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Comparative Neuroanatomical Characterization of Ventral Midbrain Grey Matter of Some Small Laboratory Mammals

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ABSTRACT

Empirical assessments of the similarities and variations of neurobiological structures in animals are the basis of comparative neuroanatomy. Animal models including small laboratory mammals are indispensable tools for neuroanatomical research. The mammalian midbrain has been described with grey matter structures including red nucleus (RN), and substantia nigra (SN) involved in important brain functions like regulation of motor and related activities. This study comparatively characterized the neuroanatomical features of the ventral midbrain grey matter (RN and SN) of three small laboratory mammals: Wistar rat, guinea pig (*Cavia porcellus*) and rabbit (*Oryctolagus cuniculus*). The laboratory mammals (n=3/species) were obtained and weighed. The brains of species were harvested and measured, and sections of RN and SN were processed for histologic and histometric assessments. Data obtained were compared among species using statistical (IBM SPSS *v*21) and imaging (MPP, AmScope, and ImageJ, US) software. Results revealed higher ($p<0.05$) values for body and brain weights with rabbits. Histologic examinations of the RN and SN *pars compacta* (SNc) revealed similarities and some variations in the species; RN and SNc presented with a variety of cell morphologies. The histometric characteristics (pyramidal cell soma area and perimeter) of the RN showed no significant difference between the species. However, SNc histometric characteristics were different ($p<0.05$) with lower mean values for guinea pigs. In conclusion, the assessed small laboratory mammalian species demonstrated similarities and variations in neuroanatomical characteristics of the ventral midbrain grey matter (SN and RN). Similarities of cytoarchitectural characteristics could be attributed to the commonality of the species' ancestry as mammals.

Keywords

Histoarchitecture, Histometry, Substantia nigra, Red nucleus, and Ventral midbrain

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INTRODUCTION

Biological assessments in the quest to identify and elucidating similarities and dissimilarities of neurobiological structures or features in different animal species, concerning functionality associated with unique behavioural patterns of these species, are the fundamentals of comparative neuroanatomy (Cosans and Frampton, 2015; Agbon *et al*., 2022; Agbon *et al*., 2023). Morphological comparison

of the central nervous system between varying species has empirically aided the taxonomy of animal species as phylogenetically related or unrelated and, long provided substantial proof for evolution (Gaucher *et al*., 2010; Miller *et al*., 2019).

In the cranial cavity of mammals lies the brain, which is the most complex structure of the body. The brain is composed of several major and minor parts essentially involved in the homeostasis of vital bodily functions. An observable cylindrical structure on the ventral aspect of the mammalian brain is the brainstem, compartmentalized by depressions into the midbrain (the most rostral portion), pons and medulla oblongata respectively (the most caudal portions) (Kandel *et al*., 2000; Kandel *et al*., 2012). Structurally and functionally, the midbrain connects with several parts (regions) of the brain and, is related to physiological characteristics including motor control, vision and hearing, sleep and wakefulness (Singh, 2009a). Generally, two major regions describe the midbrain; a narrow dorsally situated tectum and large ventrally situated cerebral peduncles (Singh, 2009a; Agbon *et al*., 2022). The ventral peduncular region presents with three parts; crus cerebri, substantia nigra (SN) and tegmentum. The red nucleus (RN) has been described as the largest nuclei (grey matter) of the tegmental part of the midbrain (Singh, 2009a; Yamaguchi and Goto, 2006; Cacciola *et al*., 2019). Essentially, the ventral midbrain grey matter (SN and RN) have been associated with the homeostasis of motor and related activities in mammals (Ulfig and Chan, 2001; Lavoie and Drew, 2002; Vadhan and Das, 2022).

The neuroanatomical description of certain brain regions, such as the ventral midbrain grey matter, concerning structural organization, behavioural patterns, evolutionary trends, and neurological status is attainable with the aid of suitable animal models (Esteves *et al*., 2018; Miller *et al*., 2019). Animal models including small laboratory mammals are vital tools for biomedical research fields (Agbon *et al.*, 2023) and empirically useful for elucidating neurological health conditions and development of possible therapies where human subjects cannot be employed (Kalueff *et al*., 2007; Ellenbroek and Youn, 2016; Esteves *et al*., 2018). Rodent and non-rodent species comprising rats (*Rattus norvegicus*), guinea pigs (*Cavia porcellus*) and rabbits (*Oryctolagus cuniculus*) are frequently used animal models in biomedicine including neuroscience research (Shomer *et al*., 2015; Keifer and Summers, 2016; Homberg *et al*., 2017; Henry *et al*., 2021). The Wistar rat, a common strain of laboratory rats, guinea pigs and rabbits have been extensively used in pharmaceutics; drug development and related therapies (Taylor and Lee, 2012; Rahmani *et al*., 2022), and is beneficial in the fields of immunology and genetics (Esteves *et al*., 2018; Shiomi, 2020; Xu *et al*., 2021). Essentially, these small laboratory mammalian species have biological similarities to humans, predominantly in their genetic composition and are thus, relevant in numerous research fields (Hickman *et al*., 2017; El-Ayache and Galligan, 2022). Across mammalian species, the brain presents with morphological variations at macroscopic and microscopic levels over close phylogenetic expanses (Miller, 1999; Seyfarth and Cheney, 2002; Snyder *et al*., 2018). Hence, the need to characterize neuroanatomical features of the ventral midbrain of these species from a comparative perspective. This could aid the identification of likely similarities and dissimilarities, and suitable species as models for certain neuroscience investigations, especially those related to motor activities and movement disorders (Rodriguez-Oroz *et al*., 2008; Philippens *et al*., 2019).

This study comparatively characterized the neuroanatomical features of the ventral midbrain grey matter (RN and SN) of some small laboratory mammals: Wistar rat, guinea pig (*Cavia porcellus*) and rabbit (*Oryctolagus cuniculus*).

MATERIALS AND METHODS

Experimental Animals

Nine male adult experimental animals composed of three common laboratory mammalian species each: Wistar rats (*Rattus spp*.), guinea pig (*Cavia porcellus*) and rabbit (*Oryctolagus cuniculus*) were obtained from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria, and transferred in plastic cages to the Neuroanatomy and Neurosciences Research Laboratory of the Department of Human Anatomy, ABU, Zaria to acclimatize for a few days before euthanasia and assessment.

Experimental Protocol

The mammals (Wistar rats, guinea pigs and rabbits; n= 3/species) were weighed, humanely sacrificed under chloroform anaesthesia and decapitated. The skulls were dissected to expose the cranial cavities from which the brains were harvested for subsequent studies (Fig. 1).

All experiments were conducted according to generally acceptable best practices, approved by the Departmental Research Ethical Committee of the Department of Human Anatomy, ABU, Zaria (P2018/BMHA/7002).

Morphological Studies

The species were measured using a digital weighing scale (Electronic Kitchen Scale SF-400, China, 0.1 g,) before euthanasia to account for absolute body weights. The harvested whole brains were weighed using a digital weighing scale (Acculab VICON; VIC-303, USA, 0.001 g). The organosomatic index (brain-body ratio) was computed as described by Amber *et al*. (2020): organ (brain) weight/ absolute body weight x 100. The observed morphological characteristics were compared between the species.

Microscopic Studies

The brain samples were fixed in a fixative (Bouin's fluid) for 72 h, sectioned coronally at the midbrain region and afterward processed using histological techniques for light microscopic examination. The histologically processed paraffin sections (microtomy at 8 µm) were stained with haematoxylin and eosin (H and E) stains and Cresyl violet (CV) to demonstrate histoarchitectural features of the ventral midbrain grey matter. Additionally, CV-stained sections were used for microscopic image analysis including schematic

illustrations of cell morphology, histometry and cell density quantification (Fig. 2).

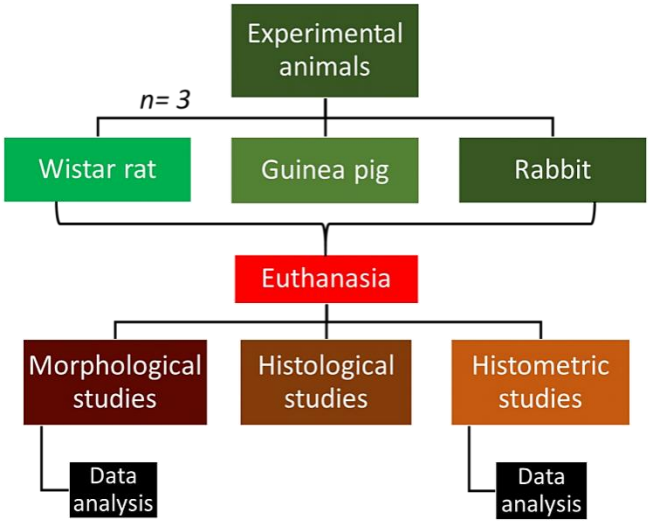

Fig. 1: Experimental Protocol

Histological Studies

The histologically processed brain sections stained with H and E, and CV stains were examined for grey matter structures of the ventral midbrain region, particularly the RN and SN pars compacta (SNc), at two microscopic magnifying powers (× 40 and 250) to demonstrate similarities and variations in the histological and cytoarchitectural features among the species. The bilateral grey matters (RN and SNc) were identified on a coronal section through the midbrain region of the rats' brain by adopting the description outlined in the Rat Brain Atlas (Paxinos and Watson, 2013); related to certain landmark structures (superior colliculi, dorsally and the hippocampi and dentate gyri, ventrolaterally). The outlined landmarks in rats were extrapolated to the other species and served as a reference for the identification and localization of grey matters of interest in these species (Fig. 2). Histological tissue processing was conducted in the Histology Unit, Department of Human Anatomy, ABU, Zaria. Microscopic examination of brain sections was conducted using a light microscope (HM-LUX, LeitzWetzlar, Germany) and capturing of micrographs using a Digital Microscopic Camera (MA 500 AmScope®, USA) was conducted in the Microscopy and Stereology Research Laboratory of the same facility.

Cell Morphology Illustration

The morphology or shape (two dimensional-2D) of varying cells observed from histological sections of the RN and SNc were illustrated schematically using an image editing software, Microsoft PowerPoint (MPP) for Windows (version 2013). A brief description of the protocol is thus: CV-stained micrographs (the CV is an excellent neuronal, cell body-specific stain (Suvarna *et al*., 2019) captured at × 250 magnification were imported into the image editing software; the MPP picture formatting tool was calibrated to select all cells from the micrographs with the following settings/ specifications: Corrections; Sharpen 50%; Brightness; -20% Contrast; +40% Colour; Colour saturation 100%; Colour Tone; Temperature 6500k; Recolour: Black and White 25%; Artistic effect; and Photocopy.

The outputs generated appeared as schematic outlines of cell profiles in black and white format on the MPP-edited micrographs. The edited micrographs were adjusted (zoomed in) for uniform dimensions set at, height: 4.25" and width: 6.53" to aid visualization and identification of cell shape types. After zooming in, the micrographs were cropped using the MPP cropping tool to outline specific cell morphologies presented with the dimensions: height: 1.33" and width: 0.88" to enhance the clarity of visualization.

The cell morphologies were identified by adopting estab-

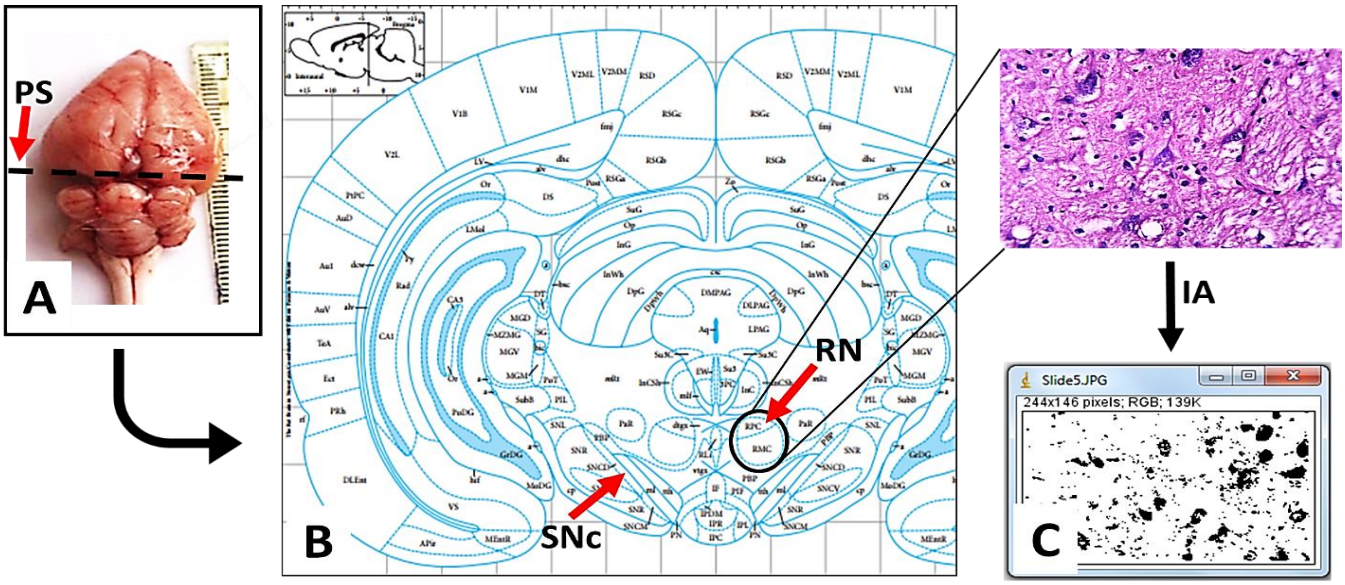

Fig. 2: Brain sectioning and identification of midbrain-tegmental structures. Rabbit's brain point of coronal section (PS; broken black line) (**A**); Schematic coronal section of the rat brain with red arrows indicating the red nucleus (RN) and substantia nigra compacta (SNc) regions of the midbrain (**B**); Image analysis (IA) for cell morphology and distribution (**C**). B (Adopted from Paxinos and Watson (2007).

lished methods described by Young and Heath (2000) and Singh (2009b) based on observed characteristic shape of cell profiles on micrographs.

Histometric Studies

Histometry is an empirical histotechnique for 2D- quantitation of cytoarchitectural features from micrographic outcomes (Asuquo *et al*., 2007; Huda and Zaid, 2007; Agbon *et al*., 2022). Histometric analysis was conducted in accordance with the method described by Agbon *et al*. (2021a) as an objective base for the comparison of pyramidal cell perikaryal size associated with its histometric characteristics (perikaryal or soma area and perimeter) (Agbon *et al*., 2021b; Agbon *et al*., 2022). The pyramidal cells of the RN and SNc were carefully identified from CV-stained histological sections (Suvarna *et al*., 2019) and histometric characteristics were measured using a light microscope with a 25/ 0.5 × objective and a micrometer slide, and a computer running imaging software (AmScope MT version 3.0.0.5, USA) according to the manufacturer's instructions.

A brief description of the protocol is thus: Two CV-stained sections per animal, per species (that is, a total of six sections per species) were selected and three different micrographic fields were randomly captured (Jelsing *et al.*, 2006; Oliveira *et al*., 2015) in the regions of interest. Pyramidal cells (ranging from 5-10 in number) that met the criteria for selection (that is, pyramidal cells, with well-outlined nuclei in the cell profiles) were randomly selected (marked) from micrographs; using the AmScope Imaging Software Polygon Tool, histometric characteristics were measured. The mean values of data obtained were computed and statistically analysed.

Cell Density Studies

The quantification of cell density (distribution) in RN and SNc was conducted by adopting the method described by Agbon *et al*. (2022). This involved assessing micrographs (captured at × 250 magnification) using a computer running image analysis software (ImageJ, NIH, US) according to the manufacturer's instructions.

A brief description of the protocol is thus: Captured micrographic fields (similar to the sampling criteria for sections/animal/species adopted for histometric analysis) were imported into the ImageJ software; the ImageJ Threshold Tool (threshold colour: Black; colour space: HSB) was calibrated to select all cells from the micrographs; modal grey values were measured and values obtained. The mean values of data obtained were computed and statistically analysed (Fig. 2 C).

Data Analysis

Data obtained were analysed using the statistical software, Statistical Package for the Social Sciences (IBM SPSS v 21.0 SPSS Inc., Chicago, USA) and results were presented in charts (using Microsoft Office Excel 2013) expressed as mean ± S.E.M. The presence of significant differences among means of the groups (species) was determined using one-way ANOVA with Tukey post-hoc test for significance. Statistical significance was set at $p < 0.05$.

RESULTS

Morphological Characteristics

The assessment of the absolute body weight of the species showed a remarkable ($p < 0.05$) difference with rabbits having weight values several times higher than that of the rats and guinea pigs. Correspondingly, a comparison of the whole brain weight between the species showed higher ($p < 0.05$) values with the rabbits. Conversely, the organosomatic index revealed a lower ($p < 0.05$) value for rabbits between the species. The guinea pig had the highest index value among the species. However, there was no difference in the guinea pig's index value when compared the rat (Table 1).

Table 1: Morphologic characteristics of studied mammals

Variable	Wistar rat	Guinea pig	Rabbit
Absolute body weight (g)	170.05 ± 7.55	351.07 ± 15.50*****	1320.15 ± 30.25**[a]**
Brain weight (g)	1.71 ± 0.15	3.90 ± 0.36*****	6.46 ± 0.20***[a]**
Organosomatic index	0.97 ± 0.42	1.13 ± 0.41	0.49 ± 0.02***[a]**

n= 3; mean ± SEM; one way ANOVA Tukey post-hoc test; ***** = $p < 0.05$ significant different when compared to Wistar rat; **a**= $p < 0.05$ when compared to guinea pig.

Histological Assessments

The histological examination of RN and SNc sections revealed the following: The RN at a lower microscopic magnifying power demonstrated similar histoarchitectural features across the species with densely packed cells including neuronal and glial cells (Fig. 3 A-C; top row). Observing closer, at a higher magnifying power revealed varying cell morphologies with distinct nuclei interspersed within the parenchyma (neuropil) of the red nuclei across the species. Additionally, some pockets of clustered cells were observed across the species. However, there was no observable difference in these cluster distributions (or frequency) amongst the species (Fig. 3 A-C; bottom row).

The SN at a lower microscopic magnifying power revealed similar histoarchitectural features and orientation across the species; three distinct layers (or parts) were observed medio-laterally; one layer with a dense cellularity and the other two with less dense cellularity. The part with densely packed cells appeared narrow with a unique crescentic orientation medial and partly dorsal to a broader and less dense part. This was identified as the compact part of the SN (SNc). The less-densely packed cells part was lateral and partly ventral to the SNc with a reticular appearance. This was identified as the reticular part of the SN (SNr). The third part, SN lateralis (SNl) with a similar appearance as SNr, was identified at the dorsal end of SNr separated by a thin layer of white matter (note: SNl is not demonstrated in the micrograph provided). At the plane of section, the SNr was laterally related to the ventral parts of the hippocampus separated by a fissure or groove (Fig. 4 A-C; top row). At a higher magnifying power, SNc presented

with different cell morphologies with distinct nuclei including neurons and glial cells, and some pockets of clustered cells across the species (Fig. 4 A-C; bottom row; see Fig. 6).

The RN and SNc revealed cytoarchitectural reactivity to CV stain. A variety of cells including neuronal cells, glial cells and interneurons with varying morphologies; shapes and sizes were identified in RN. Across the species, the following cell types were identified: basket, fusiform (bipolar), horizontal, multipolar, pyramidal, and stellate cells. There was no observable difference in the distribution and frequency of the different cell types across the species (Fig. 5). Similarly, SNc presented with a variety of cell morphologies, differing in shapes and sizes including horizontal, pyramidal, and stellate cells. Comparatively, cell distribution appeared to be similar between the species (Fig. 6).

Histometric Characteristics

The assessment of histometric characteristics (soma area and perimeter) of RN pyramidal cells revealed no significant difference between the species (Fig. 7a and 7b). However, histometric characteristics of SNc pyramidal cells were significantly different between the species with lower mean values for guinea pigs relative to the values for rats and rabbits (Fig. 8a and 8b).

Cell Distribution Analysis

Quantification of cell density (distribution) from sections of the RN and SNc revealed no remarkable difference when compared between the species (Fig. 9a and 9b).

DISCUSSION

In this study, the neuroanatomical features of the ventral midbrain grey matter, particularly the RN and SNc, were microscopically examined among three laboratory mammalian species (Wistar rat, guinea pig and rabbit) and differences compared.

The significantly higher values for mean absolute body and brain weights for rabbits relative to the other species could be associated with the obvious; larger body masses (sizes) of rabbits compared to smaller-sized rats and guinea pigs. This finding is in line with the common theory about biological species, especially in animals; the larger the body mass, the weightier the species (Agbon *et al*., 2022). Byanet and Dzenda (2014), Olude *et al*. (2016) and Ivang *et al*. (2023) reported higher mean absolute body weight values for larger laboratory mammals including *Cricetomys gambianus* (African giant rats) and *Thryonomys swinderianus* (greater cane rats or African grasscutter) compared to

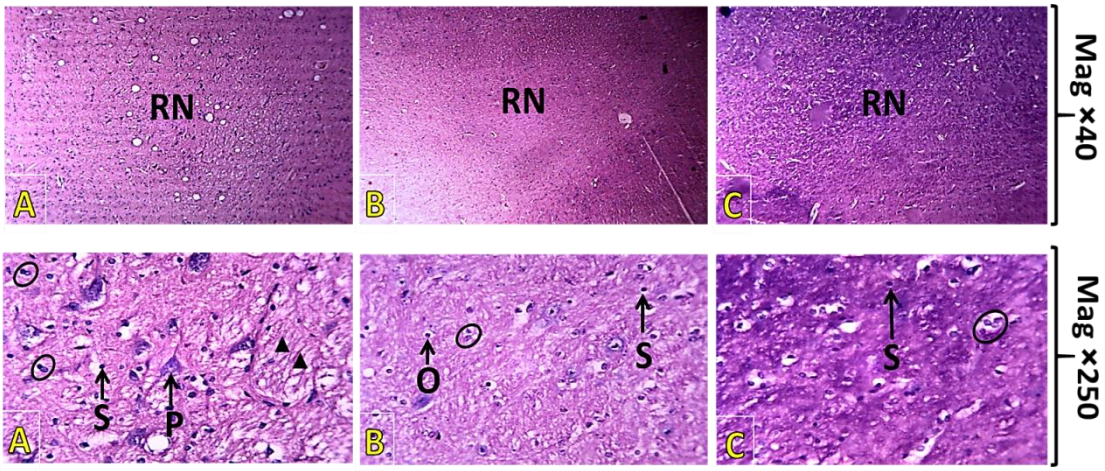

Fig. 3: Micrographs of the coronal section of the midbrain (red nucleus). Wistar rat (A), guinea pig (B) and rabbit (C); Oligodendrocyte (O); Pyramidal cell (P); Red nucleus (RN); Stellate cell (S); Synapsing cell clusters (encircled area); White matter/ nerve fibres (arrow head). Top row, × 40; Bottom, × 250. H&E Stain

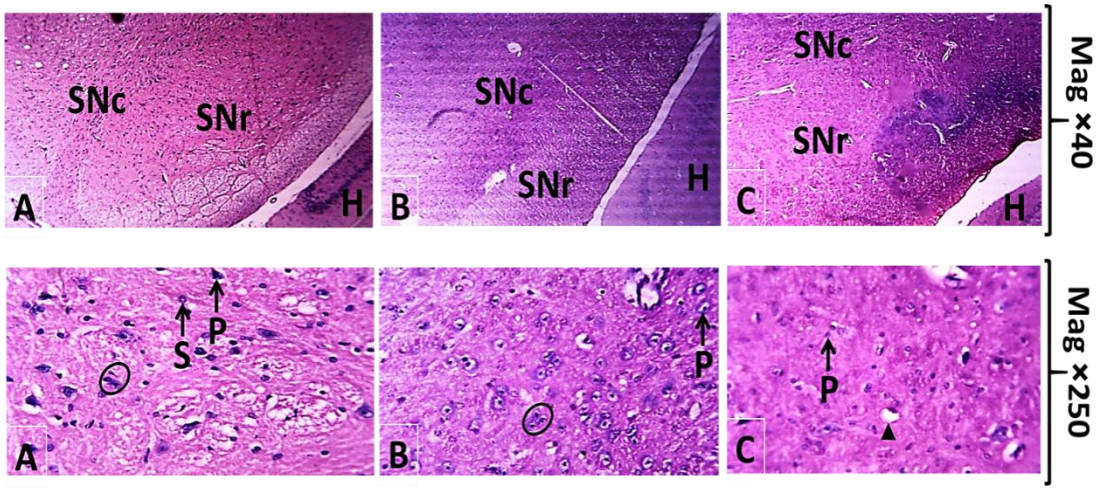

Fig. 4: Micrographs of the coronal section of the midbrain (substantia nigra). Wistar rat (A), guinea pig (B) and rabbit (C); part of hippocampus (H); Pyramidal cell (P); Stellate cell (S); Substantia nigra compacta (SNc); Substantia nigra reticularis (SNr); Blood vessel (arrow heads); Synapsing cell clusters (encircled area). Top row, × 40; Bottom, × 250. H&E Stain

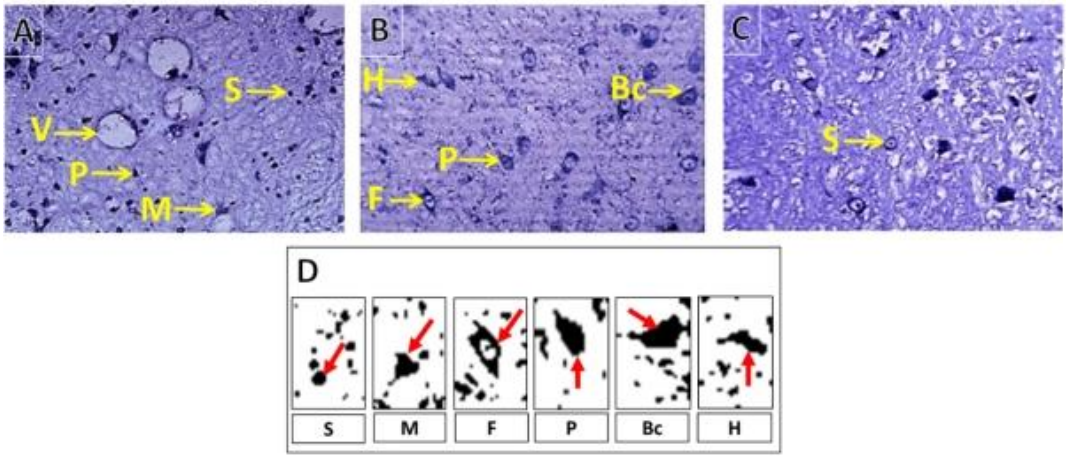

Fig 5: Micrographs of the coronal section of the midbrain (red nucleus). Wistar rat (A), guinea pig (B) and rabbit (C); Cell morphology- indicated with red arrows from schematic illustrations (D); Basket cell (Bc); Fusiform (bipolar) cell (F); Horizontal cell (H); Pyramidal cell (P); Stellate cell (S); Multipolar cell (M); Blood vessel (V). × 250. CV Stain

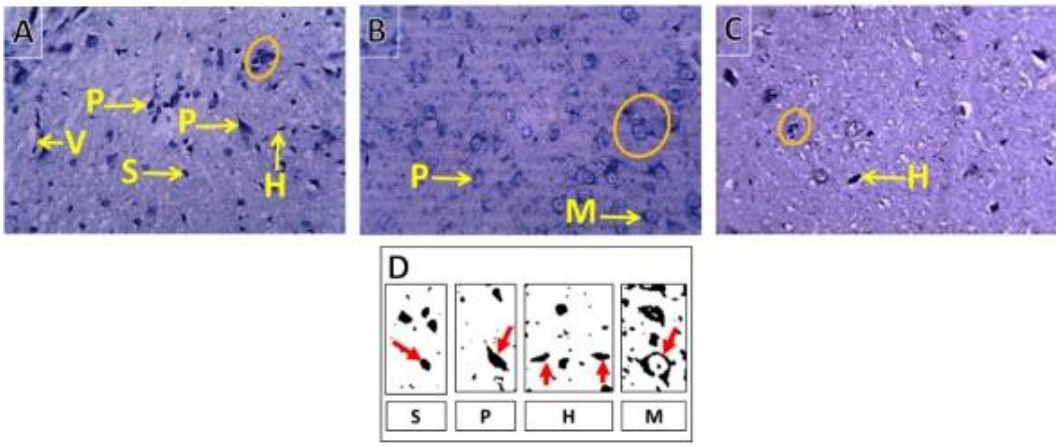

Fig. 6: Micrographs of the coronal section of the mid-brain (substantia nigra). Wistar rat (A), guinea pig (B) and rabbit (C); Cell morphology- indicated with red arrows from schematic illustrations (D); Horizontal cell (H); Multipolar cell (M); Pyramidal cell (P); Stellate cell (S); Blood vessel (V); Clustered cells (Circular outline). × 250. CV Stain

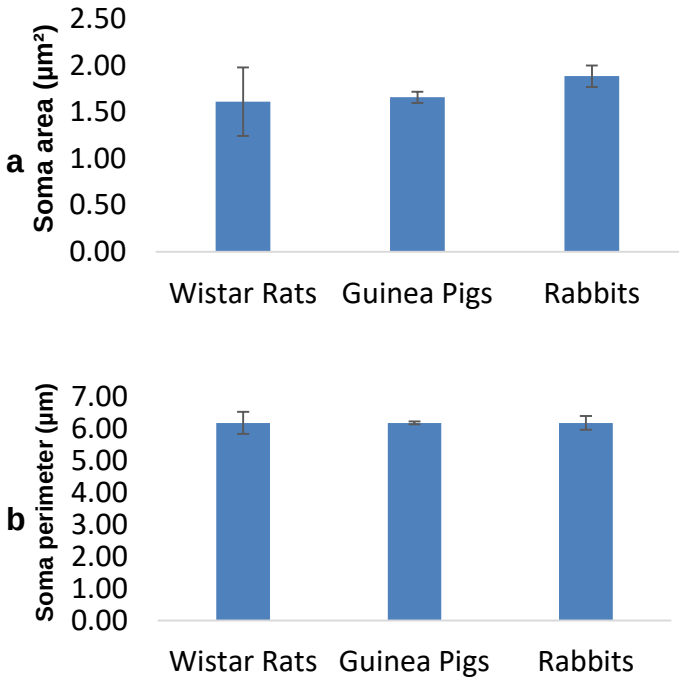

Fig. 7: Comparison of histometric characteristics of pyramidal neuron in the red nucleus of the species. a: soma area; b. soma perimeter. Mean ± SEM; one-way ANOVA; p > 0.05 - no significant difference when values were compared between species.

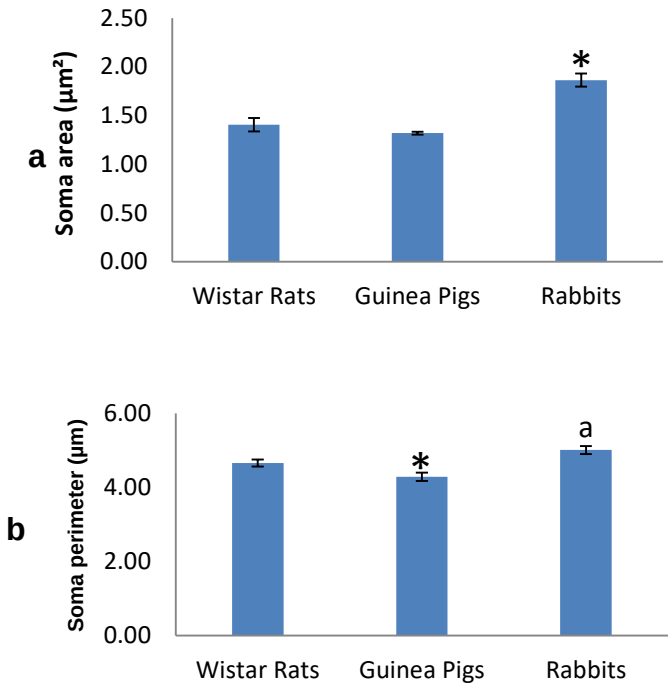

Fig. 8: Comparison of histometric characteristics of pyramidal neuron in the substantia nigra compacta of the species. a. soma area; b. soma perimeter. Mean ± SEM; one-way ANOVA Tukey post hoc test. *= p<0.05 significant difference when compared to Wistar rat, a= p<0.05 when compared to guinea pig.

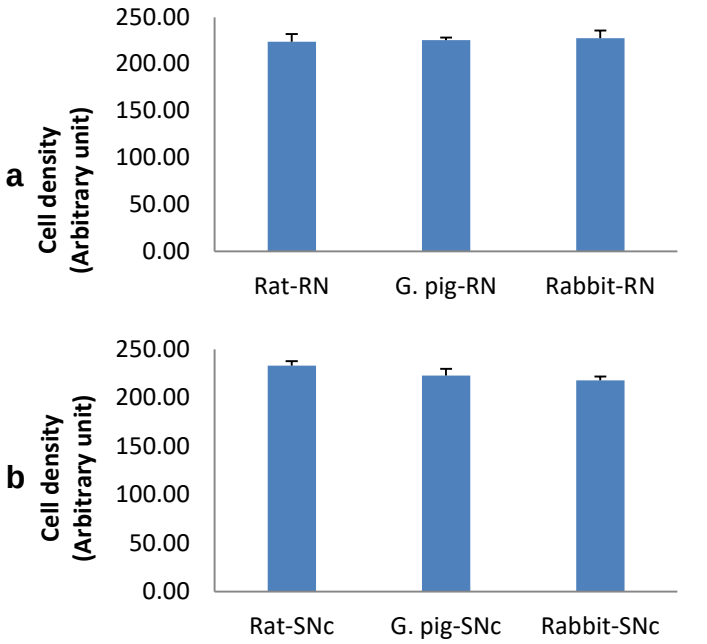

Fig. 9: Cell distribution in red nucleus and substantia nigra compacta of the species. a. Red nucleus; b. substantia nigra compacta. Mean ± SEM; one-way ANOVA; p > 0.05 - no significant difference when values were compared between species. Guinea pig (G. pig); Red nucleus (RN); Substantia nigra compacta (SNc)

smaller rodents such as mice and rats. Moreover, larger body mass has been associated with bigger and weightier bodily organs (Musa *et al*., 2016; Enemali *et al*., 2017); hence weightier rabbit brains among the compared species.

The relative brain weight differs from one taxon to another (Jerison, 1975; Seyfarth and Cheney, 2002). The guinea pig, in this study, was observed with higher values for brain-body weight ratio among the species. This finding is in line with higher values for brain-body weight ratio reported for smaller rodents including mice and rats (Russell, 1979; Olude *et al*., 2016; Agbon *et al*., 2021b) compared to that reported for larger species like African giant rats (Olude *et al*., 2016; Ibe *et al*., 2021) and African grasscutter (Ibe et al. 2017) with lower relative brain weight values. In mammalian species, larger values for relative brain weight have been associated with intelligence; providing for more intricate cognitive tasks, behavioural flexibility, escape from predators, and survival advantage (Russell, 1979; Sol *et al*., 2008; Yu *et al*., 2014). Thus, findings in this study are supportive of the well-known benefits of Wistar rats and guinea pigs as intelligent species and vital tools for neuroscience research serving as suitable animal models (Pallav, 2013; Edobor *et al*., 2021; Genzel, 2021).

The generally comparable histoarchitectural features observed in the RN across the species are suggestive of a convergent phylogenetic relationship, with similar mammalian ancestry (Mapara *et al*., 2012; Homberg *et al*., 2017). The characteristic demonstration of a variety of cells including neurons, glial cells and interneurons as integral components of the RN across the species is indicative of a typical nervous tissue (Junqueira and Carneiro, 2007; Young *et al*., 2007). The observed pockets of cells is the characteristic morphology of communicating neurons and interneurons, probably groups of functionally dependent cells involved in the homeostasis of neuronal activity (Purves *et al*., 2004; Singh, 2009a). This finding corroborates the physical and behavioural abilities of the studied species concerning motor activities including locomotion. Locomotion is an indispensable behaviour critically associated to the survival of a mammalian species, hence the need for well-organized structural components that interplay in this behavioural activity (Dyer *et al*., 2023; Forrester and Martin, 2023).

The corresponding histoarchitectural orientation of SN with distinct parts; a dorsomedially placed *pars compacta*, SNc and ventrolaterally placed *pars reticulata*, SNr observed across the species is indicative of a related mammalian ancestry (Mapara *et al*., 2012; Homberg *et al*., 2017). This finding is in line with reported morphology for SN in mammalian species including rodents. Gulley and Wood (1971) and Poirier et al. (1983) reported a similar medial-to-lateral orientation of *zona* (*pars*) *compacta* to *zona* (*pars*) *reticulata* with a dorsally placed *pars lateralis* in smaller and larger rodents including mice and rats, and two topographically distinct divisions; *pars compacta* and *pars reticulata* in primates and non-primates. The characteristic feature of the *pars compacta* with dense cellularity and *pars reticulata* with less densely packed cells is indicative of the functionality of these subparts of the SN. Gulley and Wood (1971), Lee and Tepper (2007), Paladini and Tepper (2016), and Partanen and Achim (2022) reported distinctions in the cytoarchitectural features of the different parts of the SN in rodents and other mammalian species.

The RN and SNc cytoarchitectural reactivity to CV stain, a histochemical dye with an affinity for Nissl substance within the cell bodies of neuronal cells (Suvarna *et al*., 2019; Agbon *et al*., 2021b), is indicative of normal cellular activities including biochemical and physiological processes essential for typical nervous tissue homeostasis (Suvarna *et al*., 2019; Agbon *et al*., 2022). A common cytoarchitecturally distinct cells including neurons and glial cells presenting with varying shapes and sizes as basket, bipolar, horizontal, multipolar, pyramidal, and stellate cells of RN across the species agrees with reported cell morphologies of the RN in mammalian species. Reid *et al*. (1975); Liang *et al*. (2012), and Basile *et al*. (2021) described the cytoarchitectonic of RN with differing cell morphologies in sizes and shapes for species including mice, rats, and primates. Neuronal cells of the RN play vital roles in the homeostasis of motor functionality, particularly movement of the extremities or limbs and trunk have been reported in mammalian species (Lavoie and Drew, 2002; Gray *et al*., 2005; Cacciola *et al*., 2019; Vadhan and Das, 2022).

Correspondingly, varying cells types including horizontal, pyramidal, and stellate cells identified in the SNc are characteristic of the nigral neurons and could be associated with the peculiar functionality of the SN (Danner and Pfister, 1982; Smits *et al*., 1990; Paladini and Tepper, 2016). Druga (1993) and Partanen and Achim (2022) reported morphologically distinct neuronal types having different shapes and sizes in the SN of rodent species. Additionally, several workers have reported perikaryal polymorphism in

the SN neurons of mice, rats, guinea pigs, rabbits, non-primates, and primates and associated neurons to certain physiological and biochemical (neurotransmitter) processes, sending and/or receiving inputs to -and- from several regions of the brain involved in the regulation of motor and related functions (Smits *et al*., 1990; Aumann, 2016; Poulin *et al*., 2018).

The absence of remarkable difference between the species when histometric characteristics (soma area and perimeter) of RN pyramidal neurons were compared is suggestive of comparable motor activities including locomotion. Most small mammals, including rodents share certain physical and behavioural patterns such as feeding habits, alertness and escape from predators, and social and sexual interaction that requires adopting certain body postures (Gray and Webster, 2023).

In animals, regional variation in neuronal sizes could be attributed to two commonly reported factors; the structural and functional factors (Djouhri and Lawson, 2004; Savage *et al*., 2007; Harding, 2013; Partanen and Achim, 2022). Structurally, neuronal size has been associated with the brain size of the species. On the other hand, the physiological activity of a neuron determines the size of neurons (Djouhri and Lawson, 2004; Savage *et al*., 2007; Harding, 2013; Agbon *et al*., 2023). In this study, the significantly higher value for histometric characteristics of SNc pyramidal neurons with the rabbits could be associated with the size of the brain; the rabbit's brain is remarkably bigger relative to the other species. Herculano-Houzel *et al.* (2007) reported average neuronal size is larger in mammalian species with bigger brains. This finding is in line with the reports of Agbon *et al*. (2022) that observed differences in histometric characteristics of pyramidal neurons in certain regions of the brain of compared rodent species.

Conversely, significantly lower histometric characteristics of SNc pyramidal neurons for guinea pigs relative to the two other species, rats and rabbits, could be associated with variations in certain movement patterns in the home cage including climbing, leaping and agility; observed most frequently with rats. Guinea pigs are more docile compared to smaller rodent species like rats and mice (Lee *et al*., 2014). This finding could be related to the conventional evolutionary concept of use; sizes of structures are modified by the rate of use. That is, structures used regularly are stressed and respond to hypertrophy, and those not regularly used respond to atrophy (Wackerhage *et al*., 2019; Yoganathan *et al*., 2023). In rats, a higher mobility rate could be said to task brain areas involved in motor activity thus, elaborating on the neuronal size for the species. In the wild, rabbits are known to be fast runners while guinea pigs are poor climbers (Charter and Blount, 2006). Findings in this study could be attributed to changes in behavioural patterns as a result of home cage activity following domestication as laboratory research models. Therefore, there exists variation in the histometric characteristics of SNc pyramidal neurons, but none in RN, across the assessed species.

The distribution of cells in bodily organs and tissues is critical in the homeostasis of a biological system, reflecting the functional state of the organism's organs and/or tissues fibre (Feinberg and Meister, 2015). The absence of a remarkable difference in RN and SNc cell density among the assessed species in this study is suggestive of a convergent phylogenetic relationship. Hence, there may be no variation in cell distribution of the assessed ventral midbrain grey matter across the species. However, it is important to state that, this assertion may not be completely separated from the influencing factor of the adopted methods of data collection; 2D analysis of micrographs rather 3D may provide more precision.

Conclusion

The assessed small laboratory mammalian species; Wistar rat, guinea pig (*Cavia porcellus*) and rabbit (*Oryctolagus cuniculus*) demonstrated variations and similarities in neuroanatomical characteristics of the ventral midbrain grey matter (SN and RN). Variation was observable in the histometric characteristics of SNc pyramidal neurons, but not in RN. Similarities of cytoarchitectural characteristics are a pointer to the commonality of the species' ancestry as mammals. Findings are of potential benefit in the identification of suitable models for neuroscience investigations, especially motor-related, aiding the elucidation of human-related health neurological conditions. Notwithstanding this progress, much abounds to be learned. Specific facts on comparative characteristics of the assessed grey matter using assessments including immunohistochemistry on tissues, ultra microscopy and stereological quantification for specific cell types are lacking which could increase the precision of the findings of this study.

DECLARATION

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Conflict of Interest

None declared.

Ethical Approval

All experiments were conducted according to generally acceptable best practices, approved by the Departmental Research Ethical Committee of the Department of Human Anatomy, ABU, Zaria (P2018/BMHA/7002).

Consent to Participate and Publish

Not applicable.

Authors' Contribution

Conceptualisation: ANA, ANA, FUE, RH, YMS, ZY, AEI; Data Acquisition: ANA, ANA, ZY, YMS; Data Analysis:

ANA, ANA, RH, YMS, ZY, AEI; Methodology: ANA, ANA, FUE, RH, YMS, ZY, AEI; Resources: ANA, ANA, OM, KAB, UB, FUE, RH, YMS, ZY, AEI; Supervision: ANA, ANA, ZY, AEI; Validation: ANA, ANA, OM, KAB, UB, FUE, RH, YMS, ZY, AEI; Visualisation: ANA, ANA, RH, YMS, ZY, AEI; Roles/Writing (original draft): ANA, ANA, OM, KAB, UB, FUE, RH, YMS, ZY, AEI; Writing (review and editing): ANA, ANA, OM, KAB, UB, FUE, RH, YMS, ZY, AEI.

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