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Neurone Expressions in Some Regions of Brain of the Helmeted Guinea Fowl (*Numida meleagris galeata)*: A Post-Hatch Study

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Abstract

Neurone expression in a developing brain of birds has been a near impossible scenario at several attempts in most of our research. Attempt to get the neurones in pre-hatch developing brain after many trials couldn't be achieved. This study was aimed at providing some basic information with regard to neuronal expression during developmental stages in the helmeted guinea fowl. Neuronal expressions were more pronounced at week 8 post-hatch development of the brain. The stellate neurones have soma with projection of dendrites without branching, while the basket neurones possess a soma which is relatively bigger than that of the stellate. Projections from Purkinje neurones cannot be differentiated from dendrites nor axon as a result of the darken nature. The Purkinje areas were surrounded by baskets neurones. Soma of the Golgi neurones was larger with distinct axon and dendrites. Neurones of arbor vitae display a large axon, few dendrites and an oval shaped soma.

Keywords: Neurone; Brain; Guinea fowl

Introduction

The vertebrate's nervous system is divided into two parts; the central nervous system (CNS) which consists of the brain and spinal cord, and the peripheral nervous system (PNS) which consists of the nerves that connect to the brain and spinal cord (cranial and spinal nerves)as well as the autonomic (or involuntary) nervous system [1].The brain being part of the nervous system is made up of several parts (cerebrum, mesencephalon, pons, cerebellum and medulla oblongata) which all obtain information about the internal and external environment, analyze and respond to information, store information and coordinate outgoing motor impulses to the viscera and skeletal muscles [2]. Developmentally, the central nervous system develops from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the developing embryo [3]. After neural tube closure, series of vesicles can be clearly distinguished morphologically at the anterior end indicative of an anterior-posterior axis development [4].The most anterior end of the neural tube gives rise to the procencephalon (forebrain) consisting of the telencephal on and diencephalon. The middle part of the brain is the mesencephalon, often referred to as the midbrain, while the most posterior region from the brain is the rhombencephalon, which further differentiates into metencephalon (cerebellum and pons) and myelencephalon (medulla oblongata and spinal cord [5]. Astroglia or astrocytes are characterized by a star-like appearance, having broad feet on their processes, contributing to the blood-brain barrier, and helping in the gliding of neurons during embryonic development, uptake of neurotransmitters, nourishing and protecting neurons against excitotoxicity [8].

The cerebellum, which develops from dorsal metencephalon, is foliated in mammals and birds [9]. In birds, the vermis divides the cerebellum into lobes, fissures and lobules with the primary lobules numbering I-X [10]. The cells in the cerebellar cortex are the granule, stellate, basket, Golgi and Purkinje cells with the most abundant type being the granule cells [11]. The ability of the bird to be active is based mainly upon the high sensory perception [12]. The high folding of the cerebellum influences the coordination and regulation of voluntary motor activity, sensory perception, balance in muscle tone and cognitive function [13]. The mesencephalon (midbrain) also comprises of the optic lobes which are the visual roof, often name doptictectum, in non mammalian species and superior colliculus in mammalian species [14,15]. The avian optic tectum and mammalian superior colliculus are homologous [16]. The optictectum is well developed, thick and highly laminated in birds [17,18]. The avian optictectum which exhibits more variations in position is important for motion processing, large receptive field, motion response, auditoryactivity and directional selectivity [19]. Most of the retinal ganglion cell axons in most birds with good optical acuity, project into the primary visual area, the midbrain [20]. The development of the brain is an important requirement for the survival of all birds because it controls the entire systems and most importantly, plays a vital role in skeletal movement, through continuous

sensory feedback of information concerning the effect of its action and auditory- visual precision [21]. The midbrain and cerebellum are concerned with sensori-motor (extra pyramidal) effects on the use of limbs (movement), sight (eye) and auditory (ear). All these functions depend on the type of nuclei found within these regions of the brain (cerebrum, mesencephalon and cerebellum) [22]. The survival of animals, including birds, depends on their hearing ability, sight, and precision to correctly identify, process the most important information at every point in time so as to engage their muscular activity [23]. However, basic information on the developmental features of the cerebrum, mesencephalon and cerebellum of the helmeted guinea fowl (HGF) is not fully available. Information obtained may go a long way to explain some of its peculiar behaviours.

Materials and Methods

462 fertilized guinea fowl eggs were purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State, Nigeria and other local breeders within Jos and its environs. The eggs were transported to a hatchery, still in Jos and incubated using their standard in cubation guide. During in cubation, the eggs were turned regularly (minimum of three times) each day for the first 24 days.

Three hundred and eight (308) eggs (Eleven eggs per day) for pre-hatch study were collected from days one, two, three, four, five, up to day twenty eight (28) which is the last day for pre-hatch collection. A small opening was made on the large air space area and the entire egg dropped into a labelled container of 10 percent buffered formalin for proper fixing.

For post-hatch study, one hundred and fifty four (154) eggs were allowed to hatch into young ones referred to as keeps and were removed from the incubator from day one post-hatch and kept in a cage. Immediately after hatching, brain samples were collected on consecutive days for seven days (one week). Subsequently, each week, eleven brain samples were collected for a period of seven weeks. After the period of collections, samples of histological preparation and immunoh is to chemistry were taken to the Histology Unit, NVRI, Vom, Plateau State and Department of Histopathology, Ahmadu Bello University Teaching Hospital, Shika, Zaria, respectively.

Extraction of embryo

This was done at pre-hatch using a scapel blade and clean transparent dish. The blunt side of the scapel blade was used with the egg held on the palm, and a gentle tap made on the egg until a crack was formed. Then, the crack was gently widened manually and the embryo collected in a transparent dish.

Extraction of brain

At pre-hatch, because the entire skull is soft and pliable, scapel blade and rat tooth forceps were used for extraction of the brain. At post-hatch, the keets were euthanized using Nembutal at 40 mg/b. wt. Thereafter, decapitation was made and the heads fixed in 10% neutral buffered formalin for 3 – 5 days. After proper fixation, a dissection was made at the angle of the beak up to the level of the occipital bone. The upper portion of the dissected area is pulled off gradually using the rat tooth forceps until the entire brain was exposed. The cranial nerves were severed to ease the lifting of the brain from the cranium. Some of the extracted brains were fixed in Bouin's solution (comprising of 75 ml picric acid, 25 ml formaldehyde and 5 ml glycial acetic acid) for routine staining and while others were fixed in 10% neutral buffered formalin for immunohisto chemistry.

Separation of the cerebrum, midbrain and cerebellum

The cerebellum is located on the dorsal portion of the brain stem with three peduncles: therestiform body connected to the medulla, the brachium points that connects cerebellum to the Pons and the brachium conjunctivum that connects cerebellum to the midbrain. These peduncles were severed using a scapel blade to expose the entire brainstem.

The midbrain or mesencephalon is located just caudal to the level of chiasmaopticumrostrally and at the level of ponto-mesencephallic region caudally. After the midbrain removal, the anterior portions left are the diencephalon and the cerebrum. At the boundary between the diencephalon and cerebrum, an incision was made revealing the cerebral hemispheres held together by the corpus callosum. Each hemisphere was freed by a transverse incision into the longitudinal fissure. These land marks were cut a cross using the scapel blade to extract the mid brain. Nomina Anatomica Avium was used for anatomical nomenclature [24].

Gross anatomy and morphometry

Two hundred and ninety four (196 for pre-hatch and 98 for post-hatch) brain samples were used. The weights of the whole bird, brain, cerebellum and midbrain were taken using digital electronic balance; (Model JJ1000, Max. 100 g, d=0. 01 g, e=10 g, No. 211011011098, Made in China and Analytical Weighing balance, Adventure QHAUS Corporation, Item No. AR3130, Max. Capacity= 310 g, Readability= 0. 001 g, Made in China).Length of the whole brain was taken using digital verniercaliper. Photographs of the dorsal and ventral aspects were taken using cannon digital camera (4x optical zoom lens 5. 0 - 20. 0 mm, 15. 1 mega pixels Apple, Cannon) and Digital Handheld Microscope, (Magnification1000x,5xZoom,3DstandhighspeedDSP,Madein China). Weights and lengths were all recorded in grams (g) and centimetre (cm), respectively.

Histological techniques

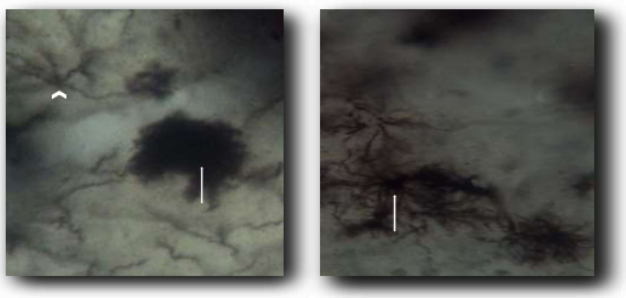
Eighty-four (84) (56forpre-hatchand28forpost-hatch)brain samples were used. For Hematoxylin and Eosin (H & E) technique, fixed brain samples were washed using tap water and dehydrated through ascending grades of alcohol (70%, 80%, 90%, 95%, 100%), within intervals of three hours each, cleared in xylene for two hours and embedded in liquid paraffin at 500 C according to standard procedures as described by Kiernan. Serial transverse sections of 5µ were made using Jungrotary microtome (Model42339, Berlin, Germany). Sections were

mounted on glass slides and allowed to dry, deparaffinized, stained, hydrated and covers lipped using diphynyl pthalate propylene xylene (DPX) as mountant. Sections were stained with H&E stains for routine general study, cresol fast violet (CFV) stainfornuclei identification. For Golgi staining technique, the modified Golgi staining technique was used; tissue blocks were taken after fixation and were treated with 2% potassium dichromate for two to five days in a dark cabinet and thereafter tissues were washed using tap water. This was followed by; dehydration, clearing, infiltration, embedding and sectioning at 25 µ. Sections were dewax and mounted in DPX. Photomicrographs of sections were taken using USB digital eyepiece (Amscope DCM 500, Resolution: 14 Mega Pixels, made in China) attached to a light microscope (OLYMPUS XSZ107BN, Ham burg, Germany),viewed and captured on the Laptop screen at different magnifications (x40, x100, x250 x400 and x560).

Results

Golgi technique for cerebellum

Post-hatch features: Using Golgi staining technique, the neurones of the molecular layer exhibited different morphology. The stellate neurone was the uppermost having a small round body, referred to as the soma, and its dendrite was observed to be filamentous, each projecting from the soma without it branching thus making it difficult to differentiate the axon from dendrites. The basket neurones were located below stellate neurones with few occasionally found between stellate neurones. The basket neurone possesses a soma which is bigger than that of the stellate. Its dendrite has numerous branches round the soma (Plate XLVI) shown in Figure 1. The Purkinje neurones were observed to be completely black or darkened, making it difficult to distinguish their various projections from the soma. Basket neurones were seen to surround the periphery of each Purkinje neurones (Plate XLVII) given in Figure 2. Golgi neurones were the only neurones to have the largest soma, compared to the neurones of the molecular, Purkinje and granular layers. Their dendrites and axons were distinct from each other unlike those of basket, stellate and Purkinje neurones. The axon of the Golgi neurone was larger exiting from one end of the soma with un branched dendrites. In the granular layer, the Golgi neurones were larger neurones while the granular neurones were more, found scattered among the Golgi neurones having a long axon, prominent soma and few cylindrical dendrites (Plate XLVIII) and (Plate XLIX) are shown in Figures 3 and 4 respectively. The arborvitae is part of the deep cerebella layer where neurone appears to display a large axon, few dendrites and a soma whose is oval in shape (Plate L).



A

Figure 1: Plate XLVI: Golgi technique showing neurones of the molecular layer of the cerebellum at week 8 post-hatch, (A)1; Purkinje neurones, Arrowhead; Soma of basket neurone, (B)Arrow; Soma ofstellate neurone. Golgi stain, X560.

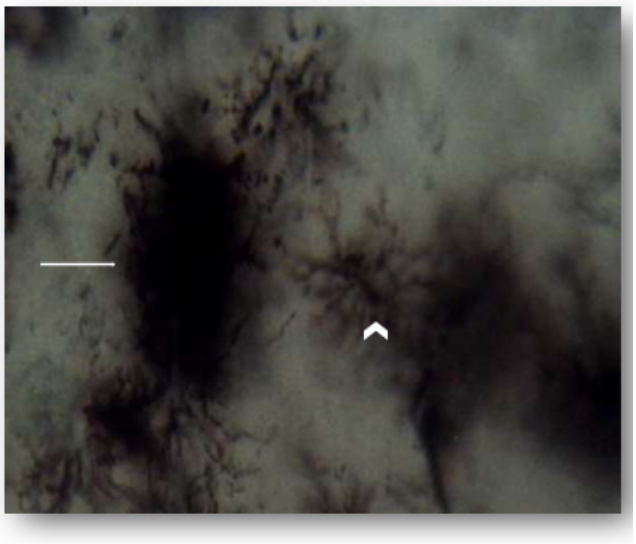


Figure 2:PLATE XLVII: Purkinje neurone of the cerebellum at week 8 post-hatch, Arrow; Purkinje neurons, Arrowhead; Soma of basket neurone, Golgi stain, X560.

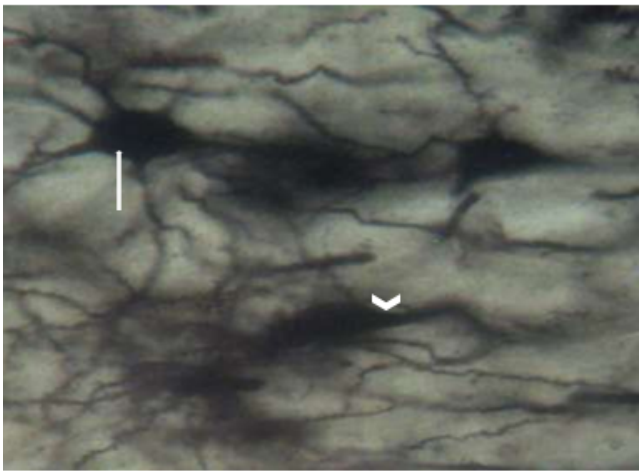


Figure 3:PLATE XLVIII: Neurone of granule layer of the cerebellum, Arrowhead; Axon of Golgi neurone,Arrow; Soma of Golgi neurone, week 8 post-hatch, Golgi stain. X560.

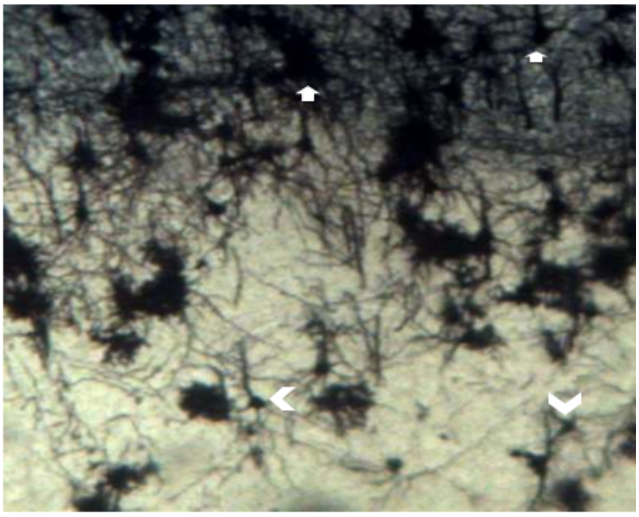


Figure 4: PLATE XLVIII: Neurone of granule layer of the cerebellum, Arrowhead; Axon of Golgi neurone, Arrow; Soma of Golgi neurone, week 8 post-hatch, Golgi stain. X560

Discussion

The basket neurone of the helmeted guinea fowl was observed to have a soma from where it had multiple processes projection. At this stage of development, it was difficult to differentiate dendrites from the axon. They were seen to send processes proximally and their terminal ends where not clear. In the mammals, the basket neurones are located in the lower third of the molecular layer. They have a typical oval soma which is large and in elephant it measures up 444 μm^2. Basket neurones are always characterized by moving horizontally a short distance before curving toward the pia surface in a typical hand fan shape. Axons can be visible in some neurones, thus making them traceable over a distance. These axons can move sometimes above the Purkinje cell layer and be seen to terminate in multiple pericellular nests with paintbrush tips around the soma of the Purkinje cells [25]. These cells have a more complex morphological appearance in human, when compared to other forms of vertebrate in terms of their dendritic disposition. In the cerebellum of the helmeted guinea fowl at week 8, the basket neurone axon is not clearly featured. It only becomes clearer during the weeks ahead.

The stellate neurone in this study is observed to have numerous branchedprocesses without a distinctive axon and distinct somata. In other vertebrates like the cats and chimpanzee, the stellate neurones are found in the uppermost portion of the molecular layer which possesses globular soma that is smaller except for the granular neurone [26]. Unlike what is seen in the cerebellum of the helmeted guinea fowl, the basket neurone is smaller than the granule neurone. The presence of their twisted processes is consistent with those reported in some vertebrates [27].

The Purkinje neurone in the cerebellum of the helmeted guinea fowl was obscured and neither the dendrites nor the axon could be seen. However, in normal presentation it is observed to have a dendrite, cell body and an axon. In the humpback whales, there exists another form of dendrite, the tertiary dendrite, whose branches tend to ascend to the pial surface in a linear, bob-twisting form, and hence, appearing convoluted [28].

Large neurones with a well-defined dendrite, cell body and an axon in the granular layer of the cerebellum were seen sending their axons downward. These axons terminated shortly after exiting from the cell body. The body was the largest in all the neurones observed in the cerebellar cortex. In the leopard and chimpanzee, the Golgi neurones were located close to the Purkinje layer, though they were found to be located in the deeper part of the granule layer [29]. They have a shapeless to a triangular-like cell body. The dendrites are sometimes thick and branched. The neurones of the granule cells had small round to oval bodies, dendrites and axons directed toward the Purkinje layer. The granule neuron extended to be more in number, smaller stand interposed with the Golgi neurones in the granule layer. In most vertebrates most especially the mammals, the granular neurones were deeply seated and were the smallest neurones. Their morphological appearance was similar to those reported by [30] and was characterized by small round cell bodies.

Conclusion

Neurones of the cerebellum for the first time to the best of my knowledge were demonstrated in the cerebellum of the helmeted guinea fowl. Most of the neurones demonstrated clear features only in the Purkinje neurons. Post-hatch expression of neurones was clearly demonstrated at week 8 of brain development.

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