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ARTICLE

Anatomical Studies on the Spinal Cord of the Greater Cane Rat (*Thryonomys swinderianus,* Temminck) I: Gross Morphometry

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ABSTRACT

Morphometric relationship between spinal cord (SC), limb dexterity and, behavioural adaptation is well established in most mammals. This study describes the gross morphology and morphometry of the greater cane rat (GCR) SC with the aim of postulating possible functional correlates. Twelve rostrocaudal linear SC parameters were generated from twelve adult GCR (6 males and 6 females). Gross morphology of GCR SC revealed an elongated, whitish roughly cylindrical mass which spanned from the foramen magnum to vertebra L_2andterminated in the coccygeal vertebrae as the *filum terminale*. Twenty seven spinal segments were recorded (Cervical 8, Thoracic 13, Lumbar 5, Sacral 1). Cervical intumescence (2.7±0.15cm length; 1.0± 0.01g weight) was located between spinal segments C_4–$T_1$and occupied nominally corresponding vertebral segments. The lumbosacral intumescence (4.2±0.20cm length; 1.90±0.20g weight) occupied spinal segments L_1-L_5 and lodged between vertebral segments T_{10}-L_1.The mean SC weight and length of the GCR measured 6.5±0.70g (5.2±0.88g in males; 7.7±0.27g in females) and 24.0±1.30cm (21.0±0.79cm in males; 26.0±1.30cm in females) respectively. Sexual dimorphism was noted in SC length, spinal segment lengths and weights of both intumescences ($p \leq 0.05$). The longer hind feet of the GCR may explain the relative higher values of spinal segment length and weight of the lumbosacral enlargement compared to the cervical enlargement. Thisstudyprovidestopographic and morphometric data on the spinal segments of the GCR, that may be useful in applied research during regional anaesthesia and diagnostic medical imaging in SC diseases. This contribution will promote the current drive of adopting the GCR as an indigenous research rat model in spinal cord injury and repair among others.\The present study is also considered to constitute a reference for future advanced morphological and physiological studies to be carried out on the GCR SC.

Keywords: spinal cord, greater cane rat, morphometry, spinal segments, vertebral formula

INTRODUCTION

The involvement of Africa in general and biomedical research is evolving with attending need to explore and adopt native animals as research models as against exotic laboratory animals (Asibey and Addo, 2000). The greater cane rat (GCR) popularly called grasscutter, is one of Africa's choice research animal (Asibey and Addo, 2000; Olude *et al.,* 2014). The GCR is also increasingly being farmed and bred because it provides protein to many West Africans. Thus, there are increased research efforts amongst African researchers aimed at better understanding and domestication of the GCR. So far, established research fields include digestive anatomy and physiology (van Zyl, *et al.,* 2004; Yapi *et al.,* 2012);, urinary and reproductive anatomy (Adebayo *et al.,* 2009;Olukole *et al.,*2009; Olukole and Obayemi, 2010);, craniometry (Olude *et al.,* 2014) and neuroanatomy (Ibe *et al.,* 2010; Ibe *et al.,* 2011; Elston and Manger, 2014). There is dearth of available information on the anatomy of the various segments of the spinal cord in the GCR.

Linear relationship between the SC morphometry, limb dexterity and, behavioural adaptation is well established (Buttler and Hodos, 2005;Akinkunmi, 2013). Morphometric differences in SC segments parameters between humans and other animal species have been documented using gross (Ko*et al.,* 2004), microscopic (Turgut *et al.,* 2007), and medical imaging (Da Costa *et al.,* 2006) methods. These morphometric results have been found useful in magnetic resonance imaging and computerized tomography as also in histopathological studies (Mitchel, 2009; Claridge *et al.,* 2010) for diagnosing and monitoring disease progress (Mitchel, 2009). The aim of this study is to describethe gross morphology and morphometry of the GCR SC. The information obtained will serve as baseline reference data and possible functions of observed features will be discussed.

MATERIALS and METHOD

All experimental protocols conformed to the ethics and guidelines on health guide for the care and use of animals in experimentsby the Federal University of Agriculture Abeokuta, Ogun State, Nigeria. Twelve adult GCRs(6 males 1.70±0.08kg and 6 females2.40±0.13kg) obtained from Pavemgo farms (Ibereko, Badagry, Lagos state, Nigeria), were used for this study. They were examined by competent veterinariansto be free of deformities or abnormalities that can interfere with this study and subsequently transported in metal cages to the Animal Care Unit, Department of Veterinary Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria.

Animal Handling and Sample Harvest

On arrival, they were stabilized for 48hours in holding metal cages designed with dark and light compartments to regulate their sleep and wake cycles. They were subsequently sedated with chloroform(0.1 ppm; Sigma-Aldrich®, Germany) and body parameters [body weight (kg), head length (cm), trunk length (cm) and tail length (cm)] taken according to Akinkunmi, 2013 and Olude *et al.,* 2014. The experimental animals were transcardially perfused using 0.9% normal saline followed by 10% buffered formalin until adequate perfusion was achieved. Thereafter, they were post-fixed in 10% formalin for 42 days. The skinandepaxial muscles were dissectedout to expose the vertebral columnand SC. The SC was carefully exposed and harvested by laminectomy following the methods of Farag *et al.,* (2012) and Akinkunmi (2013).

Gross morphology

Gross morphology of the SC was described based on itsorigin, termination, colour, contours, shape, size, intumescences and emergence of spinal nerve roots.

Gross morphometry

Twelve (12) gross SC morphometric parameters were evaluated and reportedwiththe aid of metre ruler, magnifying lens and thread. They include: SC length (cm), SC weight (g), cervical enlargement length(cm), cervical enlargement weight (g), lumbosacral enlargement length (cm), lumbosacral enlargement weight (g), inter root length (cm),

root attachment length (cm), transverse diameter (cm), dorsoventral diameter (cm), segment length (cm) and compression ratio (Farag *et al.,* 2012, Akinkunmi, 2013; Bahar *et al.,* 2013).

Definitions of morphometric parameters:

- **Head length (HL):**Measured from the rostral tip of the nose to nuchal crest (occipital bone) (cm).
- **Trunk length (TKL):**Measuredfrom the nuchal crest to last sacral bone (cm)
- **Tail length (TL):**Measuredfrom the first sacrococcygeal joint to the caudal tip of last coccygeal bone (cm).
- **Spinal cord weight (SCW):**Weightin the nearest grams (g) of the spinal cord.
- **Spinal cord length (SCL):**Measurementof the spinal cord (cm) from its rostral limit to tip of the *conusmedullaris*.
- **Cervical enlargement length (CEL) and weight (CEW):** Measurementof the length (cm) and weight (g) of the cranial expanded portion (cm) of the spinal cord.
- **Lumbosacral enlargement length (LEL) and weight (LEW):** Measurementof the length (cm) and weight (g) of the caudal expanded portion of the spinal cord.
- **Root attachment length (RAL):**Distancebetween the rostral-most and caudal-most rootlets of the same spinal nerve root (mm).
- **Inter-root length (IRL):**Distanceon the cord surface between two successive spinal nerve roots, devoid of any rootlets (mm).
- **Segment length (SGL):**Lengthof the cord extending from the rostral-most attachment of one spinal nerve root to the rostral-most attachment of the succeeding spinal nerve root (mm)
- **Dorsoventral diameter (DVD):**Distancebetween the dorsum and ventral surface of each spinal segment(mm)
- **Transverse diameter (TD):**Maximumhorizontalwidthof each spinal segment(mm).
- **Compression ratio (CR):** Expressedas the ratio of TD to DVD given by the formula: CR = DVD/TD x 100

Statistical Analysis

All data obtained were analyzed and reported as Mean ± SEM using GraphPadPrism4. Statistical significance ($p \leq 0.05$) of experimental observations was determined using independent student t-test and strength of relationship between all measured parameters was evaluated using linear regression analysis.

RESULTS

Gross Morphology:

The SC appeared whitish in colour, nearly-cylindricalin shape (Fig 1). It was situated in the vertebral canal and enveloped in three layers of meninges (dura, arachnoid and pia mater).The SC extended caudally from the foramen magnum to about the second lumbar vertebrae where it taperedto form the *conus medullaris.* The *filum terminale* extended through the sacral neural canal and terminated at the coccygeal vertebrae.Six pairs of spinal nerves constituted the *cauda equina*.

The SCdisplayed significant enlargement at two distinct regions – the cervical and lumbosacral intumescence (Figure 1). The cervical intumescence spanned from SC segments C_4 to T_1 and corresponded roughly to the brachial plexus which innervated the forelimb. This intumescence occupied its nominally corresponding vertebral segments (C_4 to T_1). The lumbosacral intumescence corresponded to the lumbosacral plexus, which innervated the hind limb. It traversed SC segments L_1 to $L_5$and occupied vertebral segments T_{10} to L_1.

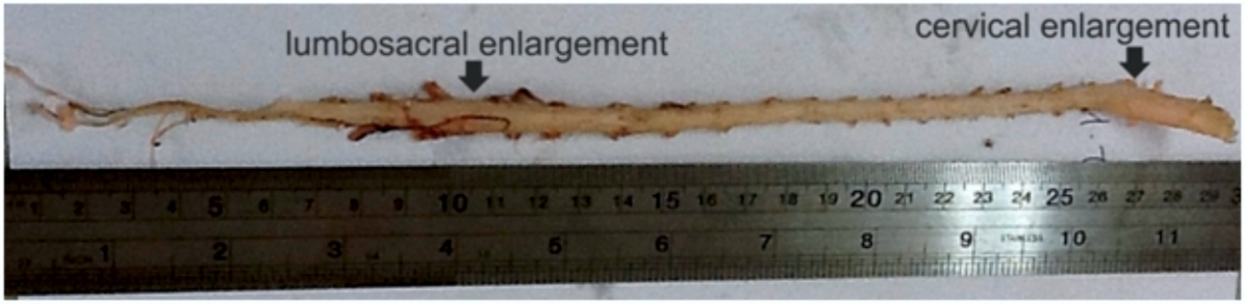

Fig. 1: Spinal cord of the GCR showing the cervical and lumbar enlargements

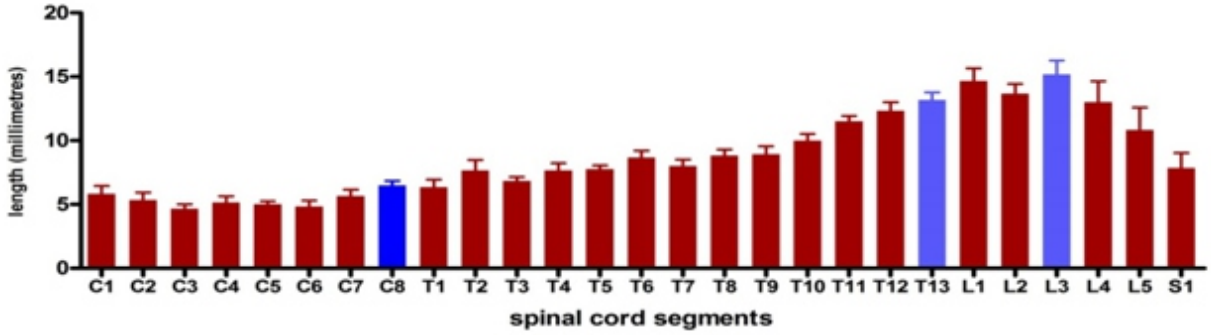

Fig. 2: Spinal segment length (mm) of the GCR. Blue bars represent regional spinal segment with the highest values (C_8, T_{13} and L_3).

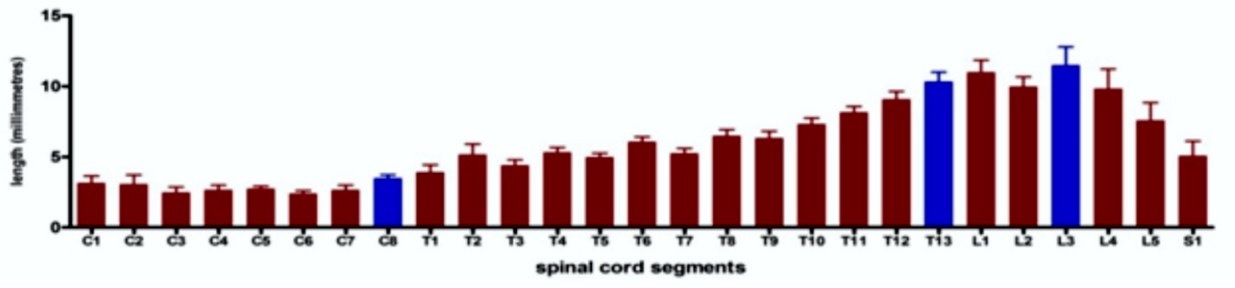

Fig. 3: Inter-root length (mm) across spinal segments of the GCR spinal cord. Blue bars represent highest values in the various spinal cord regions (C_8, T_{13}, L_3).

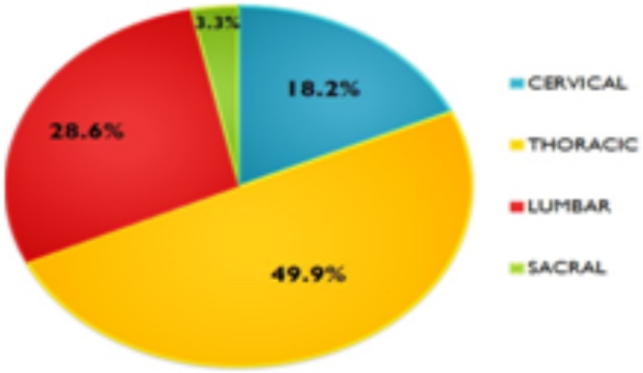

Fig. 4: Indices of spinal segment length of the GCR showing percentage contribution of the spinal segment to the spinal cord length. Highest and lowest contributions from the thoracic and sacral segments respectively

Spinal nerves are connected to the SC by means of corresponding dorsal and ventral roots, and they exit the vertebral canal through the inter-vertebral foramina. Twenty seven (27) spinal segments were recorded in the GCR (Cervical – 8, Thoracic - 13, Lumbar – 5, Sacral - 1) with their regional contributions illustrated in Figure 2.

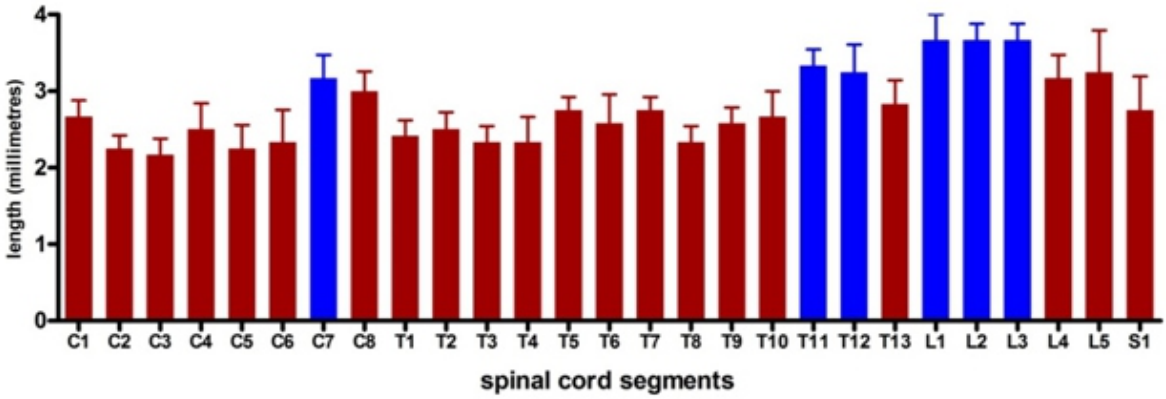

Fig 5: Spinal root attachment lengths (mm) across the spinal cord of the GCR. C_7, T_{11}, T_{12} and L_1-L_3 (represented in blue bars) had the highest values amongst respective spinal segments.

TABLE I: MORPHOMETRIC VALUES OF BODY PARAMETERS OF THE GCR

PARAMETERS	MALE Mean±SEM	FEMALE Mean± SEM	TOTAL MEAN± SEM	SIGNIFICANCE(p=0.05)
BODY WEIGHT (kg)	1.70±0.08	2.40±0.13	2.00±0.13	YES
HEAD LENGTH (cm)	11.00±0.40	12.00±0.39	12.00.±0.31	NO
TRUNK LENGTH (cm)	30.00±0.83	34.00±1.0	32.00±0.90	YES
TAIL LENGTH (cm)	17.00±1.20	20.00±0.95	19.00±0.83	NO

TABLE II: MORPHOMETRIC MEASUREMENTS OF GCR SPINAL CORD PARAMETERS OF THE GCR

PARAMETERS	MALE Mean±SEM	FEMALE Mean± SEM	TOTAL MEAN± SEM	SIG. (p≤0.05)
SC WEIGHT (g)	5.2±0.88	7.7±0.27	6.5±0.70	NO
SC LENGTH (cm)	21.0±0.79	26.0±1.30	24.0±1.30	YES
CERVICAL ENLARGEMENT LENGTH (cm)	2.6±0.28	2.8±0.18	2.7±0.15	NO
LUMBOSACRAL ENLARGEMENT LENGTH (cm)	3.9±0.15	4.6±0.27	4.2±0.20	NO

Gross Morphometry

The mean values for all body parameters of the female GCR were higher than the corresponding values obtained in males with only body weight and trunk length being statistically significant (Table I). The average SCL and SCW were 24.0±1.30cm and 6.5±0.70g respectively (Table II) and vertebral formula reported as C_7 T_{13} L_5 S_4 Cy_{20}. The lumbosacral intumescence length and weight were significantly higher than those of the cervical enlargements (Table III).

The spinal segment length yielded varying results along the different regions of the SC with peak values found at C_8, T_{13}, L_3 and S_1(Fig 2). These observations were the same for the IRL in this rodent (Fig 3) .The thoracic spinal segment had the most contribution to the SC length while the sacral spinal segments contributed the least (Fig. 4).

The values of the RAL were greatest at C_7, T_{11}, T_{12}, L_{1-3} and S_1cord segment levels (Figure 5).The TDshowed slight variations in dimension across the length of the SC with maximum values at C_7, T_1, L_3 and S_1spinal segments (Fig. 6).The TD dimensions were approximately equal in the thoracic segments. Maximum values for the DVD were noted at C_1 and C_7, T_1, L_4 and L_5, and S_1 spinal segments (Figure 7). The maximum regional CR values in the cervical spinal segments were C_1 and C_2 (70.80%); T_5 (69.30%) at thoracic segments and L_5 (61.50%) in the lumbar spinal segments (Fig. 8).

TABLE III: TEST OF STATISTICAL SIGNIFICANCE (P≤0.05) BETWEEN: (A) CERVICAL ENLARGEMENT LENGTH (CEL) AND LUMBOSACRAL ENLARGEMENT LENGTH (LSEL) AND, (B) CERVICAL ENLARGEMENT WEIGHT (CW) AND LUMBOSACRAL ENLARGEMENT WEIGHT (LSW) OF THE GCR

	CEL (cm)	**LSEL (cm)**
Mean±SEM	27.0±1.5	42.0±2.0
P value	0.0001	
P value summary	***	
Are means significantly different? (p ≤0.05)	Yes	
	CW (g)	**LSW (g)**
Mean±SEM	1.0±0.10	1.9±0.20
P value	0.0038	
P value summary	**	
Are means significantly different? (p ≤ 0.05)	Yes	

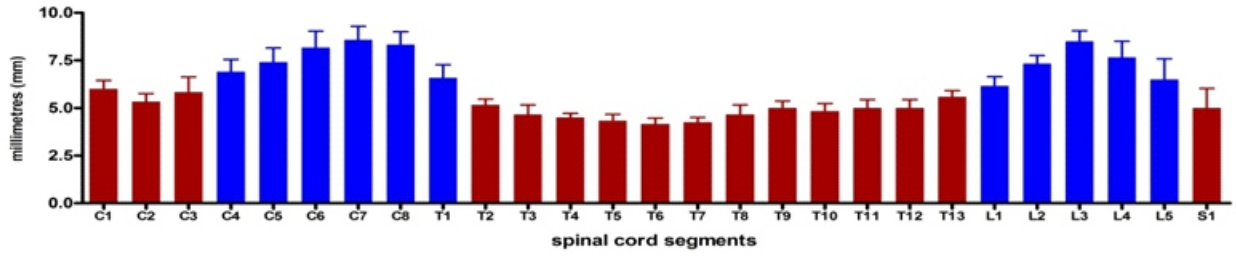

Fig. 6: Transverse diameter (mm) across the spinal cord of the GCR. Blue bars represent the cervical and lumbosacral enlargements (C_4-T_1 and L_1-L_5 respectively)

TABLE IV: LINEAR REGRESSION BETWEEN BODY AND SPINAL CORD PARAMETERS OF THE

	SC WEIGHT (G)		SC LENGTH (CM)	
Body weight (kg)	**Male**	**Female**	**Male**	**Female**
Slope	-4.7±8.1	0.17±1.7	4.9±6.8	8.2±1.5
r^2	0.25	0.009	0.34	0.97
P value	0.6653	0.9395	0.6036	0.1181
Head length (cm)				
Slope	-1.1±1.6	1.5±0.06	0.90±1.5	-0.35±7.0
r^2	0.31	1.0	0.28	0.0026
P value	0.6227	0.0253*	0.6463	0.9677
Trunk length (cm)				
Slope	-3.1±0.061	-0.098±0.23	-1.1±2.5	1.1±0.37
r^2	1.0	0.16	0.15	0.90
P value	0.0124*	0.7407	0.7435	0.2016
SC length (cm)				
Slope	0.46±1.0	-0.019±0.21		
r^2	0.17	0.0082		
P value	0.7311	0.9423		

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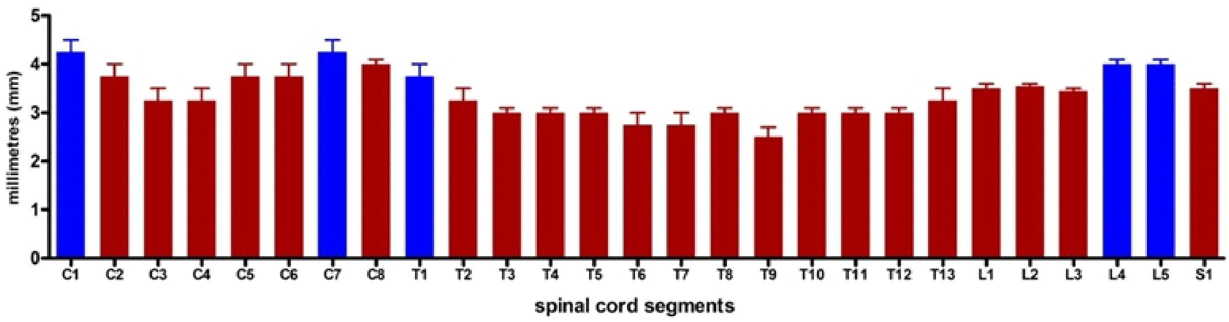

Fig. 7: Dorsoventral diameter (mm) across the spinal cord of the GCR.
Red bars represent the highest values at various regions C_1, C_7, T_1, L_4 and L_5.

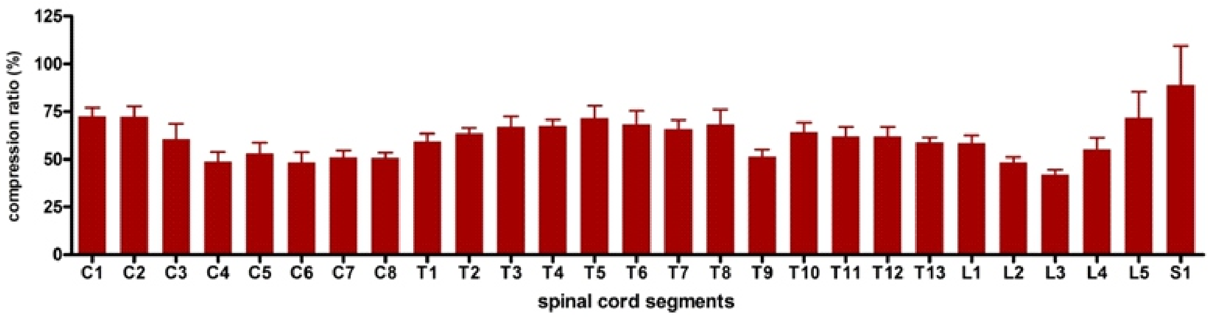

Fig. 8: Compression ratio across the spinal segments of the GCR

DISCUSSION

Anatomic description of the nervous system is important for an accurate knowledge of their morphologies, evolutionary descriptions and forms the basis for clinical, surgical and behavioural approaches (Fontenelle *et al.,* 2000, Silva *et al.,* 2002). The neural apparatus to operate the fore and hind limbs in a coordinated integrated way is present in the SC and thus, accounts for the differences between non-tetrapod and tetrapod SC (Butler and Hodos, 2005). The basic macro-anatomical features of the GCR SC presented with typical rodent SC characteristics (Hebel and Stromberg, 1976; Bjugn*et al.,* 1989; Butler and Hodos, 2005; Akinkunmi, 2013) and accounted for about 0.33% of the body weight. This is close to the value reported for the African giant rat (0.26%)) and about one-half times lesser than that of the rabbit (0.5%) (Farag *et al.,* 2012).

The GCR SC was observed to begin at a levelwith the middle of the occipital condyles, where the cranial-most rootlets of the first cervical spinal nerve root emerge. Dellmann and McClure (1975), however, reported thecranial limit of the SC to beat the foramen magnum in all domestic animals. The three meningeal coveringsof this rodent SC agrees with findings of Dellmann and McClure (1986), Carvalho *et al*. (2011) and Akinkunmi (2013). A total of 27 spinal segments were recorded for the GCR. Hebel and Stromberg (1976) detailed 34 spinal segments in the Wistar rat while Farag *et al.,* (2012) recorded 37 segments in the Rabbit.

The length of the SC in domestic animals is directly proportional to the length of the vertebral column (Bahar *et al.,* 2013). This istrue in the present study– as the GCR had higher SCL value (24.0±1.3cm; 75% of trunk length) as compared to the AGR SCL (15.63±0.32cm, 64.2% trunk length; Akinkunmi, 2013), mice (4.4cm, 55.7% trunk length; Bjugn *et al.,* 1989) and Wistar rats (34.7cm, 70.4% trunk length**;** Hebel and Stromberg, 1976; Aguh *et al.,* 2013). The relative higher values obtained in the GCR against that of other rodents could be attributed to its sheer size - dubbed the second largest rodent in Africa (Skinner and Chimimba, 2005). Sexual dimorphism (SD) was observed in the body weight, trunk length and SCL with higher values favouring the female GCR. SD in body weight is similar to reports by Byanet *et al.,*(2009) but contrary to Skinner and Chimimba, (2005). The observed variations may be ascribed to age difference or environmentally-induced phenotypic expression.

The cervical and lumbosacral intumescences of the GCR SC found between C_4- T_1 and between L_1-L_5 respectively were further corroborated with corresponding higher transverse diameters. This finding is similar toresults documented in laboratory rats (Bjugn *et al.,* 1989). The extent of the GCR cervical intumescence was the same reported for African giant rat by Akinkunmi (2013). It is, however, different from those reported in the pig C_7 - C_8 (Dellmann and McClure, 1975), C_5- T_1 in rabbits (Farag *et al.,* 2012), C_6 - T_1 in the dogs (Miller *et al.,* 1964), C_5 - T_2 in Indian sheep and donkeys (Mansour, 1980; Rao, 1990) and C_6 - T_2 in buffalo and camels (Abu-zaid, 1982; Mansour, 1983).

The lumbosacral intumescence of the GCR was entirely within the lumbar spinal segments spanning from L_1-L_5 while in the African giant rat, it is between the lumbar and sacral segments, L_2- S_3 (Akinkunmi, 2013). These enlargements at the cervical and lumbosacral segments corresponded roughly to the brachial and lumbosacral plexuses, which provide innervations to the fore and hind limbs respectively (Hebel and Stromberg, 1976; Bjugn *et al.,* 1989; Rahmanifar *et al.,* 2008). Axons to and from the SC are present in great numbers at the region of these plexi so that they cause the cord to bulge (Butler and Hodos, 2005). This is thought to contribute characteristically to limb efficiency in the GCR

as they are known to move rapidly despite having short limbs (Ajayi and Tewe, 1980). GCR have shorter fore-feet compared to their hind feet (Fitzinger, 1997) and this anatomic configuration may explain the statistical significant difference between cervical and lumbosacral enlargements.

Morphometric evaluations of the SC and its surrounding tissue have proven useful in medical imaging, as in histopathological studies (Fujiwara *et al.,* 1988; Mitchel, 2009; Claridge *et al.,* 2010). The TD and DVD of spinal segments are valuable parameters used routinely in postmortem examinations (Kamayema *et al.,* 1996; Ko *et al.,* 2004; Bahar *et al.,* 2013), diagnostic imaging studies (Ishikawa *et al.,* 2003; Da Costa *et al.,* 2006; Mitchel, 2009) and as a morphometric or diagnostic tool for SC diseases. DVD has been used in identifying lesion borders in lateral radiography - frequently preferred in the diagnosis of compressive disease of the cervical SC (Bahar *et al.,* 2013). The longest and shortest TD (C_7 and T_6) and DVD (C_1, C_7 and T_9) segments have thus been reported in this rodent.

The gross spinal CR of this rodent, documented in this study, is a vital morphometric index of its spinal segment cross-sectional shape. CR has been used to evaluate and diagnose pathological conditions in dogs (Da Costa *et al.,* 2006), horse (Bahar *et al.,* 2013) and humans (Fujiwara *et al.,* 1988; Bucciero *et al.,* 1993; Kameyama *et al.,*1996).

CONCLUSION

This workprovidestopographic and macro-morphometric reference data on the GCR spinal segments that may be useful in applied research for regional anaesthesiology and diagnostic medical imaging in SC diseases. It has also contributedto the current drive in promoting the GCR as an indigenous research rat model especially in spinal cord injury and repair.

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