Acta Scientific ANATOMY

Volume 1 Issue 6 July 2022

Research Article

Identification of Neuronal cell types and Immunohistochemical Localisation of Dopaminergic Neurons in the Spinal Cord Segment of Grasscutter (*Thryonomys Swinderianus*)

Enemali Felix Udawnojo[1]*, Iteire Kingsley Afoke[1], Uweijigho Raphael[1], Oladele Tolulope Samuel[1], Agbon Abel N[2] and Emmanuel I Odokuma[1]

[1]Department of Anatomy, University of Medical Sciences, Ondo, Ondo State, Nigeria

[2]Department of Anatomy, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

***Corresponding Author:** Enemali Felix Udawnojo, Department of Anatomy, University of Medical Sciences, Ondo, Ondo State, Nigeria.

Received: June 20,2022

Published: July 22, 2022

Abstract

Introduction: The African Grasscutter (*Thryonomys swinderianus*) is one of the few current animal models being considered for research. The animal has shown much research potential due to its similarities to other rodents.

Aim/Objective: The study aimed to provide baseline data on the types of dopaminergic neurons in the cervical spinal cord of the animal.

Materials and Methods: Six adult grasscutters (GC) (three males and three females) were acquired from a farm in Zaria, Nigeria. The animals were allowed to acclimatize for 14 days before being sacrificed. The spines were split before the lumbosacral cord segment after the vertebral column was exposed, localized, and severed for tissue analysis. Standard histological techniques were employed, and the tissues were stained with Golgi Cox. Alpha-1 antitrypsin hydroxylase was used for immunohistochemical staining. Photomicrographs were captured carried with an Olympus microscope, and the data generated were analyzed for statistical purposes using SPSS, version 21.

Results: Histochemical study revealed several neurons with various shapes, while histometry showed that these brain cells were different sizes. It was observed that neurons in male GCs were significantly larger ($P < 0.05$) than those in female GCs. Additionally, dopaminergic neurons were localized in the cervical region of the spinal cord of both sexes, just like every other rodent.

Conclusion: The histometry, histochemistry, and immunohistochemistry of GC's spinal cord reflect that of other rodents currently used as animal models for scientific research, thereby making it a suitable experimental animal for future biomedical research size relative to other rodents.

Keywords: Thryonomys Swinderianus; Dopaminergic Neurons; Congo Stain; Cervical Spinal Cord; Immunohistochemistry

Introduction

The research potential in rodent species with relatively larger sizes than rats and mice their anatomy has led researchers to develop a huge interest in using rabbits for some experiments. However, other larger rodents, such as the grasscutter, have begun to gain attention due to their unique abilities. They have exhibited a strong adaptation in using the forelimbs, mostly when eating and fighting [1]. These highlights the level of limb development and efficiency in cerebellar motor functions. It is possible that the en-

largement in the cervical segment of their spinal cord (SC), where innervations of the forelimbs are derived, may be responsible for this high-level limb mobility. Although wide varieties of GCs have been described, only two species are likely to be known. These are the greater cane rat (thryonomys swinderianus), weighing about 9 kg or more and has a head-and-body length of up to 60cm, the lesser cane rat (thryonomys gregorianus), weighing about 3.5-5 kg and may occasionally reach up to 8 kg and a body length of about 50cm [2]. The latter is currently among animal models under consideration for research in biomedical sciences. This could be because this specie has shown many research potentials in its anatomical similarities to other rodent models. Therefore, this study aimed to provide baseline data on the neuronal types and immunohistochemically localized dopaminergic neurons in the cervical spinal cord of the rodent.

Materials and Methods

Six adult GCs (three males and three females) were utilized for the study. The animals were procured from the Tamqua farm in Zaria, Kaduna state, Nigeria. They were housed in the Animal Facility of the Department of Human Anatomy, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Before the experiment, the animals were allowed acclimatization for 14 days. They were handled with best practices, as described in Ethical Guidelines for the Use of Animals in Research prepared by the National Committee for Research Ethics in Science and Technology (NENT).

Ethical consideration

Ethical permission was obtained from the ABU Committee on Animal Use and Care (ABUCAUC), and all experiments were conducted following the "Guidelines on Ethical Treatment of Experimental Animals" as proposed by the European Union directive (Directive 2010/63/EU).

Animal euthanization and sample collection

At the end of the acclimatization period, the animals' weights were obtained using a CAMRY electronic scale (EK5055, Indian) before being anesthetized with ketamine and sacrificed. The skin and epaxial muscles were dissected to enable exposure of the vertebrae column. The spinal cord was harvested by splitting the vertebra column according to the method described by Farag [3]. The cervical segment of the spinal cord was identified and dissected based on the method [4] described. The tissues were preserved in Bouin's fluid for 24 hours. Definitions of gross anatomical structures were according to standard information obtained from "rodent anatomy" by Sisson and Grossman [5].

Histometrical studies

The histometry was done using the light microscope with a 40-objective lens (×400 magnification) and a computer running image processing (AmScope MT version 3.0.0.5, USA) software for microscopy. Digital micrographs of the cervical segments of the spinal cord (stained in Golgi) were captured, and calibration measurement of microscopic features of the micrographs was conducted. The following microscopic features were considered for calibration using the software: cell area (μm^2) for neurons in the ventral horn of cervical spinal cord segments (AVHC and for neurons in the dorsal horn of cervical spinal cord segments (ADHC). Cell perimeters (μm) for neurons in the cervical spinal cord segments were also evaluated. Parts of the spinal cord where neuronal soma was evaluated include the cell perimeter of neurons from the ventral horn of cervical spinal cord segments (PVHC) and cell perimeter of neurons from the dorsal horn of cervical spinal cord segments (PDHC). The measurement process during calibrations involves: a micrometer slide containing the spinal cord tissue, which comes as 1 mm graduated in 0.01 mm was captured at the same magnification and quality (pixels). After that, the spinal cord micrograph was photographed at the same magnification. Secondly, the line tool was selected and was carefully measured from the start of one major line of the micrographs of lumbosacral segments of the spinal cord of GC from end to end; the recorded length was 649 pixels/100 um, 6.49 pixel/um. Next, the polygon tool was used to measure the area and perimeter of the desired neurons of the rodent's various sections of spinal cord segments. Values of the measurements were computed and presented automatically by the software.

Histochemical studies

Sections of the cervical region of the spinal cords were stained with Golgi Cox stain to demonstrate dendrites and axons of the various neurons in the cervical regions of the GC spinal cord. The stained tissues were finally examined for different neuronal types and shapes under the binocular light Olympus microscope (HM-LUX Leitz Wetzlar Germany) fitted with a digital camera and connected to a computer with imaging software (AmScope MT version 3.0.0.5 USA).

Immunohistochemical studies

The Bouin's fluid fixed sections were taken to water through the required standardized steps. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide, and sections were then rinsed in distilled water. Further, Epitopes were retrieved in Citrate Buffer (Ph 6) for 20 minutes, and sections were then washed in distilled water again before being incubated in alpha-1 anti-trypsin hydroxylase (diluted 1/100) for 60 minutes and washed in phosphate-buffered saline (PH 7.4). After this step, sections were incubated in Mouse and Rabbit horseradish peroxidases for 30 minutes and washed in phosphate-buffered saline (PH 7.4).

Finally, the tissue sections were incubated in 20ul of diaminobenzidine hydrochloride in 1ml of substrate buffer

for 5 minutes and washed in distilled water, counterstained in Hematoxylin for 1 minute with excess stains washed off with distilled water. The resultant slides were dried, mounted with DPX, and examined under a light microscope.

Data analysis

Data were expressed as Mean ± Standard Error of Mean. Student's *t-tests* were used as a significant test ($p < 0.05$) to determine any difference between the neuronal sizes in male and female GCs and other measured parameters. Descriptive statistics were used for the analysis of histometric data. Statistics were carried out using IBM SPSS statistics for windows version 21.0

Results

Histochemical results

The cervical region of the spinal cord showed grey matter and white matter (Figure 1), while at higher magnification, several neurons of various sizes and shapes were seen (Figure 2).

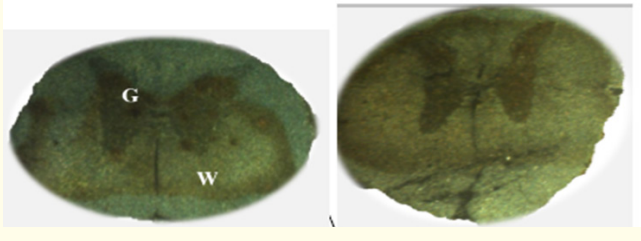

Figure 1: Sections of the cervical spinal cord segments of GC showing grey and white matters Golgi X10.

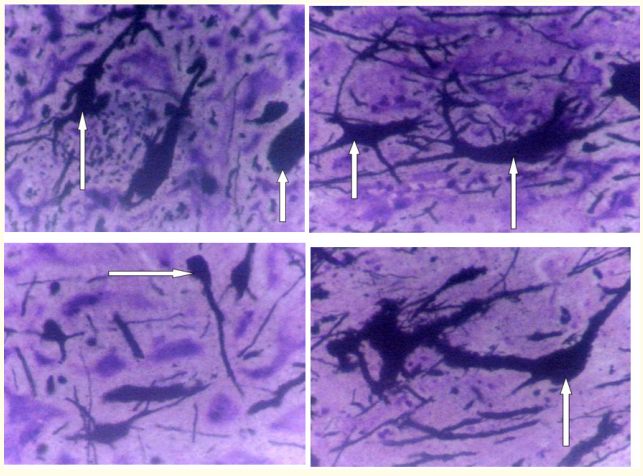

Figure 2: Sections of the cervical spinal cord segments of GC showing different neurons of different sizes and shapes in both anterior and posterior horns Golgi X250.

Immunohistochemistry

The present study showed a distribution of dopaminergic neurons (DPN) in the cervical segments of the spinal cord of GC. These neurons were localized in the grey matter of the cervical segments of the spinal cord (Figure 3).

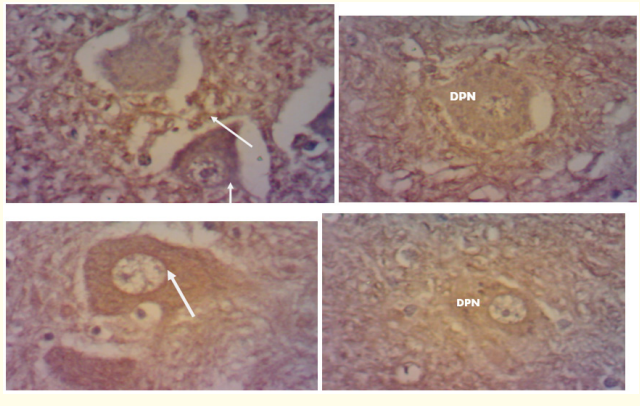

Figure 3: Sections of the cervical spinal cord segments of GC showing dopaminergic neurons in both anterior and posterior horns. Alpha-1 antitrypsin hydroxylase method X25.

Area of neurons from the ventral horn of cervical spinal cord segments (AVHC) and area of neurons from the dorsal horn of cervical spinal cord segments (ADHC). Cell perimeter of neurons from

Variables	Minimum	Maximum	Mean ± SEM
ACVH	1.65	3.71	2.56 ± 0.13
PCVH	5.05	8.08	6.36 ± 0.16
ACDH	0.51	1.40	0.83 ± 0.09
PCDH	2.91	5.07	3.86 ± 0.24

Table 1: Neuronal perimeter, P (μM) and neuronal area, A (μM^2) of neuronal cells in cervical, spinal cord segments of Grasscutter with the minimum, maximum, and mean values.

the ventral horn of cervical spinal cord segments (PVHC) and cell perimeter of neurons from the dorsal horn of cervical spinal cord segments (PDHC). Data were expressed as mean ± standard error of mean (± SEM) of values.

	Mean ± SEM	T	P-value
ACVH(Male)	4.36 ± 0.24	1.099	0.040*
ACVH(Female)	3.85 ± 0.50		
PCVH(Male)	6.19 ± 0.22	2.533	0.034*
PCVH Female	5.30 ± 0.42		
ACDH(Male)	2.36 ± 0.24	2.199	0.570
ACDH(Female)	2.85 ± 0.50		
PCDH(Male)	2.19 ± 0.22	2.733	0.764
PCDH Female	3.30 ± 0.42		

Table 2: Data analysis showing the comparison between the cell area (A) (μm2) of the cells of ventral horns of cervical spinal cord segments of GC between male and female.

Area of neurons from the ventral horn of cervical spinal cord segments (AVHC) and area of neurons from the dorsal horn of cervical spinal cord segments (ADHC). cell perimeter of neurons from the ventral horn of cervical spinal cord segments (PVHC) and cell perimeter of neurons from the dorsal horn of cervical spinal cord segments (PDHC). Data were expressed as mean ± standard error of mean (± SEM) of values, where * is significantly different ($P < 0.05$) between the male and the female GCs.

Discussion

The sections of the spinal cord of Grasscutter stained with the Golgi-Cox method revealed both the cell bodies of neurons and their axons and dendrites. Generally, a cell body gives rise to a single axon and a few primary dendrites that branch and taper along their lengths. Dendritic trees' various shapes and sizes are one of the main attributes of neurons seen in the spinal cord of GCs. Several sizes and shapes of unclassified neurons were also observed in the spinal cord. Some were with long axons, and others were observed with short axons. Neurons with long axons and other unipolar neurons are found mainly in the posterior horns of the spinal cord of Grasscutter, especially in the cervical spinal segments. Some of the neurons of the posterior horns were characterized by a spindle or fusiform cell body with few dendrites, which show a recurrent pattern of branching. These findings are similar to those observed in the kittens [6], monkeys, and Wistar rats [7].

In the anterior horns of the spinal cord, segments of GCs, short axons, and several multipolar neurons were identified mainly in the grey matter area. These local neurons are restricted to the anterior horns and are characterized by rounded or polygonal soma giving rise to three or four primary dendrites. Several secondary branches arise from a short distance from the soma with an extensive dendritic extent. Similar neuronal types have been observed by Beal and Cooper [8] and Bhardwaj [9] in lamina IV of the spinal grey matter of the macaque monkey spinal cord. These findings also follow that of Al-Saffar and Al-Haaik [10] which suggests that axons of posterior horn neurons give out few branches but still retain their structural identity while anterior horns neurons send out many branches leading to complex ramifications.

In the cervical segment of GC's spinal cord, dendritic ramifications were elaborate, giving rise to a complex communication that involves various inputs into the spinal cord circuits, as seen in the innervations of the limbs of mammals. This may explain the great dexterity of the forelimbs and greater tensile strength of the hind limbs. Our findings are remarkable and further provide insight into the morphology of the neuronal types of GCs. Furthermore, dopaminergic neurons in the spinal cord of GCs were more cervical cells clustered in the cervical and lumbosacral regions. The distribution pattern of dopaminergic neurons is closely similar to previous studies on mammals [11] but differs from the distribution pattern in the Wistar rats. The presence of dopaminergic neuronal fibers and its absence in the posterior horn of the spinal cord of Grasscutter might suggest the involvement of spinal dopaminergic neurons in the processing of nociceptive information. Another worth

mentioning finding in this study is the highly sexual dimorphism in the cell area and cell perimeter (cell size) of cervical spinal cord neurons in the cervical spinal cord of GCs. The male GCs had significantly higher values of the aforementioned histometric parameters than their female counterparts. Thus, this could help justify why the forelimbs of the male GCs are of extreme mobility compared to their females. Finally, the ventral horn of the cervical region of male GCs appears better developed by possessing more motor neurons in the ventral horn than in their female gender.

Conclusion

The data from the histometry, histochemistry, and immunohistochemistry of GC's spinal cord reflects that of other rodents currently used as animal models for scientific research, thereby making it a suitable experimental animal for future biomedical research considering its size relative to other rodents. This study has thus established baseline data for future researchers hoping to use GC for biomedical research.

Acknowledgment

The authors wish to appreciate the Department of Anatomy, Ahmadu Bello University Zaria, where most of these experimental works were carried out.

Conflict of Interest

The authors disclosed no conflict of interest of any kind. The authors solely sponsored the research work.

Bibliography

1. Aluko FA. "Qualitative characteristics of *Thryonomys swinderianus swinderianus* and *Thryonomys swinderianus gregorianus". Nigerian Journal Animal Production* 41.1 (2014): 258-263.
2. Fayenuwo JO., *et al*. "Guidelines for Grasscutter rearing. Technical Bulletin, IAR and T, Ibadan" (2013): 38.
3. Olude MA., *et al*. "Spinal Cord Studies in the African Giant Rat (Cricetomys gambianus, Waterhouse)". *Nigeria Journal of Physiological Science* 30.1-2 (2015): 25-32.
4. Farag F., *et al*. "Morphometric Studies on the Spinal cord Segments of the Domestic Rabbit *(Oryctolagus cuniculus)". Journal of Veterinary Anatomy* 5.2 (2012): 33-47.
5. Sisson S and Grossman J. "Anatomy of domestic animals". Saunders publishing Comprehensive packages (1975): 88-177.
6. Matsushita M. "Some aspects of the inter-neuronal connections in cat's spinal gray matter". *Journal of Comprehensive Neurology* 136 (1969): 57-80.
7. Todd AJ and Lewis SG. "The morphology of Golgi-stained neurons in lamina II of the rat spinal cord". *Journal of Anatomy* 149 (1986): 113-119.
8. Beal JA and Cooper MH. "The neurons in the gelatinosal Comprehensive lex (laminae II and III) of the monkey (Macaca mulatta); a Golgi study". *Journal of Comprehensive Neurology* 179 (1978): 89-122.
9. Bhardwaj R., *et al*. "Histomorphometry of Nucleus Proprius in Rat Lumbar Dorsal Spinal Horn". *Journal of the Anatomical Society of India* 50 (2001): 140-144.
10. Al Saffar FJ and Al Haaik AG. "Gross and microscopic developmental study of the local rabbit's spinal cord". *Journal of Entomology and Zoology Studies* 5.6 (2017): 2555-2562.
11. Crisp KM and Mesce KA. "A cephalic projection neuron involved in locomotion is dye coupled to the dopaminergic neural network in the medicinal leech". *Journal Experimental Biology* 207 (2004): 4535-4542.