



Anatomical Studies on the Spinal Cord of the Greater Cane Rat (*Thryonomys Swinderianus*, Temminck) II: Histomorphology and Spinal Tracings

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SUMMARY

The field of neuroanatomy and the knowledge of spinal cord, in particular, requires an accurate base on which data can be mapped. Lately, researchers have taken into consideration studies on Greater cane rat (GCR) because of its large body size and African origin. This study was designed to elucidate the histomorphologic features of the GCR spinal cord. A total of 10 adult GCR (five males and five females) raised in captivity were used for this study. Twenty-seven spinal segments (Cervical-8, Thoracic-13, Lumbar-5 and Sacral-1) were identified. Each segment was transected, processed histologically into Nissl-stained sections and observed features of the spinal cord were described. The GCR spinal segments possess the typical H-shaped inner gray matter core surrounded by an outer white matter. Variations in the shape of the central canals were observed across the spinal segments. A total of sixty-four features were delineated: twelve observed in the white matter, forty-nine in the gray matter and the remaining three were attached to the spinal cord. Laminal organisations, tracts, nuclei and motor neuron groups of the spinal cord identified were also traced and possible functions adduced in this rodent. No sexual dimorphism was observed in this study. This work has provided valuable and qualitative baseline information for understanding the laminar characteristics relevant to pathophysiological conditions of the spinal cord. It has also contributed to the knowledge of neuroanatomy of this rodent and will be valuable for spinal cord research especially in this species.

Key words: Spinal cord, Greater cane rat, Histology, Laminal organisation, Spinal tracings.

INTRODUCTION

The greater cane rat, GCR, (*Thryonomys swinderianus*), also known as the grasscutter, is one of two species of cane rats, belonging to a small family of African

hystricognath rodents (Woods and Kilpatrick, 2005). Despite been named as an agricultural pest on farmlands (Van der Merwe, 2007) and a source of food for the

African region (Matthews, 2008), the GCR is being proposed as a choice animal for general and biomedical research due to its large body size and indigenous African origin (Asibey and Addo, 2000; Mustapha *et al.*, 2015). This rodent is characteristically known for the adroit use of its limb; as they are observed to move rapidly despite having short limbs (Mustapha *et al.*, 2015). The spinal cord (SC) is equipped with the neural machinery required to operate such limb movements in a coordinated integrated way. Thus, knowledge of SC organization can be used to predict locomotor patterns (Butler and Hodos, 2005). SC neuroanatomy presents a complex repetitive arrangement whose variation is seen basically in the coronal plane (Nógrádi and Vrbová, 2013). Microscopically, the SC is composed of two discrete parts: the outer white matter and inner grey matter perforated by a central canal (Eroschenko, 2013). Although the gross morphometry of the GCR SC was recently reported (Mustapha *et al.*, 2015), no information appears to be available on the histological features of the spinal cord of this rodent.

The objectives of this study were to describe the histomorphology of the GCR spinal cord, trace observed nuclei and tracts of various spinal segments, and provide baseline research information on the histological features of the animal.

MATERIALS AND METHODS

The study was carried out using ten adult (males n=5; females=5) GCRs obtained from Pavemgo® farms, Ibereko, Badagry, Lagos State. All animals used were apparently healthy. Subsequently, they were transported in metal cages to the Veterinary Anatomy Laboratory, Department of Veterinary Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta (FUNAAB). They were stabilized for 48 hours and anaesthetized using gaseous chloroform in a closed gas chamber. Their body weights were taken and recorded

to the nearest kilogram (kg) using a Toledo Mettler® Weighing Balance (DT60J, Model: PB153-L; Serial No.: 00243365; Readability: 10grams, USA). Transcardial perfusion was achieved using the method described by Olude *et al.* (2015) and Mustapha *et al.* (2015). Briefly, a ventral midline incision was made on the chest region to expose the heart. With the aid of a scalp vein needle, normal saline (0.9%) and 10% buffered formalin were introduced sequentially into the left ventricle. A slit was made on the right atrium to allow for extravasation of circulating fluid. They were then post-fixed in 10% formalin for 42 days.

Spinal Cord Harvest and Transection

The skin and epaxial muscles were dissected to expose the vertebral spine and the SC was harvested by laminectomy (Olude *et al.*, 2015; Mustapha *et al.*, 2015). A total of twenty seven (27) spinal segments were identified and transected per animal based on methods described by Farag *et al.* (2012) and Mustapha *et al.* (2015).

Histological Processing and Histomorphology

Standard procedures were adopted for histological techniques and each segment stained using Nissl (Cresyl violet) stain (Olude *et al.*, 2014). The stained slides were examined under the light microscope (Olympus® CX21FS1) and the observed histological features of the spinal cord were described appropriately.

Photomicrography and Spinal Cord Tracings

Photomicrography

Several images of the entire section of each stained spinal segment were captured in a “left to right – top to down” manner. These images were stitched to produce a single photomicrograph of each spinal segment using AmScope® Digital Camera Software (Version: x64, 3.7.3036).

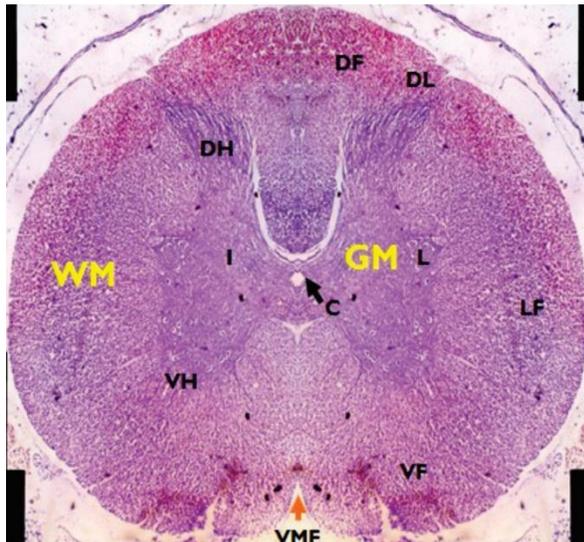


Figure 1: Photomicrograph of T1 spinal segment of the female GCR (Nissl stain $\times 400$), showing the features of a typical spinal segment including the gray matter (GM), white matter (WM), ventral horn (VH), dorsal horn (DH), lateral horn (L), intermediate substance (I), dorsal funiculus (DF), lateral funiculus (LF), ventral funiculus (VF), dorsolateral fasciculus (DL), ventral median fissure (VMF) and central canal (C)

Spinal Cord Tracings

The photomicrograph of each Nissl stained spinal segment was printed on A4 size paper. A transparent tracing film paper was then placed over each A4 print out to trace the outlines and observable structures of each spinal segment using a pencil. The final pencil drawings were then scanned with HP Scanner (Photosmart Premium All-in-one series C309); digitized and labelled appropriately with Corel Draw X5 (version: 15.0.0.489) using Watson et al. (2008) as a guide.

RESULTS

Histomorphology

Light microscopic examination of Nissl-stained histological slides of the GCR spinal segments revealed two distinct zones and a central lumen (central canal). The inner H-shaped or butterfly-shaped zone of gray matter stained dark purple while the outer

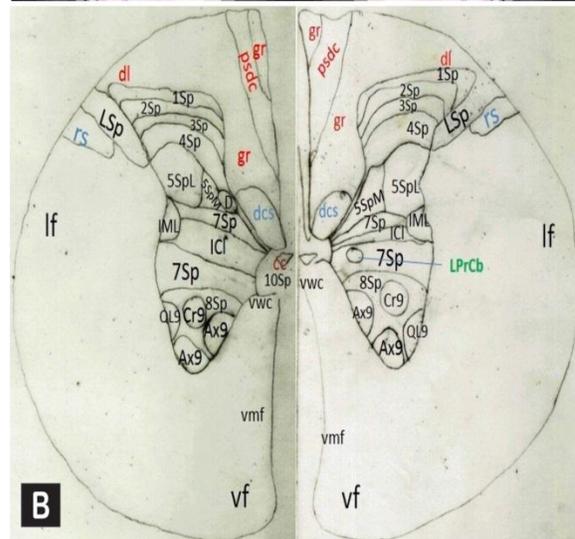


Figure 2a: L1 spinal segment of the male GCR (Nissl stain $\times 400$); **b:** L1 spinal segment tracings of the male GCR highlighting ascending tracts in red, descending tracts in blue, central canal in brown and the lumbar precerebellar nucleus in green

zone of white matter stained relatively lighter (Figures 1-8).

The gray matter was divided bilaterally into dorsal horn, ventral horn and the intermediate substance throughout the entire spinal cord length. A segment-restricted lateral horn was seen only in the thoracic and upper lumbar spinal segments. The ventral horn of the gray matter was larger than the dorsal horn in all segments, most especially at the cervical and lumbosacral

intumescence, C5 – T1 and L1 – L5 respectively (Figures 1, 2). The only exception to this observation is spinal segment C1 with larger dorsal horn compared to its ventral horn

The white matter comprised the dorsal, lateral and ventral funiculi. The dorsal and lateral funiculi were separated by a shallow dorsolateral sulcus. A ventral white commissure, located along the midline

ventral to gray matter and dorsal to the ventral median fissure, connects the right and left ventral funiculi (Figure 1).

The central canal was seen in the central core of the gray matter as a patent lumen throughout the length of the spinal cord. Shape variations of the central canal were noted across the spinal segments as that of fifth cervical segment was observed to be horizontally ellipsoidal (Figure 3).

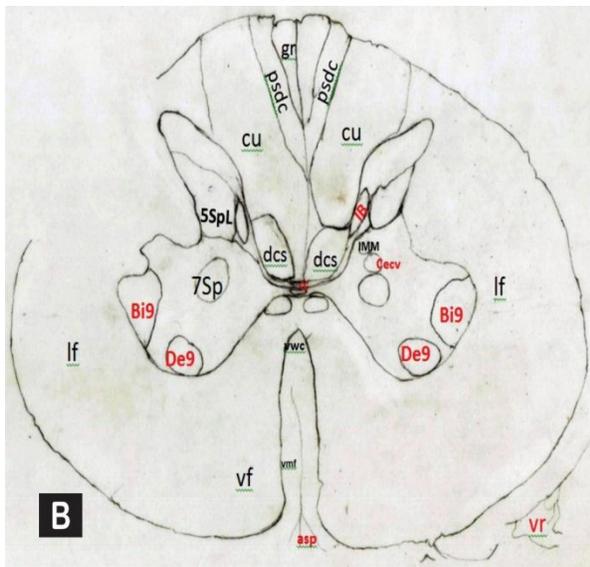
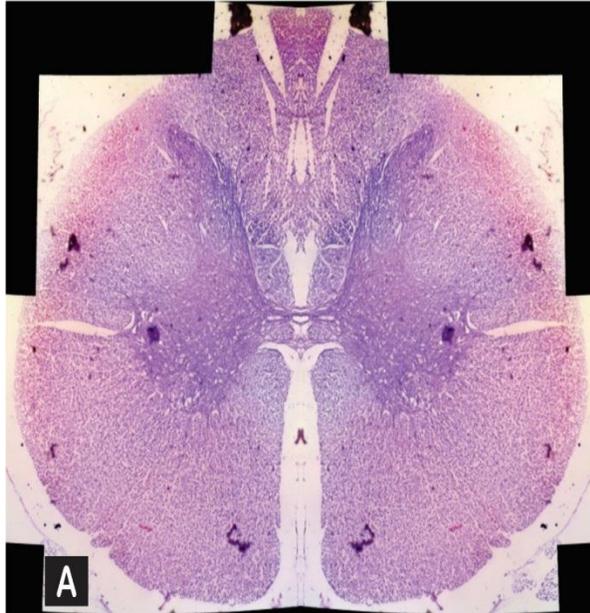


Figure 3a: Photomicrograph of C5 spinal segment of the female GCR (Nissl stain $\times 400$); **b:** C5 Spinal Segment tracing central cervical (Cecv), internal basilar (IB), central canal (cc), anterior spinal artery (asp), biceps muscle (Bi9), Deltoid muscle motor neurons (De9)

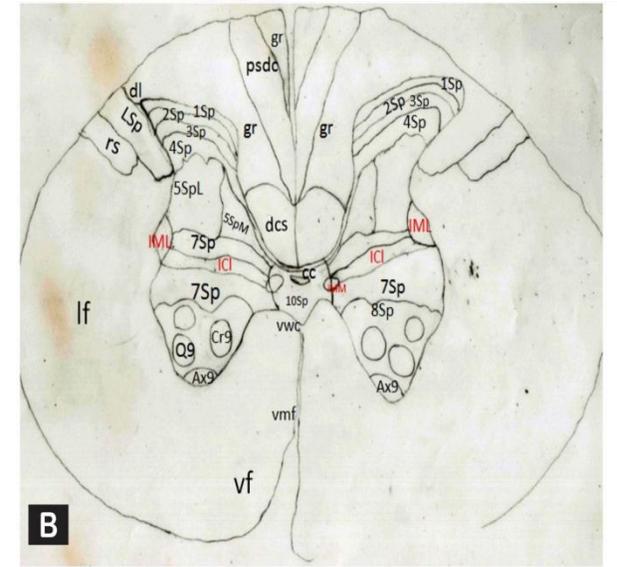
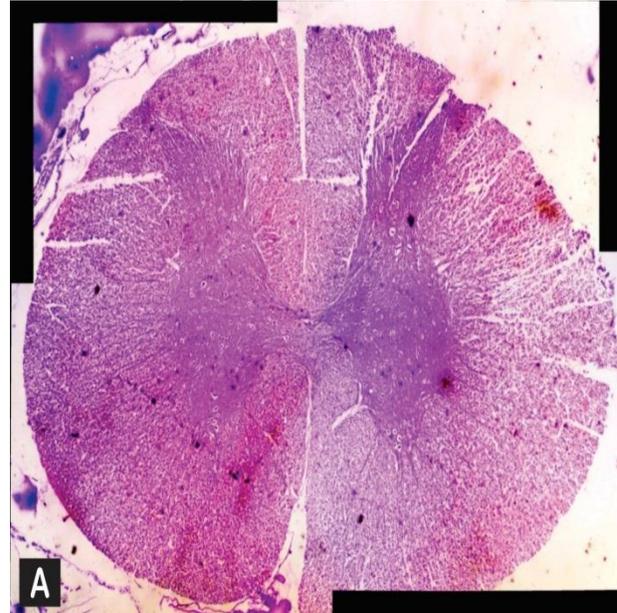


Figure 4a: L2 spinal segment of the female GCR (Nissl stain $\times 400$); **b:** L2 spinal tracings showing intercalated nucleus (Ici), intermedial column and intermedial column

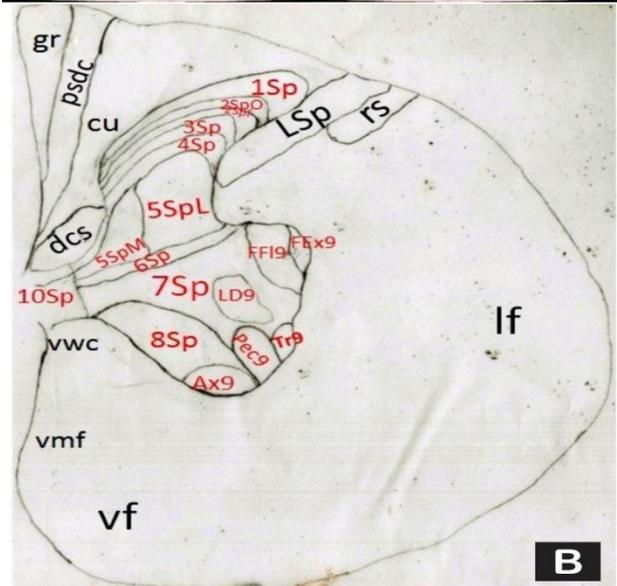
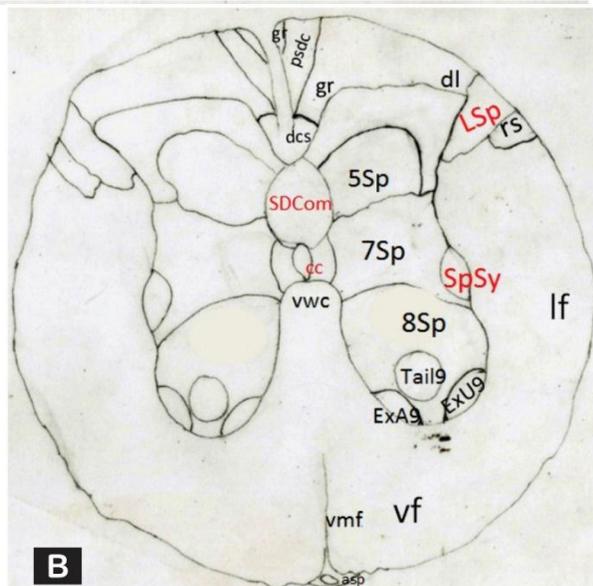
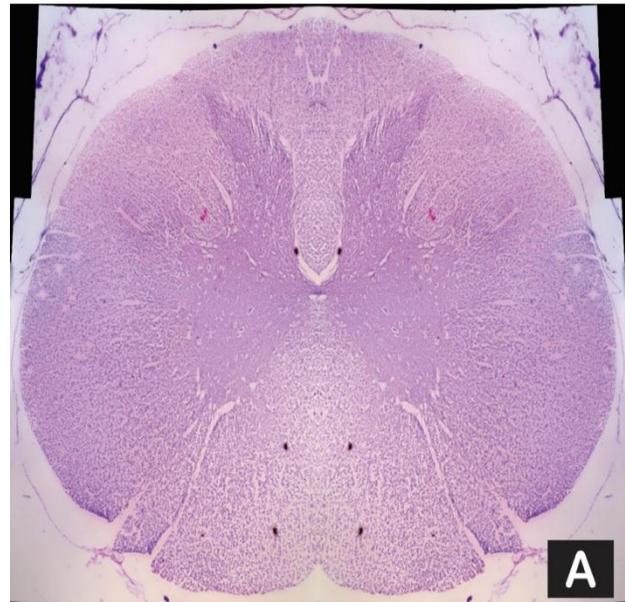
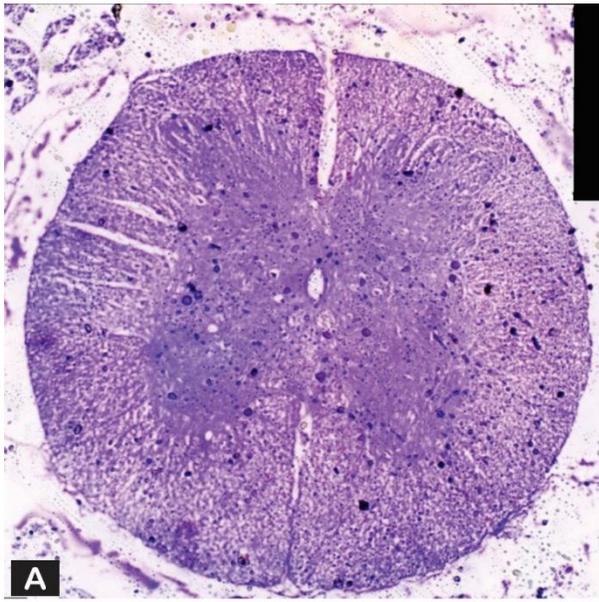


Figure 5a: S1 spinal segment of the female GCR (Nissl stain $\times 400$); **b:** S1 spinal tracings highlighting sacral dorsal commissural nucleus, sacral parasympathetic nucleus, lateral spinal nucleus and the central canal

Figure 6a: C8 spinal segment of the male GCR (Nissl stain $\times 400$); **b:** C8 spinal tracings highlighting laminae 1-10Sp and motor neurons of lamina IX labeled in red

The first thoracic segment was trapezoid to oval in shape, the fourth to eight thoracic segments were horizontally ellipsoidal while the ninth thoracic segment was circular in shape. The first lumbar segment had a horizontally oval to trapezoid shaped central canal (Figure 2) while the second and third lumbar segments had horizontally ellipsoidal canals (Figure 4). The central canal of the sacral segment was pear shaped or vertically oval (Figure 5).

Spinal Cord Tracings

A total of sixty-four structures including nuclear aggregations, laminae and tracts were identified, traced and labelled.

Nuclear aggregations

Nine spinal nuclei were identified and they include: central cervical, dorsal, internal basilar, intercalated, lateral cervical, lumbar precerebellar, lateral spinal, sacral dorsal

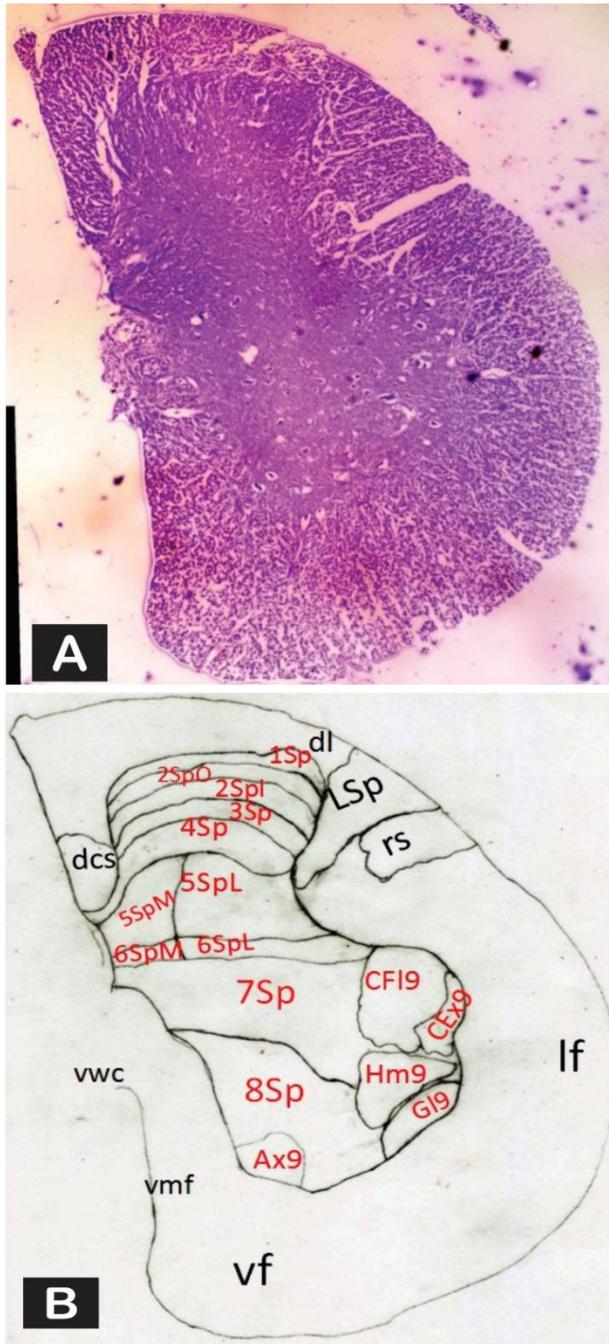


Figure 7a: L4 spinal segment of the male GCR (Nissl stain $\times 400$); **b:** L4 spinal tracings highlighting laminae 1-10Sp and motor neurons of lamina IX labeled in red

commissural and sacral parasympathetic nuclei. Of all these nuclei, only the lateral spinal nucleus was observed in all segments

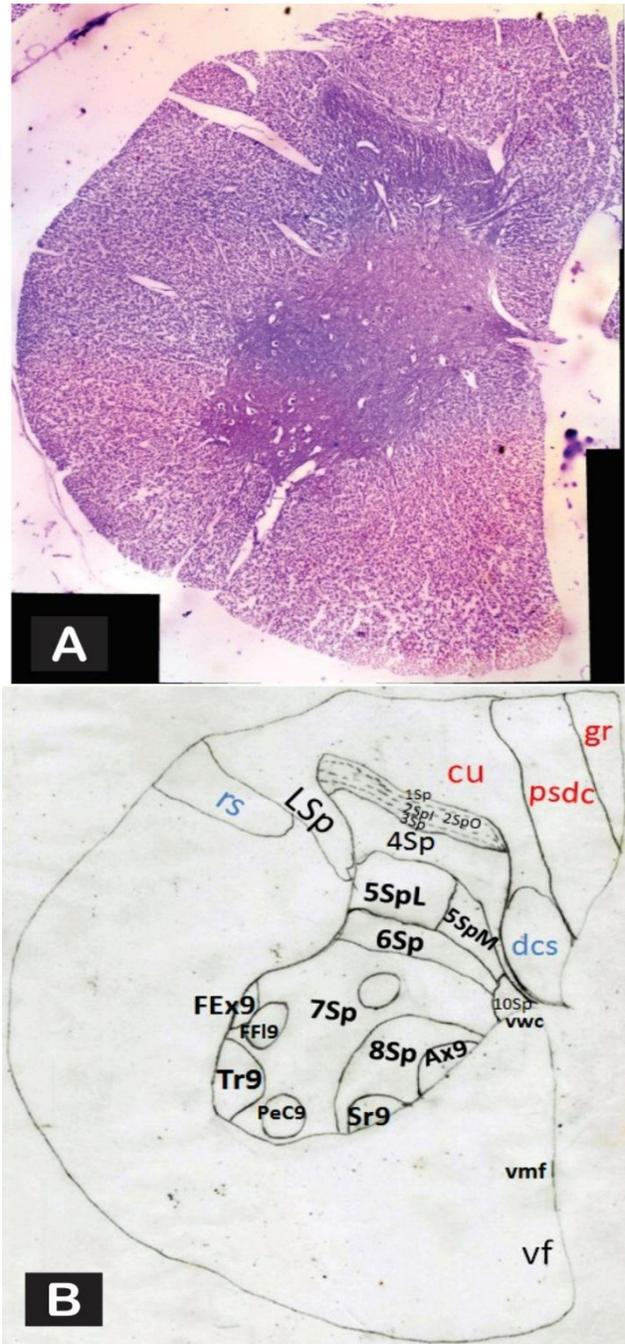


Figure 8a: C7 spinal segment of the male GCR (Nissl stain $\times 400$); **b:** C7 spinal segment of the male GCR highlighting the ascending tracts in red and the descending tracts in blue. Fasciculus cuneatus (cu), fasciculus gracilis (gr), postsynaptic dorsal column (psdc), dorsal corticospinal tract (dcs) and rubrospinal tract (rs)

(Figures 1-8). The central cervical nuclei was observed in segments C1 - C5; the internal basilar nucleus was observed only between segments C3 - C5 (Figure 3) while

List of Structures in Spinal Cord Tracings

1Sp: Lamina 1 of the spinal gray; **2SpI** :Lamina 2 of the spinal gray, inner part; **2SpO:** Lamina 2 of the spinal gray, outer part; **3Sp:** Lamina 3 of the spinal gray; **4Sp:** Lamina 4 of the spinal gray; **5SpL:** Lamina 5 of the spinal gray, lateral part; **5SpM:** Lamina 5 of the spinal gray, medial part; **6SpL:** Lamina 6 of the spinal gray, lateral part; **7SpM:** Lamina 7 of the spinal gray, medial part; **8Sp:** Lamina 8 of the spinal gray; **10Sp:** Lamina 10 of the spinal gray; **Ad9:** Adductor motor neurons of lamina 9; **asp:** Anterior spinal artery; **Ax9:** Axial muscle motor neurons of lamina 9; **Bi9:** Biceps motor neurons of lamina 9; **CC:** Central canal; **CeCv:** Central cervical nucleus; **CEx9:** Crural extensor motor neurons of lamina 9; **CFI9:** Crural flexor motor neurons of lamina 9; **Cr9:** Cremaster motor neurons of lamina 9; **cu:** Cuneate fasciculus; **D** :Dorsal nucleus (Clarke's); **dcs:** Dorsal corticospinal tract; **De9:** Deltoid motor neurons of lamina 9; **dl:** Dorsolateral fasciculus (Lissauer); **dr:** dorsal root; **ExA9:** External anal sphincter motor neurons of lamina 9; **ExU9:** External urethral sphincter motor neurons of lamina 9; **FEx9:** Forearm extensor motor neurons of lamina 9; **FFI9:** Forearm flexor motor neurons of lamina 9; **GL9:** Gluteal motor neurons of lamina 9; **gr:** Gracile fasciculus; **Hm9:** Hamstring motor neurons of lamina 9; **IB:** Internal basilar nucleus; **ICI:** Intercalated nucleus; **ICo9:** Intercostal muscle motor neurons of lamina 9; **IH9:** Infrahyoid muscle motor neurons of lamina 9; **IML:** Intermediolateral column; **IMM:** Intermediomedial column; **LatC:** Lateral cervical nucleus; **LD9:** Latissimus dorsi motor neurons of lamina 9; **lf:** Lateral funiculus; **LPrCb:** Lumbar precerebellar nucleus; **LSp:** Lateral spinal nucleus; **Man9:** Manus motor neurons of lamina 9; **Pec9:** Pectoral muscle motor neurons of lamina 9; **Ph9:** Phrenic motor neurons of lamina 9; **Ps9:** Psoas motor neurons of lamina 9; **psdc:** Postsynaptic dorsal column pathway; **Q9:**Quadriceps motor neurons of lamina 9; **QL9:** Quadratus lumborum motor neurons of lamina 9; **Rh9:** Rhomboid muscle motor neurons of lamina 9; **rs:** Rubrospinal tract; **SDCom:** Sacral dorsal commissural nucleus; **SM9:** Sternomastoid motor neurons of lamina 9; **SPSy:** Sacral parasympathetic nucleus; **Tail9:** Tail muscle motor neurons of lamina 9; **ThAb9:** Thoracoabdominal wall muscle motor neurons of lamina 9; **Tr9:** Triceps motor neurons of lamina 9; **TzSM9:** Trapezius and sternomastoid motor neurons 9; **vf:** Ventral funiculus; **vmf:** Ventral median fissure; **vr:** ventral root; **vwc:** ventral white commissure

the lateral cervical nucleus, lateral to the lateral spinal nucleus, was observed in segment C3 only. The dorsal nucleus, a well-defined oval area, was observed to span the thoracic segments and the first three lumbar segments (Figure 4). The intercalated nucleus, located within lamina VII of the spinal gray matter, was observed from T2 – L3 spinal segments and S1 segment. The lumbar precerebellar nucleus, traced in lamina VII, was observed in segments L1 and L3 (Figure 2). The remaining two nuclei, sacral dorsal commissural and sacral parasympathetic nuclei, were observed in the sacral segment (Figure 5).

Laminar organisation

Ten Rexed laminae of spinal gray matter were identified and labelled 1Sp to 10Sp.

The laminae were all consistent across the spinal segments except for lamina VI which was observed only in C5 – C8 (Figure 6) and L4 –L5 spinal segments (Figure 7). Laminae I – VI was confined to the dorsal horn of the gray matter; laminae VII and X occupied the intermediate substance of the gray matter, and laminae VIII, IX filled the ventral horn of the gray matter. Lamina I is the outermost, most dorsal and smallest while lamina VII appeared as the largest (Figure 6). The intermediolateral column, located at the lateral margin of lamina VII, was observed from segments T2 - L2 while intermediomedial column was noted in segments C3 – L2. Twenty nine (29) motor neurons of lamina 9 of the spinal gray matter were traced (Table I).

Tract tracings

Six spinal tracts broadly categorized into two (ascending and descending tracts) based on the direction of neural impulses were delineated. Ascending tracts traced include fasciculus cuneatus, fasciculus gracilis, dorsolateral fasciculus, postsynaptic dorsal column pathway while the descending tracts outlined are dorsal corticospinal tract and rubrospinal tract. The first five tracts were all located within the dorsal funiculus of the white matter while the last tract (rubrospinal tract) was situated in the lateral funiculus.

The fasciculus gracilis, dorsolateral fasciculus, postsynaptic dorsal column pathway and dorsal corticospinal tract all spanned the entire spinal cord (Figures 2 and 8). Fasciculus cuneatus was observed from segments C1 – T6, and thereafter replaced with fasciculus gracilis from T7 – S1 spinal segments (Figures 2 and 8).

Eight other features identified and mapped on the spinal tracings include the anterior/ventral spinal artery, dorsal root, ventral root, ventral funiculus, lateral funiculus, ventral median fissure, ventral white commissure and central canal. No difference was observed in the histological features of the male and female spinal cord of the GCR.

DISCUSSION

Knowledge of spinal cord anatomy provides bases for understanding and interpreting clinical implications of spinal cord injuries, cordotomy and other spinal operations (Ko

TABLE I: Observed motor neurons of lamina 9 of the spinal gray

S/N	SEGMENT OBSERVED	Motor Neurons of Lamina IX
1	C1 - L1; L4 - L5	Ax9
2	C1 - C3	IH9
3	C2	SM9
4	C3	Ph9 and TzSM9
5	C5	Rh9
6	C5	Bi9 and De9
7	C7	Sr9
8	C7 and C8	FEx9, FF19 and Tr9
9	C7 - T1	Pec9
10	C8	LD9
11	T1 and T2	Man9
12	T2 - T8	ICo9
13	T8 - T10	ThAb9
14	L1	QL9
15	L1 and L2	Cr9
16	L2 and L3	Ps9 and Q9
17	L3	Ad9
18	L3 - L5	GI9
19	L4 and L5	CEx9, CF19 and Hm9
20	S1	ExA9, ExU9 and Tail9

et al., 2004) and also in the clinical prognosis of compressive cervical myelopathy (Levine *et al.*, 2010).

The basic histological features of the GCR spinal segments observed in this study are the typical H-shaped inner gray matter core surrounded by an outer white matter and were similar to those reported for other rodents (Hebel and Stromberg, 1976; Watson *et al.*, 2008; Sengul *et al.*, 2012; Olude *et al.*, 2015). The inner H-shaped gray matter stained dark purple due to the abundant presence of neuronal cell bodies (nissl substance) while the outer zone of white matter stained relatively lighter due to scanty neuronal cell bodies in this region.

Ventral horns of the spinal gray matter are known to contain numerous motor neurons (Butler and Hodos, 2005) and receive extra axonal contributions from the brachial and lumbosacral plexi (Gruener and Biller, 2008; Sengul *et al.*, 2012; Mustapha *et al.*, 2015). The ventral horns of the gray matter in the GCR were noted to be wider than their

respective dorsal horns (except in spinal segment C1). This feature was most notably highlighted in the cervical and lumbosacral intumescence and may contribute to limb dexterity in this rodent.

Shape variations were observed in the central canal of the GCR spinal cord. In the GCR, C5 is horizontally ellipsoidal as compared to a vertical slit in laboratory rat (Hebel and Stromberg, 1976) and African giant rat, AGR, (Olude *et al.*, 2015). T1 was trapezoid to oval but vertical slit in the laboratory rat and AGR (Hebel and Stromberg, 1976; Olude *et al.*, 2015); T4 - T8 is horizontally ellipsoidal while those of laboratory rat and AGR were vertically slit (Hebel and Stromberg, 1976; Olude *et al.*, 2015); T9 central canal is circular in the GCR but vertically oval in laboratory rat and AGR (Hebel and Stromberg, 1976; Olude *et al.*, 2015); L1 is horizontally oval to trapezoid shaped but round in the laboratory rat and AGR (Hebel and Stromberg, 1976; Olude *et al.*, 2015). The functional significance of these central canal variations is however not yet known.

The lamination adopted is in accordance with Rexed, (1952) and similar to reports from laboratory rat (Hebel and Stromberg, 1976; Watson *et al.*, 2008) and mice (Sengul *et al.*, 2012). Lamina VI, observed only in spinal segments corresponding to the two intumescences, processes proprioceptive stimuli and is associated with the spinocerebellar pathway (Bassett, 2015). Lamina VII, the largest of the laminae, relays unconscious proprioception to cerebellum and is associated with the spinocerebellar and vestibulospinal tracts, sympathetic trunk and parasympathetic ganglion (Butler and Hodos, 2005, Bassett, 2015).

Observed tracts and nuclei in the GCR spinal cord were similar to those in other rodents (Hebel and Stromberg, 1976; Watson *et al.*, 2008; Sengul *et al.*, 2012; Olude *et al.*, 2015). The dorsal nucleus is correlated with sensory discrimination in the skin and development of proprioceptive

sense in the limb musculature (Zaqout and Saleh, 2013). The central cervical nuclei are essential for the coordination of movement and balance (Gruener and Biller, 2008). The rubrospinal tract is involved in the voluntary control of musculature that participates in skilled, often manipulative movement of the extremities (Hall and Guyton, 2005). The dorsal corticospinal tract is also involved in the voluntary movement of muscles of the body (Hall and Guyton, 2005). The internal basilar nucleus is associated with control of voluntary motor movements, procedural learning and routine behaviours or habits (Weyhenmeyer and Gallman, 2007; Stocco *et al.*, 2010). These nuclei appear well developed and thus, might explain the dexterous limb movements and balance shown by this rodent - documented as a good swimmer and also as a fast running, climbing and burrowing animal (Opara, 2010).

The lateral cervical, lateral spinal, sacral dorsal commissural nuclei are responsible for nociception (Rea, 2009). The fasciculus gracilis provides conscious proprioception of the lower limbs (T7-S1) and trunk to the brainstem. It also carries deep touch, vibrational and visceral pain information to the brainstem while the fasciculus cuneatus transmits fine touch, fine pressure, vibration and proprioceptive information from spinal nerves located in dermatomes C1 through T6 (Dougherty, 2010; Stocco *et al.*, 2010; Bassett, 2015). These ascending tracts are well mapped in the GCR spinal cord and putatively account for agility, psychomotor activities and quick response to danger/fright in their environment.

CONCLUSION

The histomorphology of the GCR spinal cord has been documented in this report. Major tracts and nuclei have also been identified and traced. The baseline information generated from this study provides valuable and qualitative data for understanding the laminar characteristics relevant to pathophysiologic conditions of the spinal cord. It has also contributed to the

knowledge of neuroanatomy of this rodent and will be beneficial for spinal cord research in this species.

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