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Histomorphological Study of The Pons and Medulla Oblongata of African Striped Group Squirrel (*Xerus erythropus*)

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THE study was undertaken to investigate the histological and functional relationships between the pons and Medulla Oblongata of African Striped Ground Squirrel (*Xerus erythropus*). Twenty (20) adult African striped ground squirrels were divided in to two groups: 10 males and 10 females. The ground squirrels were obtained from the surrounding villages of Zaria Local Government, Kaduna State Nigeria. Each Squirrel was euthanized using ketamine hydrochloride at dose 80mg/kg BW followed by gentle perfusion with neutral formal saline. A pair of scissors, chisel and scalpel blade was used to gently extract the brain (craniotomy). The extracted brain was fixed in bouins solution for 24hours and processed histologically. The pons and medulla are composed of conspicuous nucleus abducens, nucleus facialis and nucleus trigeminal. The nucleus facialis was found dorsal to the paramedian pontine reticular formation as group of large multipolar neurons. A conspicuous nucleus abducens was found as clusters of medium neurons located lateral to the trigeminal nucleus. In myelencephalon, the cuneate fascicles, intermediate sulcus and lateral sulcus were not evident on dorsal surface but there were grossly visible pyramids and olivary prominence on the ventral surface. Similarly, the nucleus olivary were prominent, nucleus cochlearis and spinal nucleus trigemini were well developed, nucleus hypoglossi, raphe obscurus, lateral reticular nucleus, gigantocellular reticular nucleus were all very distinct. In conclusion, the presence of distinct nuclei in the pons and medulla oblongata gives squirrels fine voluntary skills with good motor coordination and balance and good visual acuity for improved diurnal adaptation.

Keywords: African striped ground squirrel, Medulla Oblongata, Pons.

Introduction

Squirrels are mammals that belong to order *rodentia*. They are members of the family *sciuridae* and subfamily *Xerinae*, genus Xerus (African ground squirrel), species *Xerus erythropus* consisting of small or medium size rodents [1]. They are indigenous to America, Africa and Eurasia [2]. Ground squirrel has an overall uniform appearance and their fur color varies with ages and season. Striped ground squirrels are diurnal herbivores, active during the day and spend almost their entire lives on the ground, although they are capable of climbing into bushes to reach their food. The juvenile has soft grayish tan fur while adults have buffy grey to sandy brown fur and light tan or buff around the head and underside. Their ears are small and closely appressed to the head; eyes are black, large and place high on the head enabling the animal to detect approaching danger from almost any direction. Their tail has hairs much longer than those on the body, which fan out to the sides, and are multi-colored along their length, presenting a grizzled appearance [3]. They have versatile claws for grasping, climbing and digging. Their weight varies during an annual cycle but typical weight of adult is about (0.5-1) kg [4]. The squirrels live in open woodlands, grassy areas

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like cemeteries, pastures, parks, or rocky country. In Nigeria (especially south Nigeria), the squirrel is one of the most commonly consumed small mammal species (bush meat) by rural dwellers as a supplementary protein diet[1,5]. They are also used by Nigerian farmers in cultural ceremonies, for medicinal purposes (i.e. components of a anti-poison drug and as ingredients in the prevention of convulsion in children and also used to enhanced fertility in men) [5, 6].

The pons is a broad, horseshoe shaped portion of the brain consisting of large bundles of nerve fibers. The pons connects the two halves of the cerebellum and can be broadly divided into two parts; the basilar part, located ventrally and the pontine tegmentum, located dorsally [7]. A number of cranial nerve nuclei are present in the pons which includes the pontine and motor nucleus of the trigeminal nerve (CN V), located in the mid-pons while abducens nucleus (CN VI), facial nerve nucleus (VII) and vestibulocochlear nuclei (vestibular nuclei and cochlear nuclei) (VIII) are located at the lower end in the pons [8].. The pons also contains nuclei that relay signals from the forebrain to the cerebellum, along with nuclei that deal primarily with sleep, respiration, bladder control and posture [9]. The medulla oblongata is the conical part of the brain that extends from the pons to the medulla spinalis [10]. It is broad above where it joins the level of the foramen magnum [11]. The medulla oblongata is divided into two parts: an opened part (closed to the pons) and a closed part (closed to the spinal cord), the most rostral part of the medulla oblongata is the corpus trapezoideum [10]. Although, several studies have been conducted on the pons and medulla oblongata of other rodents, little or no research has been carried out on the pons and medulla oblongata of squirrels, thus this study will provide baseline data for detailed neuroanatomical descriptions of the pons and medulla oblongata of ground squirrels. This will in turn provide further laboratory research opportunities using ground squirrels as models and also provide additional information required for successful breeding as ground squirrels are a good source of protein for domestic farmers in Nigeria.

Materials and Methods

Experimental animals and management

Twenty (20) African striped ground squirrels (10 males and 10 females) were used for this study. The animals were captured live from the wild in Zaria and its environs. They were acclimatized for one month in standard laboratory cages in the animal pen of the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, Nigeria. The animals were given access to food and water *ad libitum* throughout the experimental period. They were physically examined during the pre-experimental period and only apparently healthy ones were utilized.

Morphometric parameters

The body weights of each squirrel were obtained using a weighing balance model JJ1000, USA with a capacity of 1000g and sensitivity of 0.01g. The mean length, width and depth of the pons and medulla oblongata were obtained with a vernier caliper (MG6001DC, General Tools and Instruments Company, New York; sensitivity: 0.01mm). Gross pictures were taken using canon digital camera power shot (SX170 IS) with 64-megapixel sensor (focal length: 28-448mm, 7.5cm (3.0") TFT. Histological pictures were taken using light microscope (Amscope, T120B) and a digital microscope camera (DCM 510 megapixel, ScopePhoto® China) at ×40, ×100, ×250, ×400.

Brain extraction

Each squirrel was euthanized using ketamine anesthetic at (80mg/kg bw) [4]. Each brain was perfused (intra-cardiac route) with 10% phosphate buffered formalin. Each skull was exposed after skinning and stripping off all the facial muscles within 30 minutes of euthanasia. Craniotomy was carried out through the calvaria to exposed the dura matter, which was later cut with curved pointed scissors. The falx cerebri and tentorium cerebelli were pulled from the longitudinal and transverse fissures by gentle traction. The cerebral vein was transected and at this stage, the brain was still in the cranium fixed in 10% phosphate buffered formalin for two days to enhance easy extraction as described by Ramaswamy [4].

Harvest of Pons and Medulla oblongata

The pons and medulla oblongata were isolated from the rest of the brain by gently pulling apart the two cerebral hemispheres at the occipital lobe to expose the corpus callosum. The entire corpus callosum together with septum pellucidum and the body and rostral commissure of the fornix were severed in the midline and this separates the cerebrum from the brain-stem and cerebellum. Then the floculli of the cerebellum was raised manually to expose the cerebellar peduncle which was severed starting with the laterally located brachium restiformis, followed by the middle brachium pontis and then the brachium conjuctivum. The brain-stem was free from the cranial nerves by simple trimming using a scalpel blade, an incision was made at the transverse fissure between the pons and caudal colliculi to isolate the pons and medulla oblongata from the midbrain while incision made at the pontomedullary junction separates the medulla oblongata from the pons.

Histology

The pons and medulla oblongata of squirrel were fixed at 10% neutral buffered formalin, dehydrated in a graded series of alcohol, cleared in xylene, then embedded in paraffin wax. The blocks were sectioned at 5-6 µm thickness of slice using a rotary microtome. Histological sections were stained with hematoxylin and eosin [12]. Cresyl fast violet stain was used for Nissl substance and nerve nuclei evaluation [13].

Results

Histological features of Pons and medulla oblongata of the squirrel brain

Nucleus facial: The nucleus was seen dorsal to paramedian pontine reticular formation and caudal to trigeminal nerve as group of large multipolar nuclei (Plate V). It is a relatively large nucleus with large amount of cytoplasm and the nissyl substance was clumpy and densely stain, its nucleolus is central (Plate XII).

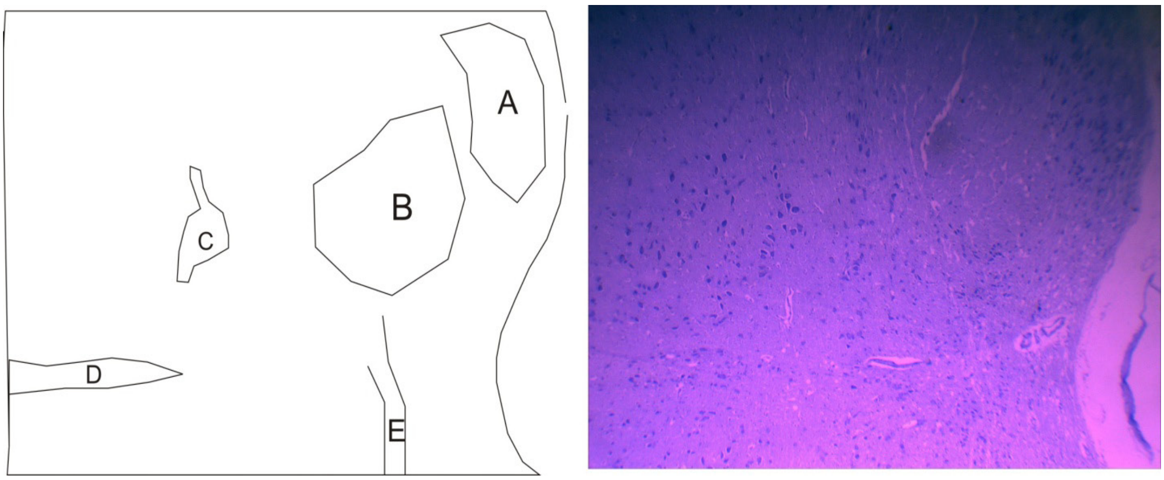


Plate V: Coronal section of the pons. A: Nucleus abducens, B: nucleus trigemini, C: Nucleus facial D: paramedian pontine reticular formation, E: trigemino-thalamic tract. (cresyl violet stains, x40).

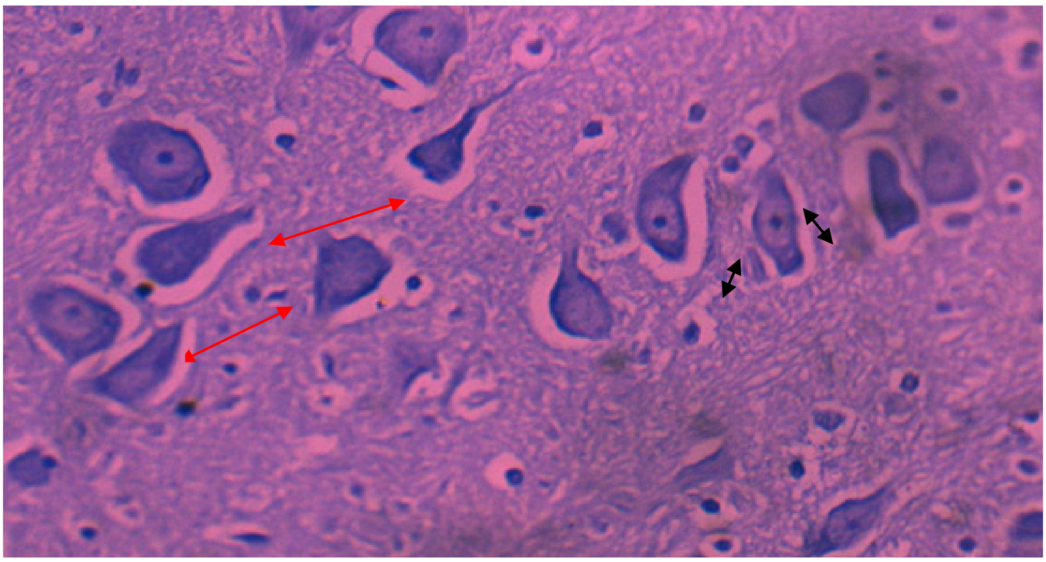


Plate XII: Coronal section of the pons showing neurons of motor nucleus of facial nerve (red) and neuron of motor nucleus of abducent nerve (black). Cresyl violet stain x400.

Nucleus abducens: It was a conspicuous nucleus found as a cluster of medium size neurons and located lateral to the trigeminal nucleus (Plate V). The nucleus was a mixture of small and medium size neurons with a large cytoplasmic nuclear ratio, densely stain with central nucleolus (Plate VI).

Nucleus and tract of trigeminal nerve: The nucleus trigemini on coronal view are evenly and uniformly organized neurons forming a bean shape (Plate I) and (Plate V). The nucleus is a mixture of the medium and large nuclei. It was bounded caudally by spinal trigeminal tract and tractus pyramidalis, dorsolaterally by nucleus hypoglossi, medially and ventromedially by nucleus ambiguus and reticular nucleus of medulla ventral respectively (Plate I).

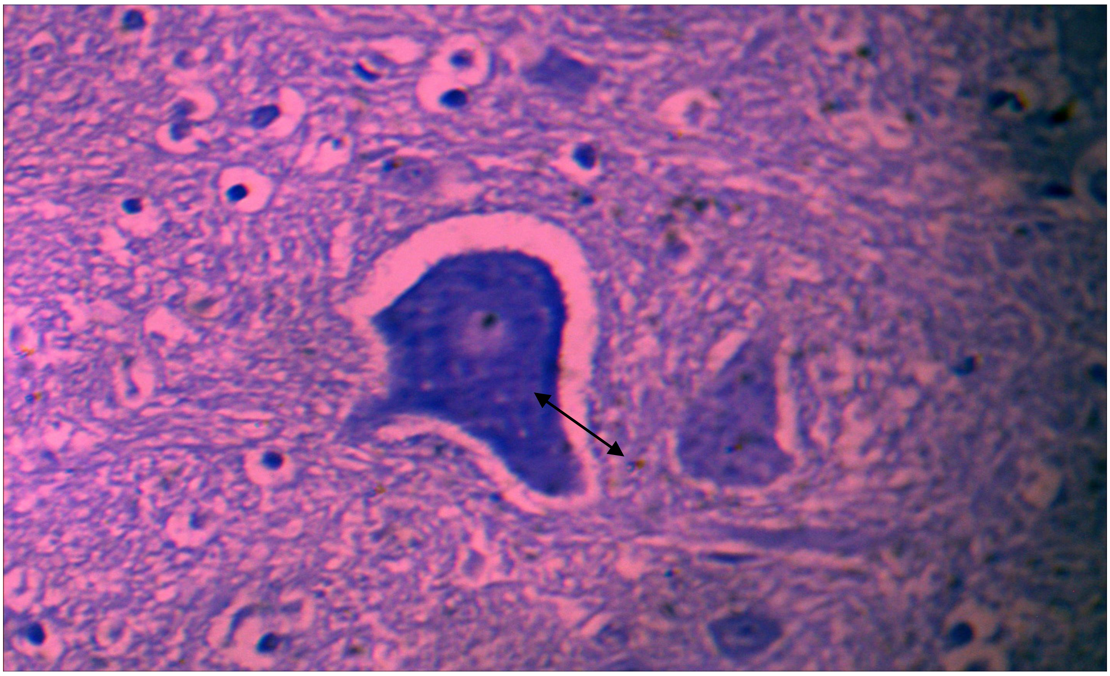


Plate VI: Coronal section of medulla oblongata showing gigantocellular reticular nucleus. Note its large and varied cytoplasmic nucleus ratio. Cresyl violet stain x250.

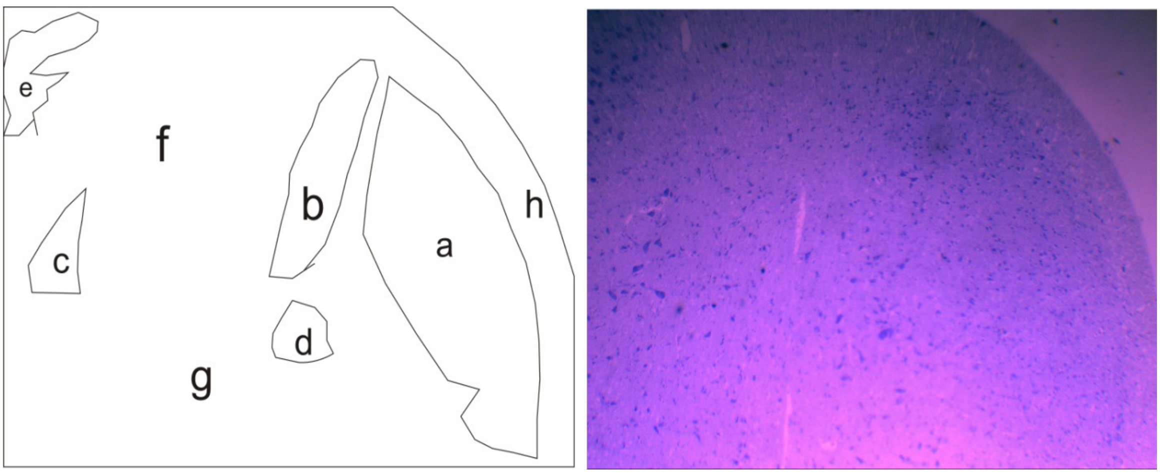
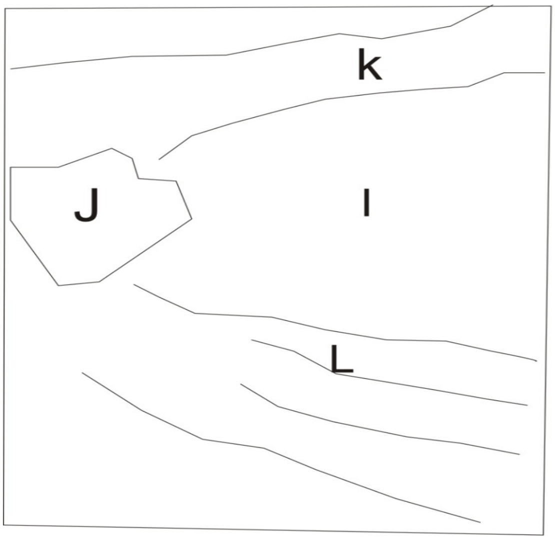


Plate I: Coronal section of medulla oblongata at 0.8mm rostral to the obex. A: nucleus spinalis and.trigemini, B: nucleus hypoglossi, C: linear nucleus of medulla, D: nucleus ambiguus, E: nucleus cochlearis, F: reticular nucleus of medulla dorsal, G: reticular nucleus of medulla ventral, H: tractus pyramidalis. (cresyl violet stain, x40).

Vestibular nucleus: These were small size nucleus located on the ventrolateral surface of the pontomedullary junction. The nucleus was found medial to the cerebellar peduncle and also ventral to pontocerebellar tract and was of spindle (fusiform) shape (Plate IV).

Nucleus ambiguus: This nucleus was most prominent at about 0.8mm rostral to the obex where it appeared as a circular group of few (15-20) oval nuclei. Its neurons were compacted, large and were found medial to nucleus spinal trigemini (Plate I). However, more rostrally the nucleus was found ventral to nucleus tractus solitarius with a pyramidal shape. It had a 1:1 nucleus cytoplasmic ratio and slight eccentric nucleolus and coarse nissyl substances (Plate X).

Nucleus olivaris: This nucleus was well stained on the ventral coronal section of myelencephalon and appeared as a large and multipolar neuron with a centrally located nucleolus and large cytoplasm. The nucleus varied between conical and oval shape with a clumpy nissyl substances (Plate VIII).



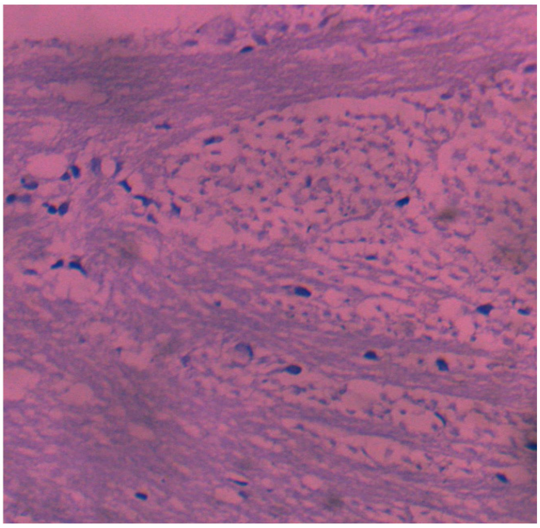


Plate IV: Coronal section of pontomedullary junction. J: vestibular nucleus, K: pontocerebellar tract, I: middle cerebellar peduncle, L: pontocerebellar fibers (Cresyl violet stain x250).

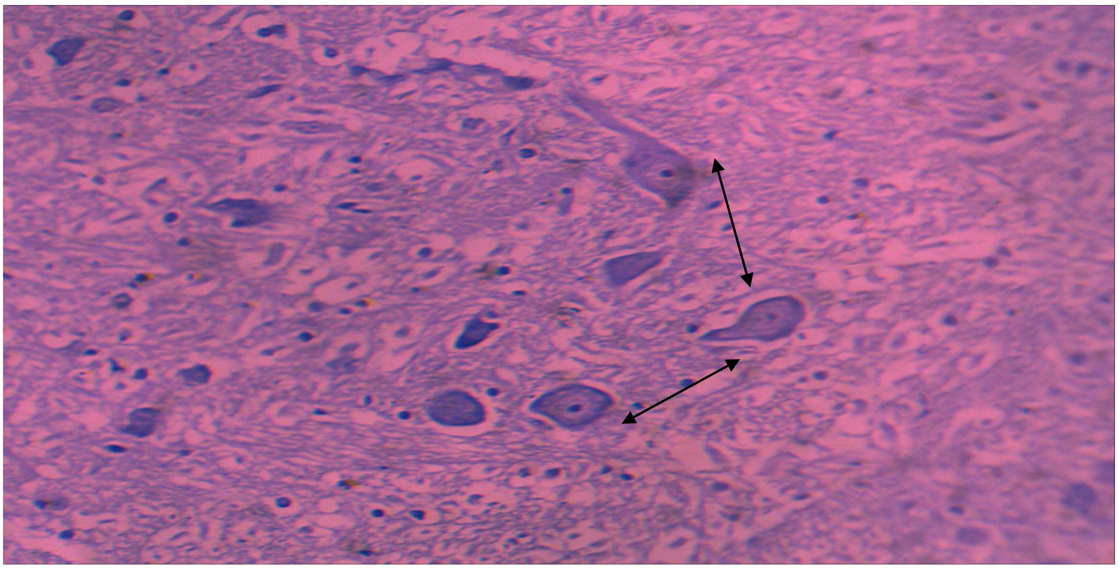


Plate X: Coronal section of medulla oblongata at 0.9mm rostral to the obex showing neurons of nucleus ambiguus. Note the 1:1 nucleus cytoplasmic ratio and slightly eccentric nucleolus. Cresyl violet stain x250.

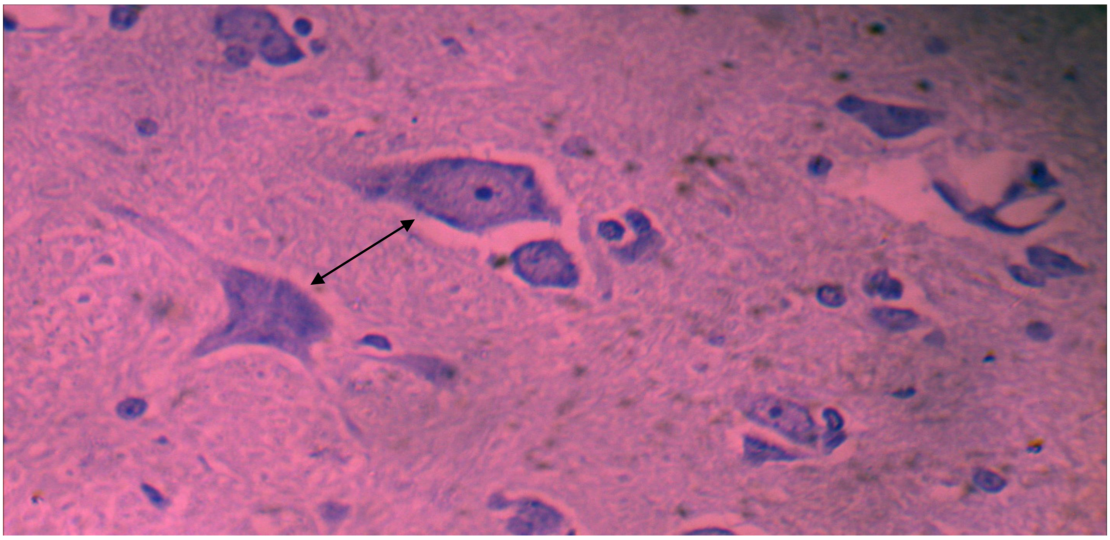


Plate VIII: Corona section of medulla oblongata showing neurons of the nucleus olivaris. Note the large and multipolar neuron in a variety of oval and conical shape. Cresyl violet stain x400.

Nucleus cochlearis: This was well demonstrated in histological section of myelencephalon that was 0.8mm rostral to the obex. It was composed of large elliptical group of nuclei with eccentric nucleoli (Plate IX). It was located on the dorsolateral portion of the medulla oblongata, dorsal to the reticular nucleus of the medulla and lateral to linear nucleus of the medulla (Plate I). Its neurons are mixture of pseudounipolar and unipolar with long dendrites (Plate IX).

Nucleus solitary tract: It was a spheroidal nucleus found at 0.9mm rostral to the obex on the open dorsomedial medulla oblongata. It was faint and lied medial to the dorsal motor nucleus of the vagus nerve (Plate II).

Nucleus hypoglossi: This nucleus was found dorsal to nucleus ambiguus and dorsomedial to the nucleus spinalis trigemini. It was found as cluster of discoid or spheroidal and multipolar neurons (Plate II). The neuron has a mixture of small and large nuclei with a small cytoplasm and centrally located nucleolus (Plate XI).

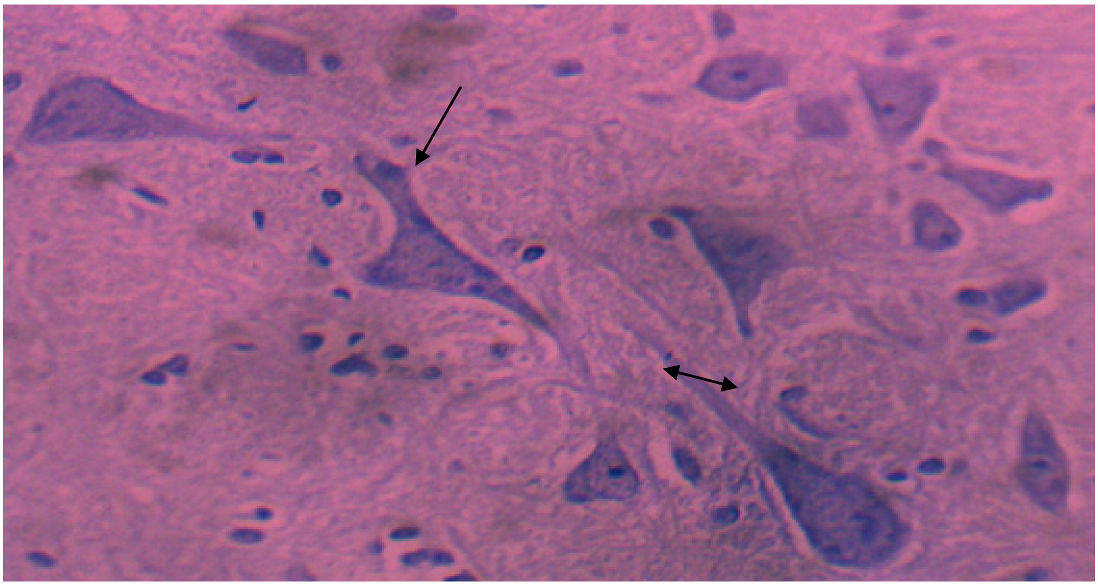


Plate IX: Corona section of medulla oblongata showing neurons of the nucleus cochlearis. Note the large pseudounipolar and unipolar neuron with elliptical shape. Cresyl violet stain x250.

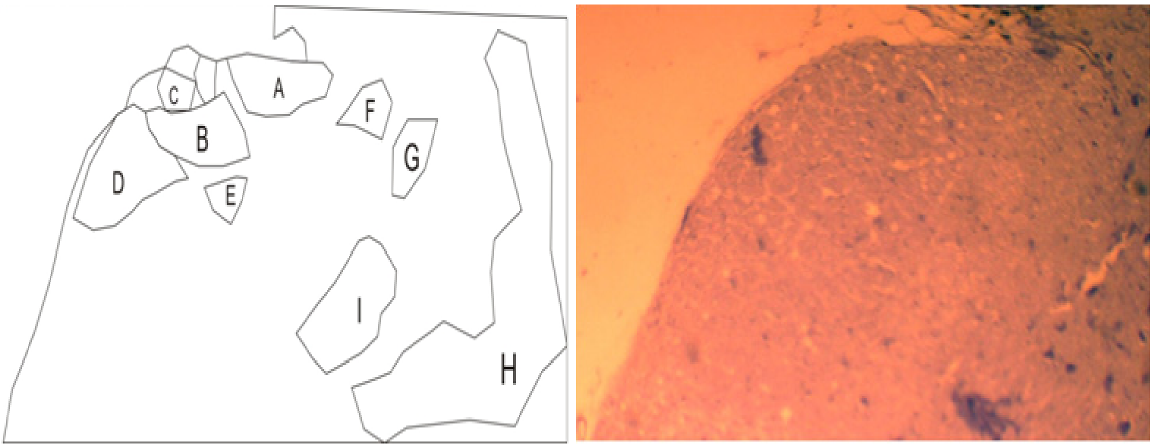


Plate II: Coronal section of medulla oblongata at 0.9mm rostral to the obex. A: dorsal motor nucleus of vagus, B: nucleus of the tractus solitarius, C: tractus solitarius, D: nucleus cuneatus, E: nucleus ambiguous, F: nucleus hyoglossi, G: nucleus of roller, H: lateral reticular nucleus, I: paragigantocellular reticular nucleus. (Cresyl violet stain, x40).

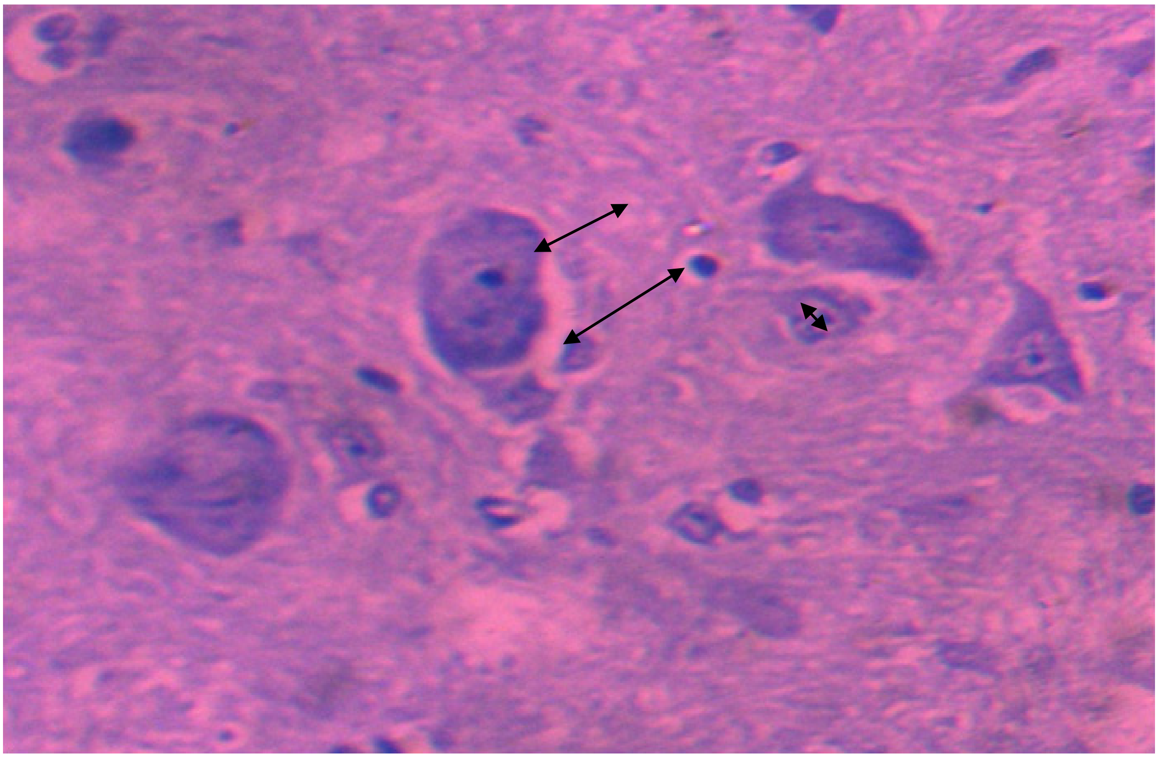


Plate XI: Coronal section of medulla oblongata at 0.8mm caudal to the obex, showing neurons of nucleus hypoglossi. Note the mixture of small and large nuclei and centrally located nucleolus. Cresyl violet stain X250.

Nucleus raphe obscures, nucleus cuneatus and lateral reticular nucleus: These nuclei were well represented in histologic section that was 0.8mm caudal to the obex. The nucleus raphe obscurus migrate medially to occupy a midline position between paramedian reticular nucleus. The cuneate nucleus was large and consist of scattered neurons. The most striking feature of the section is the very distinct nuclear cell group of the lateral reticular nucleus which was easily recognized with the nissyl stained section. The parvocellular was a cell rich region containing small fusiform and multipolar neurons (Plate III). The lateral reticular nucleus in this section are characterized by large nucleus with eccentric nucleolus and a very small cytoplasmic nuclear ratio (Plate XIII).

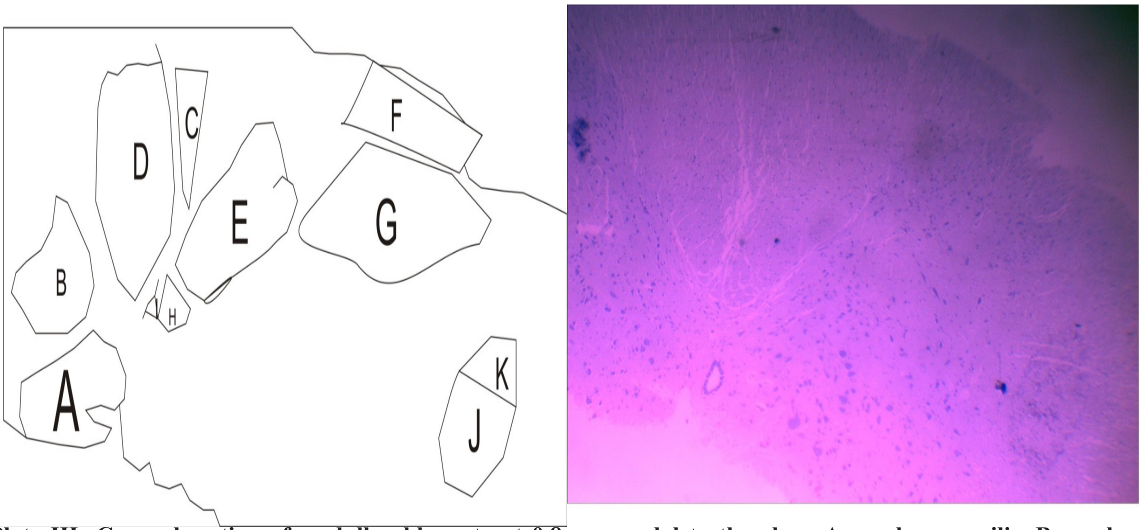


Plate III: Coronal section of medulla oblongata at 0.8mm caudal to the obex. A: nucleus gracilis, B: nucleus cuneatus, C: Raphe obscurus nucleus, D, E: paramedian reticular nucleus, F: pyramidal decussation, G: inferior olivary, H: medial longitudinal fasciculus, I: nucleus hypoglossi, J: lateral reticular nucleus, K: lateral reticular nucleus parvocellular. (cresyl violet stain, x40).

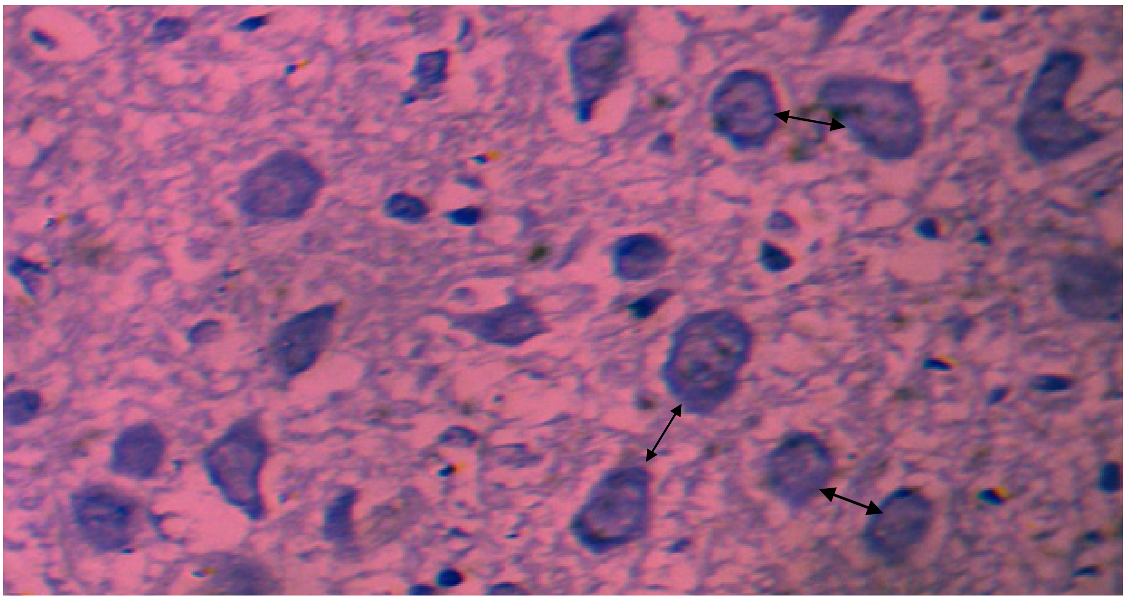


Plate XIII: Coronal section of medulla oblongata at 0.8mm caudal to the obex showing neuron of nucleus reticularis lateralis, note the spheroidal and oval shape. Cresyl violet stain x400.

Gigantocellular reticular nucleus: This has a coarse nissyl granules and large centrally located nucleus with central nucleolus. It consists of two populations of neurons which are largely multipolar and intensely stained with large nucleus (Plate VI).

Nucleus of corpus trapezoideum: It was an elongated nucleus that was well represented on the dorsal surface of the closed medulla at high magnification (Plate VII). It has coarse and densely stained nissyl substances, large cytoplasm and centrally located nucleolus.

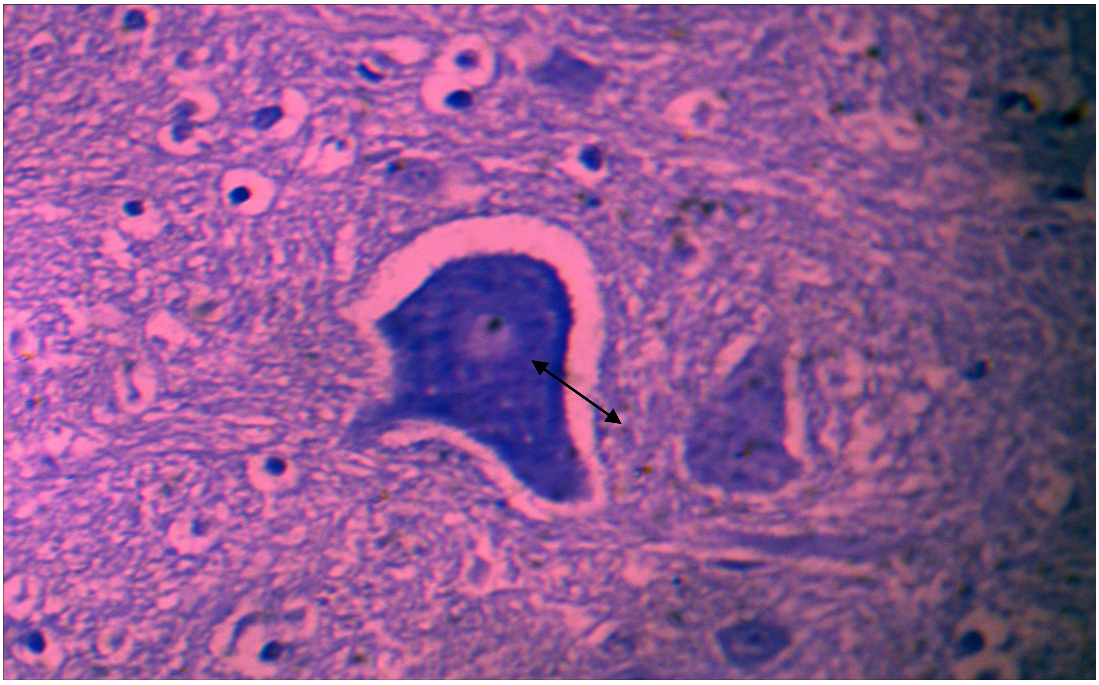


Plate VI: Coronal section of medulla oblongata at 0.8mm caudal to the obex showing gigantocellular reticular nucleus. Note its large and varied cytoplasmic nucleus ratio. Cresyl violet stain x250.

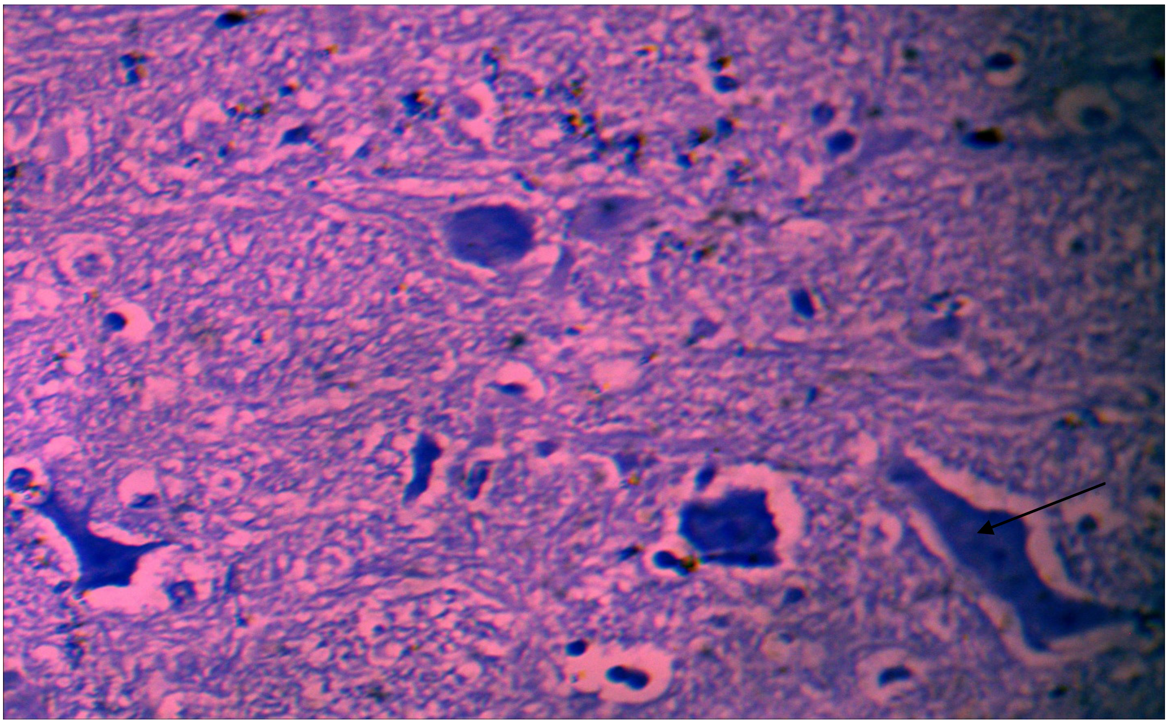


Plate VII: Corona section of medulla oblongata showing neurons of nucleus dorsalis corporis trapezoidei. Note its elongated shape, large cytoplasm and coarse nissyl substance. Cresyl violet stain x400.

Discussion

The pons and medulla oblongata are the caudal extend of the brain-stem. The pons was convex and smooth ventrally and triangular dorsally while the medulla oblongata was open rostrally and closed caudally. The presence of conspicuous olivary prominence, vestibular nucleus, pyramidal tract and the histological characteristics of the olivary nucleus in this study shows that squirrels have fine voluntary skill, good motor coordination and balance. This is similar to what was reported in grasscutter, African giant rats and wistar rat by Jones et al.[14]. Also, this finding is contrary to the report of Sricharoenvej et al.[15]. in flying fox who reported poorly developed olivary nucleus and attributed it to their poor motor coordination. Squirrels have well-developed pyramidal tracts, which is contrary to the findings of Majewska-michaska [16] who reported small pyramidal tract in guinea pigs. It can be presumable that squirrels have good motor coordination as in African giant rats and grasscutter if not better, such as symmetrical limb movement, climbing, fast runners and manus manipulation during grasping and sitting with hind limbs. The nucleus solitary tract was found rostral to the obex, this is in contrary with the findings of Ibe [17] who reported that solitary tract nucleus of African giant rat is located in the obex. The hypoglossal nucleus was found rostral to the obex at the level of the 4th ventricle, this is in agreement with the findings of Ajayi et al.[18]. who reported that the hypoglossal nucleus of grasscutter is located rostral to the obex at the level of the 4th ventricle. Defects in hypoglossal nucleus may result in uncoordinated vocalization, swallowing and chewing. The hypoglossal nucleus is devoid of numerous subdivisions but contains multipolar neurons that are mixture of both large and small, this is in agreement with the findings of Arora and Prakash [19] who reported that the hypoglossal nucleus of albino rats is devoid of numerous subdivisions. The trigeminal nucleus was very conspicuous on the lateral aspect of the medulla oblongata, this is in agreement with the findings of Dean et al. [20] who reported that trigeminal nucleus is very conspicuous on the lateral aspect of the medulla oblongata of mammals. The trigeminal nucleus has three subdivisions with no distinct bother lines, this is in agreement with the findings of Young et al. [21] who reported that trigeminal nucleus has three subdivisions in mammals. Trigeminal nerve supply sensations to the face, mucous membrane and other structures of the head and it is the motor nerve for mastication and contain proprioceptive fibers. The lateral reticular nucleus was conspicuously located caudal to the obex almost at the level of spinomedullary junction suggesting that the squirrel has fine motor function of posture, scratching and grasping with their limbs, this is in agreement with the findings of Bror and Carl-fredrik [22] who reported a conspicuous lateral reticular nucleus in cats which he said is a major precerebellar centre of mossy fiber information to the cerebellum from the spinal cord that is district from direct spinocerebellar paths and provide the cerebellum with segregated information from several spinal system controlling posture, reaching, grasping, location and scratching. The nuclei of reticular formation (raphe obscures, gigantocellular reticular nucleus, pontine reticular nucleu and parvocellular reticular nucleus) observed in this study is suggestive of good motor function, maintain good behavioral arousal and consciousness (sleep, alert, rapid eye movement), this is in agreement with the findings of Mcnamara and Nunn [23] who reported that the nucleus of reticular formation plays vital role in maintaining behavioural arousal and consciousness in mammals. Reticular formation nucleus was observed with some projections to the thalamus and cerebral cortex similar to what was reported by Thorpy and Yager [24], these projections also have projections exert some control on consciousness like alertness and sleep. injury to this reticular formation nuclei could result in irreversible coma[24]. The raphe nucleus observed in this study is suggestive of good visual acuity, this is in agreement with the findings of Lewy et al.[25] who reported that raphe nucleus plays a vital role in rapid and non-rapid eye movement, and also in sleep/wake cycle hormone secretion in rodents. The presence of conspicuous nucleus abducens is suggestive of a good visual acuity, this is in agreement with the findings of Alexander [8] who reported that the main function of abducens is to generate coordinated movement of both eyes in the same direction, however, abducens nucleus is composed of motor and interneurons. The motor neuron drives the contraction of ipsilateral rectus muscle and contraction of this muscles rotate the eyes outward (abduction) while the interneurons relay signals to the contralateral occulomotor nucleus where motor neurons drives the contraction of ipsilateral medial rectus muscles which rotates the eyes inward (adduction).

Conclusion

The presence of distinct nuclei in the pons and medulla oblongata of African striped ground squirrels gives them fine voluntary skills with good motor coordination and balance and a good visual acuity for improved diurnal adaptation.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest:
No conflict of interest

Ethical Statement

The experimental procedures were approved by the Institutional Animal Ethics Committee with an approval number of ABU/CAUC/2016/038. The present investigation was carried out at Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.

Authors contributions

All authors contributed substantially to the design, acquisition, and analysis of the study. Writing and revising for intellectual consumption were also collectively done.

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