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ANATOMY OF THE NODOSE GANGLION IN THE RAT:  
CENTRAL PROJECTIONS OF AFFERENT FIBERS IN THE HEPATIC VAGUS

A Thesis Presented

By

BONNIE E. PIPKIN

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

September 1983

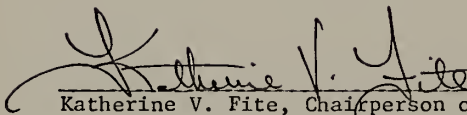
Department of Psychology


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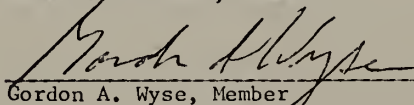
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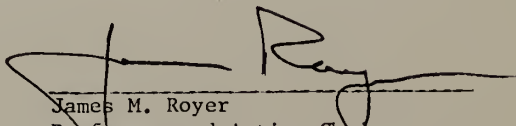
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ABSTRACT

Anatomy of the Nodose Ganglion in the Rat:  
Central Projections of Afferent Fibers in the Hepatic Vagus

September 1983

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Horseradish peroxidase (HRP) was applied to the rostral end of sectioned hepatic vagi (HV). Subsequently, a count of HRP-labeled cells in the nodose ganglia (NDG) yielded an estimate of the minimum number of afferent fibers in the HV of 139. HRP labeled cells were found only in the left NDG and were diffusely spread throughout the ganglia. No HRP labeling was found in areas of the brain previously reported to contain vagal afferent projections. In three cases small numbers of HRP labeled cell bodies were seen in the dorsal motor nucleus (DMN). The NDG were organized with cell bodies on the sides and their processes and fibers of passage in the center. The NDG have an apparent population of two cell types; large sensory neurons and smaller glial cells. However, the possibility of a population of smaller sensory cells is discussed. An average of total sensory cell counts for three NDG yielded an estimate of 9115 sensory cells. These results corroborate previous research that reported the existence of vagal afferent fibers with their cell bodies

in the left NDG, that innervate the liver. The results also provide anatomic evidence that the efferent vagal innervation of the liver emanates from the DMN. The finding that cell bodies from HV afferents are spread diffusely throughout the NDG is in disagreement with some previous reports.

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## C H A P T E R I

### INTRODUCTION

A growing body of literature suggests that there are sensory receptors in the liver that monitor various physiological processes and initiate homeostatic responses to alterations of these processes. In 1958 Tsai (70) described three presumed sensory nerve endings in the liver. Shortly after that Wolfman (71) reported that sodium excretion varies with the administration of sodium chloride in the liver, which indicates the possible existence of hepatic sodium receptors. Russek (55) proposed that the liver influences feeding behavior in dogs via hepatic glucoreceptors. Subsequently, Haberich and Ohm suggested the existence of hepatic osmoreceptors (see 24) and hepatic baroreceptors (46). In this section I will provide a brief review of the literature which is relevant to the question of whether the liver is a sensate organ. There are five major approaches that have been used to study the sensory function of the liver: behavioral, electrophysiological, nerve section, neurochemical and anatomic.

#### Behavioral studies.

Osmoreceptors. Researchers have shown that changes in the osmolarity of plasma in the liver can influence the rate of urine

flow. Using a conscious rat Haberich (24) reduced the osmolarity in the liver by infusion of water into the portal vein or the vena cava. A one percent reduction in osmolarity via the portal vein led to an increase in urine flow, but a similar reduction via the vena cava had an effect only when sustained for a longer period of time. In a later study, Haberich and colleagues (25) delivered twice-isotonic saline in the vena cava while infusing water into the portal vein and again found an increase in urine flow. However, when the procedure was reversed so that the vena cava received water and the portal vein received twice-isotonic saline, a decrease in urine flow was seen. In order to show that osmolarity rather than electrolyte concentration was producing the effect, mannitol and glucose were infused into the portal vein which also led to a decrease in urine flow. One author reported that oral or portal but not peripheral administration of hypotonic saline led to an increased urine flow (31); however, Schneider (63) was unable to replicate this work.

In 1967, Aziz (8) suggested that the decrease in urine flow following the infusion of hyperosmotic solutions in the liver is mediated by antidiuretic hormone (ADH). This hypothesis had recently been supported by a study showing that infusion of hypertonic saline into the portal vein of conscious dogs leads to a rapid increase in plasma levels that vary directly with the concentration of the hypertonic saline (14). Novin and colleagues (44) noted that hepatic afferents terminate in an area in the brain which is

two synapses from the supra-optic nucleus (SON) which manufactures, transports and secretes ADH.

Sodium receptors. In 1961, Wolfman (71) reported that sodium excretion diminished following the administration of sodium chloride in dogs with porta-caval shunts, a finding which indicates that there may be receptors in the liver that are sensitive to the sodium ion. Although this work has been replicated in the anesthetized dog (15) and cat (48), failure to replicate these experiments in conscious animals (31, 62) brings into question the significance of these proposed sodium receptors for normal physiology.

Baroreceptors. There have been two behavioral experiments that suggest the existence of hepatic receptors that respond to variations in portal blood pressure. Ohm and Haberich (46) reported that an increase in portal pressure in the rat liver initially results in anuria followed by a decrease in urine flow, while a decrease in pressure causes an increase in urine flow. However, Laing (34) found that in dogs an increase in portal pressure of less than 15 cm H<sub>2</sub>O leads to an increase in urine flow but when the pressure exceeds this point there is a decrease in urine flow.

Metabolic receptors. In 1963, Russek (55) suggested that the liver may play a sensory role in feeding behavior. He had observed that hungry dogs that were given injections of adrenaline intraperitoneally became anorexic. Later, in an effort to demonstrate that this effect is under the control of the liver rather than by a

central mechanism, Russek (56) compared the behavioral affects of hepatic portal infusions of glucose to those following injections of glucose into the jugular vein. He found that hepatic portal infusions decreased food intake in fasting dogs more effectively than did jugular injections. Although Bellinger (10) was unable to get a change in food intake in dogs in response to portal infusions of either glucose or adrenaline, a recent study by Russek (57) using larger amounts of glucose than Bellinger had used, again showed that portal but not jugular administration of the sugar leads to anorexia.

Further behavioral support for hepatic modulation of feeding comes from a number of experiments that contrast the affects of different metabolic fuels on insulin-induced feeding. It was found that administration of p-hydroxybutyrate (which is a fuel for the central nervous system but not for the liver) is less effective than fructose (a fuel that is utilizable by the liver but not the central nervous system) in attenuating insulin-induced feeding behavior (67). Granneman and Friedman (23) have shown that portal administration of fructose will block insulin-induced gastric acid secretion and electromyographic activity in the stomach if delivered before onset. Infusion of glucose (a fuel utilizable by both the liver and the brain) prevents all of the physiological and behavioral responses to insulin-induced feeding mentioned above (23, 67). Similarly, Novin et al (44) reported that infusion of 2-deoxyglucose (2DG) (an analogue of glucose which interferes

with glycolysis and causes hunger) into the hepatic portal system causes rabbits to eat sooner and in greater quantity than injections of the substance into the jugular vein. However, caution must be used in analyzing the results of this study (see 61). Since 2DG interferes with the normal metabolism of the brain, it is possible that jugular infusions attenuate feeding by debilitating the brain. Also, there is evidence that the uptake in the liver is equal for both jugular and portal routes of administration.

#### Electrophysiological studies.

Osmoreceptors. Although Niijima's study (41) showing that the rate of discharge from afferents in the hepatic vagus of a perfused guinea pig liver varied directly with the concentration of sodium chloride in the perfusate, it raised the question of whether this was a response to a change in osmolarity or to a change in sodium. In a later study Niijima, Adachi and Jacobs (2) showed that the discharge rates of units in the hepatic vagus of a perfused rat liver again responded positively to the concentration of sodium chloride but were influenced only slightly by the addition of equiosmotic urea or glucose, thereby indicating a more specific osmotic response. However, it is not clear that the effect was due to activation of osmoreceptors rather than sodium receptors. Rogers et al. (54) found units in the thalamus and pontine parabrachial nucleus that responded to portal infusions of hypertonic but not equiosmotic solutions. Novin et al. (44) recorded from the brain cells some of which

were inhibited and some of which were activated by infusion of osmotically active solutions via the hepatic portal vein. They also noted that the response of some of these cells was due specifically to saline.

Sodium receptors. Several researchers (44, 53, 62) report alterations in the firing rate of cells in the rat brain in response to intraportal infusion of hypertonic but not isotonic saline. Schmitt (62) also showed that there was no response to either jugular injections of hypertonic saline or portal infusions of equiosmotic sugars which indicates that the alterations in firing rate were in response to hepatic monitoring of changes in ionic concentration.

Baroreceptors. Andrews and Palmer (7) report that units in the hepatic vagus in both an intact dog and a perfused dog liver preparation respond to congestion of the liver. Similarly, Niijima (41) found that the discharge rate from units in the splanchnic nerve of a perfused guinea pig liver change in response to the pressure of the perfusate.

Metabolic receptors. Schmitt (62) has reported that the infusion of glucose but not sucrose into the portal vein of rats causes an alteration in the firing rates of units in the hypothalamus. Niijima (40) found that the discharge rate from the hepatic vagus of perfused guinea pig livers decreased in response to the addition of glucose but not other sugars to the perfusate. He later extended this finding to in situ preparations (42). Although, this finding was not replicated using a perfused rabbit liver, Andrews and Orbach

(6, 47) did find that this preparation yielded a change in discharge rate in response to the administration of long-chain fatty acids. More recently, Niijima (40) has reported that in rabbits, guinea pigs and rats changes in the firing rate of fibers in the hepatic vagus were seen in response to hepatic infusions of glucose, 2DG, cholecystokinin and serotonin. Similarly, Sakaguchi and Iwanaga (59) have shown that D-glucose anomers injected in anesthetized rats leads to a decrease in hepatic vagal firing rate.

#### Nerve section.

Osmoreceptors. Section of nerves known to innervate the liver has been shown to alter behavioral and electrophysiological responses to changes in hepatic osmolarity. The antidiuretic response to portal infusion of hypertonic saline was shown to decrease after the dorsal root of the sympathetic nerve was cut (4). Recording from the thalamus and pontine parabrachial nucleus, Rogers et al. (54) reported that the change in firing rate seen as a response to portal vein infusion of hypertonic saline was eliminated following right cervical vagotomy.

Sodium receptors. Passo et al. (49) found that portal infusions of hypertonic saline caused an increase in sodium excretion in the cat which was eliminated by bilateral cervical vagotomy. However, Perlmutter et al. (50) reported that the sodium excretion that was seen as a response to infusions of isotonic saline were not affected by vagotomy. Because they had shown that portal infusions were more



effective than vena caval infusions in eliciting an increase in sodium excretion, the authors interpreted the lack of effect of vagotomy to indicate the role of a humoral factor. In an electrophysiological study described above (62) section of the splanchnic nerve eliminated the response of units in the brain to portal infusions of hypertonic saline while vagotomy increased this response which suggests that both parasympathetic and sympathetic nervous systems are involved. Novin (44) has reported in an unpublished study with Martin that hypertonic saline given via hepatic-portal cannula was less effective in activating drinking after vagotomy.

Metabolic receptors. In a study that showed that fructose blocks insulin-induced feeding in the rat, Friedman (20) reported that hepatic vagotomy eliminates this response. In two studies that demonstrated that activity in the stomach is responsive to changes in the liver, section of the hepatic vagus eliminated this response. Granneman and Friedman (23) have shown hepatic vagotomy stops the ability of fructose to block insulin-induced gastric acid secretion and electromyographic activity in the rat. Hepatic vagotomy has also been shown to block the electrophysiological response of units in the gastric vagus to infusions of glucose in the portal vein (59). In an unpublished work, Sawchenko, Friedman and Gold (see 61) reported that hepatic vagotomy in female rats altered their diurnal feeding pattern. However, recently Tordoff, Hopfenbeck and Hovin (69) claimed that for male and female rats, hepatic vagotomy did not affect feeding behavior in response to insulin, 2DG, sodium or alter body weight or

patterns of diurnal feeding. In the same study, 2DG hyperphagia was attenuated by subdiaphragmatic vagotomy and hypertonic saline induced drinking was lessened by truncal vagotomy.

Neurochemical studies. Most of the neurochemical studies of hepatic innervation have shed more light on efferent rather than afferent neural activity (see 61 for a review). Evidence gained by measuring acetylcholinesterase (AChE) suggests that a cholinergic plexus is spread throughout the liver; however, the extent of the plexus is in question (52). Although AChE is usually associated with parasympathetic effectors, it has been shown to be present in sensory cells of the rabbit carotid body and may also play some sensory role in the liver. Andrews and colleagues have demonstrated that injections of AChE in the liver increase the firing rate of hepatic afferents in the rabbit (6) and the dog (7).

Anatomic studies. A number of studies have established that there is both an intralobular (37, 63, 64, 66) and vascular (11, 25, 50) intrinsic innervation of the liver. From these locations sensory fibers would be able to respond to both alterations in blood composition and more general changes in hepatic functioning. However, the anatomic evidence for sensory fibers in the liver is conflicting and circumstantial. Seto (62) failed to find any receptors in the liver but others have reported finding vascular sensory receptors (see 59). In 1958 three types of receptor endings in the liver were described by Tsai (70) as noted above.

Much of the anatomic evidence for afferent innervation of the liver comes from degeneration studies. Using this method Foley (19) estimated that approximately 50% of the splanchnic nerve of the cat consists of afferent fibers. The finding that 75-90% of the fibers in the abdominal branch of the vagus of the cat (3) and the rabbit (18) are afferent may suggest that the hepatic branch of the vagus also contains afferents. In studying hepatic nerve degeneration as a consequence of nerve section, Tsai (70) reported extensive degeneration after section of the dorsal roots distal to the spinal ganglia. Bilateral vagotomy produced less degeneration with none following section of the ventral roots or phrenic nerve. Studies that traced the degeneration of myelinated fibers in the vagus (5) found little evidence of afferent fibers; however, this finding is not unexpected since other studies have found that approximately 90% of the sub-diaphragmatic fibers are unmyelinated (17, 18, 19, 28). Recently, in an electron microscopic study of the fiber composition of the vagus nerve in the cat, Mei (37) found 30,000 sensory fibers in the infranodose vagus. After injecting horseradish peroxidase (HRP) into different locations in the liver of the rat Carobi and Magni (13) demonstrated direct afferent innervation when they found labeled bodies in the left nodose ganglion (NDG).

C H A P T E R I I  
RESEARCH OBJECTIVES

Since the vagus nerve has been implicated as a courier of hepatic information in many of the studies described above, it is of interest to know more about the organization and distribution of afferent fibers and their cell bodies within this nerve. Visceral afferent fibers of the vagus nerve have their cell bodies in the NDG with their distal processes ending upon sensory receptors and their proximal processes terminating in the central nervous system (14). The central processes are always thinner than the peripheral processes (51). For some neurons, the peripheral fiber is myelinated and the central fiber unmyelinated (27). In the cat it has been estimated that the NDG contains between 25,000 (19) and 29,000 (27) cell bodies. Ranson (51) found that in the cat the ganglion cells are unipolar neurons with axons that often take a convoluted course around the cell body. There are also some cells which are bipolar. Motor fibers of the vagus usually run along one side of the NDG with the fibers that originate from the ganglion cells in the center (22). Recording from the NDG of the cat, Mei (37) found that the cell bodies of afferent fibers from different peripheral branches of the vagus were topographically represented in the ganglion. Similarly, Carobi and Magni (13) reported that

HRP-labeled cell bodies of hepatic vagal afferents were localized in the NDG of the rat. However, using the anterograde and retrograde transport of HRP Kalia and Mesulam have recently done a thorough study of the afferent and efferent projections of the vagus complex in the cat (29, 30). Looking at the location of the cell bodies of vagal afferents they found that the soma for any particular peripheral branch of the vagus are not localized, but are spread diffusely throughout the NDG. Although they did not study the vagal afferent connections to the liver, their work serves as a helpful guide to the study of vagal central projections. They report that afferents from the vagus enter the medulla and join the tractus solitarius where they bifurcate, sending out a short ascending and a longer descending process. Most vagal afferents terminate in the caudal portion of the nucleus of the tractus solitarius where they show some specificity within its sub-nuclei. There is also evidence of fibers terminating in the area postrema (29, 30).

This study was conducted to identify the locus of termination and the location and density of cell bodies of the afferent fibers emanating from, the hepatic vagus of the rat. Retrograde axonal transport of HRP (29, 30) was used to label the cell bodies and axons of afferent fibers from the hepatic vagus. It was also an aim of this research to estimate the number of cells and describe the organization of cells and fibers within the NDG.

## C H A P T E R I I I

### METHODS

Fifty-seven adult, male, albino laboratory rats were used for the study. They were food-deprived the night before surgery. One ml of atropine was injected subcutaneously twenty minutes before they were anesthetized with sodium pentobarbital (.75 ml per 100 g of body weight). Ether was used whenever necessary during surgery to maintain adequate anesthetic levels.

The hepatic vagus was isolated by carefully following the nerve with the aid of a dissecting microscope as it traversed the esophagus to join the main trunk of the vagus. The distal length of the hepatic vagus was cut just before entry into the liver and a small section of gelfoam soaked in HRP (approximately a 50% solution) immediately placed on the nerve stump. The HRP pad was changed every 15-20 minutes to reduce dilution of HRP by tissue fluids. The HRP was applied to the transected nerve for 90 minutes.

Survival times varied from 24 to 120 hours after administration of HRP. The animal was then deeply anesthetized with nembutal and perfused transcardially. The solutions for the perfusion were 100 cc of saline with 6% dextran at room temperature; followed by 150 cc of 1% paraformaldehyde and then 1.25% gluteraldehyde and 5% sucrose in cold buffer. Both the brain and the NDC were removed and stored in a 30% sucrose-buffer solution overnight. The following

morning, the brain was blocked and the caudal section of the brain and the NDG were embedded separately in a solution of 20% gelatin heated to 40 degrees C and placed under vacuum at 15 psi for fifteen minutes. The brain was removed, frozen and cut coronally on a microtome into 40 micron sections. The ganglia were frozen and cut either coronally or longitudinally in 40-micron thick sections. The cut sections were serially ordered and placed in phosphate buffer and reacted for HRP histochemistry using a modified Hanker-Yates Method for the NDG and either the Hanker-Yates or tetramethyl bezidine (TMB) procedure (31) for the brain. The tissue was left in phosphate buffer until the sections were mounted on chrome-alum coated slides. The sections were then counterstained with cresyl-violet.

The slides were then scanned at a power of x 400 using a Bausch and Lomb light microscope to determine the location of HRP-labeled cell bodies in the NDG. Cells were counted as HRP positive only if individual grains of HRP could be visualized. This sometimes required examining individual cells at a magnification of x 1000.

Sections of the brain (caudally from the area postrema to the rostral pole of the nucleus solitarius) were scanned at a power of x 400 in an attempt to detect HRP-labeling of afferent processes from the hepatic vagus. The tractus solitarius, the nucleus of the tractus solitarius and the area postrema were systematically scanned at a magnification of x 1000.

In order to estimate the total number of sensory cells within the NDG cell counts were made using tissue sections cut longitudinally at 40 microns from three NDG that were intact (i.e., no sections missing) from the rostral to the caudal poles and stained with cresyl violet. Using a modified method of cell sampling as recommended by Jones (27) cell counts were made for two consecutive sections of every six. Sections were systematically scanned at a magnification of x 400 using a grid located in the eyepiece to control for overlapping or missing counts. A cell was counted when the nucleolus could be clearly distinguished.



## CHAPTER IV

### RESULTS

Gross organization and cell population of the NDC. The NDC was found to be organized with cell bodies to one side and their processes and fibers of passage more centrally located (Figure 1). It is composed of large cells with a diameter of approximately 40 microns which are the somata of afferent fibers, surrounded by glial cells (Figure 2). Using cases 1-3 (Table 1) estimates of the total sensory cell population for each NDC was made yielding counts of 8382, 8369, and 10,598 respectively. An average of these three cases gives an estimate of the sensory cell population of the NDC of 9115.

HRP labeling in the NDC. Following HRP administration to the hepatic vagus, HRP-labeling was only found in the left NDC. In well labeled intact ganglia HRP-labeled cells were found to be dispersed in all but the extreme sections and as far as the caudal extent of the ganglia (Figure 1). Rostrally, an occasional cell body could be seen below the main body of the ganglion (Figure 4).

Of all the cases attempted, five cases showed distinct and relatively heavy HRP-labeling (Figures 1-3). Three of these were intact ganglia that yielded a total cell count for each (see Table 1).



Fig. 1. HRP-filled cells spread diffusely in NDG.



Fig. 2. Single HRP-filled cell showing individual grains.

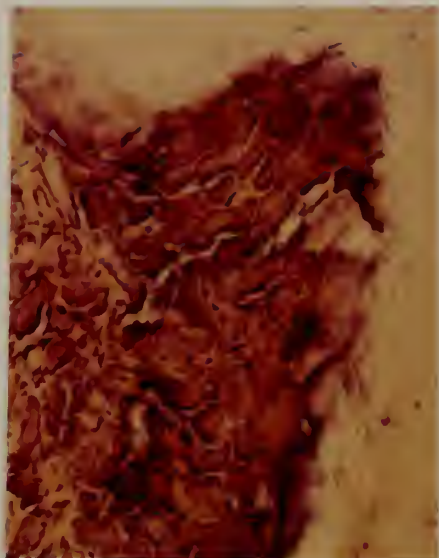


Fig. 3. Area of high density HRP-filled cells.



Fig. 4. Single HRP-filled cell caudal to NDG.

TABLE 1  
 COUNTS OF HRP-LABELLED CELLS IN NDG.

Case #	Rough Estimate	Adjusted Estimate
1	267	139
2	192	96
3	130	68
4	181	94
5	197	102

Rough estimate is the number of HRP-labeled cells counted. Adjusted estimate is the rough estimate applied to the formula below.

\* indicates cases with missing sections.

In cases 4 and 5 part of the ganglia were lost during removal but the labeling was clear and had a density consistent with the intact cases. The overall structure of the ganglia were such that it was felt the missing sections could be accounted for and an estimate of labeled cells made. Because any visualization of HRP grains was counted as a labeled cell, overestimation of cells was likely. Therefore a formula for the estimation of nuclear populations (1) was applied where P is the adjusted estimate of cell population, A is the rough count of cells, M is the thickness (in microns) of the section, and L is the average diameter of the cells, then

$$P = A \frac{M}{L + M}$$

The results are in Table 1.

HRP-labeling in the brain. Following searches of areas in the brain where visceral afferent fibers have been reported to terminate (29, 30), no HRP-labeling was found in the tractus solitarius, the nucleus of the tractus solitarius or the area postrema regardless of survival time. However, in cases 1 and 3 (Table 1) and one other case a small number of cell bodies were labeled in the area of the dorsal motor nucleus (DMN). In the best case (case 1), seven cells were seen to have HRP-labeling (see Figure 5).

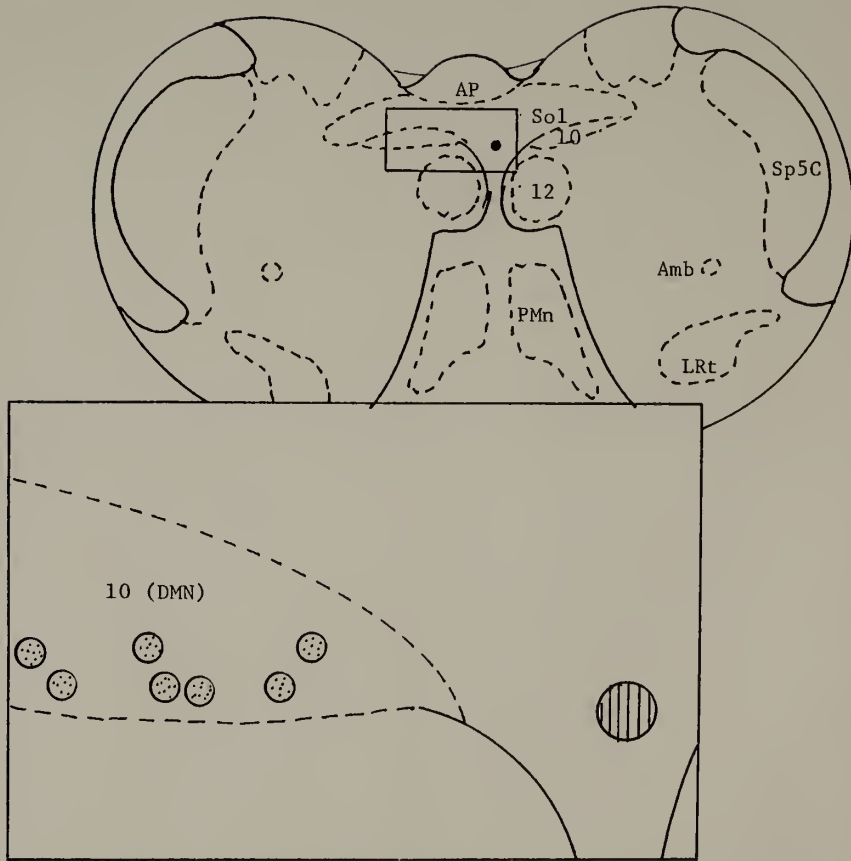


Fig. 5. Distribution of HRP-labeled cells in DMN from case 1.

## C H A P T E R V

### DISCUSSION

The NDG was found to be organized as previously described in the literature for cats (22) with the cell bodies to one side and their processes and fibers of passage occupying a more central position. There are two types of cells apparent in the NDG; large sensory cells (with a diameter of approximately 40 microns) and smaller cells which have been described as glial or satellite cells (22). However, there is the possibility that some of these smaller cells might be sensory neurons. Based on the findings of Gabella and Reese (see 20) that the diaphragmatic level of the vagus in the rat contains about 10,000 fibers and other reports that this part of the vagus is predominantly afferent (19, 28) a projection was made that the NDG in the rat would contain roughly 8,000 cells. The present study found an average of 9,115 cells in the NDG.

No HRP labeling was found in areas of the brain where other researchers (29, 30, 37) have reported finding vagal afferent fibers. In an HRP study of hepatic afferents similar to this one Carobi and Magni (13) found no evidence of central labeling for either afferent or efferent fibers from the liver. Although Kalia and Mesulam (29, 30) reported "transganglionic" labeling following application of HRP in several different branches of the vagus nerve, the small number of fibers in the hepatic vagus, the

inconsistency of HRP uptake and the delicacy of the histological reaction techniques combined to make it difficult to visualize central hepatic afferent fibers. In the course of this research both Hanker-Yates and TMB reaction techniques were used to process the brain sections. Survival times were varied from 48 to 120 hours and both darkfield and brightfield light microscopy visualization was used to examine sections. Kalia and Mesulam (29, 30) have reported success in using autoradiographic techniques to investigate central projections of vagal branches.

The finding that the cell bodies of hepatic vagal efferent fibers lie in the DMN was not unexpected considering previous reports on other vagal efferents (29, 30, 36), however, finding HRP-labeling of these cells was unexpected. After Carobi and Magni (13) failed to visualize any central labeling following hepatic injections of HRP they noted that with the estimate of the efferent population in the abdominal vagus as low as 10% (3) and the difficulty of HRP uptake "the probability of HRP uptake by efferent neurons appears indeed negligible." Although, this finding is somewhat fortuitous it does provide anatomic evidence that the efferent fibers of the hepatic vagus, like those of other vagal branches, emanate from the NDC.

In agreement with Kalia and Mesulam (29, 30), HRP-labeled cells in the NDC were found to be spread diffusely through the ganglion. However, other authors (13, 37) report that the afferent cells in the NDC are topographically organized. Mei (37) in an

electrophysiological study of vagal afferents in the cat states that cells in the NDG that responded to stimulation of different branches of the vagus were localized. Carobi and Magni (13) reported that following injections of HRP in the liver of rats, HRP-labeled cells were grouped in the rostral end of the NDG. In contrast, the present study found not only a diffuse distribution of HRP-labeled cells but occasionally a marked cell was seen rostral to the body of the ganglion (Figure 4). A possible explanation for the variance with Carobi and Magni's findings is that the NDG is organized in regards to the location or function of fibers within the liver. A simpler explanation would be that the small number of cells labeled by Carobi and Magni were not able to provide an adequate representation of cell distribution within the NDG.

The finding that the cell bodies of afferents from the hepatic branch of the vagus nerve of the rat lie only in the left NDG is in agreement with others (13, 32, 37). Since other animals studied (e.g., cat, dog, rabbit, man) show bilateral innervation via the hepatic vagus and there is no notable species-specific response reported in the rat to manipulations apparently involving hepatic vagal fibers, the functional significance of this asymmetry is questionable. This anatomic trait may, however, be useful in examining the recent claim by Tordof et al. (68) that the greater portion (if not all) of the metabolic information supplied to the brain from receptors in the liver of the rat does not travel via



the hepatic branch of the vagus but is still of vagal origin. It is the view of these researchers that the main efferent pathway from the liver to the brain is by way of the coeliac ganglia, coeliac vagus and vagal trunks. Innervation of the liver by the coeliac vagus is more complex and not as well-documented as hepatic vagal innervation. The hepatic vagus is a small discreet branch of the anterior vagal trunk (32) which receives most of its fibers from the right cervical vagus (3). If the coeliac vagus is the main route of afferent metabolic messages from the liver, it is reasonable to expect that some of these fibers would travel via the right vagus and have their cell bodies in the right NDG. However, after injecting HRP in the liver of the rat, Carobi and Magni (13) reported finding labeled cells only in the left NDG leaving the proposed alternate vagal route in need of further investigation.

The finding of HRP-labeled cells in the NDG corroborated Carobi and Magni's anatomic study showing that there are vagal afferents which innervate the liver and that their cell bodies lie in the NDG. In the present study, HRP was applied to only one branch of an apparently larger hepatic afferent system, while the procedure used by Carobi and Magni should have had access to a greater portion, if not the entire system. However, more labeled cells were presumably found (no counts are available for Carobi and Magni but they describe the number of labeled cells as small) in the present study. The most plausible explanation for this

variance is that the procedure used in the present study facilitates the uptake of HRP more effectively than that used by Carobi and Magni.

Three of the estimates of hepatic vagal afferent cells in the NDG indicate that there are approximately 100 fibers represented. However, due to the variability of HRP uptake and without any previous estimates of hepatic vagal fibers being known to the author, caution is suggested in interpreting this finding. Because of the clarity of labeling and the integrity of the tissue, case #1, which yielded the largest cell count, is recommended as the best estimate of hepatic vagal afferents. Using the adjusted cell count (139) and applying reported percentages of efferent to afferent fibers (3, 19, 38) the number of hepatic vagal efferents can be estimated to be between 17 and 35. Combining the highest estimates of afferent and efferent fibers, the total fiber content of the hepatic vagus is 175. Although the hepatic vagus in the rat has been described as small (33) the author recommends that the preceding estimate be used as a minimum fiber content in this nerve until more information is available. The procedure that Mei (37) used in an electron microscopic study of the fiber content of different levels of the vagus nerve in the cat is suitable to do a quantitative study of the hepatic vagus.

Recently, evidence supporting the existence of hepatic receptors has increased so that there is little doubt that the liver is a sensate organ. The questions of interest now are; what are the

functional correlates of hepatic receptors and what are the neural networks that mediate these functions? More specifically, in regards to the neural system that is the focus of this study; 1) what are the functional consequences of information relayed via afferent fibers of the hepatic vagus (and thus through the NDC) and 2) what are the subsequent neural pathways involved?

Although reports suggest that the hepatic vagus is not significantly involved in feeding behavior in response to metabolic challenges (2, 21, 44) there is evidence that glucose-sensitive afferents travel in this nerve (23, 40). Research indicates that the information carried in these fibers may be used to control plasma glucose levels, gastric acid secretion (GAS) and insulin secretion in the pancreas. By altering GAS and thus digestion of foodstuffs, the liver, in response to metabolic needs, could influence the influx of new fuels. In concert with this, hepatic modulation of insulin secretion would help maintain a homeostatic balance of plasma glucose.

In 1969 Niijima (34) reported that infusion of glucose in the liver caused an increase in efferent pancreatic firing rate. More recently, Niijima (40) has shown that the stimulation of the hepatic vagus leads to a decrease in firing rate of pancreatic efferent fibers. Sakaguchi and Yamaguchi (58) demonstrated that in rats with bilateral adrenalectomy, stimulation of the hepatic vagus leads to a suppression of insulin secretion in the pancreas. In juxtaposition to the theory of hepatic modulation of pancreatic and gut

functioning is a suggestion by Lauth (32) that afferent impulses from the stomach may influence hepatic and pancreatic functioning in order to prepare the system for an increase in metabolic fuels.

In summary, this study has shown that the NDG of the rat is organized with sensory cell bodies to one side and the processes and fibers of passage in the center. There is an apparent population of two distinct cell groups (large sensory cells and smaller glial cells), however, the possibility of the existence of a population of small sensory cells is noted. HRP labeling has corroborated previous reports (13, 37) of an afferent pathway between the left NDG and the liver. Hepatic representation in the NDG was found to be spread throughout the ganglion with a minimum number of sensory fibers estimated to be 139. The estimate of the total sensory population of the NDG was 9,115 cells. No labeling of afferent fibers was found in the brain, however, a small number of hepatic efferent cell bodies were discovered in the DMN. This provides the first direct anatomic evidence of hepatic vagal pathways. Although evidence suggests that the hepatic vagus is not involved in transferring information which influences feeding behavior (3, 19, 38) there is research which suggests that metabolic information which travels via the HV may be used to regulate homeostasis.

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