COMPARATIVE CYTOARCHITECTURE OF THE MIDBRAIN COLLICULI OF DEFINED AGE GROUPS IN THE AFRICAN GIANT POUCHED RAT (CRICETOMYS GAMBIANUS)

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ABSTRACT
Background: The rostral and caudal colliculi of mammalian midbrain are pivotal to vision and audition, respectively. Cytoarchitecture of these midbrain colliculi in neonate, juvenile and adult African giant pouched rats is dearth. Objective: This study compared the histology of the nuclei, neurons and laminations of the colliculi in these age groups. Methods: Thirty-six captive African giant pouched rats consisting of twelve neonates, juveniles and adults were used. Thickness of the histologic layers of the rostral colliculi and lengths of the central nucleus of the caudal colliculi were compared among the groups. Results: the rostral colliculus composed of an outermost stratum zonale, middle stratum griseum superficiale and innermost stratum griseum profundus; migratory immature neurons of radial glial cells were observed in the stratum zonale of neonates, which disappeared in juveniles and adults; the stratum griseum superficiale of neonates was characterized of already formed neurites, similar to those of the juveniles and adults. However, this layer in adults had more oligodendrocytes than in juveniles. There was significant increase in thickness of the stratum griseum superficiale and stratum griseum profundus, with increasing age (P < 0.05). The caudal colliculus contained a central nucleus, dorsal and lateral cortices, with significant increase in the length of the central nucleus with increasing age (P < 0.05); there was transformation of fibre shaft in the lateral cortex of neonates into a mixture of chain like and marshy matrix in the juveniles which completely disappeared in adults, indicative of mature lateral cortex in the later. Conclusion: The adult African giant pouched rat may have the best auditory and visual senses, followed by juvenile. The neonate has the least acuity of these senses.

Key words: caudal colliculus; histology; lamina; nuclei; rostral colliculus.
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INTRODUCTION
The midbrain tectum consists of a pair of rostral and a pair of caudal colliculi. The caudal colliculus is the auditory reflex centre. It is the largest subcortical acoustic centre (Safi and Dechmann. 2005). It controls the nucleus of the lower auditory pathway. It receives acoustic impulse from the cochlear nuclei via the lateral lemniscus, the corresponding ears as well as motor impulse via the auditory cortex (Loftus et al., 2008). It relays the impulse to its ipsilateral medial geniculate nucleus via the caudal colliculus brachium. The medial geniculate body connects the caudal colliculus to the auditory cortex. Also, the caudal colliculus integrates reflexive auditory impulses (Loftus et al., 2008). Thus, the caudal colliculus serves as an integrative station and as a switchboard, controlling the nucleus of lower auditory pathway and production of motor-auditory reflex. It is subdivided into dorsal cortex, central nucleus and lateral cortex (Loftus et al., 2008). Majority of the neurons residing within the central nucleus exhibit disk-shaped dendritic arbores that have parallel orientation to numerous afferent fibres (Gabriele et al., 2000). Thus, the size of the
central nucleus relates to its extent of development and functional significance.

The rostral colliculus has been established as the visual reflex centre. It coordinates reflexive movements of the eyes, head and neck (Versnel et al., 2009). It consists of several layers which are the stratum zonale, stratum griseum and stratum medullaria (Nomina Anatomica Veterinaria 2017). This midbrain nucleus also receives visual, tactile and auditory impulses, encodes them into a common coordinate, to guide orienting movements toward the stimulus. Thus, the rostral colliculus is necessary for reflex and voluntary-guided behaviour of the eyes.

Studies have established the architecture of the rostral colliculus in different species and compared it with the caudal colliculus to arrive at a comparison of the acuity of visual and auditory sense in mammals. Grossly, it is bigger than the caudal colliculus in the rabbit (Bensley, 2009) and hamster (Kang et al., 2002). The caudal colliculus is bigger than the rostral colliculus in pigs (Getty, 1975), cetaceans (Marino et al., 2003) and adult African giant pouched rats (Ibe et al., 2014). Ogbonnaya et al. (2022) reported a detailed gross anatomical description of these colliculi in the neonate, juvenile and adult African giant pouched rats. However, to occlude the dearth of information on the cytoarchitecture of these colliculi in this rat, and to establish the age group with the best visual and auditory senses, the present study was designed.

The aim was to compare the cytoarchitecture of the rostral and caudal colliculi in defined age groups of the African giant pouched rat. The specific objectives included (a) to compare the histo-morphology of the rostral and caudal colliculi in the neonate, juvenile and adult African giant pouched rats in order to establish which of the two colliculi is better developed in each group, (b) to compare the histo-morphology and histo-morphometry of the rostral colliculi in the groups to establish the group with the best visual sense, and (c) to compare the histo-morphology and histo-morphometry of the caudal colliculi in the groups to establish the group with the best auditory sense.

The findings of this study will be relevant to guide authors in their choice of the most preferred age of the African giant pouched rat for research involving visual and auditory senses. Similar guide has been provided for cognitive studies in the African giant pouched rat (Olude et al., 2014)) and the African grasscutter (Ibe et al., 2017).

**MATERIALS AND METHODS**

**Ethics statement**

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

**Experimental animals**

Thirty-six apparently healthy African giant pouched rats consisting twelve neonates, juveniles and adults, each, were used. The adults and juveniles were captured live from the wild, using locally constructed metal traps while the neonates were captured in burrows. All the captured animals were transported to the Histology/Embryology Laboratory in the Department of Veterinary Anatomy, MOUAU.

**Extraction of the brain**

The rats were sedated with a cotton wool mildly soaked with chloroform. Thereafter, each animal was placed on dorsal recumbency, and perfused with 4%
paraformaldehyde, through the left ventricle, as described by Gage et al. (2012). Each animal was decapitated at the atlanto-axial joint using a rongeur. Skin and muscles of the head and neck were trimmed to expose the skull. The mandible was then separated from the rest of the skull. The skull was then placed in 10% formalin for 2 days.

The brain was extracted by removal of cranial and facial bones, using thumb forceps and a pair of scissors. Extraction of brainstem and midbrain from cerebrum and cerebellum involved systematic removal of the olfactory bulbs, cerebral halves and cerebellum. Thereafter, the brainstem was freed from the cranial nerves. The midbrain was separated from the brainstem by firstly separating the third ventricle from the rostral colliculus, leaving the lateral geniculate body intact.

**Preparation of slides and staining procedure**

The samples were put into separate labeled sample bottles and fixed in 10% formalin for 3 days. Thereafter, they were dehydrated through ascending grades of alcohol, then absolute alcohol. They were cleared in two changes of xylene and infiltrated with paraffin wax at 60ºc. They were then embedded in paraffin wax, trimmed, mounted in a wooden chuck, labelled and sectioned using a Jung rotary microtome (Model 42339, Berlin, Germany) at 5µm thickness.

The floated sections were mounted on albumenized slides, dried, deparaffinised, and stained using Haematoxylin-Eosin (H&E) and thionin stains. Images of sectioned brain of rats from Paxinos and Watson’s stereotaxic atlas (Paxinos and Watson, 1998) was used as guide for nuclear organization and histological landmarks. Nuclei images were studied and photographed with a digital eyepiece (Scopetek® DCM500, Resolution: 5M pixels) attached to light microscope (OLYMPUS® – XSZ107BN, Hamburg, Germany). Neuronal types were classified, and thickness of the layers of the rostral colliculi were obtained using a calibrated ocular micrometer (LeitzWetzlar, Germany) following appropriate calibration of the light microscope with a stage micrometer (Graticules Ltd., London, U.K.). Length of the central nucleus was calculated by multiplying the number of sections in which it appeared with the section thickness (Arora and Prakash, 2006). Nomina Anatomica Veterinaria (2017) was used for nomenclature.

**Analysis of data**

All data were expressed as mean ± SEM, and presented on graphs. Values were subjected to one-way analysis of variance, followed by Turkey’s post-hoc test to determine significance of the mean difference among the three groups. Values of P < 0.05 were considered significant. GraphPad Prism version 4 (GraphPad Software Inc., San Diego, California) for Windows 10, was used.

**RESULTS**

**Histo-morphology of the rostral colliculus**

The rostral colliculus in each of group was laminated. The three identified laminae were an outermost stratum zonale (SZ), middle stratum griseum superficiale (SGS) and the innermost stratum griseum profundus (SGP) (Fig. 1: i, ii, iii).

In the neonates, the extracellular matrix of the SZ had few immature migratory neurons within glial radial fibre shafts that formed a meshwork of thick fibrous layer. These migratory neurons lacked neurites. The neurons of this layer were small, conical, but numerous. The extracellular matrix of the SGS was more densely packed with immature migratory neurons which were
bigger, but fewer than those seen in the SZ (Fig. 2i: red arrow). The meshwork of thick fibrous layer was still observed (Fig. 2i: circled areas) and immature migratory neurons within the radial glial fibre shafts of the extracellular matrix were distributed within the nucleus. The neurons were still conical, without delineation of the nucleus from the nucleolus just as was observed in SZ (Fig. 2i: black arrow). This layer was more fibrotic than cellular. The SGP had a mixture of small conical and medium-sized pyramidal neurons (Fig 3i: black arrow) with evidence of dendrites (Fig. 3i: A). This layer was the most densely stained.

In the adults, the SZ was characterized by numerous small-sized conical and oval neurons with thick processes which formed an interlocking meshwork of thin fibrous layer. The SGS was characterized by large deeply-stained pyramidal and oval neurons that were the biggest compared to those in the three layers of juveniles and neonates. The neurons in this layer were mostly bipolar than multipolar pyramidal and the meshwork observed in the SZ had disappeared. Few oligodendrocytes were seen and the nucleus was clearly delineated from the nucleolus. The SGP was characterized by a mixture of small conical and medium-sized unipolar pyramidal neurons. These neurons were fewer than those in the SGP of the other two groups. There was a well characterized fibre mass, likely, the commissural fibres of the rostral colliculus (Fig. 2 and 3: iii).
Histo-morphometrics of the rostral colliculus

In the neonates, the mean thickness of the SZ, SGS and SGP were 0.025 ± 0.02µm, 6.533 ± 0.09 µm and 1.384 ± 0.04 µm, respectively. In the juveniles, the mean thickness were 0.059 ± 0.04µm, 14.050 ± 0.27 µm and 2.579 ± 0.05 µm, respectively. In the adults, the mean thickness were 0.060 ± 0.006 µm, 15.440 ± 0.13 µm and 3.272 ± 0.10 µm, respectively. The results indicated that in each of the age groups, the SGS was significantly the thickest layer (P < 0.05), while the SGP was significantly thicker than the SZ (P < 0.05).

The graph (Figure 8) compared the thickness of each layer of the rostral colliculus among the neonate, juvenile and adult groups. The thickness of the SZ in the neonate, juvenile and adult was not significantly different (P > 0.05). However, the SGS of the adult was significantly thicker than that of the juvenile and neonate (P < 0.05) while the SGS of the juvenile was significantly thicker than that of the neonate (P < 0.05). Similar observation was made with regards to the SGP, so that, the adult rostral colliculus had the thickest values of SGS and SGP, followed by the juvenile; the neonate rostral colliculus had the thinnest values of SGS and SGP.

Histo-morphology of the caudal colliculus

The caudal colliculus of the neonate, juvenile and adult African giant pouched rats contained all the three layers of a typical mammalian caudal colliculus. These layers were the dorsal cortex (DC), the lateral cortex (LC) and the central nucleus (CN) (Fig. 4: i, ii, iii).

Histo-morphometrics of the caudal colliculus

Figure 4: Coronal section of the caudal colliculus in the neonate (i) juvenile (ii) and adult (iii) African giant pouched rat. DC: Dorsal cortex; LC: Lateral cortex; CN: Central nucleus; AS – Aqueductus Silvius. i and iii, thionin stain; ii Haematoxylin and Eosin. Scale bar - 10µm.

Figure 5: Coronal section of the lateral cortex of the caudal colliculus in the neonate (i), juvenile (ii) and adult (iii) African giant pouched rat. Black arrow – Pyramidal neurons; Red arrow: oval neuron. Thionin stain. Scale bar - 100µm.

Figure 6: Coronal section of the dorsal cortex of the caudal colliculus in the neonate (i), juvenile (ii) and adult (iii) African giant pouched rat. Black arrow – Pyramidal neurons; Red arrow: oval neuron; Block arrow – Oligodendrocyte. Thionin stain. Scale bar - 100µm.
In the neonate caudal colliculus, there was a clear delineation between the LC and the CN. The LC was not as extensive as was the DC while the CN was clearly marked out by the concentric arrangement of the cell nuclei within each caudal colliculus around the longitudinal intracolliculi sulcus which separated the two caudal colliculi. The LC was made up of a mixture of large pyramidal multipolar neurons whose nuclei were eccentric with clearly defined nucleolus (Fig 5i: black arrow) and small round non-polar neurons (Fig. 5i: red arrow).

The nucleus occupied the entire nucleolus, making it impossible for a well-defined nucleolus to be seen. The LC also contained immature migrating neurons chained together by the fibre shaft of radial glial in the extracellular matrix forming thick fibrous layers and so, this layer was more fibrous than cellular. Oligodendrocytes were invisible. The DC was characterized by numerous blood vessels, more cellular than fibrous, with the neurons ranging from multipolar pyramidal cells with nucleus that covered the entire surface of the nucleolus (Fig 6i: black arrow) to oval unipolar neurons with eccentric nucleus and so the nucleolus was delineated (Fig. 6i: red arrow). Deeply stained oligodendrocytes were evident. There was evidence of long dendrites and prominent axons. The CN was characterized by the presence of large blood vessels, few oligodendrocytes, totally cellular and mixed neurons that were either small round or large pyramidal in shape.

Figure 7: Coronal section of the central nucleus of the caudal colliculus in the neonate (i), juvenile (ii) and adult (iii) African giant pouched rat. AS – Aqueductus Silvius; Black arrow – Neurons. Thionin stain. Scale bar - 100µm

Figure 8: Comparative layer thickness of rostral colliculus in defined age groups of the African giant pouched rat (bars indicate the standard error of the mean for each column). SZ: Stratum Zonale; SGS: Stratum Griseum Superficiale; SGP: Stratum Griseum Profunde

Figure 9: Comparative length of the central nucleus of the caudal colliculus in defined age groups of the African giant pouched rat (bars indicate the standard error of the mean for each column). Columns of the same index in each period with different letters (a vs. b) are significantly different (P < 0.05)
pyramidal cells were multipolar, centric, without visible nucleolus and with evidence of axoneogenesis and synaptogenesis through the dendrites. The round cells were non-polar and also centric occupying the entire nucleolus. The CN of the neonate was the most deeply stained layer of the caudal colliculus (Fig. 5, 6 and 7: i).

In the juvenile caudal colliculus, the LC, DC and CN were markedly delineated from each other with the DC being more extensive than the LC and CN just as in neonates. However, in the LC, the immature migratory neurons were held in a mixed chain-like and meshy-like fashion by the fibre of radial glial in the extracellular matrix. The immature migratory neurons were oval in shape, with centric nucleus that lacked a clearly marked nucleolus. Axons and dendrites were absent and so, there was no evidence of axonal growth and synaptogenesis. Few oligodendrocytes and blood vessels were seen within the meshy fibre of the radial glial. In the DC, blood vessels were not evident as in that of the neonate. The neurons were a mixture of both large unipolar pyramidal and oval shaped cells with almost all their nuclei being centric with little or no nucleolus evident. This layer of the caudal colliculus was purely cellular just as in neonates and many oligodendrocytes, though faintly stained, were evident. In the CN, the neurons were a mixture of small round and large oval cells that were all eccentric with the nucleus leaving a clearly marked nucleolus. This component was entirely cellular and no oligodendrocyte and blood vessel were seen, unlike in that of the neonates (Figures 5, 6 and 7: i).

In the adult caudal colliculus, the DC and CN were not clearly delineated as in the juveniles and neonates but the LC was evident. The concentric arrangement of the CN nuclei around the longitudinal intracolliculi sulcus was absent, unlike in the juveniles and neonates. The DC and CN were extensive and elongated compared to those of the juveniles and neonates. In the LC, there were numerous conspicuous blood vessels with no observable oligodendrocyte. The neurons of the LC were mature and consisted of a mixture of many round, small sized and few oval, large sized cells. The nuclei of the round cells were centric and so the nucleoli were not clearly marked but those of the oval cells were eccentric leaving very visible nucleoli. There was no evidence of axonal growth and synaptogenesis. The fibre shaft of the extracellular matrix was now scanty and thin, resulting to the formation of thin fibrous layer. In the DC, large blood vessels were observed, just as in neonates. Also, the thin fibre shaft of extracellular matrix observed in the LC was maintained, although faintly stained, unlike in juveniles and neonates were they were absent. The neurons were either large oval or small round cells. These cells were few per field of view and were mostly eccentric, leaving an obvious nucleolus that was clearly delineated from the nucleus just as in neonates, but unlike in juveniles. Oligodendrocytes were not visible. In the CN, the neurons were also a mixture just as in juveniles and neonates. However, the neurons were either large unipolar or oval non-polar. Irrespective of the shape, each neuron had eccentric nucleus with very large nucleolus. This component of the adult caudal colliculus was cellular and the fibre shaft nature of the extracellular matrix has disappeared just as in juveniles and adults. Numerous blood vessels were observed, unlike in juveniles and neonates, but oligodendrocytes were not visible just as in juveniles and neonates (Fig. 5, 6 and 7: i).

**Histo-morphometrics of the central nucleus of the caudal colliculus**

The central nucleus was visible throughout the rostrocaudal extent of the caudal colliculus in the adult and juveniles only. The mean values of the rostrocaudal extent of the central nucleus in the neonate, juvenile and adults were represented on Fig. 9. The results revealed that the central nucleus of the juvenile and adult were, each, significantly longer than the central nucleus of the neonate (P < 0.05), while the difference in length of the central
The nucleus of the juvenile and the adult was not significant (P > 0.05).

**DISCUSSION**

Lamination of the rostral colliculus is indicative of the extent of its development in any animal. This, in turn, is extrapolated to the acuity of the visual system in such animal. Cooper et al. (2004) reported that the superficial layers of the blind mole rat rostral colliculus are collapsed to one layer, suggestive of poor vision. Conversely, Telford et al. (1996) reported that the Sprague-Dawley rats’ stratum griseum is further sub-divided into SGS, stratum opticum, stratum griseum intermediale, stratum album intermediale and SGP, suggesting a good vision. Similarly, Mensah-Brown and Garey (2006) reported six laminae in the camel rostral colliculus. They also concluded that the camel’s vision is acute. The acute vision of the camel has been previously reported by Adogwa (1985), who observed that the rostral colliculus is bigger than the caudal colliculus, thus inferring a better vision than hearing in the one-humped camel. Moreover, the camel is known to have conspicuous eyeballs. Only three layers were observed in the rostral colliculus of the African giant pouched rat from this study, irrespective of the age group. This suggests poorly developed rostral colliculus, which indicates poor eyesight. The three layers of the rostral colliculus in the neonate, juvenile and adult African giant pouched rat from this study, irrespective of the age group. This suggests poorly developed rostral colliculus, which indicates poor eyesight. The three layers of the rostral colliculus in the neonate, juvenile and adult African giant pouched rat from this study corresponded to the study conducted in the African grasscutter rostral colliculus by Ibe et al. (2019). However, the migratory immature neurons of the radial glial found in the SZ of the neonate rostral colliculus in the present study was already mature in the neonates of the African grasscutter (Ibe et al., 2019). This implies that the neonate African grasscutter may have better vision than their African giant pouched rat components. These migratory immature neurons had disappeared in the juvenile and adult African giant pouched rats in the present study. This indicated that the rostral colliculus, thus, the visual sense of the neonate is least acute, compared with that of the juvenile and adults. The presence of these immature neurons in the neonate also imply that neuronal ablation by any external assault can occur in neonates. Therefore, caution is advised in the administration of therapeutics that cross the blood-brain barrier to neonate of African giant pouched rats. They can result to abnormal development of the rostral colliculi which will subsequently affect the visual capacity of the neonates. The minute blood vessels observed in the SZ may imply minimal energy demand for cell metabolism.

The presence of well-developed neurites in the SGP of the neonates implies that axonogenesis and synaptogenesis were active. The increased number of blood vessels observed in SGP of adults compared to juveniles means an increased vascularization possibly due to increased demand of energy for cell metabolism. The presence of oligodendrocytes in the adults is due to the production of myelin sheath that will protect this part of the brain because of the hyperactive nature of this group. This increased vascularization and dominance of oligodendrocytes in the adult rostral colliculus, compared to the juveniles is also indicative of a better developed rostral colliculus in the adult. This was further validated by the significantly thicker layers of the SGS and SGP in the adults than in the juveniles. This implied that there were more neuronal and neuroglial cells in these adult layers than in those of the juveniles and neonates. This confers a better visual sense in the adult African giant pouched rat, than in the juveniles or the neonates.
The caudal colliculus of the African giant pouched rat from this study was made up of the three divisions typical of a well-developed caudal colliculus. This was observed in all the age groups, and it implies that the rodent has a good hearing ability. The developed components of the acoustic pathway, from this study, probably highlights the auditory demands of percussive foraging in the African giant pouched rat. These three divisions of the caudal colliculus in the three age groups corresponded to the findings in the African grasscutter by Ibe et al. (2019). However, the presence of the migratory immature neurons and numerous blood vessels in the neonates is suggestive of possible neuronal ablation, high energy demand for cell metabolism and rapid vascularization of this group. Therefore, external assaults including drugs which tend to cross the blood brain barrier should be avoided. The transformation of the fibre shaft of the neonates into a mixture of chain like and marshy appearance in juveniles is as a result of a gradual differentiation of these astrocyte precursors as the brain developed (Barry, 2013) and its complete disappearance in adults with thin layers suggest that at this age, no further differentiation of the neuroglial cells occur. This implied that the adult caudal colliculus was most developed, followed by that of the juveniles; the neonatal caudal colliculus was still immature. These agreed with the work of Ibe et al. (2019) on the African grasscutter partly, especially for the neonates and juveniles but differed in the adults. However, statistical comparison of the lengths of the central nucleus of the caudal colliculus indicated that the juvenile and adult were each better developed than the neonate.

CONCLUSION

The histo-morphologic and histomorphometric findings in this study have established that the rostral colliculi are poorly developed than the caudal colliculi in all the age groups. However, each of those colliculi are best developed in the adults, followed by the juveniles, and least developed in the neonates. This implies that the visual sense is less acute than the auditory sense in the African giant pouched rat, irrespective of age. However, these two senses are more acute in the adult, followed by the juvenile, and least acute in the neonate. Thus, it is advised that adult African giant pouched rat be preferred for studies centered on visual and auditory senses.

CONFLICTS OF INTEREST

The authors affirmed that there are not any conflicts of interest.

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