

Original Research Article

Histology and Brain Derived Neurotrophic Factor immunoreaction of the neurons in the *corpora quadrigemina* of the African grasscutter (*Thryonomys swinderianus* – Temminck, 1827)

Chikera Samuel Ibe¹, Suleiman Olawoye Salami², Ekele Ikpegbu¹, Mohammed Adam³

¹Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Nigeria

²Department of Veterinary Anatomy, University of Ilorin, Ilorin, Nigeria

³Department of Veterinary Pathology, University of Ilorin, Ilorin, Nigeria

Correspondence to:

Dr. Chikera S. Ibe, Department of Veterinary Anatomy, Michael Okpara University of Agriculture, Umudike, Nigeria; Tel.: +2348032882105; E-mail: ibe.chikera@mouau.edu.ng

Abstract

The African grasscutter is the second largest rodent in Africa, thus, a key component of the minilivestock industry. The study described the histological features and probed the distribution of Brain Derived Neurotrophic Factor (BDNF) in the *corpora quadrigemina* of the African grasscutter at foetal and postnatal developmental periods. Brain samples from foetuses explanted on foetal days 60 (F60) and 90 (F90) and extracted from 3 and 6-days-old pups (P3 and P6, respectively), 72-days-old juveniles (P72) and 450-days-old adults (P450) were used. They were prepared for histology and immunohistochemistry. Three laminae were distinct in the rostral colliculi at the foetal and postnatal periods; an outermost *stratum zonale*, middle *stratum griseum superficiale* and an inner *stratum griseum profundum*. *Stratum griseum intermediale* and *stratum medullarium* were not distinct. On F60 and F90, the *stratum zonale* was made of immature neurons, devoid of neurites; the central nucleus of the caudal colliculus was also made of immature migrating neurons. On P3, the neurons were already mature. The *stratum zonale* was made of medium-sized neuronal cells and thick processes. The thickness of this layer decreased with age. On P3, the caudal colliculus was made of all the components typical of a developed caudal colliculus. There was no BDNF immunoreactive cell in the *stratum zonale* at any postnatal period; a moderate BDNF-immunoreactivity in the *stratum griseum superficiale* on P3, a mild immunoreactivity on P6, none reactivity on P72 and mild on P450. The dorsal and lateral cortices of the caudal colliculus were none reactive to BDNF at any postnatal period. The results suggest a better auditory than visual capacity in the rodent.

Keywords: rostral colliculus; caudal colliculus; BDNF; African grasscutter.

INTRODUCTION

Rodents are the widest order of placental mammals as they constitute more than a half of the class (Aydin et al., 2008). The African grasscutter is a member of the sub-order Hystricomorpha and family Thryonomyidae. The family is represented by a single genus, *Thryonomys*; and two species, *Thryonomys swinderianus*, known as the greater cane rat and *Thryonomys gregorianus*, known as the lesser cane rat (Bronner et al., 2003).

The African grasscutter is one of the most preferred and most expensive bushmeat in West Africa, including Nigeria, Togo, Benin, Ghana and Côte d'Ivoire (Asibey and Addo, 2000). It contributes to both local and export earning of most West African countries (Opara, 2010). It is hunted aggressively through bush burning, resulting

in the destruction of animal life and the ecosystem. In an attempt to solve this problem, efforts are being made in the sub-region to breed and domesticate the rodent. By this, it will be more readily available, the farmers will gain economic benefit and the environmental destruction during its collection from the wild will be reduced.

Despite the current domestication of the African grasscutter, its biology has not been fully investigated. Understanding the biology of the African grasscutter may proffer information on the behaviour of the rodent, which will be valuable in its domestication. Behavioural studies by Opara (2010) and Williams et al. (2011) have proposed a more acute auditory than visual ability in the African grasscutter. Comparative gross anatomical study of the rodent's auditory and visual

reflex centres by Ibe et al. (2017) also pointed to a better auditory than visual ability in the rodent. The lack of further histological and immunohistological studies on the centres, to help support or confute these previous studies, led to the present research.

The *corpora quadrigemina* is the centre for auditory and visual reflexes, and a nerve pathway of the cerebral cortex. It contains major relay stations for neurons transmitting signals to the cerebral cortex and many reflex centres carrying sensory and motor impulses. It is composed of a pair of rostral and a pair of caudal colliculi. The rostral colliculus is the visual reflex centre, concerned with the co-ordination of reflexive movements of the head, neck and eyes, as well as focusing of the lens and visual tracking of objects (Mensah-Brown and Garey, 2006; Walton et al., 2007). The caudal colliculus is the acoustic reflex centre. It is the largest subcortical acoustic centre and the principal midbrain nucleus of the acoustic pathway (Safi and Dechmann, 2005). Nucleus of the lower auditory pathway and motor-auditory reflex are under the control of the caudal colliculus (Gelfand, 2004). It receives acoustic impulse from the cochlear nuclei through the lateral lemniscus as well as motor impulse from the auditory cortex (Loftus et al., 2008).

Several neurotrophic factors are necessary for the growth and survival of developing neurons. Studies have shown that these proteins which include neuropoietic cytokines, neurotrophins and glial cell-line derived neurotrophic factor family ligands (GFLs) have been identified in neurons of neonate rats (Ichikawa et al., 2006; Bałkowiec and Bałkowiec-Iskra, 2010). The immunolocalisation of neurotrophic factors in neurons has been used to buttress the theory of neuronal regeneration (Tarsa et al., 2010). Neurotrophins are proteins that help to stimulate and control neurogenesis (Hampstead, 2006), and BDNF is the most active in stimulating neurogenesis postpartum (Benraiss et al., 2001). The null hypothesis of the study is that BDNF is not reactive in the neurons of the rostral and caudal colliculi of the African grasscutter. Consequently, the study was aimed at describing the histological features and evaluating the neuronal plasticity in neurons of the rostral and caudal colliculi of neonate, juvenile and adult African grasscutters, using anti-BDNF.

MATERIALS AND METHODS

Experimental animals

Four nulliparous African grasscutter does of an average age of 5 months and a mature buck of 10 months age, were used for the prenatal study. Eleven foetuses were explanted on F60 from 2 does (6 foetuses from 1 doe and 5 foetuses from the 2nd doe) and 9 foetuses were explanted on F90 from the remaining 2 does (5 foetuses

from 1 doe and 4 foetuses from the 2nd doe). Furthermore, 36 African grasscutters, consisting of 9 each of 3 days old (P3), 6 days old (P6), 72 days old (P72) and 450 days old (P450), were used for the postnatal study. The mean body weights of the African grasscutters used for the postnatal studies were 109.76 ± 2.12 g, 160.11 ± 7.46 g, 273.39 ± 6.70 g and 2925.56 ± 141.96 g, for P3, P6, P72 and P450, respectively. All the experimental animals were obtained from a mini-livestock industry in Rivers State, Nigeria. The animals were transported by road, to the Histology/Embryology Laboratory, Michael Okpara University of Agriculture, Abia State, for the experiment. Prior to the experiment, they were clinically examined, and only the healthy ones were used. They were given access to fresh guinea grass, cane grass, rodent-pelleted concentrates and drinking water *ad libitum*. The cages and feeding troughs were disinfected with Milton® every day.

Ethical approval

The experimental protocol was approved by the Ethical Committee of Ahmadu Bello University, Zaria, Nigeria. Management of the experimental animals was as stipulated in the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council, USA (National Academic Press, Washington, DC: <http://www.nap.edu>).

Mating and pregnancy diagnosis

The nulliparous does were housed singly for 4 months before mating. This was to allow them attain the stipulated minimum of 7 months for sexual maturity, and also, to ensure induced ovulation. Prior to mating, the vaginal status of each doe was obtained by visual observation and recorded. Hand-mating was conducted by transferring the nulliparous does to the buck in the colony paddocks where they were left with the buck until they were mated.

The does were examined at 6 hours intervals for post-pairing perineal changes which are indicative of successful mating. These include perforation of the vaginal membrane, vulva congestion and presence of a copulatory plug in the vagina or on the floor of the cage, as reported by Addo (2002). On observing any of the signs, the females were immediately and permanently separated from the male, weighed, and the date and time of the appearance of the mating-signs were recorded.

Early pregnancy was ascertained by the use of vaginal mucosal swab, as is the routine in most commercial grasscutter farms. Yellow coloration of the swab is indicative of early pregnancy. Late gestational pregnancy was evident by change in body weight, gravid nature of pelvic region and enlarged teats.

Caesarean section

The foetuses were explanted via caesarean section. Each pregnant doe was anaesthetized using 40 mg/kg of sodium pentobarbital, intraperitoneally. The caesarean section was based on the procedure described by Bowers et al. (2001). In summary, a 3 cm caudal midline laparotomy incision was made. The mesentery and colon were displaced to expose the uterine horns. A 2-cm longitudinal incision was made along the antimesenteric border in the mid-portion of each uterine horn. The foetuses and placentas were gently extruded through the hysterotomy. Afterwards, the uterine incisions were closed with a continuous non-locking 5-0 polyglycolic acid suture, and the deep abdominal fascia as well as peritoneum was closed with 4-0 polyglycolic acid sutures. The skin was closed separately using the same material.

Brain extraction and separation of the corpora quadrigemina

Once a foetus was explanted and decapitated, the fibrous skull tissue was excised with scalpel blade and thumb forceps to expose the brain. The head was then post-fixed in Bouin's solution for 24 hours to enable easy brain extraction. After 24 hours, the samples were transferred to 10% neutral buffered formalin. The post-fixed tissues were exposed by making longitudinal cut along the mid-cranial line with a scalpel blade. The cut fibrous tissue was removed by gentle traction. This exposed the *dura mater*, which was cut with a scalpel blade along the same line. The *falx cerebri* and *tentorium cerebelli* were pulled from the longitudinal and transverse fissures of the brain, respectively, by gentle traction. This facilitated easy extraction of the brain.

For the postnatal brain samples, animals were sedated with Thiopental Sodium (Rotexmedica, Trittau, Germany; 20 mg/kg; i/p). Thereafter, their body weight was obtained with an electronic balance (Citizen Scales (1) PVT Ltd.; 0.01 g). This was followed by perfusion fixation of the brain with 4% paraformaldehyde, as described by Gage et al. (2012). The animal was decapitated at the atlanto-axial. Intact brain was extracted from the skull following craniotomy, as described by Ibe et al. (2016). Extracted brain samples were fixed for microscopic studies.

To obtain the *corpora quadrigemina*, the brainstem was separated from the cerebrum and cerebellum. In order to remove the cerebrum, the cerebral halves were laterally displaced to expose the *corpus callosum* which was cut in the midline. The two cerebral halves became free and were separated from the remaining brain parts. The cerebellum was separated from the brainstem by severing the cerebellar peduncles which were identified beneath the cerebellar lobes. To separate the *corpora*

quadrigemina from the rest of the brainstem, a horizontal incision was made at the mid-portion of the midbrain.

Histological study

The foetal and postnatal brain samples were utilised for histology, whereas only the postnatal brain samples were used for immunohistochemistry. The samples were prepared for histological study following the routine procedure for histology. Specifically, the samples were washed and dehydrated for 1 hour each in a series of 70%, 80%, 90%, 95% and 100% alcohol. The tissues were cleared in xylene, infiltrated with 50 °C melted paraffin wax (BDH Chemicals Ltd. England), blocked and labelled.

Serial coronal sections of the rostral and caudal colliculi, each, were cut at 5 µm using Jung rotary microtome 42339 (Berlin, Germany). The sections were floated on charged slides and oven-heated at 60 °C for 2 hours. The slides were deparaffinised using xylene and alcohol and thereafter, rehydrated. Every 4th section was stained with haematoxylin and eosin for general histo-architecture, and every 5th section was stained with cresyl fast violet for identification of the Nissl substance and nuclei. The slides were dried in the oven for 15 minutes and cover-slipped using DPX.

Pictures of the neuronal cells and nuclei were obtained at different magnifications, using a digital eyepiece (Scopetek® DCM500; 14M pixels) fixed to a microscope. The neuronal morphology was studied. Thickness of each lamina was also evaluated by visual observation. Terminology, consistent with the *Nomina Anatomica Veterinaria* (2012) was used to label the structures observed in the study.

Immunolocalisation of BDNF

To immunolocalise BDNF in the *corpora quadrigemina* (rostral and caudal colliculi) of the postnatal brain samples, the samples were fixed for 3 days in 10% neutral buffered formalin. Thereafter, trimmed sections of the rostral and caudal colliculi were placed in labelled tissue cassette and transferred to a semi-enclosed bench-top tissue processor TP 1020 (Leica Biosystems, Nußloch, Germany) for tissue dehydration and clearance. Tissues were embedded into galvanized moulds, at 58 °C, using melted paraffin wax (Salsa Wax) and blocked. Serial coronal sections of the block were obtained at 5 µm thick, using the rotary microtome stated earlier, and every 6th section was floated on adhesive slides. The slides were oven-heated, deparaffinised and rehydrated as described earlier. They were incubated in Citrate Buffer (Abcam PLC., Cambridge UK; pH: 6.0) at 65 °C, for antigen retrieval, washed in Tris buffered-saline and 0.05% Tween 20 (TBST 10-0028; Genemed Biotechnologies Inc., California; pH: 7.4) for 2 minutes and treated with hydrogen peroxide block and protein block from a Rabbit Specific ABC/DAB detection IHC

kit (Abcam PLC., Cambridge, UK; Product number: ab64261). The slides were washed again in Tris buffered-saline and 0.05% Tween 20 for 2 minutes, treated with the Anti-BDNF primary antibody (Abcam PLC., Cambridge, UK) at a dilution factor of 1:1000, and incubated at room temperature for 60 minutes. The slides were washed again for 2 minutes and treated with a biotinylated goat anti-rabbit antibody from the same detection kit, as the secondary antibody. Thereafter, the slides were treated with streptavidin peroxidase and 3, 3-diaminobenzidine chromogen plus diaminobenzidine substrate at a ratio of 1 drop : 1.5 ml, counterstained for 30 seconds with haematoxylin, dehydrated, dried in the oven for 15 minutes and cover-slipped using DPX.

The slides were examined under the light microscope at final magnification of $\times 400$. Brown cytoplasmic precipitates indicated positive immunoreactions. A quantitative scoring was carried out in which the number of positive neurons was manually counted. Counting was done on the entire surface area of each section. The total number of sections counted varied with the postnatal age groups. It ranged from 15, 13, 21 and 26 for P3, P6, P72 and P450, respectively. The mean number of positive neurons in all counted sections for each postnatal age was utilised for the quantification. The positive neurons were quantified as strong when more than 50% of the neurons were positive, and ascribed +++; moderate when 10 to 50% of the neurons were positive, and ascribed ++; mild when less than 10% of the neurons were positive, and ascribed +; negative when none of the neurons was positive, and ascribed -. The results were validated using positive and negative controls.

RESULTS

Histology

Description of *corpora quadrigemina* on F60 and F90 (foetal period)

The lamina organisation of the rostral colliculus was evident and of the same presentation on F60 and F90. Three laminae were distinct. These were the outermost *stratum zonale*, middle *stratum griseum superficiale* and inner *stratum griseum profundum* (Figure 1(i)). The *stratum zonale* was made of immature neurons, devoid of dendrites and axons. The immature neurons were densely parked in the extracellular matrix to form a meshwork of thick fibrous layer (Figure 1(i): SZ). It was followed by the *stratum griseum superficiale*. This layer had immature neurons which were bigger, but fewer than those of *stratum zonale* (Figure 1(i): SGS). *Stratum griseum profundum* was the thickest of all the layers. There was a mixture of small and large immature neurons in this lamina (Figure 1(i): SGP).

The caudal colliculus was made up of all the components typical of a mammalian caudal colliculus (Figure 2). The dorsal cortex and central nucleus were elongated. The lateral cortex was also evident. The central nucleus of the caudal colliculus was made of immature migrating neurons, oval in shape, lacking distinct nucleus and evidence of axonal growth and synaptogenesis. These immature neurons were held in a chain-like fashion by the fibre shaft of radial glial in the extracellular matrix (Figure 3(i)).

Description of the *corpora quadrigemina* on P3 and P6 (neonatal period)

The histological features of the *corpora quadrigemina* was the same on P3 and P6. The lamina organisation of the rostral colliculus which was observed at the foetal periods was maintained in the neonates. However, unlike what was observed in the foetal periods, the neurons of the *stratum zonale* were already mature on P3. These neurons were densely packed in the extracellular matrix to form a meshwork of thick fibrous layer (Figure 1(ii): SZ). Neurons of the *stratum griseum superficiale* and *stratum griseum profundum* were mature; the *stratum griseum profundum* was the thickest of all the three layers, made up of large unipolar and multipolar pyramidal neurons with oval nucleus and well-delineated nucleolus (Figure 4(i): line arrow). Oligodendrocytes were visible in this layer (Figure 4(i): arrowhead).

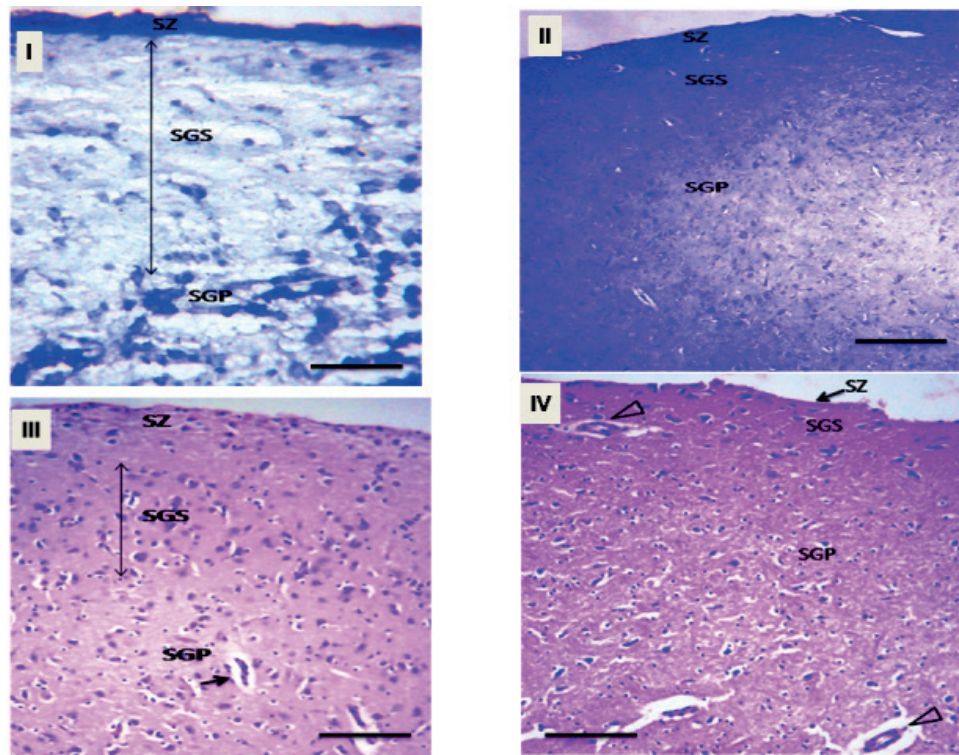
Neurons of the central nucleus of the caudal colliculus were mature at this period (Figure 3(ii): line arrow). There were a mixture of diverse sizes, but predominantly pyramidal in shape. The extracellular matrix was a mixture of chain-like strands and marshy network (arrowhead) unlike the chain-like arrangement, observed in the foetal periods. Oligodendrocytes were evident at this period.

Description of the *corpora quadrigemina* on P72 (juvenile period)

The lamina organisation of the rostral colliculus was maintained on P72. However, the *stratum zonale* had lost most of its fibrous nature, and was more cellular than fibrous. The cells resident in the *stratum zonale* were medium sized round unipolar neurons (Figure 1(iii): SZ). The *stratum griseum superficiale* was thicker than that observed in the neonates. Numerous blood capillaries (Figure 1(iii): black arrow) were observed in the *stratum griseum profundum*.

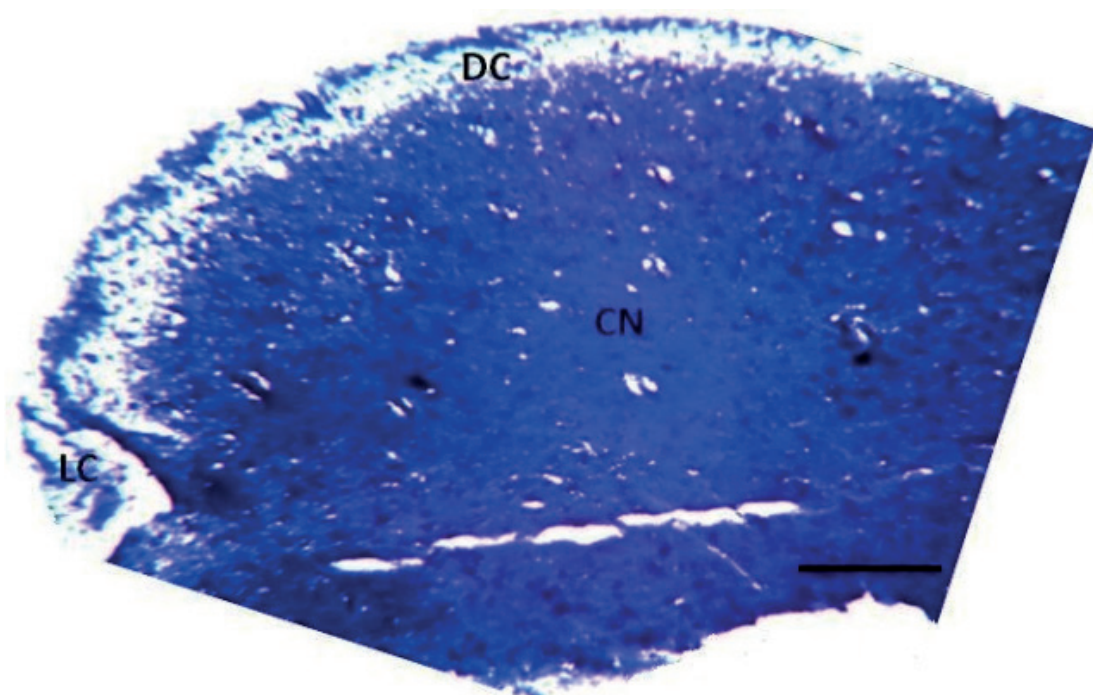
On a higher magnification, most of the pyramidal neurons in the *stratum griseum profundum* were bipolar, unlike the unipolar neurons observed in the *stratum griseum profundum* of the neonates. There were more pyramidal neurons per field, at this period, relative to those observed in the neonates (Figure 4(ii): black arrows).

Figure 1. Coronal section of the rostral colliculus of the African grasscutter brain on F90 (i), P3 (ii), P72 (iii) and P450 (iv).



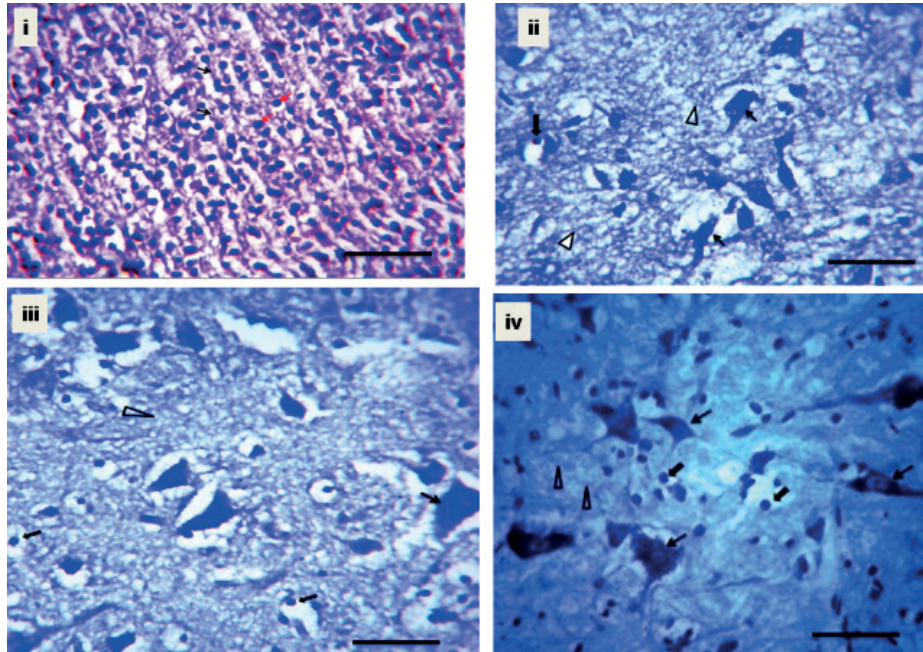
SZ: Stratum zonale; SGS: Stratum griseum superficiale; SGP: Stratum griseum profundum; black line arrow and arrow-head: Blood vessels. i: Cresyl fast violet stain; scale bar = 100 µm; ii: Cresyl fast violet stain, scale bar = 40 µm; iii and iv: Haematoxylin and Eosin stain; scale bar = 10 µm

Figure 2. Coronal section of the African grasscutter caudal colliculus of F90.



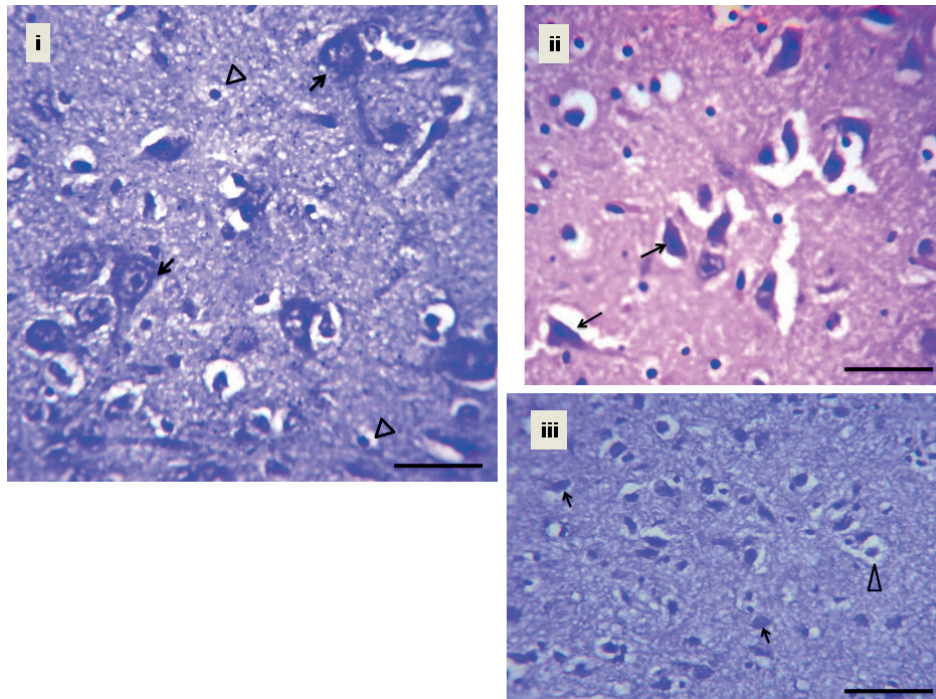
DC: Dorsal cortex. CN: Central nucleus. LC: Lateral cortex. Cresyl fast violet stain; scale bar = 40 µm.

Figure 3. Photomicrograph of the central nucleus of the caudal colliculus of the African grasscutter brain on F90 (i), P3 (ii), P72 (iii) and P450 (iv).



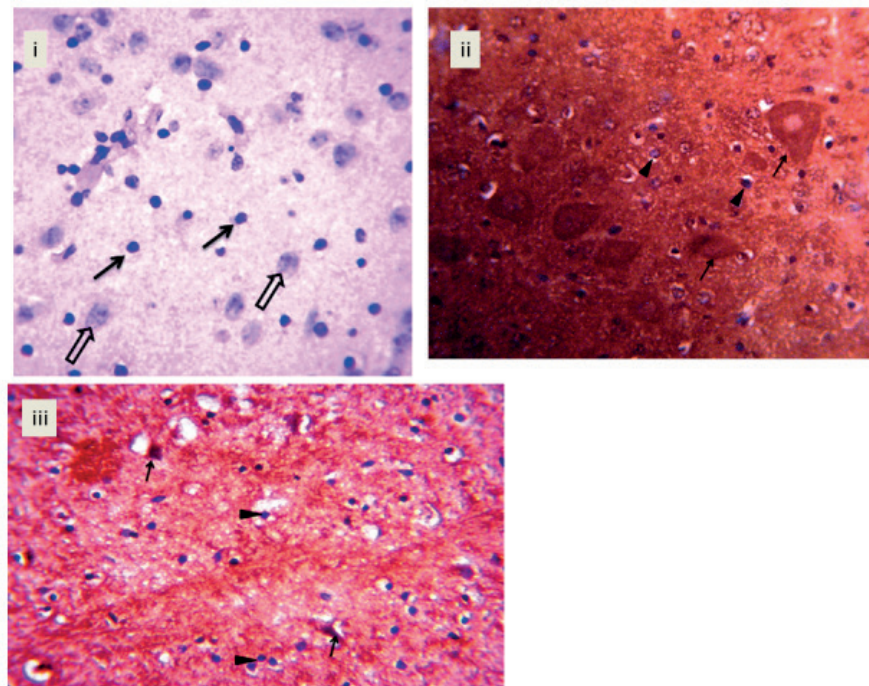
Black arrowhead on i: immature migrating neurons held in the extracellular matrix by fibre shaft of radial glial (black line arrows); Line arrow: Neurone; Block arrow: Oligodendrocytes; Arrowhead: Mature extracellular matrix. i: Haematoxylin and Eosin stain, x400; ii, iii and iv: Cresyl fast violet stain; scale bar = 100 μ m.

Figure 4. Photomicrograph of the *stratum griseum profundum* of the African grasscutter rostral colliculus on P3 (i), P72 (ii) and P450 (iii).



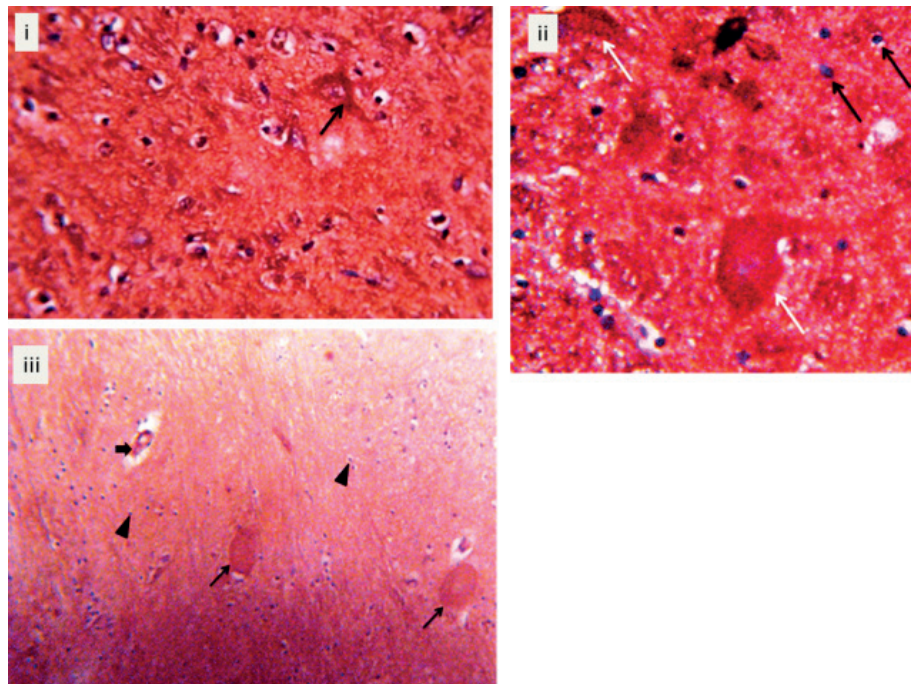
Black arrow: Neurons; Arrowhead: Oligodendrocytes. I and iii: Cresyl fast violet stain; scale bar = 100 μ m; ii: Haematoxylin and Eosin stain; scale bar = 100 μ m.

Figure 5. Immunoreaction of BDNF in the African grasscutter *stratum griseum profundum* on P3 (i), P72 (ii) and P450 (iii)



Block arrow: Negative immunoreactive neurons; Line arrow: Negative immunoreactive astrocytes; magnification: $\times 400$. ii: Line arrow: Strong immunoreactive pyramidal cells; Arrowhead: non-reactive glial cells (oligodendrocytes); magnification: $\times 400$. iii: Line arrow: Mild immunoreactive neurons; Arrowheads: non-reactive glial cells; magnification: $\times 400$.

Figure 6. Immunoreaction of BDNF in the African grasscutter central nucleus on P6 (i), P72 (ii) and P450 (iii):



i: Line arrow: Mild immunoreactive neuron; Magnification: $\times 400$. ii: White line arrow: moderate immunoreactive neurons; Black line arrow: non-immunoreactive glial cells; Magnification: $\times 400$. iii: Line arrow: Mild immunoreactive cells; Arrowhead: non-immunoreactive oligodendrocytes; block arrow: blood vessel; magnification: $\times 400$.

Neurons of the central nucleus of the caudal colliculus were mostly large, multipolar, and pyramidal, with distinct nuclei (Figure 3(iii): line arrow). The extracellular matrix was completely marshy (arrow head), unlike the chain-like arrangement observed in the foetal period, and the mixture of chain-like and marshy appearance observed in the neonates. Oligodendrocytes were also evident at this period.

Description of the *corpora quadrigemina* on P450 (adult period)

The lamina organisation of the rostral colliculus was maintained in the adult period. However, there was a continuous decrease in the thickness of the *stratum zonale*, such that it was reduced to a very thin layer in the adult period (Fig. 1(iv): SZ). The *stratum griseum superficiale* was less extensive than the *stratum griseum profundum*. There were numerous blood capillaries which were more than those observed in the rostral colliculi of the juveniles and far more than those observed in the neonatal periods.

On a higher magnification, it was observed that the neurons in the *stratum griseum profundum* were smaller in size, but more numerous than those observed in the same layer in the juveniles (Figure 4(iii): black arrow). The neurons varied from pyramidal to oval and round shapes. Oligodendrocytes were also observed in the layer.

The dorsal cortex and central nucleus of the caudal colliculi were elongated. The lateral cortex was also evident. The central nucleus was made of multipolar medium-sized neurons interspaced with small sized neurons (Figure 3(iv): line arrow). The large neurons were pyramidal, whereas the smaller neurons were a mixture of oval and circular cells. The neurons had well-delineated nuclei. The cells were embedded in a marshy extracellular substance. Oligodendrocytes were also observed.

Immunoreaction of BDNF in the *corpora quadrigemina* at different postnatal periods

The scoring of BDNF immunoreactive neurons in the different layers of the rostral and caudal colliculi is represented in Table 1 below. In the rostral colliculus, there was no BDNF immunoreactive cell in the *stratum zonale* at any postnatal period studied; there was a decrease in the number of BDNF immunoreactive cells in the *stratum griseum superficiale* such that a moderate immunoreactivity was observed on P3, a mild immunoreactivity on P6 and there was no reactivity on P72. However, on P450, the number of BDNF immunoreactive cells increased, resulting in a mild immunoreactivity. There was an increase in the number of BDNF immunoreactive cells in the *stratum griseum profundum* from a mild immunoreactivity on P3 and P6 (Figure 5(i)), to a high reactivity on P72 (Figure 5(ii)), and finally a moderate immunoreactivity on P450

(Figure 5(iii)). Only the large pyramidal neurons were BDNF immunoreactive in the *stratum griseum profundum*; the glial cells were non-reactive to BDNF.

In the caudal colliculus, the dorsal and lateral cortices were negative to BDNF at all postnatal periods. There was a moderate immunoreactivity in the neurons of the central nucleus on P3 and P72 (Figure 6 (ii)), and a mild immunoreactivity on P6 (Figure 6 (i)) and P450 (Figure 6 (iii)). The cell bodies and processes of the pyramidal neurons of the central nucleus on P6 were positive for BDNF. On P450, the BDNF immunoreactive cells were the large pyramidal neurons, while the glial cells were non-reactive.

DISCUSSION

Results of the present study showed that by F60 and F90, the neurons of the central nucleus of the caudal colliculi were still immature and migratory. This implies that these neurons are easily prone to neuronal ablation by any external insult. Thus, F60 and F90 are not safe periods for the administration of drugs or extracts to pregnant African grasscutter doe. This is to avoid abnormal development of the caudal colliculi and its resultant defects in the animal.

There was increased vascularisation of the *corpora quadrigemina* in adults than in juveniles and neonates. This may be linked to the high energy demand for cell metabolism. The progressive transformation of the extracellular matrix from the chain-like arrangement in foetal samples, due to the radial glial cells, to a mixture of chain-like and marshy appearance observed in the neonates, and finally, a completely marshy appearance in the juveniles and adults is as a result of a gradual differentiation of these astrocyte precursors as the brain develops (Barry et al., 2013).

A spatial immunolocalisation of BDNF in the *corpora quadrigemina* of the African grasscutter at different postnatal periods, and confinement of the protein to some specific midbrain neurons were recorded in this study. Most of the pyramidal neurons in the *stratum griseum profundum* were bipolar, unlike the unipolar neurones observed in the *stratum griseum profundum* of the neonates. Similarly, there was an increase in BDNF-immunoreaction from a mild status in neonates to a high value in juveniles. This is indicative of active axonal growth and synaptogenesis, as BDNF is implicated in the potentiation of synaptic plasticity (Tyler and Pozzo-Miller, 2003; Bartkowska et al., 2010). This work has further buttressed this role of the protein in the maintenance of neuronal synapse, and specific connection of neurons of the midbrain.

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 rostral colliculus

Table 1. Quantitative analysis of BDNF immunoreactive neurons in the *corpora quadrigemina* of the African grasscutter at different postnatal periods

Layers of Rostral and Caudal Colliculi	Scoring of BDNF Immunoreactive Neurons at Different Postnatal Periods			
	P3	P6	P72	P450
Stratum zonale	–	–	–	–
Stratum griseum superficiale	++	+	–	+
Stratum griseum profundum	+	+	+++	++
Dorsal cortex	–	–	–	–
Lateral cortex	–	–	–	–
Central nucleus	++	+	++	+

in the blind mole rat are collapsed to a single layer, suggestive of a poorly-developed visual system. Conversely, Telford et al. (1996) reported that the *stratum griseum* of Sprague-Dawley rats is further divided into *stratum griseum superficiale*, *stratum opticum*, *stratum griseum intermediale*, *stratum album intermediale* and *stratum griseum profundum*, suggesting a good eyesight. Similarly, Mensah-Brown and Garey (2006) recorded six laminae in the rostral colliculus of camels. They also concluded that visual system of the camel is well developed suggesting a good eyesight in the camel. The result of this study revealed only three layers in the rostral colliculus of the African grasscutter. This is suggestive of a poorly-developed rostral colliculus, indicative of poor eyesight. The caudal colliculus of the African grasscutter from this study was made up of the three divisions, typical of a developed caudal colliculus. This points to an acute acoustic system in this rodent.

There are other neuronal substrates of the visual and auditory sense, which were not subject of the present study. Some of the visual neuronal substrates are ocular sensory photo-receptors, optic nerve, optic chiasma, optic tracts, lateral geniculate nucleus, oculomotor nerve nucleus, trochlear nerve nucleus and abducens nerve nucleus, whereas some of the auditory neural substrates are cochlear nuclei, medial geniculate nucleus, superior olivary complex and medial trapezoid nuclei. The cytoarchitecture of these nuclei and other relevant neuronal substrates need to be investigated to further buttress the variation in the visual and auditory sense of the African grasscutter.

CONCLUSION

The present study has expounded the histology of the visual and auditory reflex centres of the African grasscutter. The study, which represents the first definitive immunolocalisation of BDNF in developing *corpora quadrigemina* of the African grasscutter, has added to the database of postnatal neurogenesis in rodents. Results of the study suggest a better auditory than visual capacity in the rodent, knowledge of which

may be beneficial in enhancing the breeding and domestication of the rodent. However, other neural bases of auditory and visual abilities need to be studied to buttress or disproof the present finding.

REFERENCES

- Addo P. G. (2002): Detection of mating, pregnancy and imminent parturition in the grasscutter (*Thryonomys swinderianus*). *Livestock Research for Rural Development* 14: 8–13.
- Asibey E. O. A., Addo P. G. (2000): The Grasscutter, a Promising Animal for Meat Production. In: Turnham, D. (Ed.) *African Perspectives, Practices and Policies Supporting Sustainable Development*. Weaver Press, Harare, Zimbabwe, 120 p.
- Aydin A., Yilmaz S., Ozkan Z. E., Ilgun R. (2008): Morphological Investigations on the circulus arteriosus cerebri in mole-rats (*Spalax leucodon*). *Anatomia Histologia Embryologia* 37: 219–222.
- Bałkowiec A., Białkowiec-Iskra E. (2010): Novel Approaches to Studying Activity-Dependent Regulation of Neurotrophins and Neuropeptides in Sensory Pathways from Orofacial Tissues. In: Daskalaki, A (Ed.). *Informatics in Oral Medicine*, pp. 46–63.
- Barry D. S., Pakan J. M., O'Keeffe G. W., McDermott K. W. (2013): The spatial and temporal arrangement of the radial glial scaffold suggests a role in axon tract formation in the developing spinal cord. *Journal of Anatomy* 222: 203–213.
- Bartkowska K., Turlejski K., Djavadian R. L. (2010): Neurotrophins and their receptors in early development of the mammalian nervous system. *Acta Neurobiologiae Experimentalis* 70: 454–467.
- Benraiss A., Chmielnicki E., Lerner K., Roh D., Goldman S. A. (2001): Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *Journal of Neuroscience* 21: 6718–6731.

- Bowers D., McKenzie D., Dutta D., Wheelless C. R., Cohen W. R. (2001): Growth hormone treatment after caesarean delivery in rats increases the strength of the uterine scar. *American Journal of Obstetrics and Gynecology* 185: 614–617.
- Bronner G. N., Hoffmann M., Taylor P. J., Chimimba C. T., Best P. B., Matthee C. A., Robinson T. J. (2003): A revised systematic checklist of the extant mammals of the southern African subregion. *Durban Museum Novitates*, 28: 56–95.
- Cooper H. M., Herbin M., Nevo E. (2004): Visual system of a naturally microphthalmic mammal: The blind mole rat. *Journal of Comparative Neurology* 328: 313–350.
- Gage G. J., Kipke D. R., Shan W. (2012): Whole animal perfusion fixation for rodents. *Journal of Visualized Experiments* 65: e3564.
- Gelfand S. A. (2004): Hearing, an Introduction to Psychological and Physiological Acoustics. Marcel Dekker, pp. 71–75.
- Hampstead B. L. (2006): Dissecting the diverse actions of pro- and mature neurotrophins. *Current Alzheimer Research* 3: 19–24.
- Ibe C. S., Ikpegbu E., Nlebedum U. C. (2017): Comparative morphology of the visual and auditory reflex centres in the African grasscutter (*Thryonomys swinderianus* – Temminck, 1827). *Italian Journal of Anatomy and Embryology* 122: 196–205.
- Ibe C. S., Ojo S. A., Salami S. O., Ayo J. O., Nlebedum U. C., Ikpegbu E. (2016): Cytoarchitecture and brain-derived neurotrophic factor immunolocalisation in the cerebellar cortex of African grasscutter (*Thryonomys swinderianus*). *Journal of Morphological Science* 33: 146–154.
- Ichikawa H., Yabuuchi T., Jin H. W., Terayama R., Yamaai T., Deguchi T., Kamioka H., Takano-Yamamoto T., Sugimoto, T. (2006): Brain-derived neurotrophic factor-immunoreactive primary sensory neurones in the rat trigeminal ganglion and trigeminal sensory nuclei. *Brain Research* 1081: 113–118.
- Loftus W. C., Malmierca M. S., Deborah C., Bishop D. C., Olive D. L. (2008): The cytoarchitecture of the caudal colliculus revisited: a common organization of the lateral cortex in rat and cat. *Neuroscience* 154: 196–205.
- Mensah-Brown E. P. K., Garey L. J. (2006): The superior colliculus of the camel: a neuronal-specific nuclear protein (NeuN) and neuropeptide study. *Journal of Anatomy* 208: 239–250.
- Nomina Anatomica Veterinaria (2012): International Committee on Veterinary Gross Anatomical Nomenclature, 5th Edition. Hannover Columbia.
- Opara M. N. (2010): Grasscutter I: a livestock of tomorrow. *Research Journal of Forestry* 4: 119–135.
- Safi K., Dechmann D. K. N. (2005): Adaptation of brain regions to habitat complexity: a comparative analysis in bats (*Chiroptera*). *Proceedings of the Royal Society London Series B*, 272: 179–186.
- Tarsa L., Bałkowiec-Iskra E., Kratochvil F. J., Jenkins V. K., McLean A., Brown A., Smith J. A., Baumgartner J. C., Balkowiec A. (2010): Tooth pulp inflammation increases BDNF expression in rodent trigeminal ganglion neurones. *Neuroscience* 167: 1205–1215.
- Telford S., Wang S., Redgrave P. (1996): Analysis of nociceptive neurones in the rat superior colliculus using c-fos immunohistochemistry. *Journal of Comparative Neurology* 375: 601–617.
- Tyler W. J., Pozzo-Miller L. (2003): Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. *Journal of Physiology* 553: 497–509.
- Walton M. M. G., Bechara B., Gandhi N. J. (2007): Role of primate superior colliculus in the control of head movements. *Journal of Neurophysiology* 98: 2022–2037.
- Williams O. S., Ola S. I., Boladuro B. A., Badmus R. T. (2011): Diurnal Variation in Ambient Temperature and Humidity in a Pit Pen Grass-Cutter (*Thryonomys Swinderianus*) House in Ile-Ife. *Proceedings of the 36th Annual Conference of the Nigerian Society for Animal Production, Abuja, Nigeria*, pp. 111–113.

Received: March 29, 2019

Accepted after revisions: September 18, 2019