The Organization of the Brainstem Nuclei Associated with the Vagus Nerve in the Agouti (*Dasyprocta Leporina*)

A Neurohistological Study

CM Phillips, A Odekunle

ABSTRACT

A total of six adult animals were used for the study. Following anaesthesia via intraperitoneal injection of a mixture of ketamin and bombazine in ratio 2:1, thoracotomy was performed to exteriorize the heart for intracardial perfusion. The perfusion canular was inserted into the left ventricle and animal perfused sequentially with normal saline and 10% formal saline. Following perfusion, craniotomy was performed to remove the entire brain along with the upper segments of the spinal cord. The brain specimen was then dehydrated, cleared and infiltrated with paraffin wax. The specimen was then cut in 15 micron thick serial sections. The sections were then processed for neurohistological analyses using a Nikon microscope to which was attached Nikon camera.

Analyses of the sections revealed bilateral representation of the dorsal motor nucleus of the vagus nerve in the medulla oblongata. The nucleus ambiguus, nucleus of the tractus solitarius, hypoglossal nucleus and the area postrema were also identified in the medulla oblongata. The implications of our findings are discussed in the text of the article.

Keywords: Agouti, vagal nuclei, vagus nerve

Organización de los Núcleos del Tronco del Encéfalo Asociados con el Nervio Vago en el Agutí (*Dasyprocta Leporina*)

Un Estudio Neurohistológico

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RESUMEN

Un total de seis animales adultos fueron usados para el estudio. Tras de una anestesia mediante una inyección intraperitoneal de una mezcla de ketamina y bombazina en proporción 2:1, se practicó una toracotomía para extraer el corazón y realizar una perfusión intracardíaca. La cánula de perfusión fue insertada en el ventrículo izquierdo y el animal fue perfundido de forma secuencial con solución salina normal, y 10% de solución salina formal. A continuación de la perfusión, se realizó una craneotomía a fin de extraer todo el cerebro junto con los segmentos superiores de la espina dorsal. La muestra del cerebro fue entonces deshidratada, aclarada, e infiltrada con cera de parafina. La muestra fue entonces cortada en secciones seriadas de 15 micrones de espesor. Las secciones fueron entonces procesadas a fin de someterlas a análisis neurohistológico, usando un microscopio Nikon al cual se le conecta una cámara Nikon.

Los análisis de las secciones revelaron una representación bilateral del núcleo motor dorsal del nervio vago en la médula oblonga (bulbo raquídeo). También se identificaron el núcleo ambiguo, el núcleo del tracto solitario, el núcleo hipoglosal, y el área postrema, en la médula oblonga. En el texto del artículo, se discuten las implicaciones de nuestros resultados.

Palabras claves: Agutí, núcleos vagos, nervio vago

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INTRODUCTION

The value of experimental animal studies to the field of medicine cannot be over-emphasized as they serve as sources of vital information on the complex structure of the human body systems, the mechanisms involved in several metabolic and physiological processes in the human body and as means of evaluating the efficacy of new drugs and various herbal food supplements intended for human consumption. Of all the known mammalian species, approximately half belong to the order Rodentia (1) and of these, the laboratory rat and mouse constitute over 80% of the animals utilized in medical and scientific inquiry (2, 3).

However, one of the main benefits of animal research is the ability to extrapolate information gained to aid in the understanding and controlling of human disease and though reliable in some cases, the knowledge obtained from animal models can and often has been misleading when applied to their human counterparts. Consequently, the search for species with closer anatomical and physiological characteristics to man continues. In an effort to facilitate this pursuit, the agouti, a rodent local to the Caribbean was chosen as the experimental animal.

The agouti is a neotropical rodent belonging to the family Dasyproctidae (4) and has been cited as a probable research animal by Baas *et al* (5) for four main reasons: its size [3.2 – 5 kg] (4), longevity (18 – 20 years) maintenance in captivity and its resistance to zoonotic diseases (5). Since then, several anatomical and physiological studies have been conducted on the animal (5−20). Whereas these investigations may seem diverse, they have one main objective: to add to the growing database of the animal. For any animal to enter the scientific arena as a human model, a detailed knowledge of its anatomical features and physiological processes is a prerequisite.

It is with this background that we began a project on the brain of the Agouti, focussing specifically on the neuroanatomical relationship between the brainstem and the gastrointestinal tract. The rationale for selecting this area stems from the recognition that food and nutrition are essential biological factors that must be addressed if there is to be a sustained supply of animals for economic and scientific purposes. Furthermore, no information has been documented on this crucial area in this animal. However, before the neuroanatomical relationship between the brainstem and the digestive system could be established, knowledge of the organization of the nuclei in the brainstem is necessary to better localize the distribution of labelling, especially since the brainstem of this animal was never investigated.

A review of the literature further revealed that the brainstem nuclei have been labelled and precisely localized in various mammalian species including man. For instance, afferent neurons have been demonstrated to project to the subnuclei of the nucleus of the solitary tract (NTS) and the spinal nucleus of the trigeminal (21−26), area postrema (AP), commissural nucleus and the substantia gelatinosa of the first cervical segment of the spinal cord (21−24). Efferent fibres have also been shown to originate from two main brainstem nuclei: the nucleus ambiguous (NA) and the dorsal motor nucleus of the vagus [DMNX] (21−24, 27−32). Furthermore, labelled neurons have also been localized in the reticular formation of the medulla, the nucleus dorsomedialis, nucleus retroambiguus and the nucleus of the spinal accessory nerve (21−24).

Based on these and other findings, it is speculated that the brainstem of the agouti would contain the same complement of nuclei associated with the vagus nerve.

Thus, the primary objective of the present study was to determine the topography, morphology and rostrocaudal extent of medullary nuclei associated with the gastrointestinal tract with the aim of gaining a greater understanding of this vital region. The present study placed emphasis on the DMNX, NA, NTS, AP and the hypoglossal nucleus.

SUBJECTS AND METHODS

Six adult agoutis, weighing 2.5 – 3.0 kg, of *D leporina* were utilized for this study. They were obtained from a wildlife farmer in the North Central Region of Trinidad, under the administration of the Wildlife Division of the Ministry of Agriculture. The animals were physically inspected to ensure that they were in good health. They were then housed in the controlled environment of the Animal Room. Each animal was placed in a separate cage and had free access to food and water. Food was withheld 8−12 hours prior to surgery.

All animals were sacrificed according to the guidelines of the National Institute of Health which parallel those of The University of the West Indies, Animal Ethics Committee.

Each animal was anaesthetised *via* an intra-peritoneal injection of a fresh mixture of ketamin and bombazine in a ratio 2:1, respectively (1 ml/kg). The anterior chest wall hair of the animal was removed using an electric shear. Complete sedation was then tested using toe pinch and corneal reflex. With the aid of a scalpel, toothed forceps and scissors, thoracotomy was performed and the heart was exteriorized to the anterior chest wall for transcardial insertion of the perfusion canular.

Perfusion commenced with the passage of 0.9% saline until the escaping perfusate became clear *ie* devoid of blood. The second perfusate was 800 – 1000 ml of 10% formal saline fixative. Subsequently, craniotomy was performed and the brain along with the upper two segments of the spinal cord were removed and placed in a specimen cup containing the same fixative used in perfusing the animal. After the specimen had sunk, the medulla was removed, and processed using the following sequence in an automated machine:

Dehydration	Buffered formalin	–	1 hour
	Buffered formalin	–	1 hour
	70% alcohol	–	2 hours
	95% alcohol	–	1 hour
	95% alcohol	–	1 hour
	100% alcohol	–	1 hour
	100% alcohol	–	1 hour
Clearing	Xylene 1	–	45 minutes
	Xylene 2	–	30 minutes
	Xylene 3	–	30 minutes
Infiltration	Paraffin wax	–	1 hour 30 minutes
	Paraffin wax	–	1 hour 30 minutes

Following processing, the tissue was blocked in paraffin and serial sections 15 µm thick were taken and mounted onto albumenised slides. They were then hydrated, stained and cover slipped according to the following schedule:

Hydration

Xylene 1	–	5 minutes
Xylene 2	–	5 minutes
100% alcohol	–	20 dips
100% alcohol	–	20 dips
95% alcohol	–	20 dips
70% alcohol	–	20 dips
50% alcohol	–	20 dips
Quick rinse in tap water	–	5 seconds
Distilled water	–	10 dips

Staining

Neutral red	–	5 minutes
Running tap water	–	approx 10 seconds

Dehydration

50% alcohol	–	10 dips
70% alcohol	–	10 dips
95% alcohol	–	10 dips
95% alcohol	–	10 dips
100% alcohol	–	10 dips
100% alcohol	–	10 dips
Xylene 1	–	10 minutes
Xylene 2	–	10 minutes

The slides were then viewed and analysed with the Nikon microscope. Identification and localization of the nuclei were done with the aid of a Stereotaxic atlas (33). Photographs were taken using a Nikon camera coupled to the microscope.

RESULTS

The Dorsal Motor Nucleus of the Vagus Nerve (DMN)

The dorsal motor nucleus was present bilaterally throughout the rostrocaudal extent of the medulla and extended rostrocaudally from 1.2 mm rostral to 3.67 mm caudal to the obex. It was difficult to determine the mediolateral extent of the nuclei at any point since the neurons extended irregularly into the adjacent reticular formation. The nucleus composed multipolar cells, fusiform cells and fork-shaped cells and was devoid of melanin pigment. Furthermore, the cells were uniformly distributed throughout the rostrocaudal extent of the nucleus, although as one moved rostrally, the cells became more numerous.

At the beginning of its caudal end, it was difficult to differentiate the DMN from the hypoglossal nucleus (HN) and the DMN was located lateral to the central canal. However as one proceeded rostrally, the nuclei separated into two distinct populations (Fig. 1-A) and the cells of the DMN were visibly smaller than those of the HN throughout its extension. At this point, the nucleus was elliptical in shape with its long axis in the horizontal plane.

Between 3.67 and 2.80 mm caudal to the obex, the cells of the DMN were sparse and scattered and had an average size of 15.5 µm (Fig. 1-A). Conversely, the cells increased in number, as well as size when approaching the obex rostrally. Closer to the obex, the nucleus was located dorsolateral to the central canal, ventromedial to the NTS and continued as a horizontal band of cells however, its long axis was now diagonally placed (Fig. 1-B).

At and rostral to the obex, the nucleus became more dorsal, lying beneath the ependymal layer of the floor of the fourth ventricle and was closely related to the NTS (Fig. 1-C). At the rostral end of its extension, the nucleus gradually went from elliptical to rounded shape before its termination at 1.2 mm rostral to the obex (Fig. 1-D).

The Nucleus Ambiguus (NA)

The nucleus ambiguus was found in the reticular formation, close to the ventrolateral border of the medulla. It contained large multipolar cells (average size: 26.9 µm) which were larger than the cells of the DMN and had numerous Nissl granules. Rostrocaudally, the nucleus was not a continuous column and contained irregular cell populations. Rostrally, the nucleus was composed of two large multipolar neurons, approximately 23.12 and 30.98 µm (Fig. 2-A) that were very close and compact. However, as one proceeded caudally, the nucleus was composed of irregularly arranged cell groups that were scattered within the reticular formation (Fig. 2-B). As a result, the rostrocaudal extension of the nucleus could not be determined.

The Nucleus Tractus Solitarius (NTS)

The nucleus of tractus solitarius was not seen throughout the rostrocaudal extent of the medulla but became visible at approximately 0.8 mm caudal to the obex and extended to 2.41 mm rostral to the obex. At its first appearance (caudal the obex), the nucleus was located lateral to the DMN and dorsolateral to the HN. There was a fair amount of neurons, whose average size was 15.68 µm, and the nucleus was a distinct population of cells (Fig. 3-A).

Closer to the obex, the sub-nuclei could be distinguished. The tractus solitarius also became prominent at this level and the nucleus was located dorsolateral to the DMN

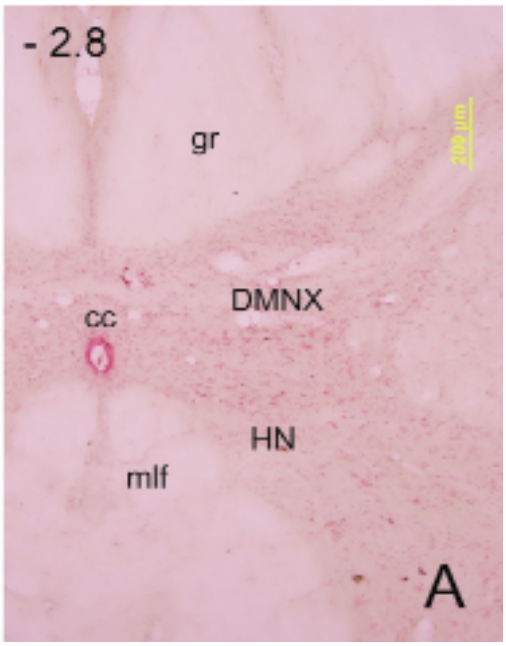

FIG. 1A

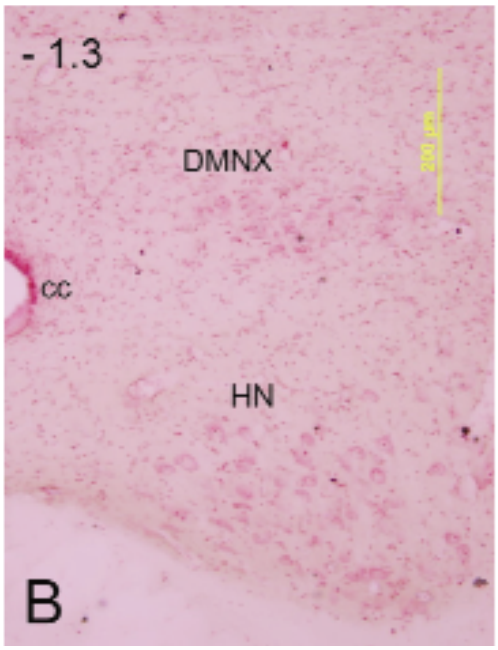

FIG. 1B

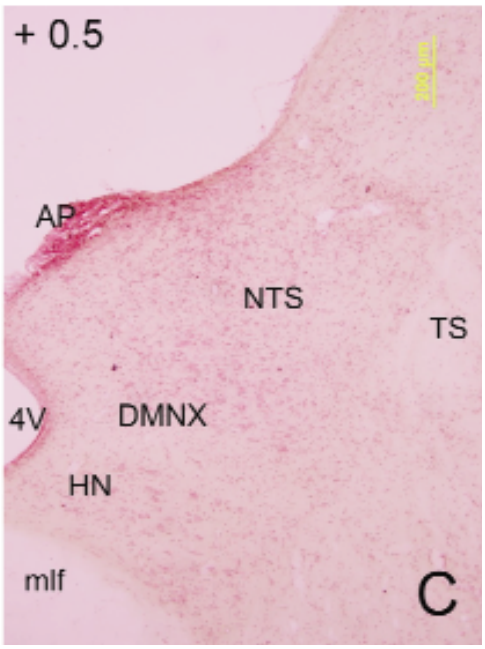

FIG. 1C

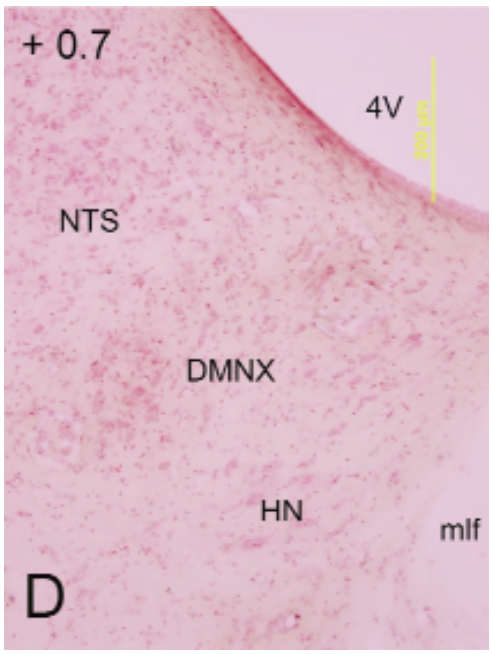

FIG. 1D

Fig. 1: Photomicrographs of transverse sections of the brainstem showing the DMN.

and medial to the tract (Fig. 3-B). The solitary complex contained these main nuclear subgroups: lateral, dorsolateral and dorsomedial.

At the level of the obex and proceeding rostrally, the nucleus became less distinct and was located ventral to the floor of the 4th ventricle. The nucleus was not clearly distinguished from the reticular formation and neither were the sub-nuclei recognized (Fig. 1-C).

The Area Postrema (AP)

The area postrema was readily identifiable as it was intensely and brightly stained. The "cells" were of various irregular shapes and there were prominent gaps and spaces within the substance of this nucleus. Caudal to the obex, the area postrema was seen as a single group, in the dorsomedial aspect of the medulla, dorsal to the central canal of the closed part of the medulla. The general shape was triangular with its apex directed towards the central canal. Staining here was

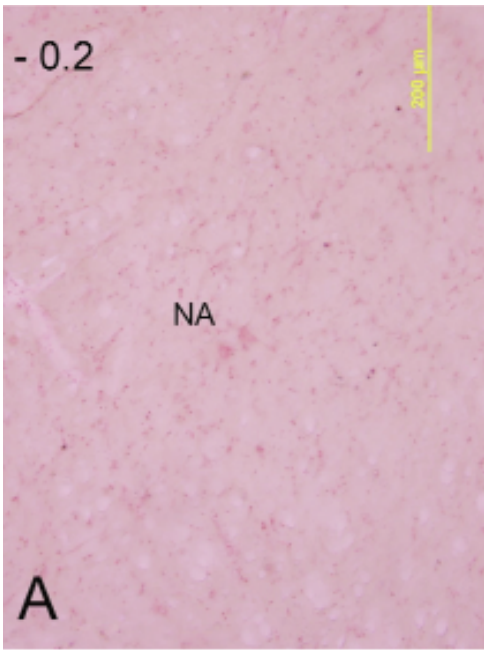

FIG. 2A

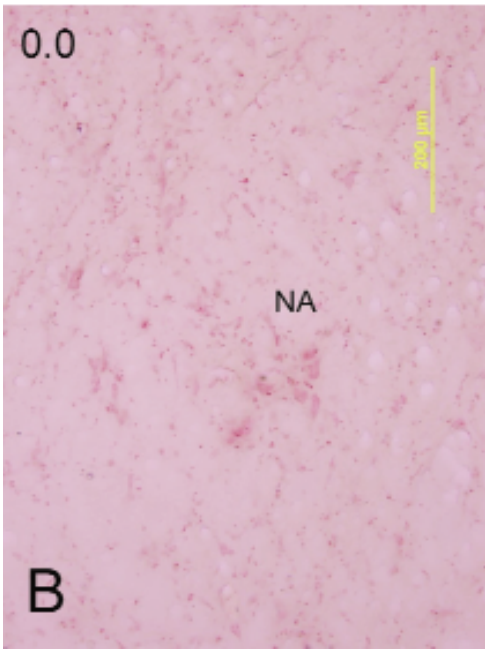

FIG. 2B

Fig. 2: Photomicrographs of transverse sections of the brainstem showing the NA.

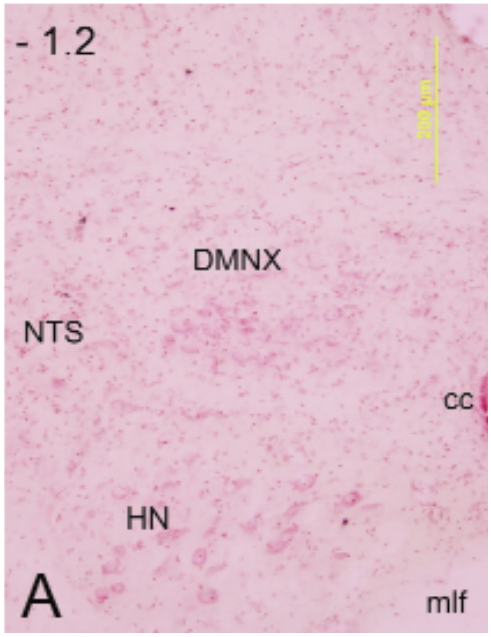

FIG. 3A

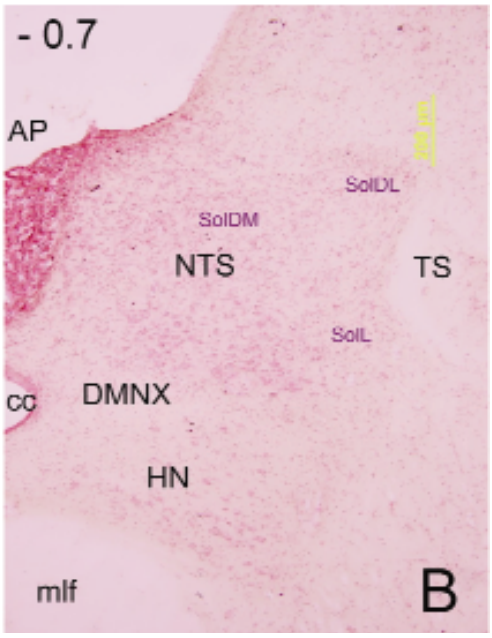

FIG. 3B

Fig. 3: Photomicrographs of transverse sections of the brainstem showing the NTS.

intense but not as vivid as sections taken closer to the obex (Fig. 4-A and B). At and above the obex, the area postrema was located bilaterally, on either side of the 4th ventricle in close relation to the NTS (Fig. 1-C). The AP extended from 0.8 mm caudal to 0.3 mm rostral to the obex and was the most densely stained nucleus in all the sections analysed.

The Hypoglossal Nucleus (HN)

Whereas the hypoglossal nucleus does not contribute to vagal fibres, it serves as an important landmark in the organization of nuclei in the medulla oblongata. This nucleus was located ventral to the dorsal motor nucleus of the vagus and lateral to the central canal of the closed part of the medulla oblongata. It contained numerous multipolar cells which were larger

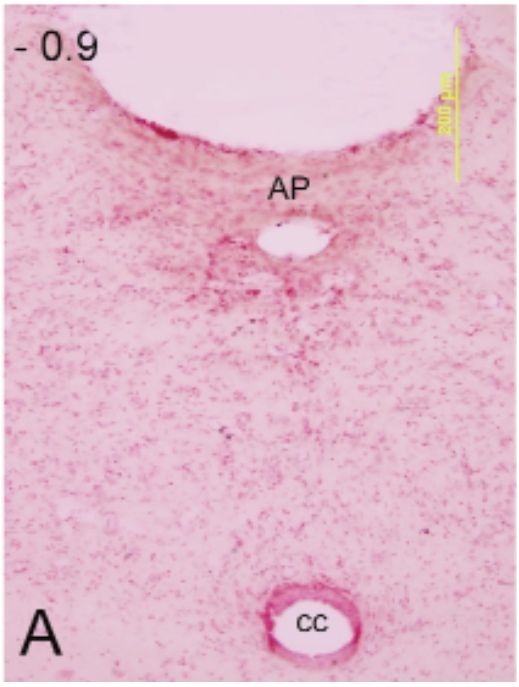

FIG. 4A

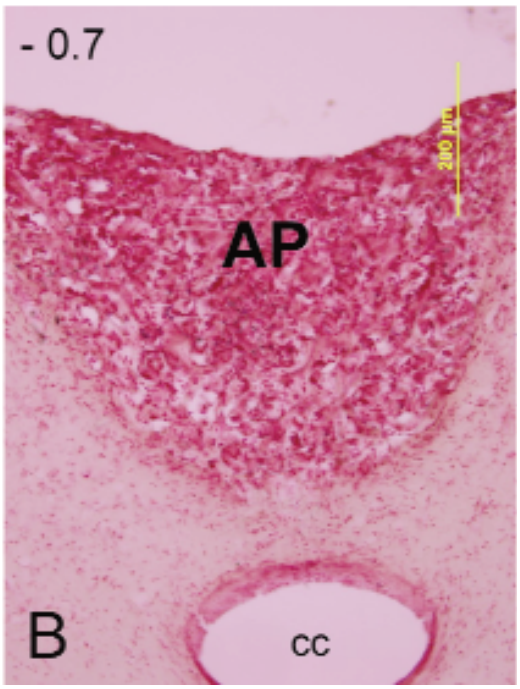

FIG. 4B

Fig. 4: Photomicrographs of transverse sections of the brainstem showing the AP.

4V = Fourth ventricle; AP = Area postrema; CC = Central canal; DMN = Dorsal motor nucleus of the vagus nerve; GR = Nucleus gracilis; HN = Hypoglossal nucleus; MLF = Medial longitudinal fasciculus; NA = Nucleus ambiguus; SolDL = Dorsolateral subnucleus; SolDM = Dorsomedial sub-nucleus; SolL = Lateral subnucleus; TS = Tractus solitarius

than those of the DMN (average size 28.5 µm) [Fig. 1-B and 3-A].

DISCUSSION

The primary objective of the current study was to determine the nuclear organization of gastrointestinal related nuclei in the agouti, a new experimental animal species. The present observations have demonstrated for the first time ever the morphology, topography and extent of the nuclei which contribute to the fibres in the vagus nerve in the agouti. The central finding was that there exists, no difference in the nuclear complexity of this area when compared to other rodent species.

All the nuclei identified appeared to be similar morphologically and topographically to those previously described for the laboratory rat (33−37), mouse (38) and house musk shrew (39). These findings are also consistent with those reported for the ferret (28, 30, 40−45). Furthermore, the present study found no nuclei that have been reported in the rat that was not present in the agouti. Our findings constitute a significant addition to the fast growing data base on the agouti. In addition to the four reasons given in the introduction section of this report for adoption of the agouti as a laboratory animal, the present study has also revealed that the nuclei investigated in the study are much larger in size than those of other rodent currently being used as laboratory animals. By virtue of the larger size of the nuclei, it will be easier to identify and manipulate them in the agouti compared with the smaller rodents. This is a positive score in the proposal aimed at adopting this species as a laboratory animal.

In conclusion, although the rostrocaudal and mediolateral extent of some of the nuclei could not be determined, this study has successfully characterized the distribution of the brainstem nuclei associated with vagal gastrointestinal innervation. Specific labelling of the cells using modern neuronal tracing techniques would provide more conclusive data on the precise delineation and extension of each nucleus. Our laboratory is engaged in a study aimed at providing irrefutable proof of the characterization of these nuclei, as indeed gastrointestinal nuclei, using neuronal tracing neurohistochemical techniques. Furthermore, the retrograde labelling approach by intramuscular injections of neuronal tracers into segments of the gastrointestinal tract would facilitate precise determination of the boundaries between the various nuclei projecting through the vagus nerve to the gastrointestinal tract. This approach can also be applied to other body systems innervated by the vagus nerve.

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