualitatively similar at different stimulus strengths. In some instances, medial and lateral stimulations were repeated at several points in the A-P plane.

Aortic, Vena Caval Occlusion.

This procedure was similar to that for BCO. After electrodes had been positioned in suitably reactive points in the medulla, the chest was opened on the right side and loops of umbilical tape were passed around the descending aorta and posterior vena cava. The phrenic nerve was separated from the latter so as not to include it in the loop, and care was taken to avoid trauma to nerves and other structures in the area. The ends of each loop were passed through glass tubing of suitable length and, when carefully withdrawn through the tubing, produced the desired degree of occlusion. In these experiments, the arterial catheter was introduced well into the thoracic aorta so that its tip was rostral to the level of aortic occlusion.

At the initiation of occlusion, desired pressure levels were ordinarily attained within 5 to 10 seconds, although in some cases the snare had to be continually tightened to counteract reflex changes in pressure towards control levels. Stimulation was applied in the range of 18 to 85 seconds after the onset of pressure change, although in most experiments this interval was in the range of 20 to 35 seconds. Control and recovery stimulations were usually done 1 to 2 minutes before and after the occlusion stimulation.

In all of these experiments, both vagi were intact. In most cases, each procedure was repeated one or more times, sometimes at different voltages and, with few exceptions, results were at least qualitatively the same.

CHAPTER IV

RESULTS

Exploration.

The results of a preliminary exploration series are shown in figure 1. As indicated in the legend, the bradycardia has been classified as follows: a five second stimulation giving a reduction of heart rate by 120 beats per minute or more was designated as a +4 response; a rate reduction of 30 beats per minute was designated as a +1 response, intermediate values were accordingly assigned, and a score of 0 was entered for the absence of a response as well as for those few instances when sympathetic effects were obtained.

The results of each experiment were tabulated and average values were calculated for each stereotaxic point; that is, the bradycardia scores were totaled and then divided by the total number of stimulations at that point. Final average values less than +0.50 were treated as "no response" and are not indicated in figure 1. Values between +2.1 and +3.0 are designated as marked responses, those greater than +0.50 but less than +1.1 are classed as slight responses, and values +1.1 to +2.0 have been termed moderate responses.

STIMULATION POINTS in the CAUDAL MEDULLA

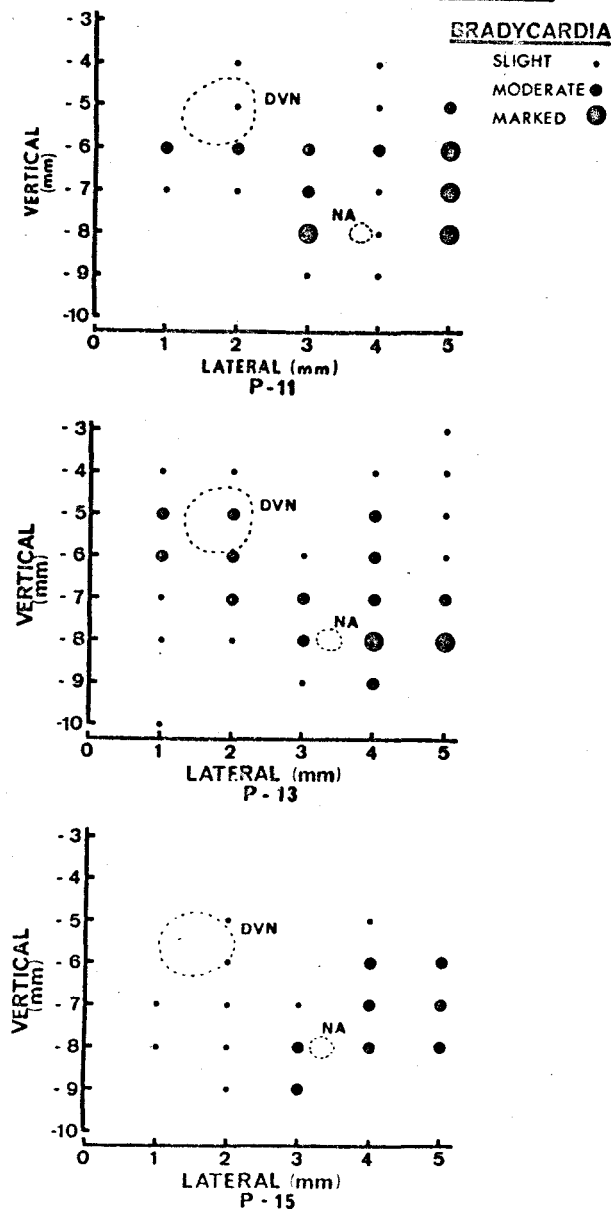


Figure 1. Composite results of exploration of the medulla in a series of 11 cats; P15 corresponds approximately to the level of the obex. The approximate locations of the vagal motor nuclei have been outlined and the relative degrees of bradycardia have been indicated. Intersects in the grid giving no slowing have been left blank.

It is apparent that bradycardia can be elicited from a number of loci in the medulla. The points giving most pronounced bradycardia are localized in the region of NA, although it is possible that this activity may simply represent a convergence of vagal cardiac fibers prior to their exit from the medulla. Some degree of concentration of activity is also apparent in the region of DVN and NTS, especially at Pl3.

It is not possible to say with certainty that these stimulations have activated only nuclear structures and not afferent, as well as efferent fibers. Likewise, it is not possible to accurately define the volume of nervous tissue which is directly stimulated by the electrode; however, the observation that a response can often be obtained from a given locus but not at points 1 mm above, below or lateral to it suggests that the effective volume of a 7 volt, 0.2 msec stimulus may be approximately 1 mm³.

Bradycardia was sometimes obtained in this discrete fashion with 7 volt, 0.2 msec stimulation. Such a case is illustrated in figure 2. Here, after an initial exploration at LR 1 yielding a small number of active points, the region of the single strongly reactive point was reexamined. It can be seen that, although a good response is obtained from approximately the level of DVN (V-5) the response is attenuated when the electrode bank is at V-4 or V-5.5. Stimulus intensity was 6 volts at V-4 and V-5. At the latter site, 7 volts gave a comparable bradycardia, but slowing was negligible at 5.5 volts. Stimulus strength of 7 volts produced the mild slowing indicated at V-5.5, although 8 volts

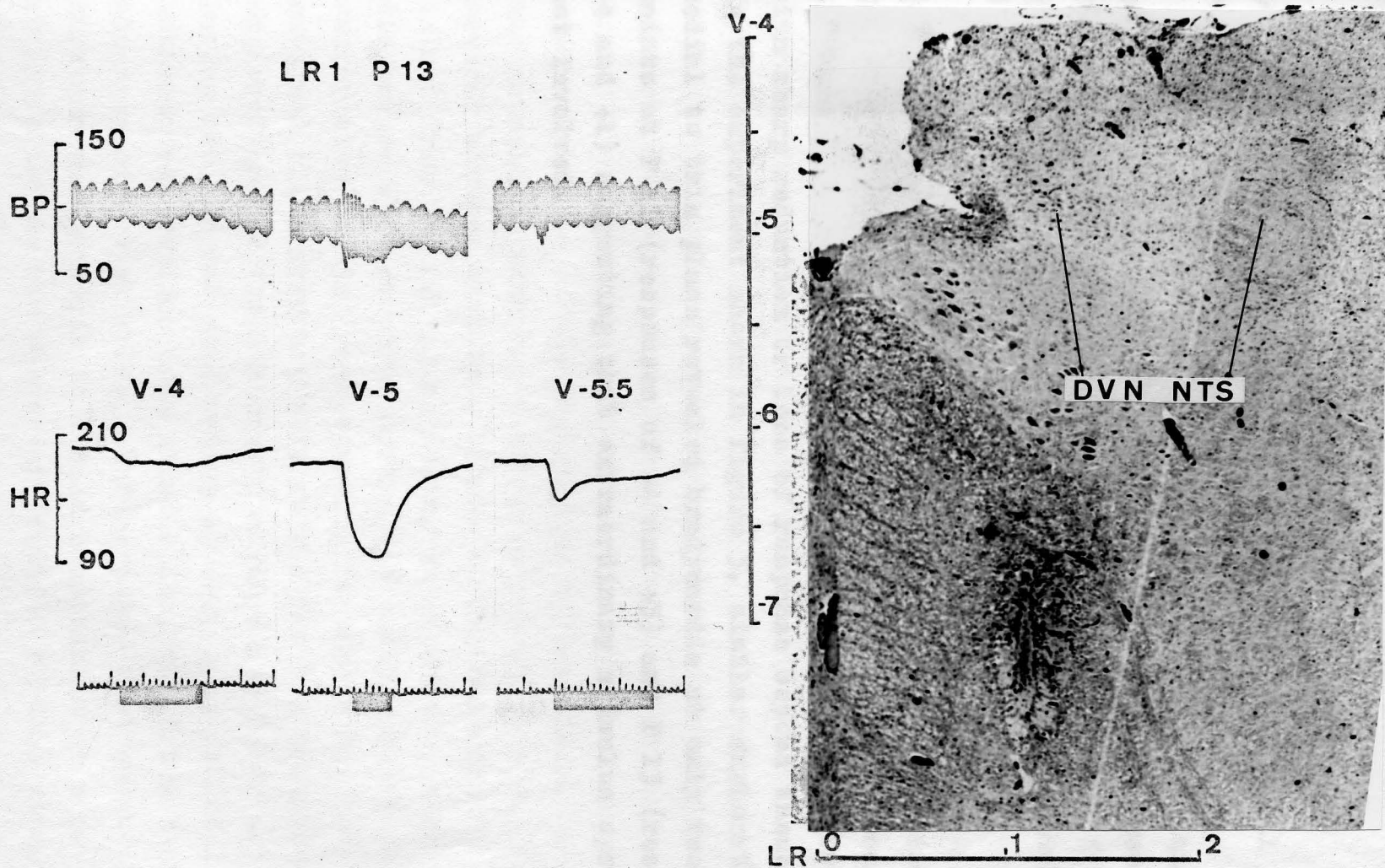


Figure 2. Cat 34, 2.4 kg. Stimulus localization, showing responses at various LR 1 electrode placements in a plane about 1 mm rostral to the obex. Lesion is at LR 1, V-7. Additional details in text.

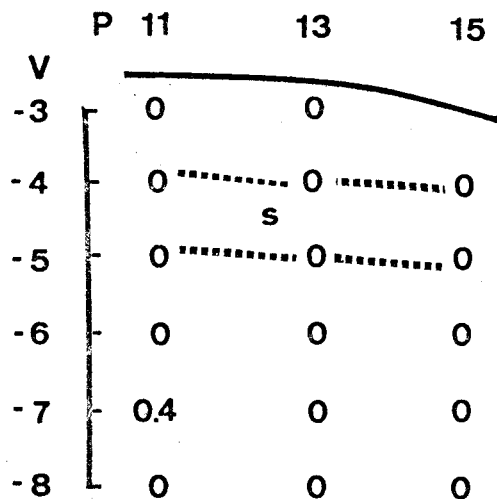
evoked a strong bradycardia. Threshold effects will be discussed in greater detail below.

Because of the elongate shape of the lesion, it is not possible to fix the vertical coordinate with precision, but correlation with measurements made at autopsy of the medulla in situ provides an additional reference point. Accuracy is probably on the order of 0.5 mm.

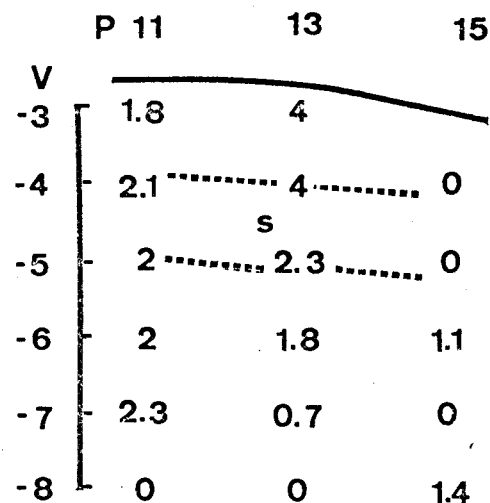
In some experiments localization of reactivity (using a 7 volt stimulus) was less discrete, as shown in figure 3B. Strong bradycardia was more commonly localized to a region of 2 mm (i.e. three points each separated by 1 mm) with sharp reduction or loss of response beyond these points. In the experiment shown in figure 3, similar exploration 1 mm medial to this plane revealed bradycardia at only two vertical points at P 11 (responses of +4 and +3) and P 13 (responses of +4 and +1) suggesting that extraordinary stimulus spread is not involved.

Reversal.

It was observed in some experiments that when the electrode bank was reset to a position from which marked bradycardia had previously been elicited, subsequent stimulation was ineffective; moreover, there were numerous instances when loci that were once unresponsive to stimulation later produced good bradycardia. This phenomenon has been termed "reversal". In some cases this may be due to inexact replacement of the electrodes but in other cases this can be ruled out. Figure 3 shows a pronounced example of this extensive reversal. Figure 3A shows numerous points at LR2 to be unresponsive to a 5 second stimulus of 7 volt, 0.2 msec, 30 cps pulses. The EKG indicated the possibility of hypoxia during the course of spontaneous respiration (depressed S-T segment, inverted T wave) in the course of an earlier exploration of the LR1 plane. The EKG returned to normal, however, within 1 minute of the institution of artificial ventilation and coincident with the start of a sympathetic discharge of several minutes duration. Three strong responses were obtained from the LR1 plane during this period. Some sympathetic discharges also occurred during the early part of the initial exploration of LR2 (corresponding to figure 3A) but heart rate and blood pressure were stable for the remainder of this exploration and the subsequent exploration shown in figure 3B. During the initial exploration of LR2, some skeletal motor activity was evident upon stimulation, verifying the relative adequacy of the stimulus. Furthermore, a stimulus of 2 msec duration was employed to obtain bradycardia at some points at LR2 prior to the second exploration of this plane (at 0.2



A



B

Figure 3. Cat 25,2 kg. An example of reversal. A indicates the results of exploration of the medulla in a plane 2 mm lateral to midline; top surface of the medulla is indicated at V-3, and the obex is located at about P 16. The solitary complex "s" is shown by the broken lines. Numerals indicate the degree of bradycardia, as explained in text. B illustrates the markedly different results of a later exploration of the same points.

msec duration) shown in figure 3B. This strong stimulation was also tested at LR1, prior to the first LR2 exploration, and was ineffective at V-10 but caused cardiac arrest near the dorsal surface of the medulla. Survey at LR4 also showed numerous points giving good bradycardia, but subsequent examination at the midline, LR1 and LR2, as well as LR4 showed greatly reduced activity. Thus a third exploration of LR2 revealed greatly diminished reactivity in relation to that shown in figure 3B, consisting of several points giving responses of mild intensity. Extensive reversal of this type was also observed in one other experiment (Cat 28) of the series of eleven exploration experiments and there are several similarities. A medial plane (LR1) explored at the beginning of the experiment was devoid of bradycardia responses. During this initial exploration institution of artificial respiration reduced the heart rate from 220 to 160 beats/min (although a high heart rate per se is certainly not incompatible with good bradycardia). Prior to the second exploration, which gave responses quite comparable to those of figure 3B, bradycardia was elicited from various points in this plane with stimulus pulses of 2 and then 0.5 msec duration. Towards the end of the experiment, a few points in the L4 plane also indicated enhanced responses when exploration in this plane was repeated.

Most of the other experiments in the "exploration" series illustrated this reversal to some extent, i.e., at least a quantitative change was observed in two or more vertical loci and one or more lateral planes. The change was usually an increase in the bradycardia response, and the effect appears to be prominent in the early part of the experiment. Moreover,

some points which gave strong bradycardia or arrest after having been previously unresponsive were tested with stimulations of reduced voltage and it was found that good responses were sometimes obtained with 4 or 5 volt pulses.

Voltage: Threshold.

In most of the experiments conducted subsequent to those of the "Exploration" series, it seemed appropriate to reduce the stimulus voltage. This was done to better localize stimulations, and also served to reduce or abolish sympathetic and/or skeletal motor activity when these accompanied bradycardia responses. Thus, an initial survey at LR1 and LR4 was conducted at a number of loci using a six or seven volt stimulus, and electrodes were subsequently positioned in one of the most reactive vertical positions. Voltage was then reduced until a moderate bradycardia was evoked. It was found that in this manner, a stimulus of 3 to 5 volts, 0.2 msec. and 20 cps. usually resulted in a reduction of heart rate in the range of 30 to 90 beats per minute (i.e. a bradycardia of +1 to +3 intensity).

It was generally observed that a stronger stimulus was required to elicit responses from sites 1 mm lateral to midline than from those in the LR4 plane. Of perhaps greater significance is the observation that a change in stimulus intensity by 0.5 to 1 volt caused a dramatic rather than gradual change in response when stimuli were at about threshold intensity.

Such pronounced changes are illustrated in figure 4. It can be seen that reduction of the LR1 stimulus from 3.4 to 2.8 volts caused a reduction in response of more than 60%. In other experiments, even larger response alterations were seen with smaller voltage changes and the same effects were seen when voltage was increased from a subthreshold level. Comparable changes were also seen as the LR4 stimulus was

reduced.

The tendency for a higher voltage being required to produce a given degree of slowing at the LRL electrode was seen in nearly all experiments. Very little decrement in response occurred over the intermediate voltage range for the LRL series shown here. This type of reaction was seen in several experiments, sometimes at LR4, and suggests that bradycardia might be attributed to a circumscribed group of fibers well within the effective radius of the stronger stimulation, as well as within that smaller area activated by lower voltage. It is also possible that the group of fibers activated have a quite narrow threshold range.

The photomicrograph of figure 4 illustrates the electrode positions and it can be seen that the tip of the LRL electrode closely approximates, but is medial to DVN. This has been the case in many of the experiments, especially with respect to those electrodes more rostral to the obex, and this medial placement is considered to be a useful compromise in view of the proximity of NTS. Since this particular horizontal section is dorsal to the center of DVN (i.e. its greatest radius) a part of this nucleus is closer to the electrode than the photomicrograph indicates.

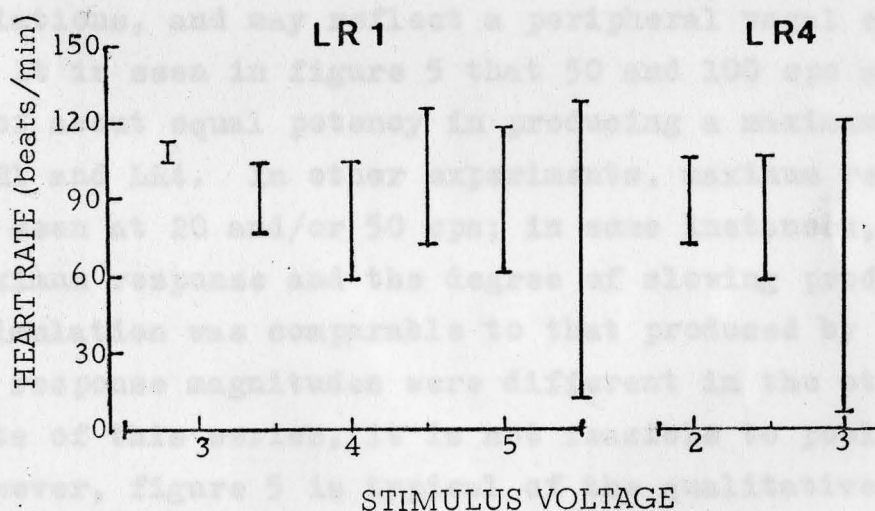
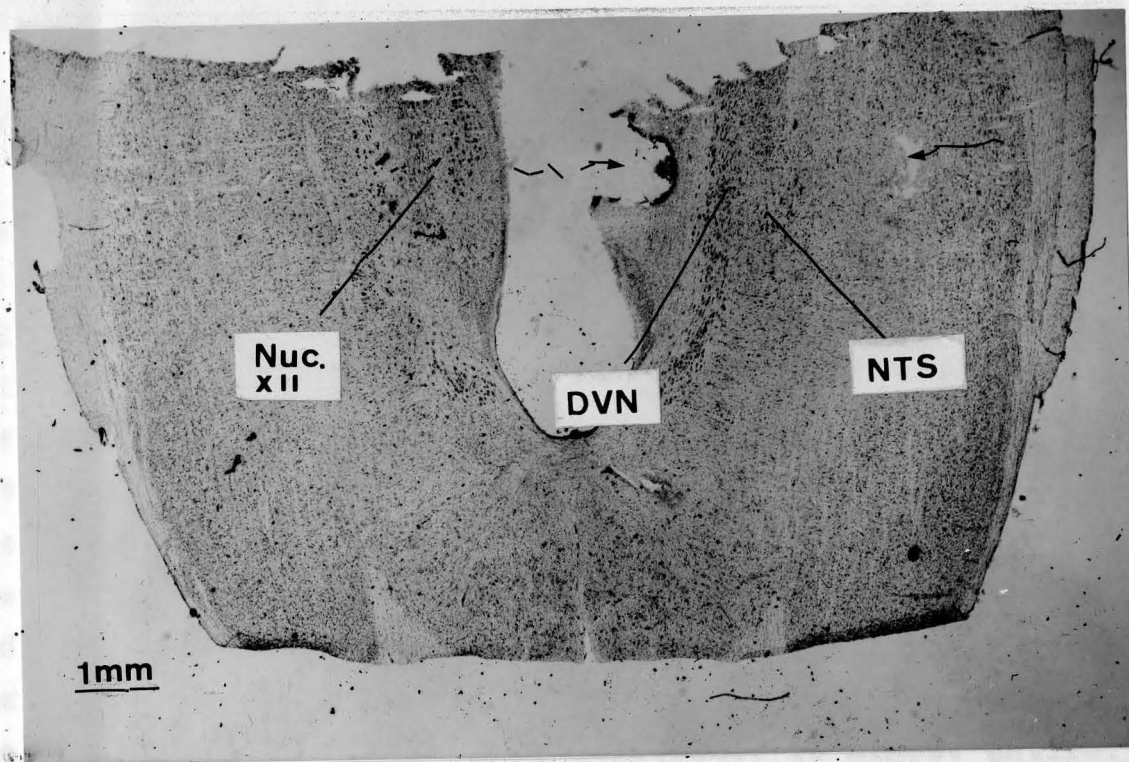


Figure 4. Cat 101, 2.5 kg, showing bradycardia responses to stimulation at various voltages. The photomicrograph shows LR1 and LR4 electrode positions (arrows). This section is approximately at the level of the LR1 electrode tip and 1 mm above that of the LR4 electrode. The graph shows heart rate before (top of bar) and during (bottom of bar) a 5 second train of 0.2 msec pulses at 20 cps.

Stimulus Frequency.

Although it was not intended to do an extensive study of stimulus parameters, it was felt that an examination of the responses to various stimulation frequencies at the LRL electrode as compared to those at LR4 might be of some value. Figure 5 shows one of six such experiments. The order of stimulation was varied from experiment to experiment, although the 20 cps stimulus was usually employed first and then repeated at the end of the series to confirm that the preparation had not deteriorated. In the experiment illustrated here, it can be seen that the final 20 cps test at LR4 (extreme right of lower graph) produces a larger response than the earlier stimulation. This difference is almost entirely accounted for by the difference in control heart rates for the two stimulations, and may reflect a peripheral vagal effect.

It is seen in figure 5 that 50 and 100 cps stimulations are of about equal potency in producing a maximum effect at both LRL and LR4. In other experiments, maximum responses were also seen at 20 and/or 50 cps; in some instances, 50 cps gave a maximum response and the degree of slowing produced by 20 cps stimulation was comparable to that produced by 100 cps. Since the response magnitudes were different in the other five experiments of this series, it is not feasible to pool the data. However, figure 5 is typical of the qualitative pattern seen in all animals.

It is also evident in figure 5 that in this particular experiment the difference between the 20 and 50 cps responses is marked at LRL but not at LR4. We do not, however, consider this to be a meaningful distinction between the two

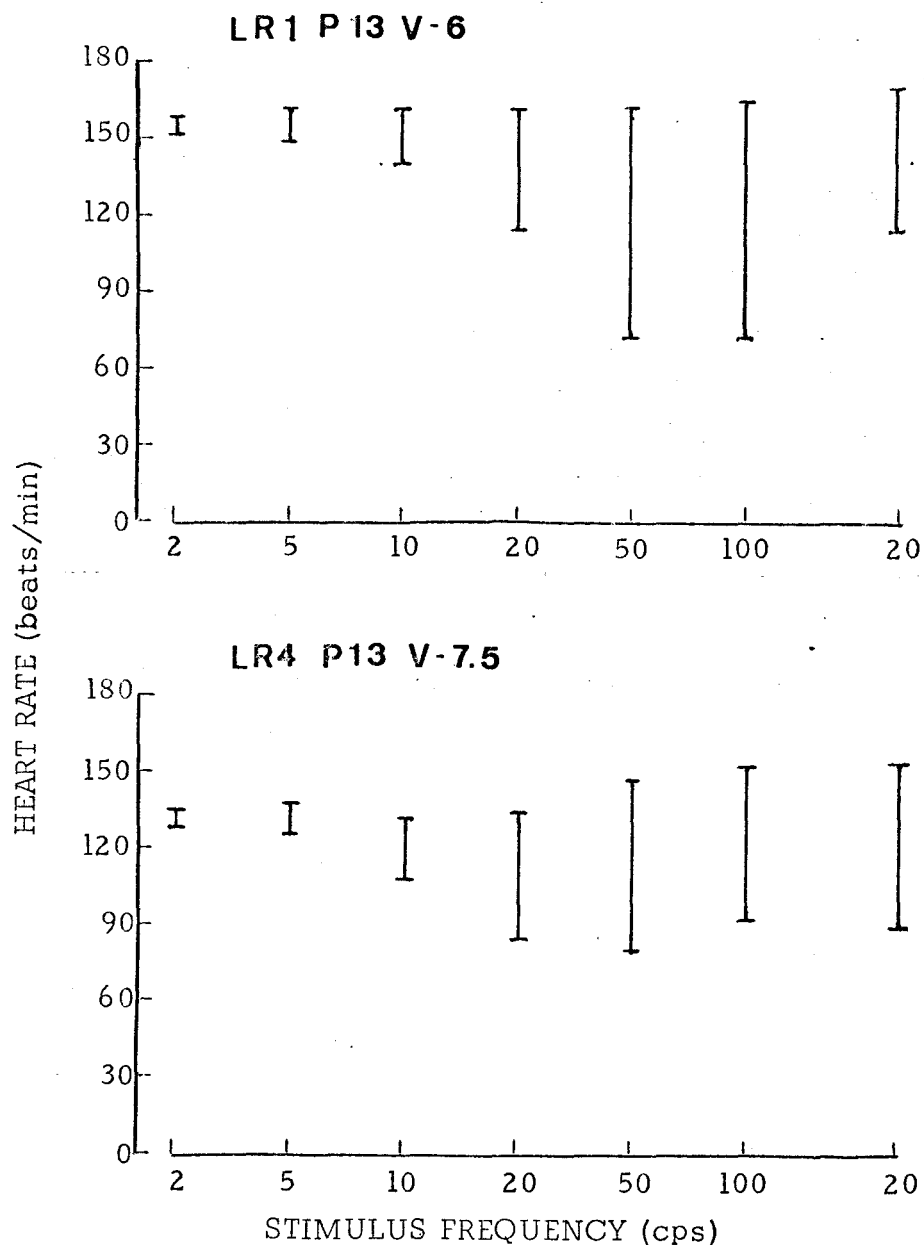


Figure 5. Cat 87, 2.5 kg. This figure shows heart rate before and during stimulation at various frequencies in a plane approximately 1mm rostral to the obex. all stimulations consisted of a 5 second train of 0.2 msec pulses with amplitude constant at 4 volts for the LR1 electrode and 5 volts at LR4.

sites since such differences did not occur in a consistent fashion. When these differences were seen, they sometimes occurred at LR4, and in cases where a series was repeated at a different stimulus intensity, the differences were minimized or abolished.

Since the synapse (or, more properly, the fine pre-synaptic terminal) is generally considered to be the part of a reflex arc most susceptible to fatigue (Patton, 1965) it was anticipated that, if the LRL electrode effects were mediated by means of a synaptic pathway more extensive than that activated by the LR4 electrode, this would be manifest especially at the higher stimulation frequencies. Figure 5, typical of the experiments in this series, suggests no such differentiation between LRL and LR4.

Because some other experimental procedures in this study involved the use of 100 cps stimulation, it has been possible to compare responses to 20 and 100 cps stimulations at LRL and LR4 loci in a total of 16 animals. These results are presented in table I, which shows the number of cases in which the bradycardia response to 100 cps stimulation was increased, decreased or unchanged in relation to 20 cps stimulation of the same point. These results were complicated by a relatively strong skeletal motor response to 100 cps stimulation in approximately one third of the animals. A further complication was the occurrence of a strong or moderate pressor response during or after the 5 second stimulation. This was seen in about half of the LRL stimulations, but in only two instances at LR4.

It is evident that in only a few cases did the 100

TABLE I

Type and Frequency of Occurrence of
Bradycardia Responses to 100 cps Stimulation
as Compared to Responses Evoked by 20 cps.

=====

Lateral Electrode Position	Number of Cases ¹		
	Increased	Decreased	Unchanged
LR1	6 (2.7)	3 (1.8)	10 (2.3)
LR4	7 (3)	3 (1.3)	10 (2.8)

¹ Values in parentheses indicate the average value of the posterior (P) electrode coordinate in mm rostral to the obex.

cps stimulation cause a reduction in response and, most frequently the response was unchanged from that evoked at 20 cps. Response increases were often substantial (as were the decreases) and in many cases the rate measured during the 100 cps stimulation differed by 30 beats/min or more from that measured during 20 cps stimulation. In most individual animals, the response change at LR1 was qualitatively different from that in the same posterior plane at LR4, but no meaningful trend was evident.

Electrode locations in the A-P axis ranged from 0.5 to 4 mm rostral to the obex. At LR1, the average position for the electrode in those six cases in which the 100 cps response was increased in relation to the 20 cps response was 2.7 mm rostral to the obex. In the three cases of decreased response, the average electrode position was 1.8 mm rostral to the obex, and in the ten instances when the responses were the same, the average position was 2.3 mm in front of the obex. Corresponding values at LR4 were 3 mm (7 cases), 1.3 mm (3 cases) and 2.8 mm (10 cases), respectively. It appears that a trend does exist at 1 mm lateral to midline - that is, a rostrally placed electrode would tend to produce a greater bradycardia at 100 cps, while the LR1 electrode nearest the obex would tend to produce a stronger bradycardia at the lower stimulation frequency, and an intermediate electrode would produce about the same slowing at 20 or 100 cps. Further analysis of the data does not completely support this relationship, however. A total of seven stimulations at LR1 were conducted within 0.5 to 1.5 mm of the obex and of these, only two exhibited a diminished response to the 100cps stimulation.

It is also seen in table I that electrode loci for both increased and unchanged responses were approximately 3 mm rostral to the obex, while in three instances of decreased response, the average electrode position was closer to the obex.

The data from these frequency studies are of limited value in determining the nature of neural elements involved in bradycardia responses. The tendency for 100 cps stimulation to produce pressor responses at LRI is an additional complication. With the exception of voltage studies, this series is the only one in this study in which an attempt has been made to compare responses at a given point to two fundamentally different stimuli.

Bilateral Carotid Occlusion.

The results of stimulation before and during BCO in a series of fifteen cats are shown in Tables IIA, IIB and III for electrode positions at LR1 and LR4, respectively. In almost every instance both loci were tested in a given animal with both electrodes in one of the posterior planes. In some cases two of the posterior planes were tested and, if qualitative differences were observed, duplicate entries have been made. Most entries in these tables represent two or more trials of a given point and sometimes testing was done at various voltages. The nature of the response was not dependent on a change in adequate stimulus voltage. In a few cases, a change in the type of response was observed as trials were repeated. If the change was repeatable, a dual entry has been made for the experiment in the tables, whereas if it was seen but once, in relation to several consistent responses of another type, it was disregarded.

Prior to a discussion of the analysis of results, it should be mentioned that the criterion of "change" in response was defined as a difference in heart rate of 18 beats per minute or more. The minimum change, in this definition, is of borderline significance, but nevertheless such small changes were seen consistently on a number of occasions and are probably real regardless of their physiological importance.

As shown in Tables IIA, IIB and III, experiments were categorized accordingly as BCO did or did not produce tachycardia. These groups were further classified with respect to the effect of BCO on the magnitude of stimulus-

Tables IIA and IIB; Effects of bilateral carotid occlusion (BCO) on the response to stimulation in the right medial (LR1) and right lateral (LR4) medulla. Experiments have been categorized accordingly as the BCO procedure increased (\uparrow), decreased (\downarrow) or did not affect (-) the degree of bradycardia; and also with respect to the presence (TP) or absence (TA) of tachycardia following BCO. Additional details in text.

TABLE IIA

Effects of BCO on LRI Responses.

=====

Expt.	<u>LRI</u> <u>Control</u>			<u>LRI</u> <u>BCO</u>			Obex (mm)	X
	△	HR	BP	△	HR	BP		
28	69	162	95	78	172	130	2	S
46*	44	200	128	48	206	175	2	S
50	30	240	130	30	240	175	0.5	S
56	78	270	106	75	270	134	3	I
-TA 67+	50	175	115	48	175	130	3,5	S
69	87	190	100	87	190	130	4	I
71*	68	172	115	80	185	140	1	I
77	87	255	102	90	265	112	1	I
85	60	125	119	60	130	185	0.5	I
MEAN		199	112		204	146		
57+	60	156	100	60	168	190	2,4	S
-TP 67	78	168	115	78	180	130	3	S
↑TA 70	54	216	100	73	212	115	0	I
↑TP 68	30	148	125	48	178	160	3	I
49	87	235	118	51	235	140	1	S
↓TA 51	116	180	80	92	170	142	4	S

Experiment Column:

* indicates pentobarbital anesthesia.

+ indicates similar results from 2 or 3 A-P electrode loci.

△ indicates the decrease in heart rate (beats per minute) measured during the stimulation period.

HR = Pre-stimulation heart rate in beats per minute.

BP = Pre-stimulation blood pressure in mm.Hg.

Obex Column indicates the electrode position (in mm.) rostral to obex.

X Column indicates the condition of the left vagus; I = intact.

S = sectioned.

TABLE IIB

Effects of BCO on LR4 Responses.

=====

Expt.	△	<u>LR4</u> <u>Control</u>		△	<u>LR4</u> <u>BCO</u>		Obex (mm)	X
		HR	BP		HR	BP		
28	94	152	97	97	160	132	0	S
48*	115	160	107	124	170	158	2	S
50+	78	240	130	68	236	175	0.5, 2.5	S
51	25	180	80	28	184	142	4	S
-TA 67+	84	175	115	80	171	130	3, 5	S
69	60	190	100	60	190	130	4	I
70	28	212	100	29	203	115	2	I
71*	100	172	115	110	182	140	1	I
85	34	130	119	28	138	185	0.5	I
MEAN		179	107		180	145		
-TP 57+	100	150	100	100	170	190	4	S
↑TA 77	76	265	100	114	264	115	1	I
↑TP 57+	60	150	100	80	170	190	2	S
68	42	150	125	78	190	160	3	I
↓TA 49	135	235	118	51	235	140	1	S
67+	84	168	110	66	168	125	1, 3	S

Experiment Column:

* indicates pentobarbital anesthesia.

+ indicates similar results from 2 or 3 A-P electrode loci.

△ indicates the decrease in heart rate (beats per minute) measured during the stimulation period.

HR = Pre-stimulation heart rate in beats per minute.

BP = Pre-stimulation blood pressure in mm.Hg.

Obex Column indicates the electrode position (in mm.) rostral to obex.

X Column indicates the condition of the left vagus; I = intact.

S = sectioned.

evoked bradycardia, which was either enhanced, diminished or unchanged.

In the majority of cases, BCO had no effect on the magnitude of bradycardia produced by stimulation. These groups (-TA and -TP) illustrate a rather wide range of control heart rates and varying degrees of slowing. Not shown are the results of various stimulation intensities in a single preparation; the responses were qualitatively the same whether strong or relatively mild degrees of slowing were evoked.

Generally, BCO produced moderate pressor responses, although concomitant rate increases were mostly small even in cases where control heart rate was relatively low.

The role of the vagi in determining the nature of the response is not quite clear. In all but one instance of increased slowing during BCO (+TA and +TP groups) both vagi were intact. However, both nerves were also intact in nearly half of those experiments in which BCO was without effect on vagal bradycardia. The left vagus was not intact in those few instances when a decreased response to stimulation of central vagal structures was obtained.

Table IIA also indicates that the location of the electrode in relation to the obex does not necessarily correlate with the type of response obtained. It is likely that the region of the obex contains many relay fibers concerned with cardiovascular reflexes (Grill and Reis, 1968) and it is possible that the LRL electrode within 1 to 2 mm of the obex will activate some solitary tract fibers. In two of the four cases where the LRL electrode provoked a response during BCO which differed from the control response, the electrode was

within 1 mm of the obex; in the other two experiments, however, electrodes were 3 and 4 mm rostral to the obex. In four of the eleven experiments where the response to LRL stimulation was unaffected by BCO, the electrodes were within 1 mm of the obex. In those few cases where two or three of the posterior planes were tested, the same type of response was usually obtained at each plane. Experiment 67 is an exception with respect to the LR4 plane; testing at 1 and 3 mm rostral to the obex at first showed the responses to be decreased during BCO, and later testing at 3 and 5 mm rostral to the level of the obex showed responses to be unaffected by BCO.

Table III is a restatement of some of the results shown more completely in Tables IIA and B, arranged accordingly as BCO was without effect on the LRL response, or altered it. In the eight experiments in which the LRL response was unchanged during BCO, the LR4 response was altered in three experiments, although in two of these, the LR4 change was not consistent in all trials. The LRL response was altered (increased or decreased) in four experiments, while in only two of these animals was the LR4 response altered.

On the basis of these experiments, it is difficult to say conclusively whether the number of BCO-induced changes at LRL was significant with respect to the number of changes at LR4. Were it not for the inconsistencies in experiments 57 and 67 at LR4, it could be said that there is a slightly greater incidence of BCO-induced change in response in the LRL plane. It may be significant that in these two experiments the responses at LR4 were unchanged at sites 4 and 5 mm rostral to the level of the obex, but change did occur at 1

TABLE III

Summary of Effects of BCO
on Bradycardia Responses.

=====

<u>Expt.</u>	<u>LR1</u>	<u>LR4</u>
28	-TA	-TA
50+	-TA	-TA
57+	-TP	-TA, ↑ TP(20)
67+	-TA, -TP	-TA, ↓ TA(-18)
69	-TA	-TA
71*	-TP	-TA
77	-TA	↑TA(38)
85	-TA	-TA
68	↑TP(18)	↑TP(36)
70	↑TA(19)	-TA
49	↓TA(-36)	↓TA(-84)
51	↓TA(-24)	-TA

This table summarizes the results shown in Tables IIA and B in which both LR1 and LR4 were stimulated. Symbols are as described in those tables. Values in parentheses indicate the difference in control vs. BCO bradycardia, in beats per minute for those instances in which BCO altered the response to stimulation.

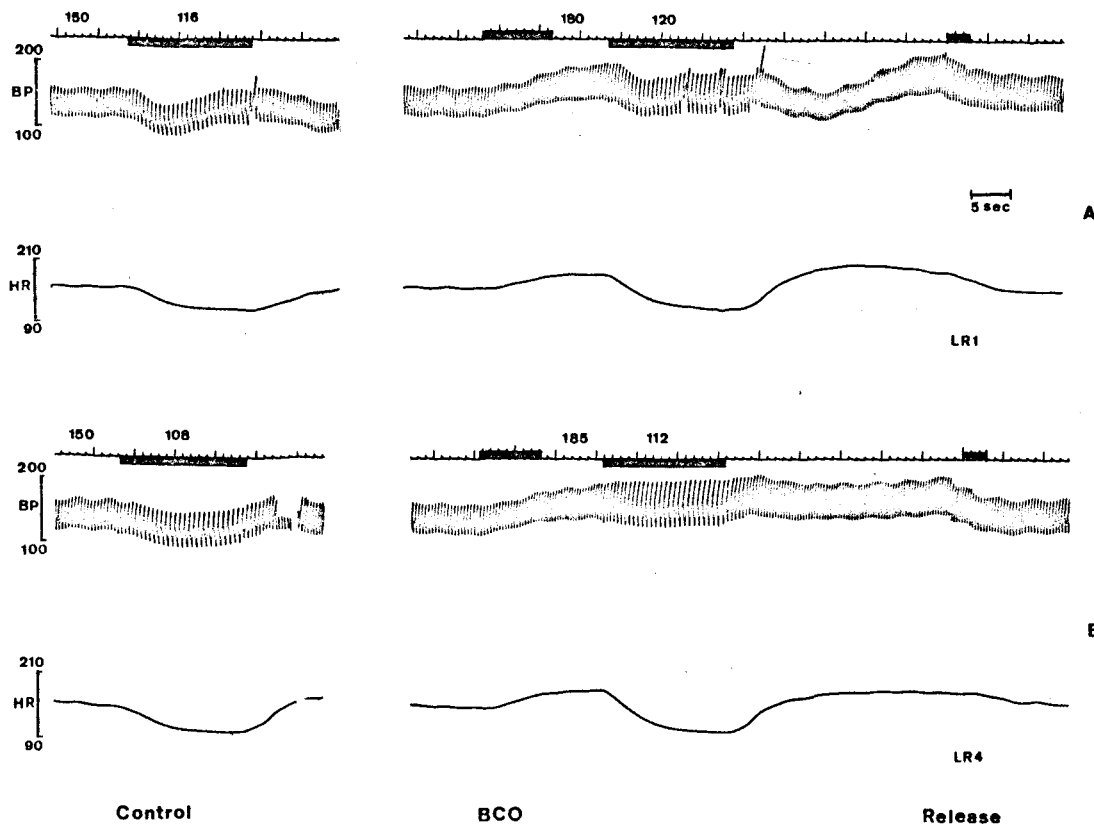


Figure 6, cat 68, 3 kg. Stimulation before and during BCO. Panel A: stimulation at LR1, P13 (3 mm rostral to the obex) at about the top surface of the medulla. Panel B: stimulation at LR4, P13 at approximately the same vertical level as the LR1 electrode. From above downward are shown time and event marker, blood pressure (mm Hg) and heart rate (beats/min.). Values above time signal indicate heart rate during corresponding periods. Event marker below the time line indicates stimulation. The pair of signals above the time line indicate onset and release of BCO.

and 2 mm rostral to this level, while at 3 mm (experiment 67) responses were both changed and unchanged. It is thus possible that stimulation of afferent intramedullary vagal fibers may have been involved at LR4 at the levels close to the obex, although stimulation at this level did not always influence responses.

It must be concluded that BCO can impose conditions such that stimulation at both one and four mm to the right of midline may elicit a changed response. A number of possible mechanisms will be treated in the discussion.

Figure 6 illustrates the effect of BCO on stimulation at LRL and LR4 at a level 3 mm rostral to the obex. At both electrode loci, BCO caused a distinct tachycardia and resulted in an enhancement of the effect of stimulation. It is also of interest that in the stimulation of LRL, during BCO, the degree of enhancement of bradycardia is nearly exactly equivalent to the degree of tachycardia brought about by BCO. Repetition of these stimulations after both vagi had been cut was without effect on heart rate.

Aortic and Vena Caval Occlusion.

Results of experiments on the effect of aortic or vena caval occlusion on bradycardia responses to medullary stimulation are shown in tables IV A and B, and a typical series of stimulations is shown in figures 7A and B. Figure 8 summarizes the results shown in these tables, and presents a comparison of the bradycardia responses before and during occlusion of a great vessel. Reduction in heart rate (Δ_c) produced by a control stimulus is subtracted from the slowing (Δ_o) produced by an identical stimulation during occlusion, and the resultant for each of a series of six experiments is plotted in figure 8. A positive value for $\Delta_o - \Delta_c$ indicates a response during occlusion that was stronger than the control response.

It is seen in figure 8 that, in the LR1 series of stimulations, occlusion of the aorta caused a substantial bradycardia increase in one of five experiments (cat 100) and vena cava occlusion resulted in a pronounced reduction of response in two of five experiments, 100 and 103. In all three instances, the change was in the predicted direction; an increased response was observed with elevated pressure in the carotid and aortic baroreceptor areas, and a smaller response was elicited when pressure in these regions was reduced. Two relatively large response changes were seen at the LR4 position (both in experiment 103) and again were in the predicted directions.

In addition, there were three instances of 12-beat bradycardia differences at LR4. These are of borderline significance; one of these represents only 10% change from the

Tables IV A and B show the heart rate response to medullary stimulation at LR1 or LR4 before, during and after occlusion of the aorta (table IV A) or vena cava (table IV B). In all cases, both electrodes were in the same posterior plane, as indicated in the "Obex" column. Heart rate and blood pressure measurements shown in these tables were made immediately prior to the 5 second stimulation period, and the rate measured during this stimulation was subtracted from the control rate to give the quantity Δ , a measure of the slowing produced by a stimulus. Recovery periods of at least 1 - 2 minutes were interposed between stimulations.

TABLE IV A

**Effect of Aortic Occlusion
on Bradycardia Responses**
=====

Expt.	Obex ¹	<u>Control</u>			<u>Occlude</u>			Time ²	<u>Recovery</u>			
		HR	BP	△	HR	BP	△		HR	BP	△	
<u>LR1</u>	94 ³	3.5	186	107	48	180	187	44	20	180	112	48
	100 ^{3,5}	3	172	85	58	162	107	90	25	174	96	96
	101	3	122	73	50	122	117	54	20	116	72	48
	102 ⁵	0.5	144	83	56	126	100	54	50	150	87	54
	103 ^{4,5}	0.5	156	72	54	144	118	48	20	159	83	51
<u>LR4</u>	94 ³	3.5	178	113	60	177	183	60	20	174	117	62
	97 ³	1	144	72	66	144	145	54	30	138	72	60
	100 ^{3,5}	3	180	91	120	162	106	108	35	175	91	115
	101	3	102	87	45	102	125	46	20			
	102 ⁵	0.5	140	82	26	120	108	24	27	138	72	24
	103 ^{4,5}	0.5	162	90	36	156	131	54	20	174	82	30

¹Obex column indicates electrode position (in mm) rostral to obex.

²Time column indicates delay (in seconds) between onset of pressure change and onset of stimulation.

³Decamethonium has been administered in these experiments.

⁴Pentobarbital anesthesia.

⁵Partial reflex recovery of blood pressure upon occlusion.

TABLE IV B

Effect of Vena Cava Occlusion
on Bradycardia Responses

=====

	Expt.	Obex ¹	<u>Control</u>			<u>Occlude</u>			Time ²	<u>Recovery</u>		
			HR	BP	Δ	HR	BP	Δ		HR	BP	Δ
<u>LR1</u>	94 ³	3.5	180	107	48	186	69	48	25	186	107	48
	100 ^{3,5}	3	177	83	57	186	58	36	63	180	92	54
	100 ^{3,5}	3	174	102	62	182	62	70	45	166	97	52
	101	3	102	77	45	104	45	44	20	104	82	44
	102 ⁵	0.5	135	83	52	160	40	52	55	140	83	44
	103 ^{4,5}	0.5	168	90	50	180	40	18	23	174	90	48
<u>LR4</u>	94 ³	3.5	186	107	69	180	63	68	35			
	97 ³	1	138	72	60	144	40	66	25	150	75	54
	100 ^{3,5}	3	183	96	123	180	54	114	25	174	96	111
	101	3	104	85	42	102	45	42	18	100	78	43
	102 ⁵	0.5	138	77	30	150	42	18	35	138	82	22
	103 ^{4,5}	0.5	170	82	14	176	42	20	30	170	67	20
	103 ^{4,5}	0.5	164	72	36	176	55	14	85	167	83	42

¹Obex column indicates electrode position (in mm) rostral to obex.

²Time column indicates delay (in seconds) between onset of pressure change and onset of stimulation.

³Decamethonium has been administered in these experiments.

⁴Pentobarbital anesthesia.

⁵Reflex recovery of blood pressure during occlusion.

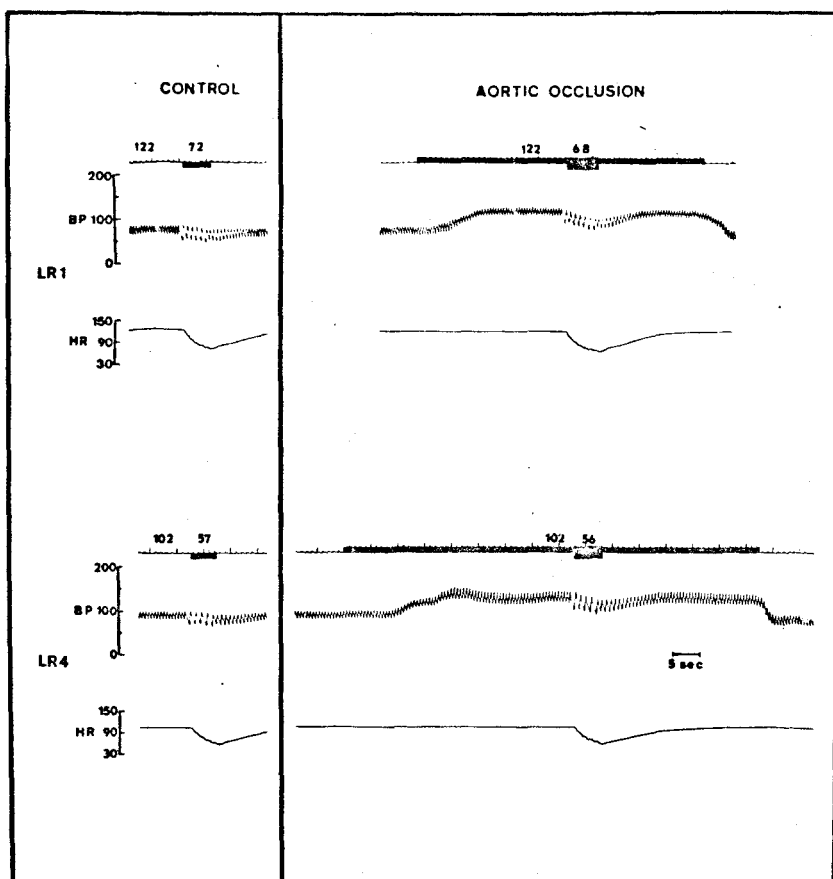


Figure 7A. Cat 101, 2.5 kg. An example of stimulation at LR1 and LR4 before and during occlusion of the thoracic aorta. From above downward are shown time and event marker (upper signal indicates occlusion, lower indicates stimulation) blood pressure in mm Hg and heart rate in beats/min. Heart rate counts are also shown above the time and event marker. Recovery periods between "control" and "occlusion" stimulations range from 90 to 165 seconds.

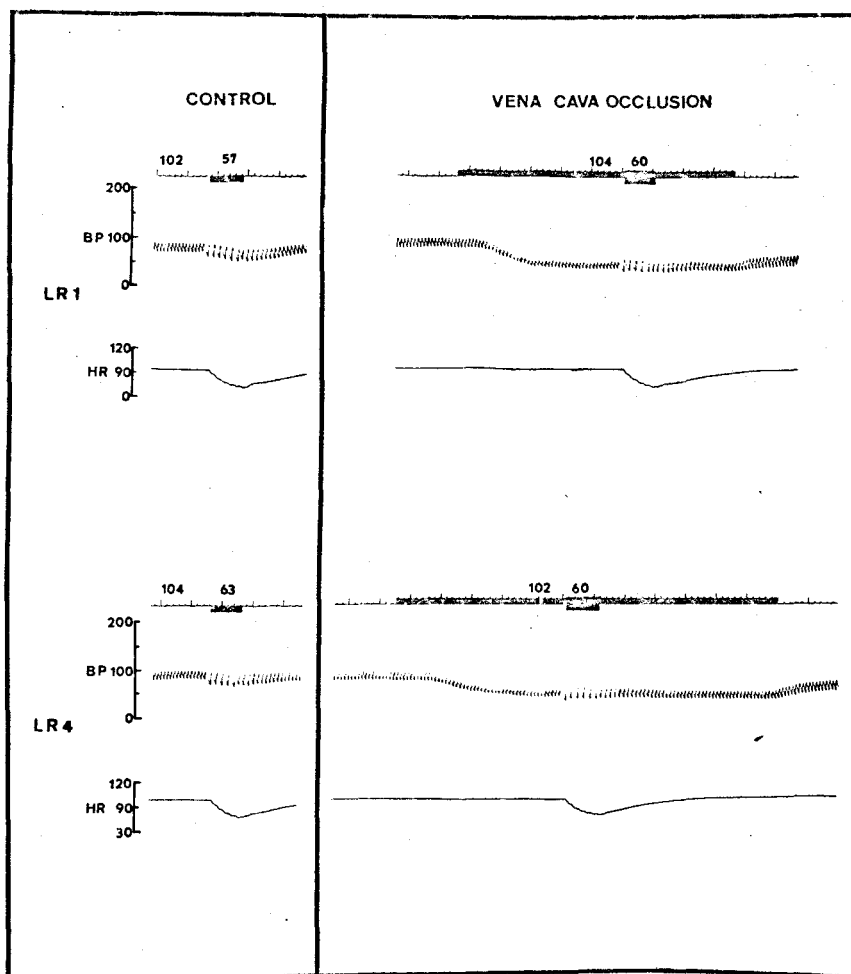


Figure 7B. Same cat as in figure 7A. An example of stimulation at LR1 and LR4 before and during occlusion of the inferior vena cava. Heart rate counts are shown above the time and signal marker, and the tracings are as described in figure 7A.

control response. In all three cases, comparison with the poststimulation recovery response shows differences of seven beats or less. This alteration of the recovery response was also observed after the aortic occlusion for the LRI stimulation in experiment 100 (see table IV A). Here, the heart rate was reduced by 58 beats per minute during the control stimulation, by 90 beats per minute when the stimulation was delivered during aortic occlusion, and by 96 beats per minute when the stimulus was applied 135 seconds later, during the recovery period. This might suggest a shift in response type, although the following stimulation, applied after an additional 115 seconds, produced a slowing of 62 beats per minute. This phenomenon, possibly a prolongation of vessel occlusion influences for a minute or longer after release of the snare, was seen in a few other stimulations not shown in table IV. These changes were seldom as great in magnitude as that shown for experiment 100. When this unusual recovery was observed during the course of the experiment, repetition of the stimulation showed that normal responses sometimes occurred within two minutes of release of occlusion.

This atypical type of response is reported as an incidental observation, and no attempt was made to further study it. It would appear, however, that in evaluating responses evoked during occlusion, it is probably better to compare them to stimuli applied before, rather than after occlusion since in some cases, the effects of occlusion may persist for one or two minutes.

Figure 8 shows that the LRI electrode was close to the obex in only one of those two animals whose responses were

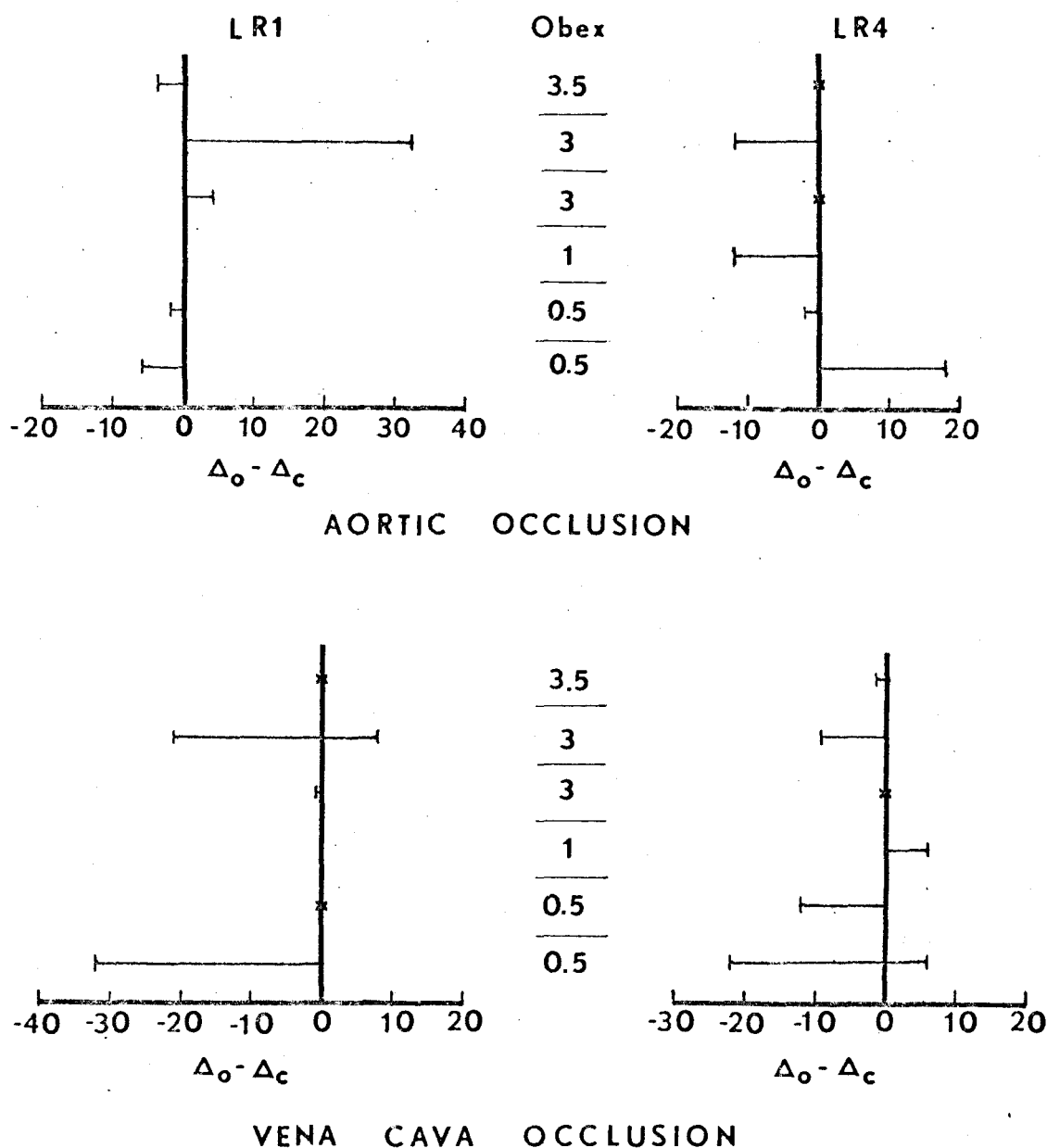


Figure 8. This figure shows the difference ($\Delta_O - \Delta_C$) of control (Δ_C) and great vessel occlusion (Δ_O) responses to a constant stimulus at LR1 or LR4 for each of the 6 experiments shown in tables IV A and B. In the obex column, between the plotted values for each experiment, is shown the corresponding location of the LR1 and LR4 electrodes in mm rostral to the obex. Where the values of Δ_O and Δ_C are identical, an "X" has been entered on the ordinate, and in those cases where repeated testing gave responses opposite in sign, both values have been entered at the same position on the ordinate.

influenced by occlusion. In the other experiment, the electrode was approximately 3 mm rostral to it. Significant changes at LR4 occurred in a single animal and here the electrode was 0.5 mm rostral to the level of the obex. It is possible that afferent vagal fibers from the aortic arch or other cardiovascular afferents were affected by this electrode in their course through the medulla. In summary, great vessel occlusion caused alteration in the response to LRL stimulation in two of five animals, while the response at LR4 was altered in only one.

In some of the experiments indicated by footnote 5 in tables IV A and B, occlusion was accompanied by a strong recovery of blood pressure, sometimes accompanied by reflex heart rate changes. It was sometimes necessary during these occlusions to gradually tighten the snare in order to maintain pressure at a constant level. In these experiments, however, an excessive degree of tightening was required for maintenance of a constant pressure change. Even this procedure was not capable of maintaining the pressure changes in some animals. Furthermore, it was seen that rapid release of occlusion in these animals (especially aortic occlusion) usually caused an initial, strong excursion of pressure beyond the control pressure level, suggesting moderate to strong reflex vascular compensation, and relatively good reflex responsiveness. However, in four animals in which BCO alone was done as a test of reflex excitability, there was a mean pressure increase of 18% and in no case was there a significant rate increase.

In all occlusion experiments, stimulation was repeated after section of the right vagus. These stimulations caused no decrease in heart rate. Thus it is clear that

TABLE V

**Aortic Occlusion and Reflex Bradycardia
Before and After Vagisection.**

=====

Expt.	Vagus		Peak BP	End BP	Peak HR	End HR	
	R	L					
100	I	I	157	108	185	156	29
	S	S	142	130	192	180	12
101	I	I	160	160	110	66	44
	S	I	142	142	118	110	8
103	I	I	172	142	170	140	30
	S	I	157	125	165	150	15

Table V illustrates the result of aortic occlusion procedures (without electrical stimulation) before and after vagisection in order to evaluate the role of the vagi in reflex responses to aortic occlusion. Shown are the heart rate (HR) and blood pressure (BP, in mm Hg) at the onset (Peak) of occlusion and just prior to release of occlusion (End) after approximately one half minute. Vagus columns indicate the condition of the right and left vagus, intact (I) or sectioned (S) and the delta column indicates the difference in heart rates as measured at the beginning and end of occlusion.

bradycardia responses were uncomplicated by sympathoinhibitory effects. The only significant pressure change evoked by these stimulations was a 35 mm Hg rise in pressure produced by the LR4 electrode in experiment 103.

In three experiments the aortic occlusion procedure was repeated after vagisection to determine the role of the vagus in the reflex response to occlusion. Results are shown in table V. Vagisection sharply reduced compensatory bradycardia to levels of borderline significance. Bilateral vagotomy in experiment 100 strongly reduced reflex pressure change, but right vagisection in experiment 103 was virtually without effect on vascular compensation during aortic occlusion.

These testing procedures were done at the end of the experiment; comparison of table V with table IV A shows that, in general, these final test procedures involved more complete aortic occlusion than that employed during electrical stimulation procedures. It was intended to avoid the production of excessive pressures during the course of stimulation procedures since overdistention might have had an adverse effect on the heart and great vessels. Also, as expected, the larger pressure increases tended to produce a more pronounced reflex bradycardia. Two types of reflex bradycardia were seen. One developed slowly and gradually increased during the course of occlusion. In the other a relatively strong bradycardia, such as that of experiment 101 in table V, occurred within a few seconds of the pressure peak. This latter type of bradycardia was generally seen only with higher peak pressures (greater than those attained in stimulation testing) and may be related to the magnitude of pressure increase, the rate of pressure

increase, an alteration in cardiac sensitivity to efferent vagal activity or some combination of these factors.

The occurrence of reflex bradycardia at higher aortic occlusion pressures is further confirmation of the preparations' responsiveness. Although aortic occlusions performed for the purpose of stimulation testing were apparently not always of sufficient magnitude to evoke reflex slowing, it seems reasonable to consider these occlusions "subthreshold" in nature and appropriate to the testing purposes involved here.

Cardioacceleration following vena cava occlusion was observed less frequently than reflex responses to aortic occlusion, and was of lesser magnitude since most of the "control" pressure values in table IV B are in the range of 70 to 85 mm Hg. This is not surprising in view of the generally held opinion that the carotid sinus does not function at mean pressures less than about 60 mm Hg.

Post Stimulation Bradycardia.

In approximately one-third of more than 60 experiments in which exploration was carried out at LRL and LR4, a response to stimulation was observed which involved a relatively prolonged post stimulation bradycardia (PSBC). This form of bradycardia was repeatable in a reactive preparation, although a change in electrode position frequently abolished it. In nearly every instance, the electrode which elicited PSBC was in the LRL plane, within one mm of the obex and close to the dorsal surface of the medulla. In a few instances, PSBC was obtained at sites further rostral to the obex and was sometimes seen at two LRL loci. However, in every animal in which PSBC was observed, normal bradycardia and recovery were also recorded, often at other LRL loci. In five experiments, PSBC was also elicited at LR4. These responses were not as consistent in their occurrence as the LRL responses, and were seldom repeatable.

PSBC was produced most readily by 100 cps stimulation and was usually accompanied by a moderate to strong pressor response. Figure 9 (panel B) shows PSBC following 100 cps stimulation at the level of the obex and is atypical in that a pressor response is absent. Normal slowing was observed 2 mm rostral to this electrode (panel A) although later in the experiment, PSBC was also obtained at this electrode. Figures 10 and 12A show more typical PSBC responses to 100 cps stimulation. In some experiments, PSBC was produced by 20 cps stimulation (figure 11) and in these experiments 100 cps was also effective.

The PSBC was frequently accompanied by a stimulus-

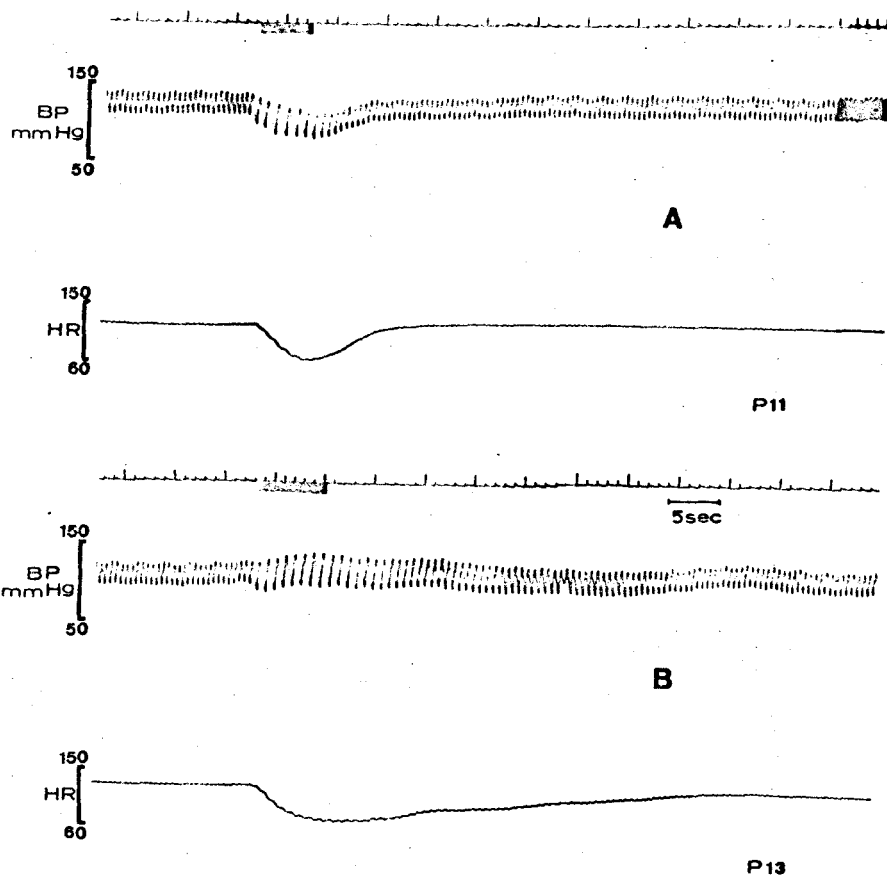


Figure 9 . Cat 91, 2.5 kg. An example of post-stimulation bradycardia. In each panel, from above downward, are shown time and signal marker, blood pressure and heart rate. Panel A; stimulation at P 11, LR1, V-7.5 (3 mm rostral to obex) shows stimulus-bound slowing and ordinary recovery. Panel B; stimulation at P 13, LR 1, V-7.5 shows stimulus-bound slowing and a recovery phase that is greatly prolonged.

evoked pressor response of varying magnitude, but this kind of response has been observed both with and without a rise in blood pressure. Such an experiment is shown in figure 10, in which progressive reduction of voltage substantially reduced pressor response while PSBC was still evident (although insignificant at 3.8 volts).

It is seen in figure 9 that PSBC follows a stimulus-bound slowing, although in most cases PSBC involved a greater reduction in rate than that provoked during stimulation, in addition to a prolonged recovery. Another type of response has been observed, in which the heart rate is unchanged or slightly elevated during stimulation. Shortly after cessation of stimulation, the PSBC response occurs (figure 11, LRI stimulation). This pattern was commonly seen when the stimulation produced a pressor response.

Because of the many instances in which PSBC has followed a stimulus-evoked pressor response, especially with 100 cps stimulation, and since stronger stimulation resulting in greater pressure increases is accompanied by greater PSBC, it is important to determine whether or not this phenomenon is simply reflex in nature and dependent only on the increase in pressure. The finding that PSBC can be elicited in some animals from the region of the obex in the absence of a pressor response supports the proposal that PSBC can be a separate entity. Several other observations are consistent with this idea.

In a few cases where PSBC was associated with a pressor response, the reactive point was tested at various voltages. Weaker intensities producing no pressure change

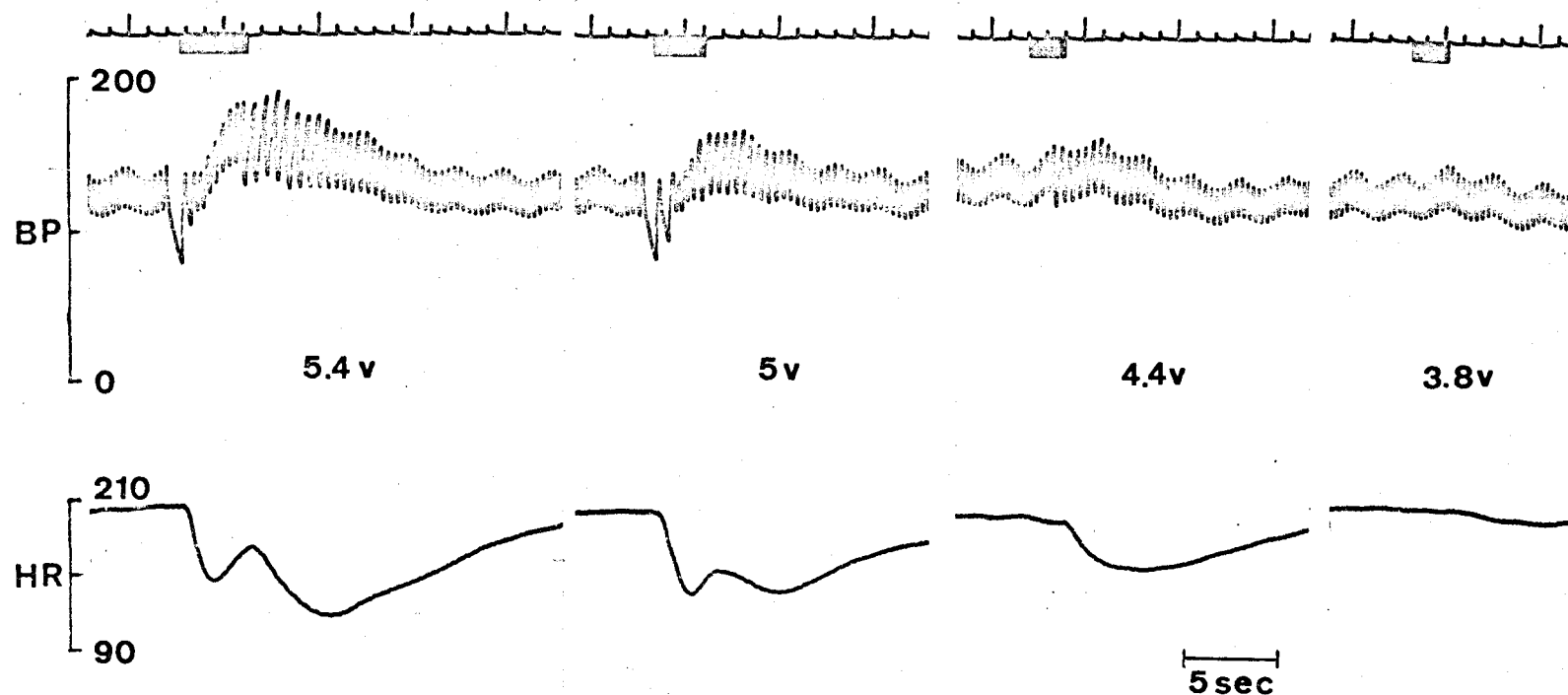


Figure 10. Cat 92, 4 kg. Effect of reduced stimulus voltage on PSBC associated with a pressor response. This stimulating electrode was visually positioned at the level of the obex after partial removal of the cerebellum, 2 mm lateral to midline and approximately 0.5 mm below the surface of the medulla. Stimulation frequency was 100 cps and the voltage (v) is indicated for each stimulation.

were usually followed by either a brief arrhythmia (evident in the pressure tracing as a small number of irregular beats) or an attenuated PSBC, as seen in figure 10. A 5.4 volt, 100 cps stimulation resulted in a strong pressor response (accompanied by some stimulus-bound slowing) and distinct PSBC. The PSBC was still present at 4.4 volts, while the pressor response was barely evident. Weaker stimulation (3.8 volts) had no apparent effect on blood pressure, but a small post-stimulation bradycardia persisted. Thus, these findings support the fact that PSBC is at least partially independent of an increase in blood pressure.

Figure 10 also shows that the rate irregularity evident during the stronger stimulations is not produced by the 4.4 volt stimulus although PSBC is still quite apparent. It is possible that separate mechanisms are involved here, with the threshold for stimulus-bound slowing at 100 cps between 4.4 and 5 volts in this experiment. Stimulation of this point at 4.8 volts and 20 cps produced a modest PSBC (intensity about +1) with a 15 mm Hg pressure increase and no rate change during stimulation.

In one experiment in which PSBC was associated with a strong stimulus-bound pressor response, the latter was abolished by dorsal quadrisection of the lower medulla caudal to the stimulating electrode. Although control blood pressure was reduced, the stimulation subsequently produced PSBC with a negligible pressor component.

Figure 11 illustrates PSBC associated with a pressor response (LR1 stimulation) while a comparable pressor response at LR4 is not associated with PSBC. It has been

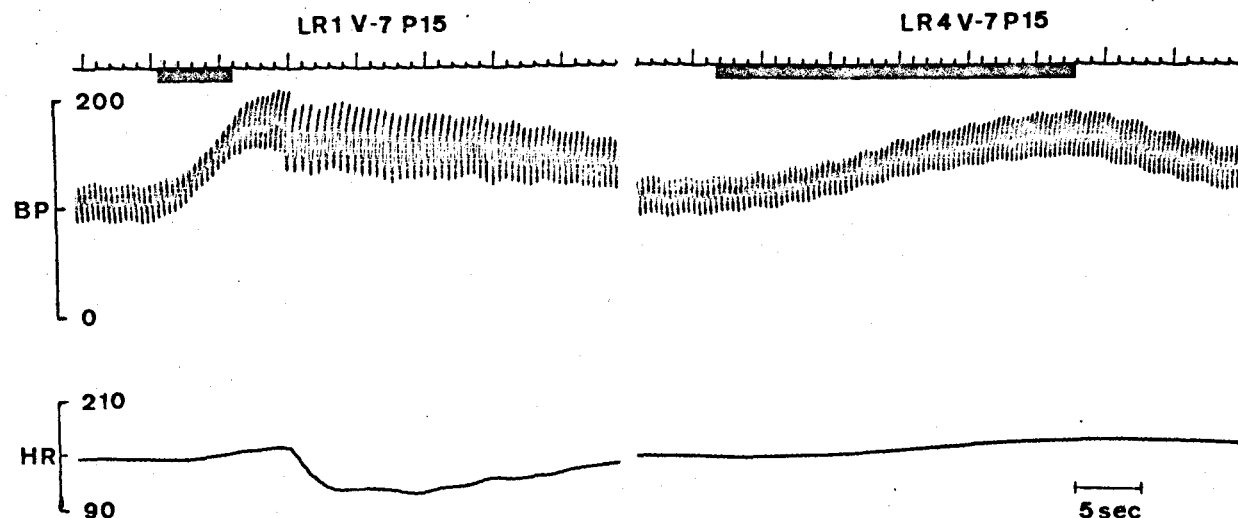


Figure 11. Cat 57, 2.5 kg. An example of PSBC after 20 cps stimulation, accompanied by a pressor response. Prolonged stimulation at LR 4 produces a pressor response nearly as great as that seen at LR 1 but without any bradycardia. The LR 1 electrode, at the level of the obex, is on the dorsal surface of the medulla. The left vagus is cut in this preparation; stimulus intensity is 7 volts.

previously mentioned that LR4 pressor responses were relatively infrequent in relation to those at LR1; nevertheless, there are several experiments in the PSBC series in which pressor responses here were seen independently of PSBC.

BCO provides another means of attaining increased systemic pressure, and in a number of experiments in which BCO was suddenly released, bradycardia accompanied recovery in only two instances, and in these cases this was not observed until the later part of the experiment with systolic pressure during BCO in the range of 200 mm Hg. As a rule, recovery from the pressor responses to BCO did not involve significant bradycardia but was quite similar to the recovery shown in figure 12A, panel B.

As a means of further evaluating the role of the carotid sinus barosensory reflex in relation to PSBC, stimulation which produced PSBC was repeated during BCO in eight animals. In three of these the contralateral vagus was sectioned, and BCO regularly abolished PSBC. However, on a number of occasions after electrical stimulation during BCO, a prolonged slowing similar to PSBC appeared when occlusion was released. This occurred at pressures relatively low in relation to those normally required to produce such distinct bradycardia through simple reflex mechanisms. This pattern was suggestive of an effect that outlasted the stimulation by 15 seconds or more. In numerous tests on the five cats with both vagi intact, PSBC was abolished only occasionally. Figure 12 A is somewhat atypical and shows the nearly complete removal of PSBC during BCO with vagi intact although there is evidence of some skipped beats in the poststimulation period. Note that

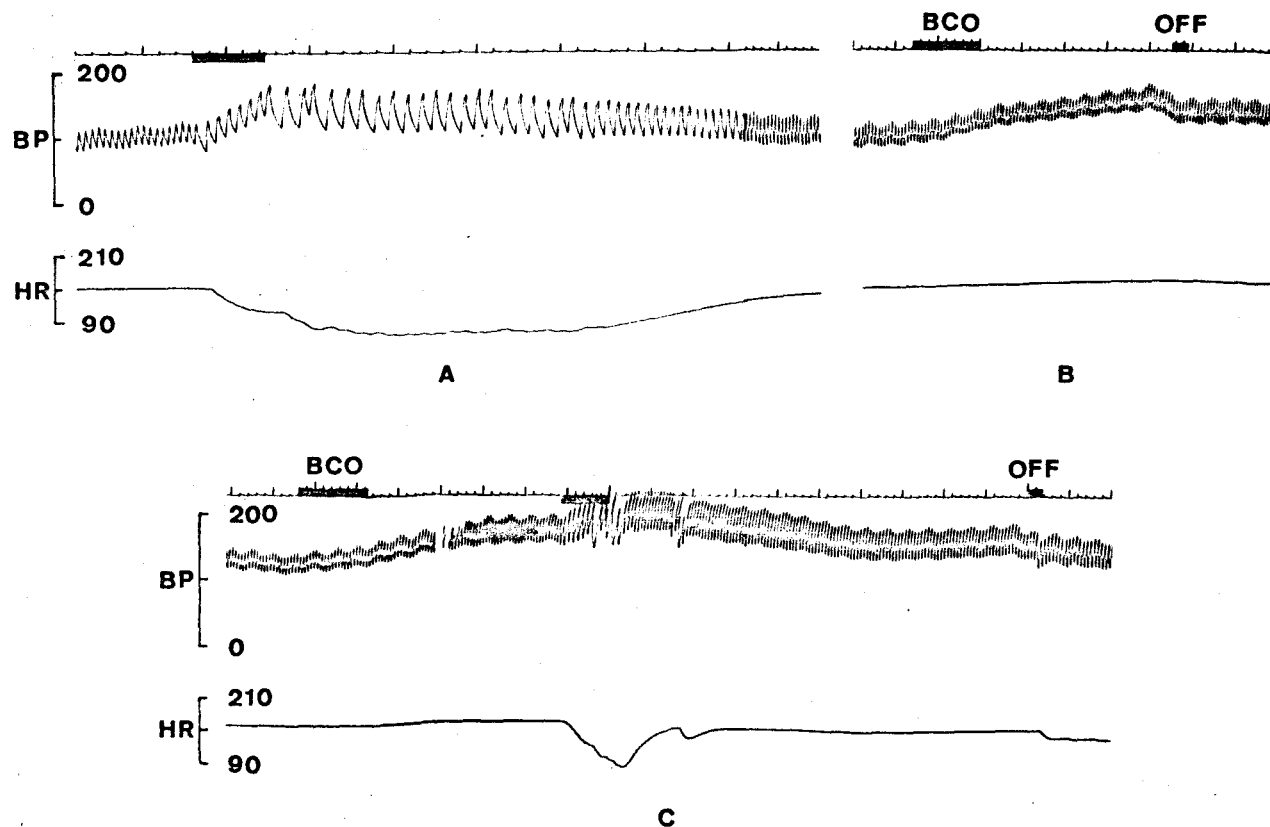


Figure 12 A. Cat 89, 2.5 kg. Effect of BCO on PSBC. Panel A shows PSBC following a 4.5 volt, 100 cps stimulation at LRI just below the surface of the medulla and 1 mm rostral to the obex. Panel B shows the pressor response to BCO (released at "off") and panel C illustrates virtual absence of stimulus evoked PSBC in the presence of BCO. Time marker at 1 and 5 sec intervals.

stimulus-bound bradycardia is unaffected by BCO, and only slight bradycardia follows release of occlusion. Subsequent testing showed BCO was no longer completely effective in abolishing PSBC and in a few instances, PSBC was seen following BCO release when occlusion resulted in a particularly large pressor response. In most cats with intact vagi, PSBC was either unaffected or only partially reduced by BCO.

Although these BCO results support the view that barosensory elements are involved in PSBC, its occurrence in the absence of any pressor response, as in figure 9, and other relevant findings discussed above caution against a simple explanation. Since it has been observed in some experiments that BCO or stimulus-evoked pressor responses do not in themselves necessarily result in PSBC, but that BCO may inhibit this response it is possible that the barosensory mechanism(s) may be sensitized by the stimulation which results in PSBC. This would explain the usual failure of pressor responses elicited by means other than LRI stimulation to evoke PSBC as part of the compensatory mechanism, and is strongly supported by the finding that, when aortic arch receptors are partly eliminated by contralateral vagisection, protection of carotid barosensory areas from a rise in pressure eliminates PSBC. Furthermore, PSBC was sometimes deferred until BCO release. It is possible that the nature of this sensitization may involve compensatory mechanisms effected by rate alterations, or may specifically enhance bradycardia reflex mechanisms. Certainly one cannot neglect the possible role of the central connections of carotid and aortic bodies especially since some PSBC responses were accompanied by a stimulus bound pressor response combined with

bradycardia.

Interaction between stimulation of a PSBC locus and BCO responses is shown in figure 12B. The stimulating electrode was positioned at the site which evoked the PSBC of figure 12A. Reduction of voltage to a level having no obvious effect on control pressure or rate (panel A) and no effect during BCO (panel B) was nevertheless capable of reducing the BCO reflex if occlusion was applied during a prolonged stimulation of this point. Attenuation of the BCO pressor reflex may be due to sensitization of the aortic baroreceptor reflex or inhibition of the BCO reflex. This may be mediated by the medial reticular formation. It is also possible that this weak stimulus does not activate those elements responsible for PSBC and has its effect on the BCO response by an entirely different mechanism.

It is obvious that the preceding discussion has not considered all possibilities involved in PSBC as related to BCO; many possibilities do exist, and the very nature of our observations precludes any more than tentative suggestions as to the mechanism involved in PSBC. However, from the evidence presented it appears that PSBC is not a peripheral vagal phenomenon, but is the result of specific brain stem stimulation in a region designated by many investigators as a central point for baroreceptor reflexes. Larger pressor responses enhance PSBC, but since stimulation of this region in certain cases results in PSBC in the absence of a pressor response, this phenomenon is not a simple barosensory reflex to a stimulus-induced pressure rise. The prolonged nature of PSBC, and its appearance only after BCO release suggests a prolonged central discharge and possibly a polysynaptic pathway.

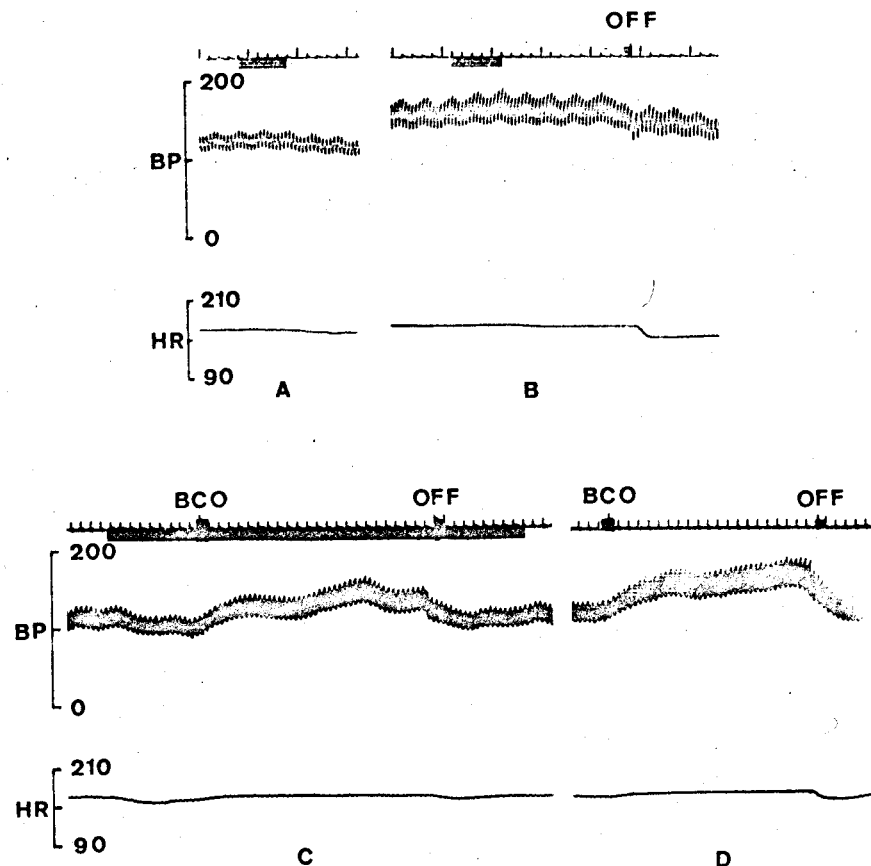


Figure 12 B. Interaction of weak stimulation of an active point and BCO response. Electrode is at same locus as in figure 12A but stimulus intensity has been reduced to 3 volts. Panel A shows a control stimulation, B illustrates stimulation during BCO which was applied 65 sec earlier; release of occlusion is indicated by "off". Panel C illustrates an attenuated BCO response during a weak electrical stimulation of the medulla (shown by a constant stimulus marker) and panel D shows a control BCO response. Time marker at 1 and 5 sec intervals.

However, it is possible to rule out a long, ascending pathway since PSBC was seen in animals after mid or post-collicular transection. The presence of normally recovering bradycardia evoked from other loci largely eliminates the possibility of inadequate acetylcholine destruction at the periphery.

Sympathoinhibition can be ruled out since in four experiments, PSBC was abolished by vagisection. Although PSBC was observed in some animals which had been Nembutalized, in three animals PSBC was abolished by intravenous Nembutal at a dose of 5 mg/kg. This suggests blockage of a polysynaptic circuit, especially since evoked pressor responses were only slightly affected, but it is difficult to evaluate the role of baroreceptive reflexes since Nembutal administration caused a substantial drop in control blood pressure. A given pressor response would be expected to have a less potent effect through barosensory mechanisms at reduced control pressure.

Brain Stem Transection.

Consideration has been given to the possibilities a) that stimulus evoked bradycardia produced in the medulla was mediated by pathways projecting rostral to the medulla; b) that if LRL responses are in fact due to stimulation of NTS mechanisms, these in turn might be subject to influences from higher levels and thus subject to change after brain stem transection.

In eight cats, the brain stem was transected at the mid-collicular level using a blunt knife. This procedure resulted in a transient hypertension followed by a prolonged hypotension and prolonged bradycardia. Responses after this procedure were equivocal; usually points which produced bradycardia prior to transection were subsequently depressed or inactive. Some of these were at LRL, others at LR4, and sometimes neither was responsive. In other cases, LRL and/or LR4 electrodes produced slowing comparable to that seen during control periods. No distinct pattern with regard to medial versus lateral electrode loci was evident. Quantitation was difficult since post transection heart rate and blood pressure were usually changed; furthermore, knife transection usually causes the brain stem to separate at this level by a few mm and there is the possibility of a slight shift in electrode-brain stem relationship. This would be especially important when the electrode tips were positioned close to the dorsal medullary surface.

Because of the possible shortcomings of this method, electrolytic transection was carried out in a series of five cats at the level of the pontomedullary junction after a series

of control stimulations had been done. This was accomplished by inserting a bank of three electrodes into the brain stem, positioned 1, 3 and 5 mm lateral to the midline, between 6 and 8 mm posterior to the interaural reference. Radiofrequency current was passed through each electrode for a period of from 45 to 90 seconds, and the bank was moved up from the ventral part of the brain stem in 2 mm steps after each series of lesions was completed. When the entire side of the brain stem had been thus destroyed the procedure was repeated on the opposite side. The entire procedure usually required 60 to 90 minutes. Postmortem examination of the fixed brain stems revealed extensive regions of tissue destruction. Visible evidence of damage was not, however, contiguous from one electrode tract to the next. Thus it is not known whether transection was complete, and it is possible that some functional fiber groups may have traversed the level of transection.

Previously reactive loci were tested at various intervals for periods as long as two hours after transection. Removal and replacement of stimulating electrode banks, which was necessary for placement of the transecting banks of electrodes, showed responses to be accurately reproduced; this is, however, possible source of error when it is desired to make quantitative comparisons of bradycardia. For this reason, after replacement of the stimulating electrodes at previously reactive points, stimulation was often applied at various levels around these loci.

Responses to electrical stimulation at LR1 and LR4 are shown in table VI. In four of the five animals, post-transection bradycardia responses were generally decreased

TABLE VI

Effect of Electrolytic Transection at the Level
of the Pontomedullary Junction on
Bradycardia Responses to Stimulation.

=====

Expt.	Cardiac slowing, beats/min*			
	Control	<u>LR1</u> Transected	Control	<u>LR4</u> Transected
58	100	78	132	90
76	90	106	86	78
80	38	24	80	56
81	68	39	48	21
82	155	146	172	147
MEAN	90	79	102	78

*In some cases these values were obtained at vertical loci others than those from which "control" responses were obtained.

(in most instances by 10 to 30%) both at LR1 and LR4. Responses were increased in one animal at LR1 by 25% and unchanged or decreased at LR4. In most cats, two antero-posterior levels were stimulated, so the general finding of depressed bradycardia was substantiated at four loci in most animals. Designation of a decreased response does not necessarily imply a response from the identical vertical coordinate, but rather relates the maximum bradycardia response that was obtained after exploration. Thus, after transection, bradycardia as great as the control bradycardia was rarely seen at any location.

With one exception, heart rate after transection was close to the control rate, and in all instances blood pressure was only slightly lower than levels prior to transection.

A larger number of electrolytic transections might provide more confidence for quantitative comparisons if a rigorous exploration was conducted before and after this procedure. However, since the general trend of these five animals indicated that transection produced a general depression at LR1 as well as LR4, it appeared that either the experimental approach or the technique would not provide a basis for differentiation and no further experiments of this type were carried out.

Pentobarbital Administration.

King, Naquet and Magoun (1957) among others have suggested that the mechanism of action of barbiturates is by blockade of polysynaptic pathways. Thus, if bradycardia responses in the present experiments were largely due to afferent or internuncial elements of cardiovascular reflex arcs, it was reasoned that pentobarbital might interfere with these responses.

In a series of eight animals, intravenous pentobarbital (10 mg/kg or one or more doses of 5 mg/kg) was administered, usually towards the end of the experiment. The effect of pentobarbital on bradycardia responses is shown in figure VII. Quantitative comparison of reactive sites at LRL and LR4 was made from responses immediately prior to and following drug administration. Seven of these animals showed a reduced bradycardia response after pentobarbital. Ordinarily, responses of +2 or +3 intensity were diminished by 0.5 to 1.5. In instances where several doses of the drug were given, bradycardia was sometimes completely abolished. Pentobarbital always caused a mild to moderate fall in blood pressure and frequently reduced heart rate.

On the basis of these experiments it is not possible to suggest any trend in response changes which would differentiate LRL from LR4 responses. It is possible that the depressant action of pentobarbital is entirely a peripheral effect, as suggested by Koppanyi, Dillie and Linegar (1935). Furthermore, Kerr and Dunlop (1954) have claimed that central vagal pathways serving respiration, despite their multineuronal character, are highly resistant to blockade. On the other hand, Nakazato and Ohga (1966) studied evoked responses in the cat

TABLE VII

Effect of Small Doses of Pentobarbital
on Bradycardia Responses.

=====

Expt.	Cardiac slowing, beats/min			
	Control	<u>LR1</u> Pentobarb.	Control	<u>LR4</u> Pentobarb.
43	105	90	120	105
44*	60	30	60	30
58	66	24	98	48
59	30	12	63	21
63	81	81	40	40
67	51	18	90	42
85	64	24	96	50
88	30	18	51	30
MEAN	60	37	77	45

* Pentobarbital anesthesia.

vagus after central stimulation of the contralateral vagus, splanchnic or sciatic nerve. They reported that pentobarbital, 15 mg/kg, abolished most components of vagal responses.

Curare Administration.

Peiss and Manning (1959) have suggested that d-tubocurarine has a specific action to inhibit bradycardia responses following electrical stimulation of DVN. They ruled out a site of action at peripheral vagal ganglia or at the myocardium since the dosages employed were without effect on the response to stimulation of the peripheral vagus. On the basis of their suggestion, we sought to determine whether curare administration might enable a differentiation between LRL and LR4 responses.

In a series of eight cats, after the electrodes had been positioned at suitably reactive points, testing was repeated after the administration of d-tubocurarine, 100 or 150 mg/kg. via the right carotid artery. Almost without exception, LRL and LR4 responses of +1 to +3 intensity were decreased by approximately equal degrees as shown in table VIII. In several instances a single dose of curare was not effective in blocking bradycardia and one or more additional doses had to be given to obtain this effect. It is not known whether this is due to a biologic variability within the animals tested, differences among the batches of curare used, or some other factor or factors. Serial stimulation in several animals has shown the time course of recovery from the curare effect to be of the order of 15 to 30 minutes. However, LRL and LR4 responses appeared to recover equally.

The statement of Peiss and Manning (1959) that this blockade effect is not manifest in the presence of strong bradycardia has been confirmed. In our experiments, it was observed that strong bradycardia (+4 intensity) was unaffected by a dose of curare which reduced weaker responses at other

TABLE VIII

Effect of Curare on Bradycardia Responses.

=====

Expt.

Cardiac slowing, beats/min.

	<u>LR1</u>		<u>LR4</u>	
	Control	Curare	Control	Curare
43	90	24	90	18
54	60	14	90	51
55	51	15	120	78
58	39	18	69	48
59	42	21	66	48
61	120	48	90	21
70	48	24	60	36
90	75	45	90	60
MEAN	66	26	110	45
% Depression	61		59	

LR1 and LR4 electrodes in the same animal.

The original finding of Peiss and Manning (1959) with relation to DVN susceptibility to curare might suggest that this effect was due to the blockade of central synapses if their bradycardia was, in fact, due to stimulation of NTS elements. However, the consistent finding that LR4 responses were equally blocked by curare negates this argument unless it is assumed that all LR4 responses are also due to afferent stimulation.

Vagotomy.

Most of the animals in these studies were tested to ascertain that evoked slowing was mediated entirely by the ipsilateral vagus. In a total of 23 animals with the left vagus cut, one or more active sites were tested at LRL (17 animals) and/or LR4 (21 animals) after section of the remaining vagus. In no instance was bradycardia produced at a previously active site after bilateral vagotomy, even when testing was done at increased stimulus intensity. One or more active sites at each plane were tested before and after right vagisection in a series of ten animals with the left vagus intact. In one of these, the bradycardia response survived ipsilateral vagisection although it was markedly attenuated. It was produced at an LRL site which also evoked a pressor response and PSBC. Strong stimulation of this locus after section of the left vagus resulted in a pressor response accompanied by a modest cardioacceleration. In three experiments in which reactive sites were studied in preparations with both vagi intact, section of the left (contralateral) vagus was without effect on bradycardia.

Stimulation of loci giving bradycardia rarely caused cardioacceleration after right vagotomy, and in the majority of cases produced no change in pressure exclusive of that due to slowing. In a few instances depressor responses were seen after ipsilateral vagotomy, and pressor responses sometimes occurred, especially with 100 cps stimulation.

CHAPTER V

DISCUSSION

The literature review has pointed out the anatomical complexity of central vagal relationships, whereby numerous neural systems are related to vagal nuclei, and conversely, vagal fibers have been traced to many parts of the central nervous system not ordinarily associated with this nerve. This complexity is probably understated in relation to the information which might be obtained if the connections of secondary and higher neurons could be readily traced. Furthermore, a review of the early literature has shown the inability of conventional anatomical techniques to definitely establish the role of DVN in relation to cardiac fibers; it is evident that current controversy regarding the role of DVN in relation to vagal cardiac fibers dates back to the last century. Modern technological advances have probably been best employed in relation to this problem by Calaresu and Pearce (1965a) with their measurement of electrical activity of DVN units. It is possible, however, that their stimulation technique, which failed to elicit slowing from DVN, was overly refined. Although their electrodes evoked appropriate activity when positioned in the hypoglossal nucleus (Calaresu and Pearce, 1965b) its cells are considerably larger than those of DVN. Bradycardia was obtained by these workers after NTS stimulation; cells in this nucleus are comparable in size to those of DVN, but it is conceivable that stimulation of even a very few NTS fibers

would produce discharge in a relatively large number of efferents because of the polysynaptic nature of NTS pathways.

It was largely on the basis of these latter considerations that the present experiments were undertaken. The use of relatively large electrodes was considered mandatory if the failure to obtain slowing with more discrete stimulation (Calaresu and Pearce, 1965b) was due to inadequate activation of a number of fibers sufficient to produce bradycardia. Because the larger electrode necessarily activates a greater volume of nervous tissue, its use is probably complicated by a number of factors relating to the anatomical arrangement of vagal nuclei. These will be briefly summarized.

The fifth cranial nerve may constitute an important afferent path in the production of reflex bradycardia under certain conditions. Hunt (1899) cited a statement by Tigerstedt that afferent stimulation of this nerve can produce slowing of the heart. Brodie and Russel (1900) attributed the bradycardia following chemical irritation of nasal mucous membranes to an afferent path along the nasal branch of the trigeminal nerve. Allen (1934-35) reported a similar role of the fifth nerve but claimed that sympathoinhibition was largely responsible for bradycardia, although his use of barbital anesthesia may have strongly depressed vagal responsiveness. Andersen (1966) has recently suggested that the trigeminal is a major afferent pathway for diving bradycardia in the duck, possibly serving chemoreceptors in the nasal area. Butler and Jones (1968), however, suggested that areas innervated by the ninth and tenth nerves are of primary importance in this regard.

Since medullary stimulation may involve sensory pathways, consideration must be given to the role of rostral structures, since they may affect vagal activity or actually mediate bradycardia. In the present experiments, the possibility that either electrode bank produced slowing by a path which relayed through rostral areas is unlikely, especially in view of the transection experiments described above. It is possible, however, that bradycardia resulted from stimulation of pathways descending from higher cardiovascular centers. Glasser (1962) suggested that portions of the pons influence medullary cardiovascular activity in a fashion similar to pontine modulation of respiration.

It is possible to elicit bradycardia and other parasympathetic effects by stimulation of the anterior hypothalamus. Wang and Ranson (1941) reported bradycardia produced by stimulation of the preoptic region, although some slowing persisted after vagotomy. Crosby and Woodburne (1951) described the dorsal longitudinal fasciculus (DLF) as a "series of periventricular ascending and descending fascicles from the preoptic and hypothalamic levels to the caudal end of the brainstem". The DLF is in proximity to DVN and has interconnections with it as well as MTS. Crosby and Woodburne (1951) suggested that this pathway mediates hypothalamic outflow to the parasympathetic system and may thus mediate the parasympathetic effects described by Wang and Ranson (1941). Cheatham and Matzke (1966) also described sparse connections between the hypothalamus and DVN on the basis of Nauta studies following hypothalamic lesions. The pathway described, however, was in the reticular formation, in which they ob-

served diffuse degeneration.

Numerous authors have traced at least some vagal fibers to the reticular formation. The work of Crill and Reis (1968) is of particular significance since they described reticular formation input from the carotid sinus nerve. One might surmise that these fibers subserve cardiovascular adjustments that are mediated by the reticular formation. The precise stimulation experiments of Kovalev and Bondarev (1962/3) lend support to the idea that parts of the reticular formation may be related to cardiovascular adjustments in a highly specific fashion. The work of Chai, Mu and Brobeck (1965) also implicated the lateral reticular formation, although it is difficult to understand why thermal stimulation produced ipsilateral vagal slowing while electrical stimulation at the same point did not.

Other studies have shown that appropriate afferent stimulation of a peripheral nerve, such as the sciatic, sometimes interfered with efferent vagal activity (McDowall, 1931 and Iriuchijima and Kumada, 1964). The type of fibers involved and their central relationships remain obscure. Akert and Gernandt (1962) described a hierarchy of competitive influences converging on vagal nuclei; respiratory center activity has priority in the determination of vagal outflow, followed by vestibular and limbic activity, vagal afferent and trigeminal afferent impulses in that order. These authors defined this blockade as an occlusion phenomenon. Furthermore, they cited previous studies which indicated that vestibular effects on the cardiovascular system are rather weak.

It is evident that the LRL electrode, placed in or near DVN, may also stimulate elements of NTS. Its possible

activation constitutes an important complication of LRL stimulation. Other structures which may be importantly influenced by the stimulating electrode include the dorsal longitudinal fasciculus, the medial reticular formation and the genu of NA. Windle (1933), in his description of NA, suggested that in the adult cat the genu may not be present to the extent that is seen during development; migration may leave only remnants of the genu close to DVN.

Electrode placements at LR4, intended to stimulate either NA or vagal efferent fibers, may also have stimulated cardiovascular afferents destined for NTS. Other possible complications include the nucleus and tract of the fifth nerve, as well as the lateral reticular formation. Little can be said about the cardiac efferents reported to be in the bulbar accessory nerve (Sperti and Xamin, 1960) if indeed they should be treated separately from the vagus in this context.

In spite of the anatomical complexities, the studies reported here have used relatively large stimulating electrodes for the reasons outlined above. Since most of the nearby nervous structures which might also give rise to bradycardia are involved in cardiovascular reflexes, consideration has been given to the possibility that their responses might be so identified on this basis.

Sherrington (1929) summarized his findings with regard to activation of skeletal motor reflexes. It was shown that appropriate stimuli, applied simultaneously, might produce a response greater than their sum when applied individually because of central summation of subliminally excited fields. Overlap of these fields was likely to activate neurons which

had been only weakly excited by a single stimulus; the possibility of occlusion was also recognized. In this instance, there is an overlap of neurons activated by either stimulus and the net response when two appropriate points are simultaneously stimulated is less than the sum of responses to individual stimulations.

It seems reasonable to speculate that cardiovascular reflexes are in this sense similar to somatic reflexes - that is, activity in a group of afferents which leads to the firing of cardiovascular efferents might also produce a "peripheral" pool of subliminally stimulated and/or inhibited efferents (or internuncials). Under these altered conditions, the response to an electrical stimulation should be changed to the extent that its subliminal fringe overlaps neurons with appropriately changed thresholds. If the electrode primarily activates preganglionic rather than afferent or internuncial fibers, modification of the threshold of cardiovascular reflexes (by BCO, for example) should have little or no effect on the response to electrical stimulation.

There is ample precedent for the proposal that cardiovascular reflexes are subject to summation phenomena. In addition to studies mentioned above, those of Bach (1952) Prout, Coote and Downman (1964) Reis and Cuenod (1965) and Raiciulescu and Bittman (1966) have specifically shown that stimulation of the reticular formation influences cardiovascular reflexes, and inhibition of the BCO reflex by means of the ventromedial reticular formation was observed by several groups. Baccelli, Guazzi and Libretti (1965) described the interaction of pressoreceptive and chemoceptive reflexes with

diencephalic rage mechanisms, and suggested that the central excitatory state may be an important determinant of the size and magnitude of autonomic reactions, although it is probably often obscured by anesthesia.

Hoff, Breckenridge and Spencer (1952) also implicated the reticular formation and suggested that it and pontine structures serve to integrate vagal reflexes. Gellhorn's well known "autonomic tuning" (1957) may also be a manifestation of summation phenomena in the autonomic nervous system. Indeed, he has demonstrated (1960) both spatial and temporal summation for the sympathetic system although in the parasympathetic system, temporal summation was rarely seen. Scott and Reed (1955) described vagal facilitation and occlusion effects while comparing stimulation of the right or left carotid sinus region (by manipulation) with simultaneous stimulation of both carotids. Samonina and Udelnov (1964) have also demonstrated summation effects in the frog. Mild distension of the bladder and/or stomach enhanced a simple bradycardia response to an extent greater than that predicted on the basis of addition of the individually applied responses.

Hilton (1966) stated that enhancement of cardiovascular reflexes after various procedures may represent a general (rather than cardiovascular specific) alteration of autonomic reactivity. Furthermore, he suggested that the pressor component of the rage response may be effected largely by an inhibition of carotid sinus pressoreceptor reactivity, possibly triggered by chemoreceptors.

One of the most readily available procedures for alteration of cardiovascular homeostasis is BCO, and comparison

was made of responses evoked before and during occlusion.

A prominent complication is that the pressor response to BCO is opposed by aortic arch receptors. It has been pointed out that in the dog (Glick and Covell, 1968) and rabbit (Alexander and DeCuir, 1963) aortic receptors are of equal or greater importance compared to those in the carotid sinus in the control of heart rate. Glick and Covell (1968) also reported that when opposite changes were simultaneously imposed on the two areas, the regular response was cardiac slowing. It was further stated that in no instance did changes in carotid sinus pressures affect heart rate more than comparable changes in the aortic arch.

Because of the possibility of species differences, these considerations can be applied to the cat only with some reservation. Section of the left vagus should eliminate some aortic arch afferents, but in most of these animals the response elicited during BCO was unchanged from the control response. In the cases where responses in unilaterally vagotomized animals were changed, these were (with one exception) altered in the direction predicted on the basis of the carotid sinus as the predominant sensory region. In the majority of animals with both vagi intact, BCO was without effect on evoked bradycardia. The few changes which were observed were all augmented bradycardia responses. Although small in number, these changes are consistent with the proposal that with intact vagi, aortic arch receptors may exert influences on central barosensory elements equal to or greater than those elicited from carotid sinus receptors.

It is difficult to appraise the role of the carotid bodies, except to state that their anoxia would provoke brady-

cardia responses (MacLeod and Scott, 1964), perhaps accompanied by pressor responses. Prolonged occlusion did not result in responses to stimulation that were in any way different from those evoked after normal occlusion periods. This argues against the importance of cumulative anoxic effects in the brain and carotid bodies as manifest in the bradycardia response to stimulation. In the present studies the effects of anoxia which might have occurred during the first 15 seconds of occlusion have not been evaluated.

Neil, Redwood and Schweitzer (1949) claimed that chloralose (but not pentobarbital) usually inhibits the carotid sinus barosensory mechanism in the cat but not in the rabbit or dog. However, Douglas and Schumann (1956) presented evidence indicating the presence of two types of barosensory fibers in the carotid sinus nerve, and did not substantiate all the findings of Neil's group (1949). Bergmann and Rosenblum (1968) recently presented evidence of two types of pressure receptor in the carotid sinus, in support of the finding of Douglas and Schumann (1956).

In the present BCO experiments it is readily apparent (see Table III) that in most cases BCO was without effect on the magnitude of evoked responses. It is possible that in those animals with both vagi intact, the failure of BCO to influence bradycardia responses was due to an exact balance between aortic arch and opposing carotid sinus influences. It is unlikely that this was the case in every instance since responses in most of the left vagotomized animals were also unaffected by BCO. The more reasonable possibility appears to be that responses were unchanged

because a suitable pool of subliminally altered neurons was not present distal to the stimulating electrode. This, in turn, could indicate either that evoked bradycardia was mediated by neurons in a pathway that was unaffected by BCO or that the involved fibers were vagal preganglionics, i.e. neurons of the final common pathway. Although these experiments do not permit a distinction between the last two possibilities, the abundance of cardiovascular relays in the region of the LRL electrode favors the likelihood that if synaptic mechanisms are involved in bradycardia, these will be affected by BCO.

Response changes at LR4 were seen nearly as often as at LRL; the most likely explanation is that these LR4 responses were due to the stimulation of intramedullary sensory fibers, probably from the ninth or tenth cranial nerves. Since response changes at LR4 did not necessarily occur in cats showing LRL changes, it is neither probable that changes were due to some peripheral effect, nor that they represented a generalized alteration of individual animals' responses due to BCO.

The use of great vessel occlusion as a conditioning procedure eliminates a major complexity inherent in the BCO procedure in that pressure changes in the two principal barosensory regions are in the same direction. In addition, the alteration of pressure can be graded at will. Nevertheless, other complications may arise, notably as a result of stretch receptors known to be present in the heart and great vessels and classically manifest in the Bainbridge reflex. It is also possible that changes in the pulmonary vascular bed

following occlusion of the aorta or vena cava might give rise to activity in cardiovascular afferent fibers. However, Kinnison and associates (1965) reported that in the dog, reduction of pulmonary arterial pressure caused only respiratory changes and had no effect on heart rate. It is also possible that distension of the heart may alter myocardial response to a given neural or humoral stimulus. Moreover, Goetz (1965) suggested that there are intrinsic cardiac reflexes whereby a moderate increase in right heart pressure caused an increase in rate while further pressure increase caused a fall in rate. These effects were independent of vagotomy and sympathetic blockade.

Because of these considerations, occlusion procedures were conducted in a conservative fashion as described above. Drastic pressure excursions were not produced until the end of experiments in which it was desired to evaluate pure reflex responses in the absence of electrical stimulation. Perhaps because of this precaution, it would appear that alteration in myocardial responsiveness and/or intrinsic cardiac reflexes did not occur as a result of the occlusion procedures; however, if these changes did occur, they should be of equal importance in the LR1 and LR4 trials (since occlusion was always of comparable magnitude) and thus cancel each other in relation to LR1 - LR4 comparisons.

It is likely that vascular occlusion procedures gave rise to afferent cardiovascular input over a number of pathways. In light of the small magnitude or absence of rate changes in most cats at the height of occlusion, the pressure stimuli can be described as of threshold or subthreshold in-

tensity in relation to reflex bradycardia. According to Samonina and Udelnov (1964) who described vagal subliminal fringe summation effects in the frog, weak stimuli are best suited to the demonstration of these phenomena.

Results of experiments in which control responses were compared to those evoked during aortic occlusion are consonant with findings obtained in the BCO series. In most cases, an imposed alteration of blood pressure - whether an increase or decrease - had no effect on the bradycardia produced by electrical stimulation. In two animals, bradycardia changes occurred as a result of occlusion. These were in directions predicted on the basis of the change in blood pressure and are consistent with the proposal that response reinforcement was due to recruitment of neurons in a subliminal fringe which had undergone threshold alteration as a result of the pressure change. Many of the considerations which were presented in relation to the BCO experiments are applicable to the great vessel occlusion experiments as well. On this basis, it is proposed that those electrode placements which do not show response changes during occlusion probably signify stimulation of the vagal final common pathway.

There are numerous descriptions in the literature of post stimulation slowing which appears to be quite similar to the PSBC described in our results. In most cases, these responses have followed stimulation of preoptic and/or anterior hypothalamic regions (Wang and Ranson, 1941; Gellhorn, 1959 and 1960a and b; Sperti, Midrio and Xamin 1962). The latter group attributed this effect to the ipsilateral vagus. Manning and Cotton (1963) also evoked a post stimulation

slowing from the midbrain reticular formation which was usually manifest as a relatively prolonged arrhythmia. This was abolished by vagotomy, but was also abolished by stellate ganglionectomy. Buryak (1964) claimed that stimulation of vagal nuclei disturbed coronary circulation and led to arrhythmias, but presented no evidence for this statement. Calaresu and Pearce (1965b) described a "slight, late cardiac slowing" as the only bradycardia observed when DVN was strongly stimulated in an attempt to produce slowing. Since these stimuli produced hypertension, it was reasoned that the slowing was induced reflexively from baroreceptor areas. Varma (1966) readily evoked bradycardia from the region of DVN and reported that slowing "lasted in some preparations up to 10 seconds after the stimulation had been stopped." Most of his cats had been pretreated with reserpine.

The report of Manning and Cotton (1963) suggested that the observed arrhythmia resulted from a simultaneous barrage of sympathetic and parasympathetic impulses on the heart. This cannot fully explain our findings since PSBC was seen in the absence of any apparent sympathetic outflow. Electrocardiographic recordings were made in only one PSBC experiment. These indicated that PSBC could occur as a sinus rhythm, although in some instances, there were bouts of ventricular rhythm; spontaneous conversion to sinus rhythm did not necessarily abolish PSBC, however.

It appears that a PSBC response indicates involvement of NTS fibers, or perhaps related afferents. The slow recovery from PSBC suggests a prolonged discharge or reverberating circuit. Sherrington (1929) stated that stimulation of

afferents may provoke repetitive discharge, and Anderson and Berry (1956) and Crill and Reis (1968) have both presented evidence of polysynaptic mechanisms in NTS on the basis of afferent electrical stimulation of the ninth and tenth cranial nerves. Moreover, McDowall (1931) and Wechsler and Pace (1968) reported prolonged slowing after afferent vagal stimulation. The occurrence of stimulus-bound slowing together with an evoked pressor response in some PSBC responses suggests a chemoreceptor pathway, and is not unlike changes seen in the diving response.

On the basis of the occurrence of PSBC, it is concluded that NTS excitation is involved in some, but not all instances of evoked bradycardia. Although it was seen in about one third of all experiments, this may be an underestimation of its frequency of occurrence since 100 cps stimulation was usually more effective in producing PSBC but was not used in all experiments. In twenty experiments in which 100 cps stimulation was employed (sometimes evoking pressor responses at LRI without PSBC) PSBC was observed in nine cases.

On the basis of vascular occlusion experiments and the occurrence of PSBC, it appears that a distinction can be made between responses mediated by polysynaptic paths and those produced by an uncomplicated path, presumably the final common pathway. Occlusion experiments, particularly those employing BCO, may fail to demonstrate significant changes in evoked responses if subliminal pool alterations are of inadequate magnitude. In this respect, PSBC appears to be a more reliable indicator of the incidence of bradycardia due to stimulation

of polysynaptic paths.

The experiments of Varma (1966) are of special significance in relation to bradycardia evoked from the dorsal medulla and to the role of synaptic as opposed to possible pre-ganglionic mediation. His main objective was to characterize the central nervous system transmitter that might be involved in vagal bradycardia relays. Following decerebration, cerebellectomy and high spinal transection, the dorsal medulla was explored with a small stimulating electrode. Sites producing bradycardia were tested before and after the local application of various drugs to the medulla. Local atropine markedly reduced or abolished evoked slowing in eight preparations, but had little or no effect in six others. Physostigmine or neostigmine application potentiated bradycardia responses to stimulation in fourteen of sixteen experiments. Atropine interacted with these drugs in an antagonistic fashion. Locally applied hexamethonium was effective in blocking the bradycardia response in only two of seven preparations; the quantity of drug may not have been adequate, but it is also possible that central synapses are less sensitive to this agent than peripheral ones.

Varma (1966) verified that the locally applied substances did not affect vagal responses at the periphery, but said little with regard to the limitations of his method which might be imposed by diffusion processes. Nevertheless, his final conclusion is that vagal bradycardia evoked by medullary stimulation may be mediated by a central synaptic pathway (with acetylcholine as the transmitter) or by stimulation of the final common pathway (presumably DVN). This nicely

compliments our findings; however, it is difficult to quantitatively reconcile Varma's (1966) anticholinesterase data with his atropine findings and also with our general results. His anticholinesterase experiments suggest that nearly all responses are mediated by cholinergic synapses in the region of the electrode. Results of his studies in which atropine was employed, as well as our findings, indicate that central synapses are involved in a considerably smaller number of responses.

Perhaps the most perplexing of our findings involved the observation which we have termed reversal. A dramatic example of this is shown in the explorations of figure 3. In instances in which extensive exploration was not undertaken, reversal was sometimes observed in a small number of sites, usually in the LRL plane. In other animals, the tendency for bradycardia responses to be of greater magnitude as the experiment progressed was probably also a manifestation of this phenomenon.

It is well recognized that an inversion of response may be produced by suitable choice of stimulus parameters, especially frequency (Douglas and Schumann, 1956). Samonina and Udelnov (1965) claimed that vagal activity is capable of increasing or decreasing heart rate depending on the number of fibers activated. However, the type of inversion related to variation of stimulus parameters is probably due to stimulation of functionally different groups of fibers and thus unlike the reversal we have encountered.

Many of the studies mentioned in the earlier part of this discussion illustrate the wide variety of influences which may influence vagal activity and responses. Gellhorn's tuning phenomenon might be closely allied to reversal. He has shown, for example, that stimulation of a given point in the hypothalamus can provoke a pressor or a depressor response, depending on the level of systemic blood pressure during drug induced pressure changes (Gellhorn, 1960b and 1964). Tuning has also been established when the conditioning procedure was central stimulation of a mixed nerve (Gellhorn, 1960b) and Iriuchijima and Kumada (1963) have shown that afferent stimulation of the dog sciatic nerve may inhibit tonic and reflex vagal activity, as does pentobarbital. Gellhorn also pointed out (1960b) that the level of anesthesia may be an important determinant of tuning. However, McDowall (1931) claimed that, in spite of adequate anesthesia, the initial surgical preparation for an experiment produces a sympathetic discharge which endures for 30 minutes to an hour and which inhibits cardioinhibitory but not depressor responses. From their description of methods, Gunn and associates (1968) apparently have applied a relatively small number of DVN stimulations in each cat. A reversal effect might account for their failure to elicit slowing from the cat DVN, although it was evoked from NTS and NA, as well as from the dog DVN.

McDowall (1931) also stated that bradycardia responses are more readily obtained in some cats than others, and that these cats are most frequently encountered in winter and autumn but not in early spring. It is thus possible that seasonal variation (or some other factor which determines this)

may account for failure to obtain bradycardia responses from DVN. However, in relation to the work of Gunn et al. (1968) it is then difficult to explain the slowing obtained from other nuclei in the same animal. In our experience, there have been two or three occasions over the course of more than two years when a number of poorly responsive animals have been encountered in consecutive experiments. It is unknown whether this represented some form of seasonal variation or a diseased state prevailing in a group of cats. No attempt was made to categorize experiments according to season since some cats were obtained locally but others came from a different part of the country and climatic differences would probably obscure seasonal effects.

Consideration was given to the possibility that abnormal ventilation might influence vagal responses and perhaps result in reversal. Published reports on the relationship between ventilation are in many cases contradictory. Young and associates (1951) stated that hypercapnia and reduced pH enhance the cardiac effect of cervical vagal stimulation, but this effect is inhibited by concomitant hypoxia. Mohamed and associates (1961) claimed that both hypercapnia and hypoxia enhanced vagal effects on the heart.

During the course of our experiments, care was taken to maintain adequate ventilation as judged by the maintenance of normal blood pressure. On a few occasions, ventilation was increased and/or decreased for varying periods in normally responsive animals and in those which were considered poorly responsive. Although quantitative response changes were sometimes seen, these were not produced in a regular fashion,

and on the basis of our experiments it is not possible to generalize with regard to the effects of ventilation on evoked vagal responses. However, it can be said that excessive under - or overventilation for periods of five minutes or more did not produce qualitative changes similar to those seen in reversal.

There are many possible causes for reversal. In most of the present experiments it was not seen in a dramatic fashion but often as a gradual change from mild to stronger responses over the course of a three hour study. In some cases, however, exploration revealed little or no activity initially, with an increase in responsiveness after subsequent testing; LRL placements gave this type of response more often than LR4 placements. It has not been possible to determine the cause of reversal, but it may bear some relationship to the failure of some workers to obtain slowing from DVN.

Analysis of the comparative threshold studies at LRL and LR4 must be done with cautious appreciation of the drawbacks inherent in the technique of electrical stimulation. There may be important differences in brain tissue characteristics at each locus; furthermore, different results may simply reflect electrode placements relative to active elements. Differences in individual electrodes could also account for apparent threshold differences, but this is not likely in the present experiments since interchanging LRL and LR4 electrode banks in a few experiments did not change the observation that, for a given stimulus voltage, LRL responses were weaker than LR4 responses. It is also possible that LRL responses were weaker simply because these electrodes were consistently

further away from responsive elements than was the LR4 bank. However, exploration in a plane closer to LR4 (i.e., LR2) rarely produced responses that were markedly greater than those seen at LRL. This is evident in figure 1, which also substantiates the statement regarding LRL and LR4 response magnitude.

In spite of the agreement between the voltage-threshold findings and the results of exploration, only limited conclusions can be drawn regarding the thresholds of the neural elements involved. These results may simply indicate that afferent and/or efferent neurons responsible for bradycardia are relatively grouped at LR4 but dispersed at LRL.

Since figure 1 indicates a reactive "pathway" across the medulla, it is possible that this pathway represents the genu of NA rather than efferents from DVN. However, if Windle (1933) was correct in stating that the genu of NA migrates ventrolaterally away from DVN in the adult, and these fibers mediate bradycardia, LR2 electrode placements should more readily elicit slowing. Since this was usually not the case, and because responses as specific as that in figure 2 were sometimes seen, it appears that LRL bradycardia cannot be attributed entirely to the genu of NA.

We further conclude that some, but by no means all bradycardia responses at LRL can be attributed to DVN. It appears that bradycardia resulting from NTS stimulation can often be attributed to a polysynaptic pathway especially with 100 cps stimulation which appears to be the more effective way of producing PSBC. This, in turn, is probably the best

criterion of an NTS (or other) polysynaptic response. It is conceivable that extensive study of individual animals to find the optimum level of just subthreshold great vessel occlusion in each case would more readily illustrate the facilitatory and inhibitory effects described above. In this manner, it might be possible to obtain closer agreement with regard to the incidence of NTS stimulation as revealed by the presence of PSBC.

On the basis of LR4 threshold voltage observations, as well as the exploration studies, we cannot exclude the possibility that NA (as well as DVN) gives rise to vagal cardiac fibers. Although not consistent with generally accepted anatomical teaching, it is a reasonable possibility on the basis of Sperti and Xamin's (1960) report with regard to the role of the eleventh nerve in bradycardia responses (although they cite DVN as the origin of its cardiac fibers).

If our observations with regard to thresholds at the two nuclei are valid expressions of a physiological difference, the threshold difference may be partly related to the small size of DVN cells in relation to those in NA. Since cell body sizes probably reflect the relative diameter of their axons, the conflicting findings with regard to the diameter of efferent vagal fibers to the heart may simply be due to the presence of both large and small diameter cardiac efferents. The report of Heinbecker and Bishop (1935-6) that an increase in intensity of vagal stimulation to activate C fibers in the cat increased the bradycardia attributed to B fibers by only ten per cent does not necessarily reflect their importance when they are physiologically activated. This is especially

so if these C fibers are sometimes functionally activated independently of B fibers.

The failure of Calaresu and Pearce (1965b) to elicit slowing from DVN is probably due to their failure to stimulate an adequate number of cardiac fibers because of the small size of their bipolar electrodes, which were spaced 300 micra apart.

Unless reversal effects were prominently involved in the cat experiments of Gunn's group (1968) it is difficult to reconcile their findings with those of the present experiments. Their electrodes were slightly smaller than ours (26 gauge needle compared to our 22 gauge needle) but stimulus parameters were otherwise comparable. Their maximum stimulus intensity was 9 volts; in our experiments, the most reactive points routinely gave moderate to strong slowing with a 4 to 5 volt stimulus. It is remotely possible that their stimuli were just subthreshold for cat DVN cells but adequate for the larger DVN cells in the dog.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Although it is commonly understood that the dorsal vagal nucleus (DVN) is the origin of vagal cardiac efferents, a study of the literature indicates continuing dispute on this point for more than a century. Several recent studies using a variety of techniques have cast additional doubt on the role of DVN in the cat. In some cases, nucleus ambiguus (NA) was implicated as the origin of cardiac efferents. It has also been proposed that, in instances when electrical stimulation in the region of DVN resulted in bradycardia, the slowing was actually due to stimulation of cardiovascular reflex arcs in the nearby nucleus and tractus solitarius (NTS).

In the present study carried out on 73 cats, responses to stimulation in the region of DVN and a lateral area near NA (and the emergent vagal rootlets) were compared before and during various physiological testing procedures. Blood pressure changes were accomplished by bilateral carotid occlusion or occlusion of the abdominal aorta or inferior vena cava. It was reasoned that such changes would activate cardiovascular reflexes and cause appropriate changes in subliminal fringe neuron pools. If bradycardia was effected by electrical stimulation of NTS elements, alterations in the subliminal

fringe area should be manifest by an appropriate change in stimulus evoked bradycardia. However, if slowing resulted from stimulation of vagal preganglionics, blood pressure alterations should have little if any effect.

The following observations and conclusions have been made:

1. Distinct bradycardia responses were obtained from the region of DVN, although in some cases this was not manifest until a given plane was explored two or more times. This apparent reversal may explain the failure of some investigators to obtain slowing from this region.
2. Great vessel occlusion or BCO was usually without effect on stimulus-evoked bradycardia, but predicted changes in response were seen in other instances.
3. In approximately one third of all experiments, stimulation near the obex resulted in an unusual bradycardia, with a slow recovery suggestive of a prolonged discharge. This was often accompanied by a stimulus bound pressor response, and maximum slowing usually occurred after the end of stimulation. These responses may also indicate NTS stimulation, perhaps involving chemoreceptor pathways.
4. The anatomical proximity of DVN and NTS has complicated previous studies of the role of these nuclei in bradycardia responses to stimulation. Our experiments indicate that the DVN does supply vagal efferent fibers to the heart in the cat.
5. The testing procedures used in this study have enabled us to attribute some bradycardia responses to NTS.
6. The role of NA as an additional source of vagal

cardiac efferents cannot be ruled out. Consideration has been given to the possibility that both DVN and NA supply efferent cardiac fibers of two different sizes.

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APPROVAL SHEET

The dissertation submitted by Joseph T. Ponessa has been read and approved by four members of the Dissertation Committee.

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

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

17 January 1969
Date

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