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Spinal Cord Studies in the African Giant Rat *(Cricetomys gambianus*, Waterhouse)

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Summary: The African giant rat, AGR, is known for advantageous behavioural patterns among which are cognition and dexterous locomotion. This study investigated the morphological, morphometric and possible functional aspects of the AGR spinal cord (SC) anatomy. Ten adult (5 males and 5 females) AGR were used to determine the gross and histological features of the SC which were typically of rodent features. The mean SC weight and length given as 2.50±0.24g and 15.87±0.24cm respectively for the male and 2.32±0.16g and 15.40±0.61cm for the female showed no sexual dimorphism ($p<.05$). A positive linear relationship between the tail length and SC weight were found in both sexes ($r = 0.81$ males; $r = 0.95$ females) suggesting significant contribution of the filum terminale to SC weight. Forty-three internal structures including nuclear aggregations and tracts were traced. Eight nuclear aggregations of neurons involved in nociception and limb coordination were observed to be prominent and larger than in laboratory rats. Same was noted for the dorsal, ventral and lateral funicular tracts which control the limbic system. This study provides morphometric baseline research information and delineates the functional aspects of the AGR SC anatomy. The information provided further strengthens the drive proposing the AGR as an indigenous research model for regional anaesthesia and locomotor disease.

Keywords*:* African giant rat; spinal cord; spinal tract; nuclei; spinal segment; morphometry.

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INTRODUCTION

Rodents are the largest mammalian order with about fifty percent of the species of mammals being rodents (Sheet, 1989).The African giant rat (AGR) also known as giant pouched rat, is by size one of Africa's largest rodents and is arguably becoming Africa's most intriguing rodent because of its scientific attributes such as the detection of landmines (Verhagen *et al*., 2003) and also in the medical diagnosis of pulmonary tuberculosis (Weetjens *et al.,* 2009), disease vectors (Durnez *et al.,* 2008), potential pest species status (Peterson *et al.*, 2006) among many others. Earlier investigations on the AGR were centered on reproduction and sustenance in captivity (Oke and Oke, 1999; Akinloye, 2009) while recent studies have focused on interpreting function from morphology (Olude *et al.*, 2009; Ibe *et al.,* 2014). Information on the CNS however, is sparse with a brain bias (Ibe *et al*., 2010). The SC anatomy has received much less attention (Vera and Meyer-Siegler, 2003). This study therefore, was undertaken to add to the meagre research data on the gross and histological anatomy of the SC and interpret functional behaviour from the anatomical knowledge of the AGR SC.

MATERIALS AND METHODS

Animals

Ten adult (5 males and 5 females) African giant rats *(C. gambianus)* were obtained from the wild, stabilized in holding cages designed with dark and light compartments to regulate the sleep and wake cycles of the rats. The average body weight of African giant rats was 0.96±0.05kg for males and 0.91± 0.09kg for females.

Animal Handling

Experimental procedures conformed to the rules and guidelines issued by the University of Ibadan, Ibadan, on health guide for the care and Use of Animals in Experiments.
Animals were anaesthetized by chloroform inhalation. Gross morphometric parameters (body weight, head length, trunk length and tail length) were immediately measured before animals were perfused transcardially

with 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS), pH 7.4 and post-fixed in 10% formalin for 1 week. The SC was exposed by dissections and laminectomy, harvested and measured using metric instruments. Sections from cervical, thoracic, lumbar, sacral and coccygeal segments were then taken for histology at 5 pm, routinely stained with Thionin stain. Slides were viewed under light microscope (Leica Model DME Microscope, Model: 13595XXX, Leica Microsystems) and images captured with Canon© Power shot S70 camera (PC 1087, No. 033102132). Photomicrographs obtained were traced onto a tracing paper using an HB graded pencil.

Statistical Analysis

All data were analyzed and expressed as mean and standard error of mean using Graph pad prism 5. Statistical significance was determined using t-test and linear regression (P≤ 0.05).

RESULTS

Morphometry

The mean SC weight and length were recorded as 2.41±0.14g and 15.63±0.32cm respectively. The mean body measurements and SC measurements were greater in the males than in the females but were all statistically insignificant (Table 1). There was positive relationship between the trunk length and the SC length which was significant for females (p =0.0141) but not for males (p= 0.1999). The strength of relationship between the tail length and SC weight were significant in both sexes (p= 0.037 males, p= 0.0044 females) and were both positively correlated (Figure 1). There was positive relationship between the trunk length [TKL] and the SC length [SCL] which was significant for females (p =0.0141) but not for males (p= 0.1999). The strength of relationship between the tail length [TL] and SC weight [SCW] were significant in both sexes (p= 0.037 males, p= 0.0044 females) and were both positively correlated.

Table 1. Body and Spinal Cord measurements of the AGR ***(C. gambianus)***, Mean ±SEM

PARAMETERS	MALE	FEMALE	OVERALL
Body weight (kg)	0.96 ± 0.05	0. 91± 0.091	0.93± 0.05
Trunk length (cm)	24.70±1.30	24.10 ±1.23	24.40 ±0.85
Tail length (cm)	33.80 ±1.56	33.20 ±0.98	33.5± 0.88
Head length (cm)	6.94±0.31	6.78± 0.34	6.86 ±0.22
SC weight (g)	2.50±0.24	2.32± 0.16	2.41± 0.14
SC length (cm)	15.87 0.24	15.40 ± 0.61	15.63± 0.32

P≤0.05

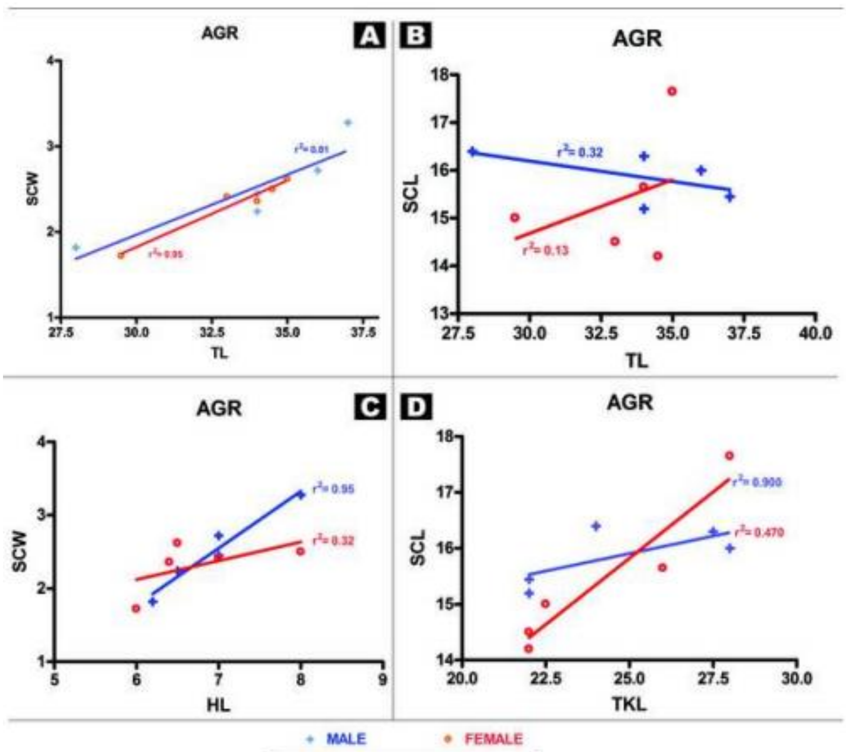

Figure 1: Graphical Representation of the Linear Regression of: (A): Spinal Cord Weight [SCW] versus Tail Length [TL]; (B): Spinal Cord Length [SCL] versus Tail Length [TL]; (C): Spinal Cord Weight [SCW] versus Head Length [HL] and (D): Spinal Cord Length [SCL] versus Trunk Length [TKL]

Figure 2: Dorsal view of the SC of the AGR (arrows) after laminectomy

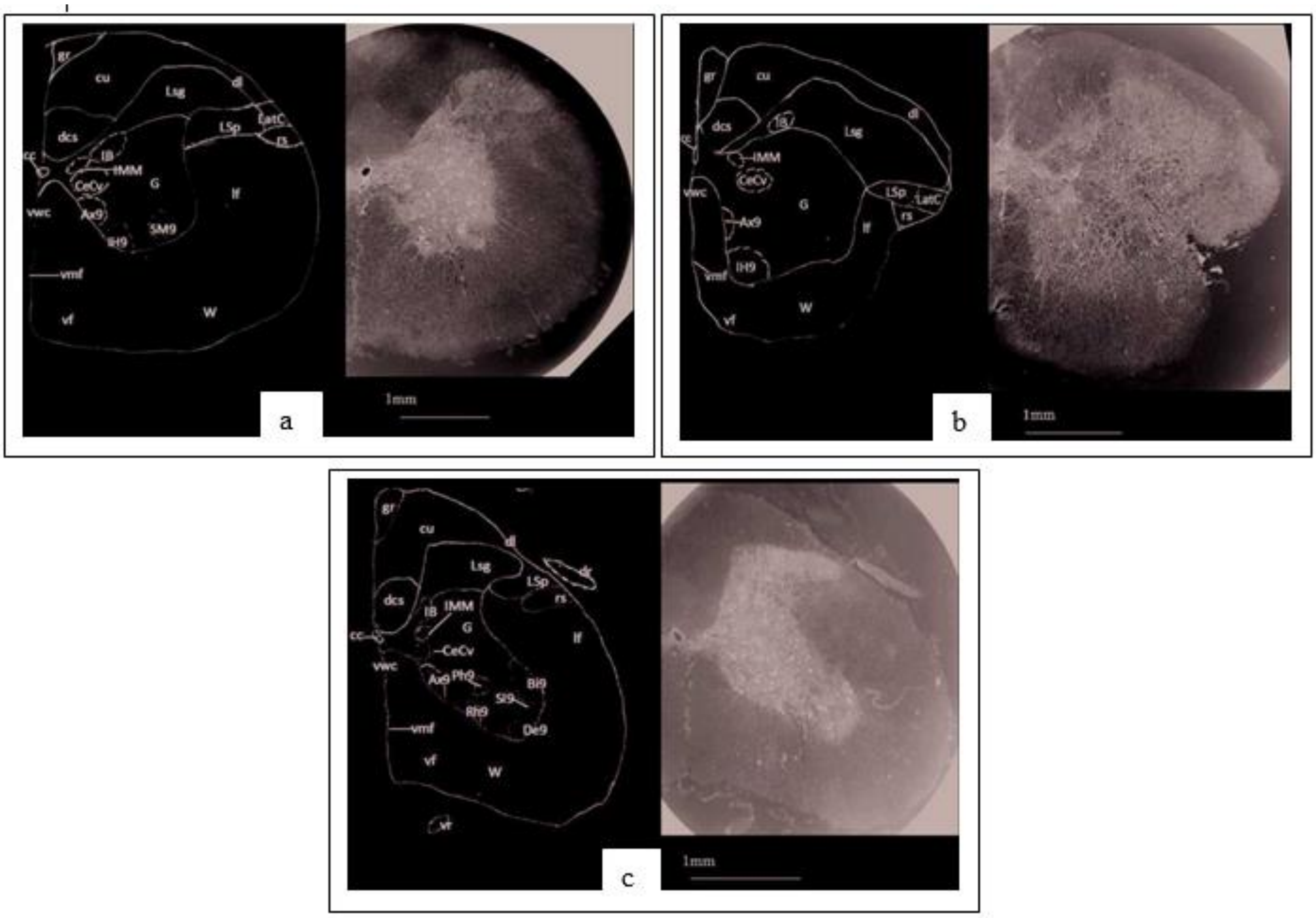

Figure 3. **(a)** The first cervical spinal segments highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph. **(b)** The second cervical spinal segments highlighting the nuclei and tracts. *Left panel*: Pencil tracing; *Right panel:* photomicrograph. **(c)** The fifth cervical spinal segments highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph

Gross morphology

The SC appeared as a whitish cylindrical, tube-like structure situated in the vertebral canal extending from the foramen magnum and continuing as the conus medullaris at vertebra L_4 (n=9) and L_5 (n=1) before terminating as the filum terminale in the coccygeal vertebrae (Figure 2). The SC was covered by the dura matter, which traversed its entire length while the spinal nerves emerged from the SC and exited the vertebral canal through the intervertebral foramina. The cervical enlargement, which contributed to the brachial plexus, spanned from C_4 to T_1 SC segments and was within the nominally corresponding vertebrae (C_4 to T_1) in all animals. The lumbosacral enlargement, which contributed to the lumbosacral plexus that innervates the hind limb, extended from SC segments L_2 to S_3 and was found about the vertebral levels of T_9 to T_{12}.

Histomorphology

Transverse sections of the SC revealed the typical central canal, gray and white matter. The gray matter, stained deep purple with Thionin, had the typical “H” or “butterfly” shape with a reticular formation complex at the lateral area of the dorsal horn. The ventral horn of the gray matter was larger than the dorsal horn being widest at the lumbosacral segments followed by the cervical segments and then the thoracic; the coccygeal segments being the narrowest.

Shape variations in the central canal were observed across the SC segments. The central canal of the first cervical segment appeared as a vertical slit (Figure 3a), the second to the sixth cervical segments were horizontally oval (Figures 3b-c) while the seventh and the eighth cervical segment appeared circular in shape. In the thoracic segments, the first and second segments were vertical slits while the others were vertically oval in shape (Figures 4a-c). The first and second lumbar segments were circular in shape (Figure 5a) while the other lumbar segments, sacral and coccygeal segments were vertically oval in shape (Figures 5b, 6a-b, 7).

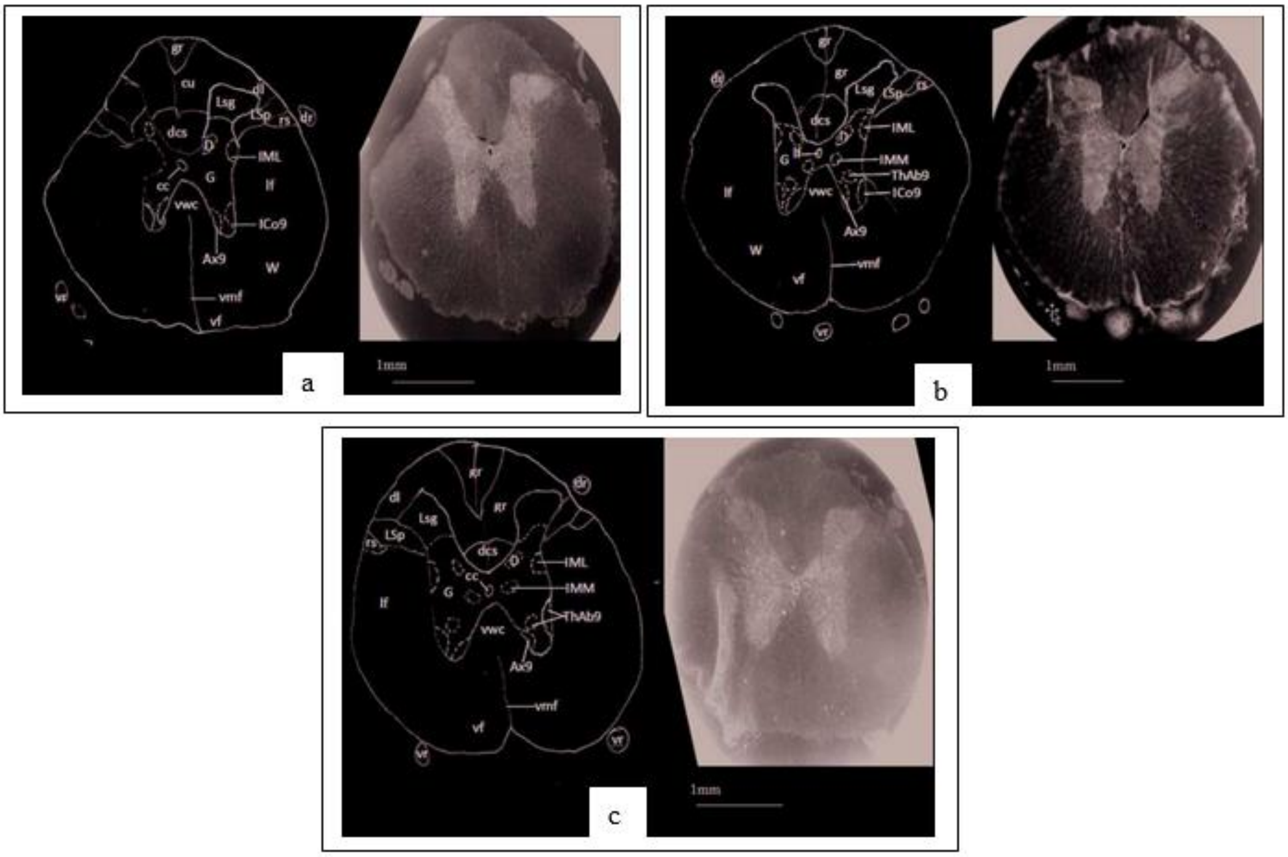

Figure 4. **(a)**The fourth thoracic spinal segments highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph. **(b)**The eighth thoracic spinal segments highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph. **(c)** The twelfth thoracic spinal segments highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph.

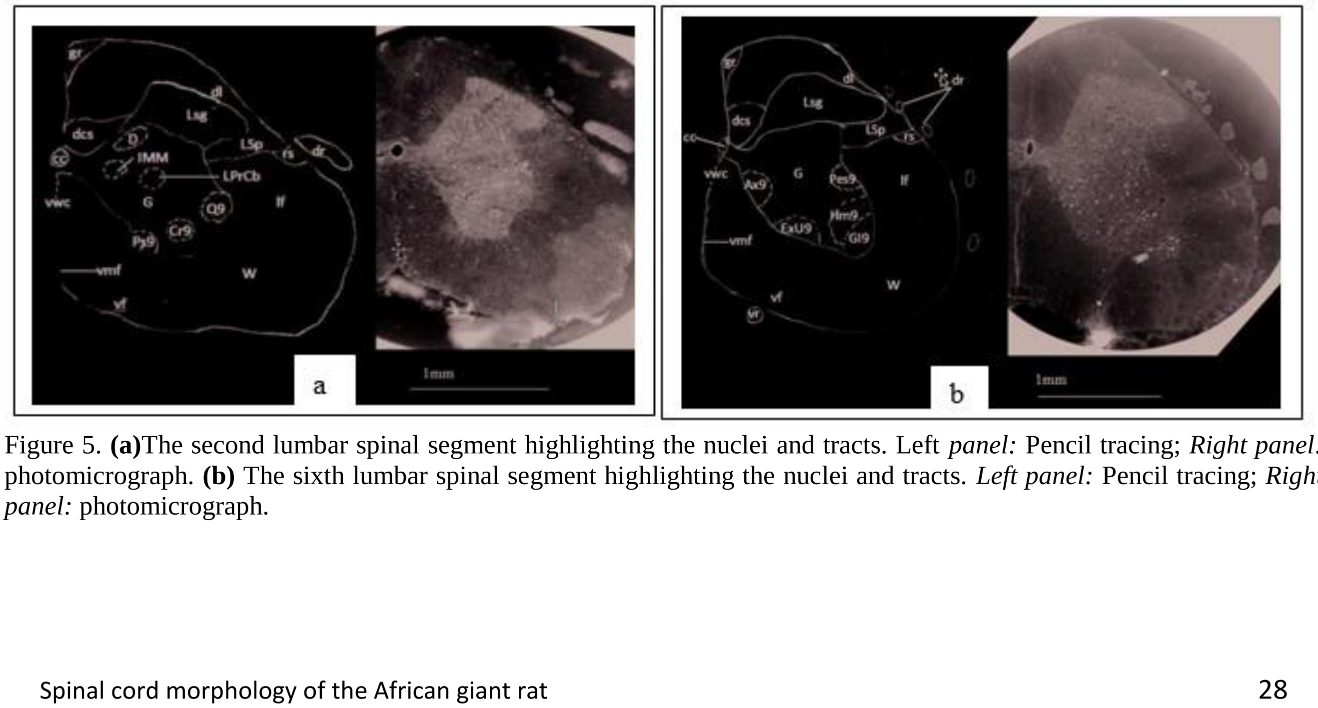

Figure 5. **(a)**The second lumbar spinal segment highlighting the nuclei and tracts. Left *panel:* Pencil tracing; *Right panel:* photomicrograph. **(b)** The sixth lumbar spinal segment highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph.

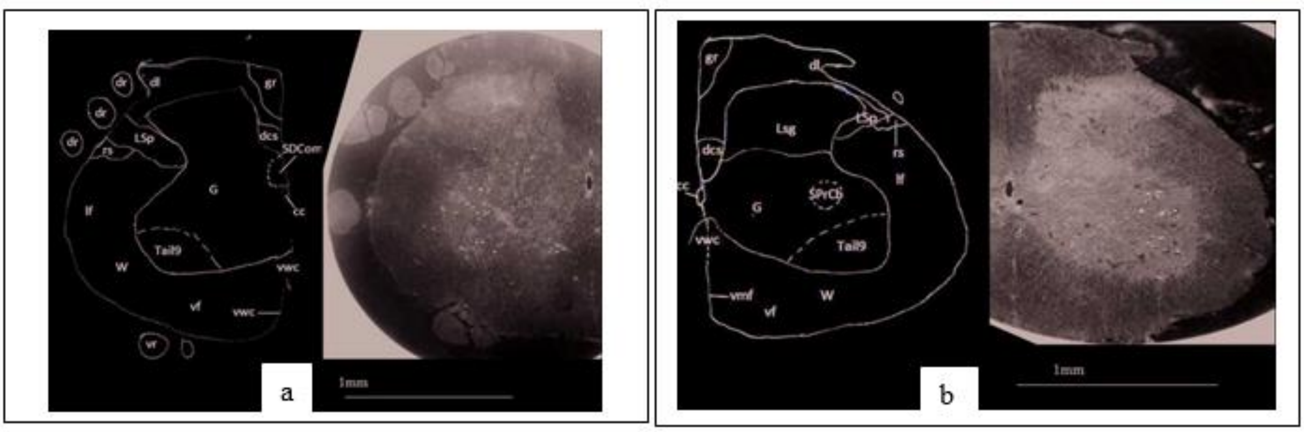

Figure 6. **(a)**The second sacral spinal segment highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph. **(b)** The fourth sacral spinal segment highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph.

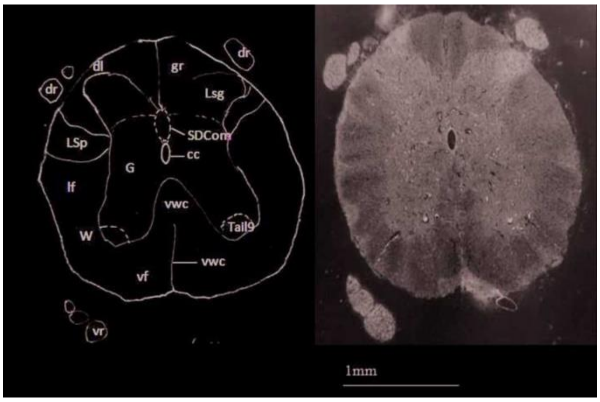

Figure7. The second coccygeal spinal segment highlighting the nuclei and tracts. *Left panel:* Pencil tracing; *Right panel:* photomicrograph.

Table 2. Abbreviations and definitions of structures of the SC traced

Abbreviation	Definition
Ax9	Axial muscle motor neuron of lamina 9
Bi9	Bicep motor neuron of lamina 9
Cc	Central canal
CeCv	Central cervical nucleus
Cr9	Cremaster motor neuron of lamina 9
Cu	Cuneate fasciculus
D	Dorsal nucleus (Clarke)
Dcs	Dorsal corticospinal tract
De9	Deltoid motoneuron of lamina 9
Dl	Dorsolateral fasciculus
Dr	Dorsal root
ExU9	External urethral sphincter motor neuron of lamina 9
G	Gray matter
GL9	Gluteal motor neuron of lamina 9
Gr	Gracile fasciculus
Hm9	Hamstring motor neuron of lamina 9
IB	Internal basilar nucleus
ICo9	Intercostals muscle motor neuron of lamina 9
IH9	Infrahyoid muscle motor neuron of lamina 9
IML	Intermediolateral column
IMM	Intermediomedial column
Lsg	Laminae of spinal gray matter
LatC	Lateral cervical nucleus
Lf	Lateral funiculus
LPrCb	Lumbar paracerebellar nucleus
Lsp	Lateral spinal nucleus
Pes9	Pesmotor neuron of lamina 9
Ph9	Phrenic motor neuron of lamina 9
Ps9	Psoas motor neuron of lamina 9
Q9	Quadriceps motor neuron of lamina 9
Rh9	Rhomboid muscle motor neuron of lamina 9
Rs	Rubrospinal tract
SDCom	Sacral dorsal commissural nucleus
SI9	Supraspinatus and infraspinatus motor neurons of lamina 9
SM9	Sternomastoid motor neurons of lamina 9
SPrCb	Sacral precerebellar nucleus
Tail9	Tail muscle motor neuron of lamina 9
ThAb9	Thoracoabdominal wall muscle motor neuron of lamina 9
Vf	Ventral funiculus
Vmf	Ventral median fissure
Vr	Ventral root
Vwc	Ventral white commissure
W	White matter

Nuclear tracing

Forty-three (43) anatomical structures including nuclear aggregations and tracts were traced (Table 2). It describes the abbreviations and definitions of structures of the SC traced out, which represents the list of structures in the histological pictures.Tracts of the dorsal, ventral and lateral funiculi were observed in the white matter. The dorsal funiculus consisted of the gracilis fasciculus, cuneate fasciculus and also a descending tract - the dorsal corticospinal tract. The gracilis fasciculus extended all through the SC segments, the cuneate fasciculus were indistinct at the caudal spinal segments (lumbar to the coccygeal segments) while the dorsal corticospinal tracts ran through the cervical to the sacral spinal segments and became indistinct at the coccygeal spinal segments (Figures 3 - 7). The lateral funiculus also consisted of a descending tract - rubrospinal tract (Figures 3b, 3c). Eight nuclei namely central cervical, dorsal (Clarke's), internal basilar, lateral cervical, lumbar paracerebellar, lateral spinal, sacral dorsal commissural and sacral precerebellar nuclei were identified and traced (Figures 3a-b, 4a-c, 5a-b, 6a-b, 7).

The SC appeared as a whitish cylindrical, tube-like structure situated in the vertebral canal extending from the foramen magnum and continuing as the conus medullaris at vertebra L_4 (n=9) and L_5 (n=1) before terminating as the filum terminale in the coccygeal vertebrae (Figure 2). The SC was covered by the dura matter, which traversed its entire length while the spinal nerves emerged from the SC and exited the vertebral canal through the intervertebral foramina. The cervical enlargement, which contributed to the brachial plexus, spanned from C_4 to T_1 SC segments and was within the nominally corresponding vertebrae (C_4 to T_1) in all animals. The lumbosacral enlargement, which contributed to the lumbosacral plexus that innervates the hind limb, extended from SC segments L_2 to S_3 and was found about the vertebral levels of T_9 to T_{12}.

DISCUSSION

The basic features of the AGR SC were typical of rodents (Hebel and Stromberg, 1976; Bjugn *et al.*, 1989). The SC accounts for about 0.26% of body weight of the AGR. It is smaller than the rabbit which weighs 5-7g (about 0.5% body weight) (Farag *et al.*, 2012) but greater than that of the horse which weighed 250-300g (about 0.06% body weight) (Nickel *et al.*, 2004).

Bjugn *et al.* (1989) reported the SC length of mice as 4.4cm (55.7% body length) which is smaller than that of the AGR 15.63cm (64.2% body length). SC length has also been described in several animals amongst which are: 34.7 cm in Wistar rats (70.4% body length) (Hebel and Stromberg, 1976; Aguh *et al.*, 2013); 34.7 cm in rabbits (99.1% body length) (Farag *et al.,* 2012); 53.8 cm in goats (61.6% body length) (Kahvecioglu *et al*., 1995); 167.2 cm in horses (68.6% body length) (Sadullah *et al.*, 2013); 106.8 cm in donkeys (53.4% body length) (Ocal and Haziroglu, 1988); 61.5 cm in brockets (64.7% body length) (Lima *etal.*, 2010).

The positive linear relationship ($p \leq 0.05$) between the tail length and SC weight of males and females established that the variability observed in the SC weight may be explained by the tail length in both sexes, indicating that the bulk of the filum terminale contributed significantly to the weight of the SC. This was further substantiated by a relatively higher tail: body length ratio of 1.07 in the AGR compared to 1.00 in mice, (Brian and William, 2000) 0.47 in fox squirrel, 0.87 in red squirrel, 0.50 in California ground squirrel (Virginia, 2008) and 0.43 in greater cane rats (Fitzinger, 1995).

The anatomical location of the cervical and lumbosacral enlargements - between C_4 and T_1 and between L_2 and S_3 respectively - were typical with laboratory rats (Bjugn *et al.*, 1989). These anatomical positions seem characteristic of rodents as several authors have reported slightly different positions in most domestic animals. The cervical enlargement is found between C_7 and C_8 in pigs (Dellmann and McClure, 1975), C_5 and T_1 in the rabbits (Farag *et al.*, 2012), C_6 and T_1 in the dogs (Miller *et al.*, 1964), C_6 and T_2 in buffalo and camels (Abu-zaid, 1982; Mansour, 1983) and C_5 - T_2 in Indian sheep and donkeys (Mansour, 1980; Rao, 1990). While the lumbosacral enlargement lies between L_2 and S_3 spinal segments in AGR, the following positions have been documented in domestic animals: between L_2 and S_1 in the donkeys (Mansour, 1980), L_6 and S_1 in camels (Mansour, 1983), L_4 and S_3 in rabbits (Farag *et al.*, 2012), the last three lumbar and first two sacral in buffalo (Abu-zaid, 1982), L_4 and S_1 in sheep (Rao, 1990), L_6 and L_7 in the pigs and L_4 and S_1 in dogs (Dellmann and McClure, 1975).

The enlargements at the cervical and lumbosacral segments provide innervations to the fore and hind limbs respectively; contributing to the brachial and lumbosacral plexuses (Bjugn *et al.*, 1989; Rahmanifar *et al.*, 2008). Worthy of note is that the extents of the enlargements, which began earlier in spinal segments, are more extensive in rodents and may characteristically add to limb efficiency than other mammals. Thus; regional anaesthesia for surgical maneuvers in the AGR can be readily achieved based on the knowledge of the extent and anatomical locations of these enlargements (Jonathan and Gerbrand, 2005).

The ventral horn of gray matter coordinates the motor neuron; this explains its relative bigger size compared to the dorsal horn (Gruener and Biller, 2008). The ventral horn appears wider at the cervical region and the lumbosacral region than other segments. This corresponds to the cervical and lumbosacral enlargements (Gruener and Biller, 2008). The AGR uses its tail to dig, defend itself and has been reported to stand on it (personal observation). It is also known to burrow more with the forelimbs and shows high locomotor dexterity (Ajayi, 1977). This probably accounts for the ventral horns of cervical and lumbosacral regions being more developed than other segments.

The dorsal (Clarke's) and central cervical nuclei are particularly essential for the coordination of movement and balance (Gruener and Biller, 2008). These nuclei appeared well developed in the AGR and might explain the dexterous limb movements and balance shown by the AGR. The AGR also has been documented as a fast running, burrowing and shovelling rodent (Olude *et al,* 2010). The lateral cervical, lateral spinal, sacral dorsal commissural nuclei are responsible for nociception (Rea, 2009) and are therefore important for their defence. The internal basilar nuclei are responsible for voluntary motor control and procedural learning relating to routine behaviours and habits (Weyhenmeyer and Gallman, 2007). The AGR has been shown to adapt well to training to detect landmines and diagnose Tuberculosis (Weetjens, 2010). The sacral pre-cerebellar nucleus also relays unconscious proprioception motor (lower extremities and trunk) feedback to the cerebellum.

Shape variation of central canal and tracts observed in the white matter. The central canal of each segment and the tracts observed in the white matter of the SC of the AGR were similar to that of rats (Watson *et al.*, 2008).

Conclusion

This study documents baseline data on the morphometric and morphologic features of the SC of AGR, thus contributing to the knowledge of anatomy of the AGR and providing useful information on its regional anaesthesia. It also strengthens the drive in adopting the AGR as a convenient indigenous research model and could assist further researches especially in the study of SC diseases/injuries within the African context.

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