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MORPHOLOGIC AND MORPHOMETRIC EVALUATION OF THE SPINAL AND PERIPHERAL NERVES OF THE BRACHIAL PLEXUS IN THE AFRICAN PALM SQUIRREL (*EPIXERUS EBII*)

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ABSTRACT

Purpose: Brachial plexus biology in the African palm squirrel was explored to fill information dearth on the anatomy of the rodent's nervous system. Principal methodologies: Fourteen adult African palm squirrels (six males and eight females) obtained from the wild were fixed in 10% formalin solution for 48hr and the nerves originating from brachial plexus were carefully dissected. Cotton blocks soaked in 2% hydrogen peroxide were placed on the ventral roots of the plexuses for better visualization of plexus elements and tracing. Key findings: The brachial plexus of the African palm squirrel comprised of the caudal branch of the ventral ramus of the fifth cervical spinal nerve (C5) to the cranial branch of the ventral ramus of the first thoracic spinal nerve (T1). The caudal branch of ventral ramus of C5 and cranial branch of ventral ramus of C6 formed the cranial trunk. The ventral ramus of C6 was bound to the cranial branch of the ventral ramus of C7 to form the middle trunk, while the caudal branch of the ventral ramus of C7 and cranial branch of ventral ramus of T1 constituted the caudal trunk. The peripheral nerve trunks and their respective spinal nerve origin (in parenthesis) were cranial pectoral (C5, C6), suprascapular (C5, C6), subscapular (C5, C6), axillary (C6, C7). Others were musculocutaneous (C6), radial (C6, C7), median (C7, T1) and ulnar (C7, T1). Morphometric analyses indicated that T1 and ulnar nerve were the longest spinal and peripheral nerves, respectively. Also, the musculocutaneous and axillary nerves, were, each, significantly higher in the male than in the female rodent. Conclusion: These results were compared with other rodents to establish species variations.

Keywords: African palm squirrel, brachial plexus, peripheral nerves, thoracic limbs ***Correspondence:** ibe.chikera@mouau.edu.ng,+2348032882105

INTRODUCTION

Rodents are a diverse group of mammals that are characterized by their continuously growing incisors which they use for gnawing, and about 40% of all mammal species are rodents [1]. The African palm squirrel is a rodent of the family Scuridae [2]. It is well adapted to living on palm trees, feeding on fruits, seeds, insects, building nests out of leaves and other materials [3]. It is active during the day and is known for its fast and agile movements. The species is generally solitary, although it may form small groups during the breeding season. It hoards food in tree cavities or underground burrows for later consumption [4].

The brachial plexus is an interconnecting network of nerves that provides innervation to the forelimb region [5]. In vertebrate animals, it is formed by the ventral rami of the cervical and thoracic spinal nerves [6]. Although this formation varies in different species, these spinal nerves exit the spinal cord through the intervertebral foramina and divide into a dorsal and ventral rami. The ventral rami form a network of nerves that pass through the neck into the axillary region. The nerve roots are the initial components of the brachial plexus and they emerge from the spinal cord. The nerve roots combine to form nerve trunks and cords. These cords then give rise to several peripheral nerves which provide innervation to various muscles and regions of the forelimb [7].

There is no research currently on the structure of the brachial plexus in the African palm squirrel, thus, comparative studies with other rodents are unavailable. Moreover, some differences have been noted in the brachial plexus of different rodents [8, 9]. Therefore, it is possible that the structure of the brachial plexus in the African palm squirrel may differ, when compared to other rodents. These differences may include the number and arrangement of nerve roots and trunks. The need to provide this information necessitated the present study.

The distribution of the brachial plexus of the African palm squirrel as compared to those of other rodents can provide insights into the evolutionary history of these species and their relationship to the environment as well as their adaptation to their habitat. Such adaptation includes the biomechanics of their movements, necessary to inform conservation efforts and domestication strategies. The study will also serve as a lead for future diagnosis of neuromuscular dysfunctions [8], and guide for local and regional anesthetic nerve block techniques in this rodent. Consequently, this study aims to identify and trace each spinal nerve that makes up the brachial plexus of the African palm squirrel from their point of origin, identify and trace the peripheral nerves that emerge from the brachial plexus and the muscles they innervate. Furthermore, the study aims to determine the length of each spinal and peripheral nerve of the brachial plexus of this rodent and report any observed sexual dimorphism in the above results.

MATERIALS AND METHODS

Study location

Fourteen (14) adult African palm squirrels were captured, 6 males and 8 females. They were sourced from the wild within Umudike, in Abia State, Nigeria. Umudike is located between latitude 5.4508°N and longitude 7.5416°E. The squirrels were transported in a wooden cage, measuring one-meter cube, to the Histology/Embryology Laboratory of the Department of Veterinary Anatomy, Michael Okpara University of Agriculture Umudike, Abia State, where the study was carried out.

Ethical clearance

Ethical approval was obtained from the Research Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike. The Ethical Approval Reference Number is MOUAU/CVM/REC/202117.

Exposure of spinal nerves

The squirrels were anaesthetized with a mild chloroform soaked in cotton wool in a confined container and weighed using an electronic weighing balance. Their nose-rump and tail lengths were measured using a meter rule. They were euthanized with a high dose of gaseous chloroform by increasing time of contact between the squirrels and cotton wool soaked in chloroform. They were dissected to remove the skin and were immersed in 10% formalin for 48 hours.

Each squirrel was placed on a dorsal recumbency and dissected to expose the sternum, using tissue forceps, scissors and surgical blades. Bilateral ventral paramedian incisions were made parallel to the sternum. This enabled disarticulation of the ribs from the chondrosternal joints. The thoracic cavity was exposed, and thoracic viscera were eviscerated. Thereafter, the forelimbs were carefully dissected and reflected laterally. The muscles around the intervertebral foramina were transected to allow visualization of the cervical and thoracic nerve roots. These spinal nerves that contributed to the formation of the plexus emanating from the plexus were carefully exposed.

Tracing the spinal and peripheral nerves of the brachial plexus

Cotton blocks soaked in 2% hydrogen peroxide were put in place to facilitate the visualization of the nerves and their tracing from their origin. The origin, organization and distribution of the ventral rami of the spinal nerves of the brachial plexuses in the subjects, irrespective of sexes were studied. Peripheral nerves of the brachial plexus were identified and dissected, following the patterns of the different arrangements of the brachial plexus. Photographs for illustration of morphologic results were obtained using 12.0megapixel Kodak[®] digital camera (Eastman Kodak[®]). Definitions of gross anatomical structures were based on standard information on rodent anatomy [9]. For the terminology, the Nomina Anatomica Veterinaria [10] was used.

Morphometric evaluation of the spinal and peripheral nerves

The length of each spinal and peripheral nerve of the brachial plexus was measured with a digital vernier caliper (British Whitworth tools, UK; sensitivity: 0.01 mm). Mean values were compared between males and females using student t-test to establish sexual dimorphism. Relationship between nose-rump and spinal nerve lengths was tested using regression analysis. Values of $P \leq 0.05$ were considered significant. The statistical software employed was the graph-pad prism Version 5.

RESULTS

The spinal nerves of the brachial plexus

The brachial plexus of the African palm squirrel was constituted of the caudal branch of the ventral ramus of the fifth cervical spinal nerve (C5) to the cranial branch of the ventral ramus of the first thoracic spinal nerve (T1). Thus, the fifth, sixth and seventh cervical spinal nerves (C5, C6 and C7, respectively) and first thoracic spinal nerve (T1) contributed to the brachial plexus in the African palm squirrel. The caudal branch of ventral ramus of C5 and cranial branch of ventral ramus of C6 formed the cranial branch of ventral ramus of C6 mas bound to the cranial branch of the ventral ramus of C7 to form the middle trunk, while the caudal branch of ventral ramus of C7 and cranial branc



Plate I: Ventral view (right arm) of the brachial plexus in African palm squirrel

C3-C7: 3rd -7th cervical vertebrae; T1: 1st thoracic vertebra; CrT: Cranial trunk; MT: Middle trunk; CdT: Caudal trunk.

Distribution of the peripheral nerves of the brachial plexus

The cranial pectoral nerve originated from the cranial part of the cranial trunk of the ventral roots of C5-C6 to innervate the superficial pectoral muscle (Plate II). The suprascapular nerve also originated from the ventral roots of C5-C6. After emerging from the caudal part of the cranial trunk of C5-C6, it passed between the subscapular and supraspinatus muscles, and exited at the lateral surface of the scapula. It continued through the supraspinatus and infraspinatus muscles, innervating them. The subscapular and axillary nerves originated from the ventral roots of C6-C7. It was divided into two nerves, the cranial and caudal subscapular nerves. They originated from the point that the branch coming from cranial trunk joined the middle trunk innervating the subscapular muscle (Plate III).

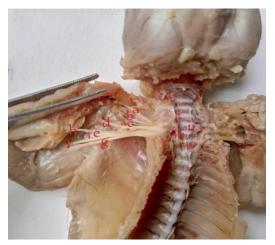


Plate II: Ventral View of the Brachial Plexus (Right Arm) of the African Palm Squirrel, Showing the Peripheral Nerves

i: Cervical spinal nerve root 5; ii. Cervical spinal nerve root 6; iii: Cervical spinal nerve root 7; iv: Thoracic spinal nerve root 1; a: Cranial pectoral nerve; b: Suprascapular nerve; c: Subscapular scapular nerve; d: Musculocutaneous nerve; e: Axillary nerve; f: Median nerve; g: Ulnar nerve; h: Thoraco-dorsal nerve; i: Radial nerve.



Plate III: Ventral View of the Brachial Plexus (left arm) of African palm squirrel showing the Peripheral Nerves

a: Suprascapular nerve; b: Cranial subscapular nerve; c: Musculocutaneous nerve; d: Axillary nerve; e: Radial nerve; f: Median nerve; g: Ulnar nerve

The axillary nerve originated from the middle trunk and after a short distance, gave out two branches; the first branch innervated the subscapularis muscle, while the second branch innervated the teres major muscle. This nerve coursed to the lateral face of the scapula innervating the teres minor and deltoideus muscles. The musculocutaneous nerve originated from the ventral root of C7. It provided branches to the coracobrachialis, brachial and biceps brachii muscles after arising from the middle trunk; it lies dorsal to the axillary nerve.

The radial nerve was also formed from the ventral roots of C7–T1 (Plate II). After emerging from the middle trunk, dorsal and cranial to the median nerve, it provided branches to the three heads of the triceps brachii, the tensor fasciae antebrachii and the brachialis muscles; at the elbow joint, the radial nerve subdivided into deep and superficial branches. The deep branch of the radial nerve inserted between the brachioradialis and the origin of the extensor carpi radialis muscles, innervating the former and distributing to all craniolateral musculature of the antebrachium (*extensor carpi radialis, extensor digitorum lateralis, ulnar lateralis* and *abductor digit I longus*). Superficial branch separated into the digital

dorsal common III and IV nerves on distal of antebrachium.

The median nerve originated from the ventral roots of C7–T1, while the ulnar nerve arose from the ventral root of T1 (Plate II). The median nerve arising cranial to the ulnar nerve was responsible for the innervation of the pronator teres, flexor carpi radialis, superficial digital flexor (superficial and deep part), and deep digital flexor muscles. The ulnar nerve originated from caudal trunk and provided branches to the skin (cutaneous caudal nerve of the forearm), anconeus, flexor digitalis, flexor carpi ulnaris, and the cutaneous palmar surface.

The thoraco-dorsal nerve provided branches to the latissimus dorsi muscle; the long thoracic nerve distributed to the thoracic ventral serratus muscle; the lateral thoracic nerve gave off branches to the cutaneous muscles of trunk. The table below is a summary of the origin of the peripheral nerves and the muscles they innervate in the African palm squirrel.

Table 1: Summary of the spinal nerve origin and muscles innervated by the peripheral nerves of the brachial plexus of the African palm squirrel

| S/N | Peripheral nerves | Origin | Innervated muscle |
|-----|-------------------|--------|---|
| 1. | Cranial pectoral | C5, C6 | superficial pectoral muscle |
| 2. | Suprascapular | C5, C6 | supraspinatus and infraspinatus |
| 3. | Subscapular | C5, C6 | Subscapular |
| 4. | Axillary | C6, C7 | subscapularis, teres major, teres minor and deltoideus |
| 5. | Musculocutaneous | C6 | biceps brachii, brachialis and coracobrachialis |
| 6. | Radial | C6, C7 | Brachialis, triceps brachii, tensor fasciae antebrachii, extensor carpi |
| | | | radialis, extensor digitorum lateralis, ulnar lateralis. |
| 7. | Median | C7, T1 | flexor carpi radialis, pronator, flexor digiti III, flexor digiti IV |
| 8. | Ulnar | C7, T1 | skin, anconeus muscle, flexor carpi ulnaris and interossei, flexor |
| | | | digitalis V. |

Morphometric studies on the spinal and peripheral nerve of the brachial plexus

The African palm squirrel weight obtained in this study ranged from 160 - 190g, irrespective of their sex. Table 2 is a summary of the mean values of the lengths of nose-rump, tail and spinal nerves that constituted the brachial plexus in the African palm squirrel from the present study. The difference in the lengths of the nose-rump as well as that of the tail between the male and female rodents was not statistically significant (P > 0.05). Similarly, the difference in the lengths of each of C6, C7 and T1 between sexes was not significant (P > 0.05). However, the C5 was significantly longer in the male than in the female African palm squirrel (P < 0.05). Within each sex, the difference between any two spinal nerve lengths was statistically significant (P < 0.05). Consequently, T1 was the longest spinal nerve while C5 was the shortest spinal nerve in each sex.

Table 2: Sexual dimorphism in the lengths of the body and spinal nerves of the brachial plexus in the African palm squirrel (Mean \pm SEM)

| Sex | Length of parameter (mm) | | | | | | | | |
|---------------------------|--------------------------|--------------------|------------------------------|-------------------|-----------------------|-------------------------|--|--|--|
| | Nose-Rump | Tail | C5 | C6 | C7 | T1 | | | |
| Female $(n = 8)$ | 205.13 ± 9.15 | 164.75 ± 11.81 | $4.63 \pm 0.15^{a^{\ast}}$ | 8.23 ± 0.12^{b} | 8.67 ± 0.07^{c} | 9.49 ± 0.10^{d} | | | |
| (n = 6) Male $(n = 6)$ | 196.33 ± 12.35 | 202.33 ± 18.98 | $5.11\pm0.30^{\text{e}^{*}}$ | 8.39 ± 0.07^{b} | $8.66\pm0.08^{\rm c}$ | 9.78 ±0.09 ^d | | | |

*Values within a column with asterisk superscript are statistically significant (P < 0.05)

a,b,c,d Values within a row with different alphabet superscript are statistically significant (P < 0.05)

Table 3 is a summary of the mean lengths of the peripheral nerves that emanated from the brachial plexus in the African palm squirrel from this study. The difference in mean value of each of all the peripheral nerves between the male and female African palm squirrels was not significant (P > 0.05). In each sex, the ulnar nerve was the longest nerve as the difference in its length and that of any other

peripheral nerve was statistically significant (P < 0.05). Similarly, the thoracodorsal nerve was the shortest in both the male and the female rodents, as the difference in its length and that of any other peripheral nerve (except the axillary nerve) was statistically significant (P < 0.05).

| Sex | Length of peripheral nerve (mm) | | | | | | | | | |
|---------|---------------------------------|-------------------|-----------------------|--------------------------------------|-------------------|-----------------------|---------------------|-------------------|--|--|
| | Suprascapular | Subscapular | Thoracodorsal | Musculocutaneous | Radial | Axillary | Ulnar | Median | | |
| | nerve | nerve | nerve | nerve | nerve | nerve | nerve | nerve | | |
| Female | $6.60\pm0.09^{\rm a}$ | 6.49 ± 0.10^{b} | $6.15\pm0.16^{\rm c}$ | $8.29\pm0.27^{\text{d}}$ | 14.83 | 7.13 ± | 20.08 | $18.80 \pm$ | | |
| (n = 8) | | | | | ± | $0.64^{a,b,c,d}$ | ± | 0.35 ^g | | |
| | | | | | 0.49 ^e | | 0.49^{f} | | | |
| Male | $6.46\pm0.16^{\rm a}$ | 6.33 ± | $6.03\pm0.24^{\rm c}$ | $8.58\pm0.30^{\mathrm{a,b,c,d,e,f}}$ | 14.57 | 7.67 ± | 20.03 | $18.90 \pm$ | | |
| (n = 6) | | 0.16 ^b | | | ± | 0.76 ^{c,e,h} | ± | 0.53 ^j | | |
| | | | | | 0.78 ^g | | 0.54 ⁱ | | | |

Table 3: Sexual dimorphism in the lengths of peripheral nerves of the brachial plexus in the African palm squirrel (Mean \pm SEM)

^{a,b,c,d}Values within a row with different alphabet superscript are statistically significant ($P \le 0.05$).

DISCUSSION

The brachial plexus of the African palm squirrel is formed by the ventral rami of C5, C6, C7, and T1 spinal nerves. However, the brachial plexuses of other rodents, such as Mongolian gerbil, mole rats, porcupines, African giant pouched rat and chinchillas, have been reported to have different contributions from these spinal nerves. For instance, the African palm squirrel does not have contributions from the T2 spinal nerve as observed in the Porcupine [1]. While the African giant pouched rat's brachial plexus is formed from C5 to T1 [7], similar to that observed in the mole rat [11], but the C8 spinal nerve is not observed in the African palm squirrel from the present study. These differences in brachial plexus formation may reflect variations in the limb function of different rodent species such as digging, climbing, running, swimming, and food manipulation. Most of these rodents tend to have more complex brachial plexuses with contributions from more spinal nerves.

In this study, it was observed that the peripheral nerves that arose from the brachial plexus in the African palm squirrel that supply the muscles proximal to the cubital joint (suprascapular, subscapular, cranial pectoral, axillary, and musculocutaneous muscles) originated from the cranial and middle trunks (C5, C6, and C7) and were mostly monosegmental. In contrast, the other peripheral nerves, which included the radial, median, and ulnar nerves, extended to the distal limb areas and supplied the extensor and flexor muscles of the carpus and digits. These nerves had their origins in C7, and T1 and were plurisegmental. These findings suggest that the brachial plexus of the African palm squirrel is more complex than the brachial plexuses of other rodents, such as mole rats and gerbils. This is likely because the African palm squirrel engages in a wider range of activities, such as digging, climbing, running, and food manipulation. These activities require a more complex and versatile upper limb function, which is reflected in the more complex brachial plexus. The origin of the suprascapular nerve was from C5 and C6 in the African palm squirrel, similar to the African giant pouched rat [7], Chinchilla [12] and porcupine [1], but different from the Spix's yellow toothed cavy which was formed by C6 and C7 [13] and the gerbil which was formed by C4 and C5 [14].

The subscapular nerve was formed by the ventral rami of C5 and C6 in porcupines [1] and white New Zealand rabbits [6] which are similar to that observed in the African palm squirrel. The thoracodorsal nerve was formed by the ventral rami of C5 and C6 in the African palm squirrel which was similar to that of porcupines [1] and white New Zealand rabbits [6]. The musculocutaneous nerve arose from C6 in the African palm squirrel, from C7-C8 in white the New Zealand rabbit [6], from only C7 in chinchilla [12] and in the African giant pouched rat, it was observed to be between C7-C8 [7], similar to what was observed in the white New Zealand rabbit. The axillary nerve originated from C6 and C7 in the African palm squirrel, but from the C5 and C6 in the red squirrel [1] and the gerbil [14].

The radial nerve in the African palm squirrel originated from C6 and C7, but from C7–T1 in the African giant pouched rat [15], and from the ventral roots of C8, T1 and T2 in the Chinchilla [12]. The ulnar nerve was formed by the ventral roots of C7 and T1 in the African palm squirrel, similar to that of the African giant pouched rat [7], the porcupine [1] and the gerbil [14], but at variance from the report in the chinchilla [12] and white New Zealand rabbit [6] which arose from T1 and T2. The median nerve originated from the ventral roots of C6 to T1 in the African palm squirrel, similar to that of the African giant pouched rat [7]. In the gerbil, it was formed from C7-T1 [14].

Morphometric analysis indicated that T1 spinal nerve was the longest spinal nerve of the brachial plexus in the African palm squirrel. This may be due to their contribution to the nerves that extend the forearm. The thoracodorsal nerve was the shortest of the peripheral nerves. This may be connected to the fact that it innervates the latissimus dorsi, positioned very close to the brachial plexus. The Ulnar nerve was significantly longer than any other peripheral nerve. It was traced along the arm, running from the brachial plexus through the medial part of the upper limb,

innervating muscles that control movement of the carpus and metacarpus.

Furthermore, there was sexual dimorphism in the mean lengths of the musculocutaneous and axillary nerves, with the value of each of the nerves being significantly higher in the male than in the female. These differences could be related to the varied sizes of the two sexes. It could also be related to the varied degree of activity of the sexes. The male African palm squirrel may be more active than the female in scavenging, climbing and jumping. This may be responsible for the increased size of the nerves of their brachial plexus.

CONCLUSION

This study demonstrated the morphology of the brachial plexus and the distribution of its nerves in the African palm squirrel. Nerves originating from the brachial plexus displayed some significant differences in African palm squirrel compared with the African giant pouched rat, gerbil, chinchilla, white New Zealand rabbits and porcupines. This study has provided a better understanding about the origin and distribution of the brachial plexus in the African palm squirrel, which has not been studied before. This will be useful in future research and clinical applications.

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