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Comparative evaluation of cerebellum development between Japanese quail and albino rats

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Abstract

The surrounding environmental conditions are affected the cerebellum development among different vertebrates. In this study, we attempt to evaluate the differences in cerebellar histogenesis between Japanese quail and albino rats (post-hatching postnatal developmental assay). Four ages from each animal species (6 individuals for each age) were used in this study. These ages included 1, 7, 16, and 30 days for quail and 1, 7, 21, and 60 days for rats. The different age stages vary for each species. Blood samples were collected from sacrificed experimental animals to estimate the antioxidants. Then the animals were dissected to remove the cerebellum for histological and transmission electron microscope investigations. The histological results revealed that the cerebellar cortex of Japanese quail reaches full histogenesis before PHD-7, while the full histogenesis of the rat cerebellum occurs after PND-7. Likewise, the cerebellar cortex of rats showed obvious branching of folia with age progress if compared with that of the Japanese quail. Comparatively with Albino rats, the cerebellar cortex cells of Japanese quail showed remarkable myelination and lysosomes but less density of RER and mitochondria.

Keywords: Quail, albino rats, cerebellar development, histogenesis, TEM.

INTRODUCTION

In the central nervous system (CNS), the neurogenesis stages involve cell production, differentiation, and migration for the integration with the circuitry of the existing neurons **[1, 2]**. It has been well documented that, the neurogenesis of the adult mammalian brains is confined to the subventricular area of the lateral ventricle and the subgranular area of the hippocampus **[3, 4].** The new neurons of the avian brains are produced along the lateral ventricle walls, and then move to the different regions of the telencephalon **[5,6].** Since avian species can sophisticate cognition and social and motor behaviors, thus that they have enlarged and complicated brains **[7].**

Precocial or altricial animal species can be categorized during birth or hatching based on the level of their behavioral dependence on parental care and maturation development. Examining the mode of development is crucial for the deep understanding of the evolutionary pathways that result in the adaptation of different animal species and their interaction with the environment, as well as serving as a means to shed light on the knowledge origins **[8].** In altricial species, the sensorial systems mature during the postnatal stage, where they depend on their mothers to survive. Also, they are usually blind, and cannot regulate their body temperature. While the precocial species can move freely and stand

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instantly after birth. So, they require a shorter time for maternal nursing [9].

This work was conducted using two different species, Japanese quails (*Coturnix Japonica*) as well as albino rats (*Rattus norvegicus*). As Japanese quails exhibit many special characteristics like, rapid growing up, limited living space with less feed requirements, and high disease resistance, therefore They were chosen as an ideal species model [10]. Furthermore, they are one member of the Galliformes order that is considered a precocial avian species [11]. For rats, they have an altricial pattern of development [12].

MATERIALS AND METHODS

1. Experimental design

This study focused on the evaluation of comparative anatomical, histological, and ultrastructural aspects for post-natal (PND) development of the brain for two different species: male Japanese quail *Coturnix japonica* (order: Galliformes, family: Phasianidae, and male albino rat *Rattus norvegicus* (order: Rodentia, Family: Muridae). This study included PND 1, 7, 14, 21, and 60 for rats, while for quail the study was applied on PHD 1,7,16 &30 for Japanese quail. The offspring of rats were obtained from the animal house faculty of Pharmacy, Mansoura University. On the other hand, the quail offspring were obtained from the veterinary Faculty, at Mansoura University. The offspring from both species were investigated accurately to discriminate any diseases and abnormalities. The healthy offspring at different ages were transferred to a comparative lab, then anesthetized for collection of the blood samples and dissected to remove the whole brain. The blood was centrifuged at 3000rpm and processed for evaluation of biochemical analysis. The brain from each species age was divided into two portions, one of these portions was used for histological and immunohistochemical investigators while the other portion was used for the evaluation of proliferating cellular nuclear antigen (PCNA).

The present work studies the postnatal development of the cerebellar cortex, of two different species: male Japanese quail and male albino rat. Japanese quails were used at constant ages of 1, 7, 16, and one month old. While albino rats were used at 1, 7, 14, 21, and two months old (sexual maturation age) [13]. Each age from two studied species was represented by ten individuals.

The two studied species (at different ages as previously mentioned) were gently euthanized and then dissected following the rules of the guidelines of Experimental Animal Ethics Committee of Mansoura University, code number: Sci-Z-ph-2021-63. After that, Blood samples were collected and processed for biochemical parameters. Once dissected, the whole brain was carefully excised for evaluation of the histological, ultrastructural, and immunohistochemical investigation and exploring the apoptosis variation within the different ages of the two species.

2. Histological investigations:

After euthanasia, brain (cerebellum) samples of the two selected species at different ages were separated and immediately fixed in neutral 10% formalin (PH 7.4), then dehydrated in ascending series of ethyl alcohol, cleared in xylene followed by impregnating with paraffin wax (58–62°C). After this, the samples of the tissue blocks were cut at a thickness of 5 µm, deparaffinized, rehydrated, and stained using hematoxylin and eosin following the standard methods of ***Bancroft et al*** [14]. Finally, histological sections were examined and photographed using a bright field Olympus light microscope (BX Series, Japan) connected to a digital camera.

3. Transmission electron microscopic (TEM) investigation

TEM examination was focused only on the cerebellar cortex at PND 16 and 30 for Japanese quail while that examination was applied at PND21 and 60 for rats. Cerebellar tissue sections were fixed in cacodylate buffered glutaraldehyde (2.5%, pH 7.4), post-fixed in 1% buffered osmium tetra-oxide for 2 hours, then dehydrated using an ascending series of ethyl alcohol, then cleared in propylene oxide followed by embedding in epoxy–resin. Subsequently, ultrathin sections were cut and collected on copper grids. These sections were stained using lead citrate and uranyl acetate, and examined by using a Joel 1000CX transmission electron microscope (Mansoura University EM unit).

4. Immunohistochemical labeling of caspase-3 in the cerebellar cortex

The cerebellum paraffin blocks of both quails and rats at different stages were cut at 5 µm thick, then mounted onto positively charged slides, de-paraffinized, and then rehydrated in a descending series of ethyl alcohol, after which they were washed in PBS. The activity of endogenous peroxidase was inhibited using 3% H_2O_2 in methanol at room temp for 40 minutes. The sections of the tissue samples were retained and processed for antigen retrieval by digestion in 0.05 % trypsin at room temperature. The sections were incubated for 45 min with diluted 1:10 monoclonal primary antibody (Anti-caspase3; clone DO-7 Dako) after washing in TRIS buffered saline (TBS), pH 7.6. Slides were then rinsed in PBS and subsequently incubated in the presence of the secondary antibody for 20 min. For all sections, the complex sites were shown brown using 3, 3 diaminobenzidine tetrahydrochloride with fresh hydrogen peroxide substrate. The cerebellar sections were counterstained with Mayer's hematoxylin, mounted, and photographed by phase-contrast light microscopy. Incidences of cellular accumulations of caspase3 protein were determined.

Image analysis: The incidence of cellular accumulations of **caspase-3** proteins was determined for each age species. Additionally, the images were analyzed on an Intel® Core I7®-based computer using Video Test Morphology® software (Russia) with a specific built-in routine for area, % area, measurement, object counting, and contact Angle.

5. Flow cytometric detection of Proliferating cellular nuclear antigen (PCNA) in the cerebellum:

Cell suspensions from the cerebellar tissue at different ages of both rats (7, 21, 60 days old) and quail (7, 16, and 30 days old) were prepared with Tris-EDTA buffer (pH7.4) (Sigma-Aldrich Co.). Centrifugation of the cell suspension lasted for 10 minutes at 1,500 rpm, then fixed in ice-cold 96-100 % ethyl alcohol at 4 ºC overnight, after which re-suspended in PBS containing 50 µg/mL propidium iodide (PI) (Sigma-Aldrich Co.). Given that the analysis relied on a measurement of 10,000 cells for every sample. Cerebellum samples from each age were processed into single-cell suspensions, and 1.5–3 $X10^6$ cells were subsequently stained for expression of the designated lineage markers (PCNA). The investigation of the DNA/PCNA relationship was conducted using a FACS-can flow cytometer (Becton Dickinson, San Jose, CA). The DNA/PCNA distributions were shown in dot plots using a 1,023 x 1,023 channel array. The examination of the cells was conducted using the Cell FL1-H computer software developed by Becton Dickinson. The quantification of PCNA was performed using specific cell cycle phases using gating analysis of the data based on DNA content. The experiments were replicated a minimum of three times.

6. Statistical analysis:

The data was presented using analysis of variance (ANOVA) followed by post hoc analysis with the SPSS (version 15) software package for Windows. Differences among calculated values are considered significant at a significance level of $p < 0.05$.

RESULTS

1. Histological investigations of cerebellum

At PHD-7, 16, and 30 days of Japanese quail, the cerebellar cortex appeared well-differentiated into three distinct layers: the outer molecular layer, the inner granular layer, and an intermediate layer of Purkinje cells. The outer molecular layer showed sparsely distributed basket cells with their characteristic basophilic cytoplasm. The molecular layer increases in thickness with age. The granular layer displayed a high density of deeply stained basophilic granular cells. This layer decreases in thickness with age. Purkinje cell layer localized at the junction between the granular and molecular layers with its characteristic acidophilic large pyramidal-shaped cells. Purkinje cell processes extend into the molecular layer, and their bodies rest on the granular layer. Regarding the differences among the three layers, the molecular layer increased in thickness while the granular layer showed the reverse with the progression of age. Likewise, Purkinje cells showed a remarkable increase in their size (Figure 1).

At PND-7 of rats, the cerebellar cortex showed a remarkable difference with PH-7day of Quail

whereas it is differentiated into four distinct cell layers: the outer granular layer, the molecular layer, the Purkinje cell layer, and the inner granular layer. In addition, the basket and granular cells appeared more basophilic stained if compared with those of Quail. At PND-14, the cerebellar cortex showed remarkable fusion between the outer and inner granular layers to become one layer of granular cells while the molecular layer migrated to the outer and the Purkinje cells became oval and situated at the junction of two layers. With age progress (PND-21 and two months), the cerebellar cortex showed remarkable branching of folia as well as the Purkinje cells became larger with their characteristic acidophilic cytoplasm (Figure 2). Comparatively, the cerebellar cortex of Japanese quail reaches the full histogenesis before PHD-7 while the full histogenesis of rat cerebellum occurs after PND-7. Likewise, the cerebellar cortex of rats showed obvious branching of folia with age progress if compared with that of Japanese quail.

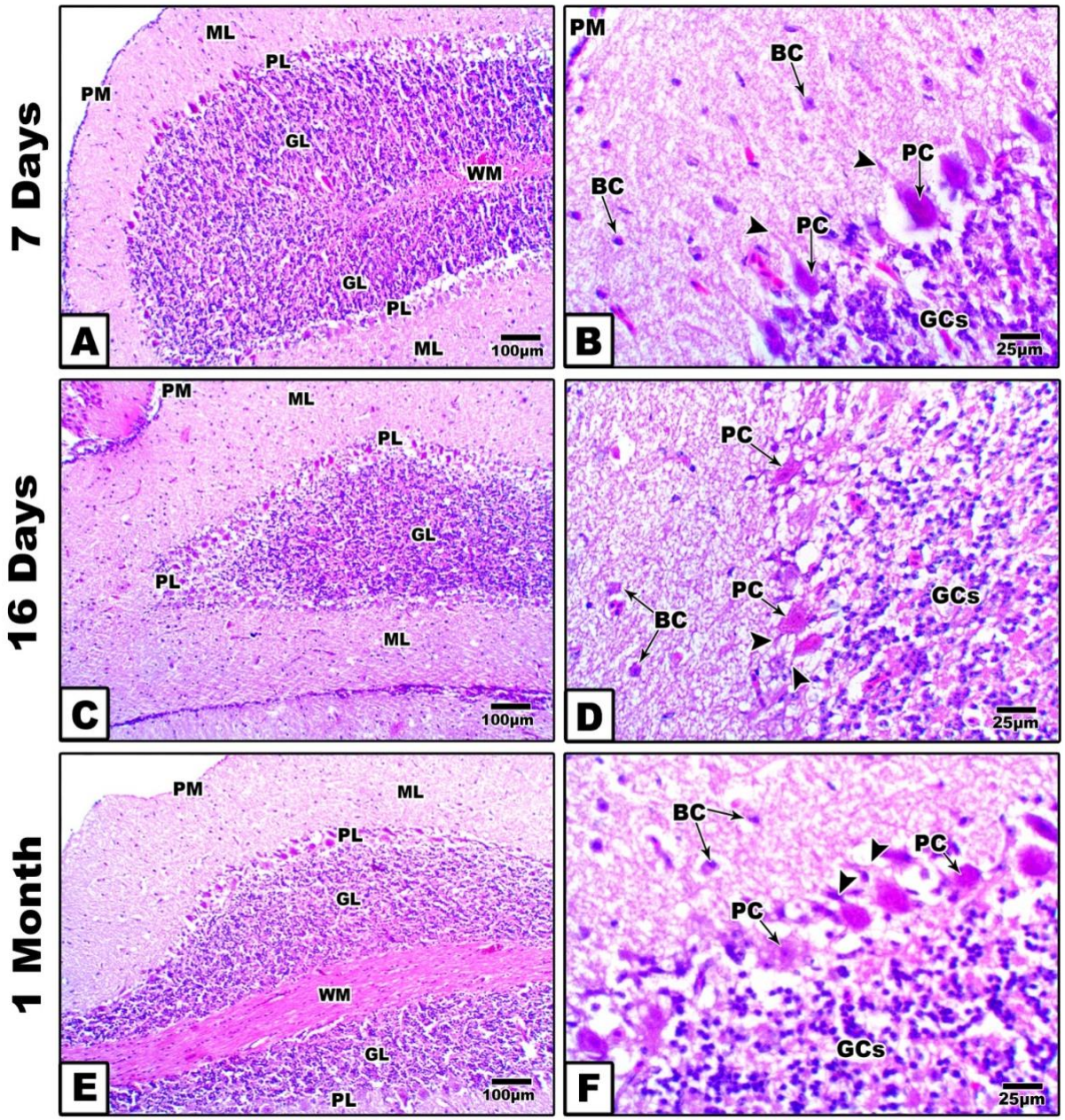

Fig1. Images of histological sections from the cerebellum of Japanese quail at post-hatching days 7, 14, and30 show the three distinct cell layers of the cerebellum: the molecular layer (ML) that is enclosed by pia matter (PM) and contains sparsely distributed basket cells (BC), the Purkinje cell (PC) layer with their pyramidal form cells, and the granular layer (GL) with darkly stained packed granular cells (GCs). An obvious decrease in the thickness of ML and a decrease in the thickness of GL also appear with age. The white matter (WM) is indicated in the core of the folium. The arrowheads point to the dendrites of Purkinje cells.

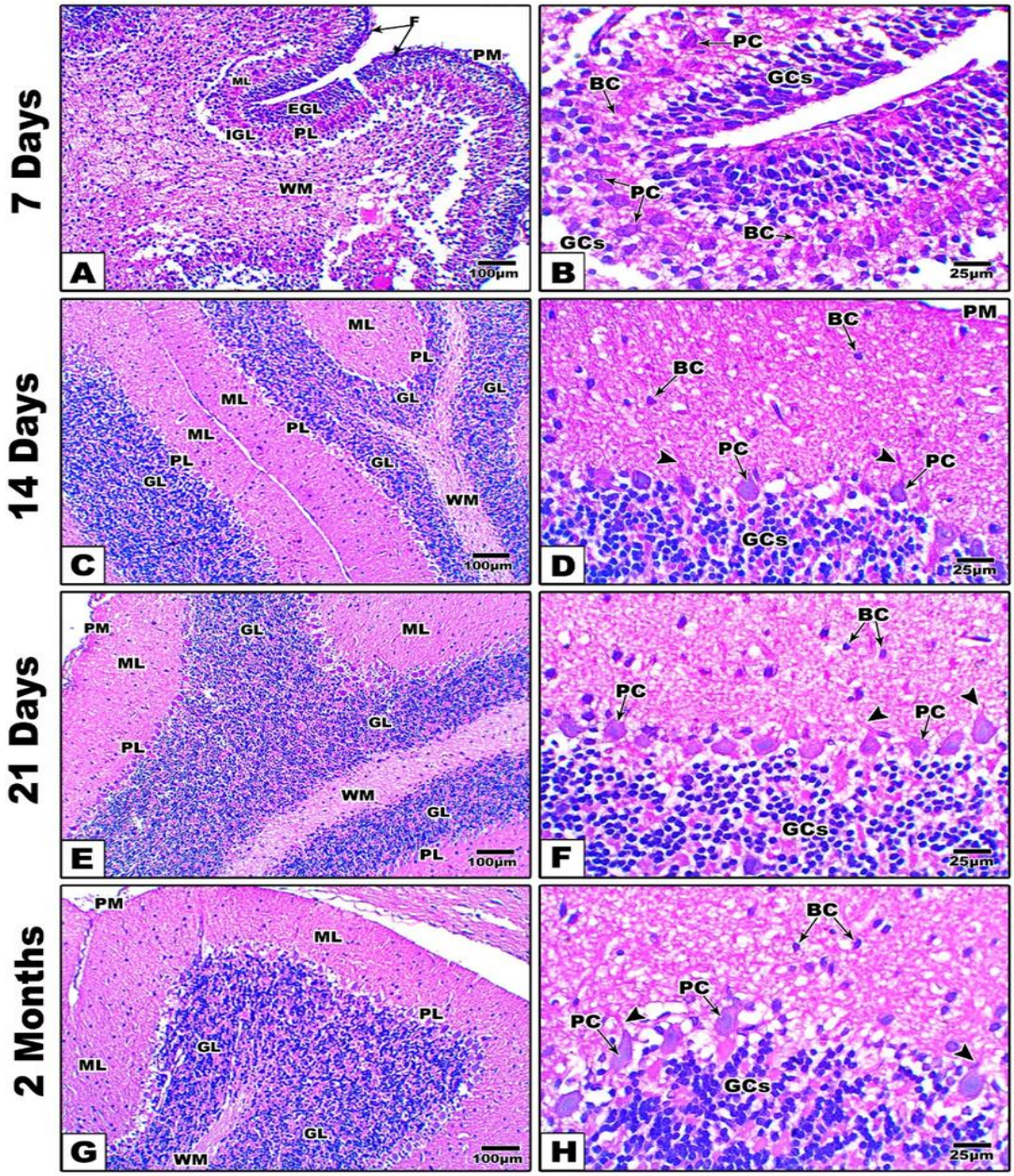

Fig2. Histological micrograph of the cerebella of albino rats at post-natal days (7, 14, and 21) and two months of age. At PND-7, the cerebellar cortex reveals incomplete histogenesis of the granular layer, which is still separated into the external granular (EGL) and inner granular (IGL) layers. At PND 14, 21, and 60, the three distinct cell layers of the cerebellum are well differentiated into the outer molecular layer (ML), which is enclosed by pia matter (PM) and contains sparsely distributed basket cells (BC), the middle Purkinje cell (PC) layer with its oval-shaped cells, and the granular layer (GL), with darkly stained packed granular cells (GCs). The white matter (WM) is indicated in the core of each folium. The arrowheads point to the dendrites of Purkinje cells.

2. Ultrastructural observation of the testis

Our TEM investigations included the three major types of cerebellar cortex cells (basket, Purkinje, and granular cells) for only two ages of each species (PHD16 and 30 for Japanese quail and PND21 and 60 for albino rats). At PHD-16, the cerebellum basket cells showed a high density of rough endoplasmic reticulum (RER), oval mitochondria, and remarkable myelination (Figure 3, A). The granular cells appeared condensed and darkly stained with characteristic multiple and small-sized mitochondria, lysosomes, and little RER (Figures 3, B&C).

Purkinje cells represented the largest cell type in the cerebellar cortex and showed remarkable myelination, especially at the junction with the molecular and granular layers. Additionally, the cytoplasm showed widely distributed mitochondria and RER (Figure 3, D). At PHD30 of Japanese quail, the myelination decreased while the density of the mitochondria, lysosomes, and RER were markedly increased if compared with those of PHD-16 (Figures 3, A1–D1).

Regarding the TEM investigation of albino rats' cerebellum, the three categories of cerebellar cortex cells showed a high density of large mitochondria and obvious condensation of RER however, the myelination and lysosomes were poorly found (Figure 4).

Comparatively with Albino rats, the cerebellar cortex cells of Japanese quail showed remarkable myelination and lysosomes but less density of RER and mitochondria.

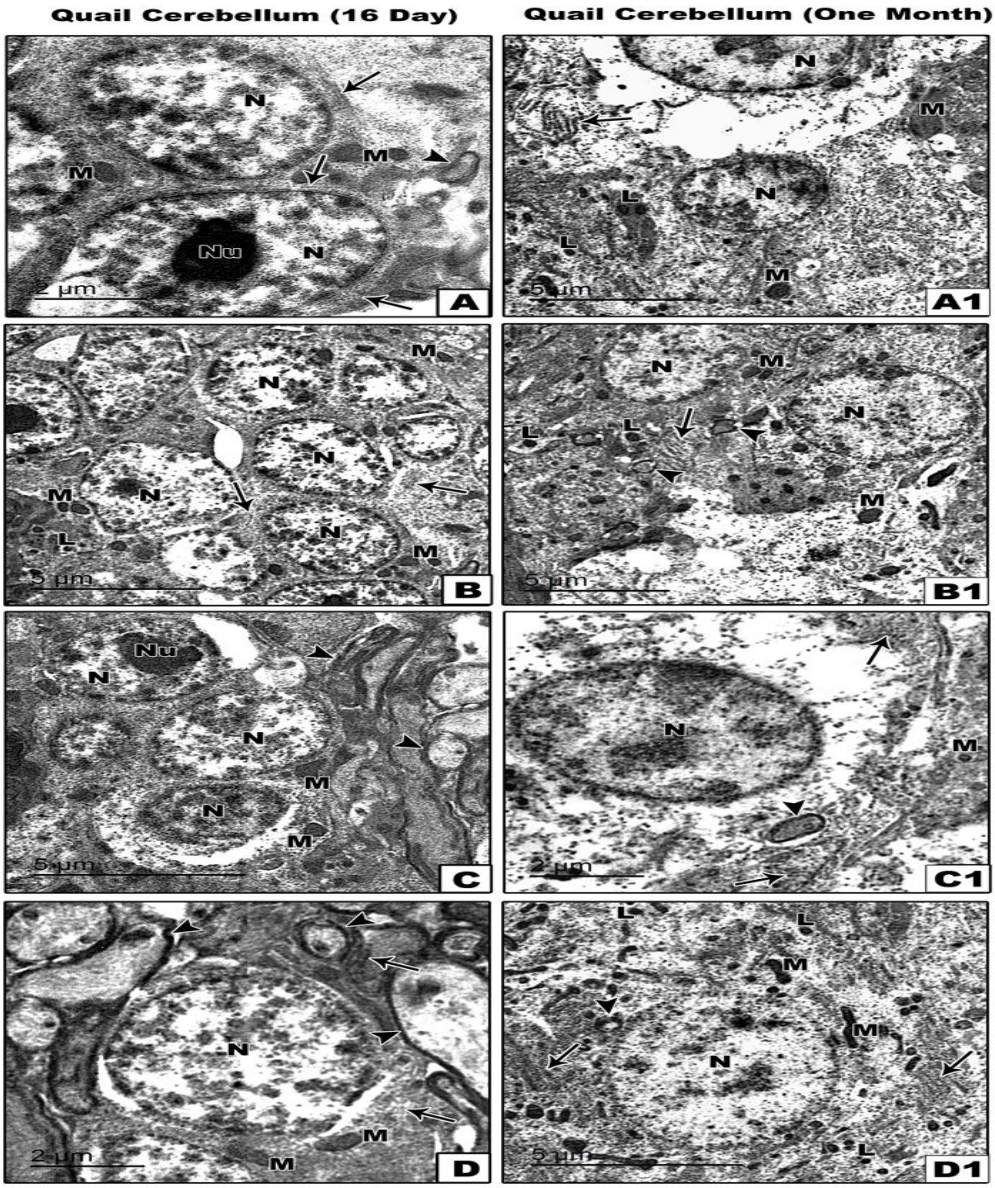

Fig 3. Transmission electron micrographs (TEM) of the cells of cerebellar cortex from Japanese quail at PHD-16&30. Panel (A, A1): basket cell, panels B, B1&C, C1: granular cells, and panels D, D1: Purkinje cell. N: nucleus, M: mitochondria, L: Lysosomes, myelinated axons (arrowheads), and rough endoplasmic reticulum (arrows).

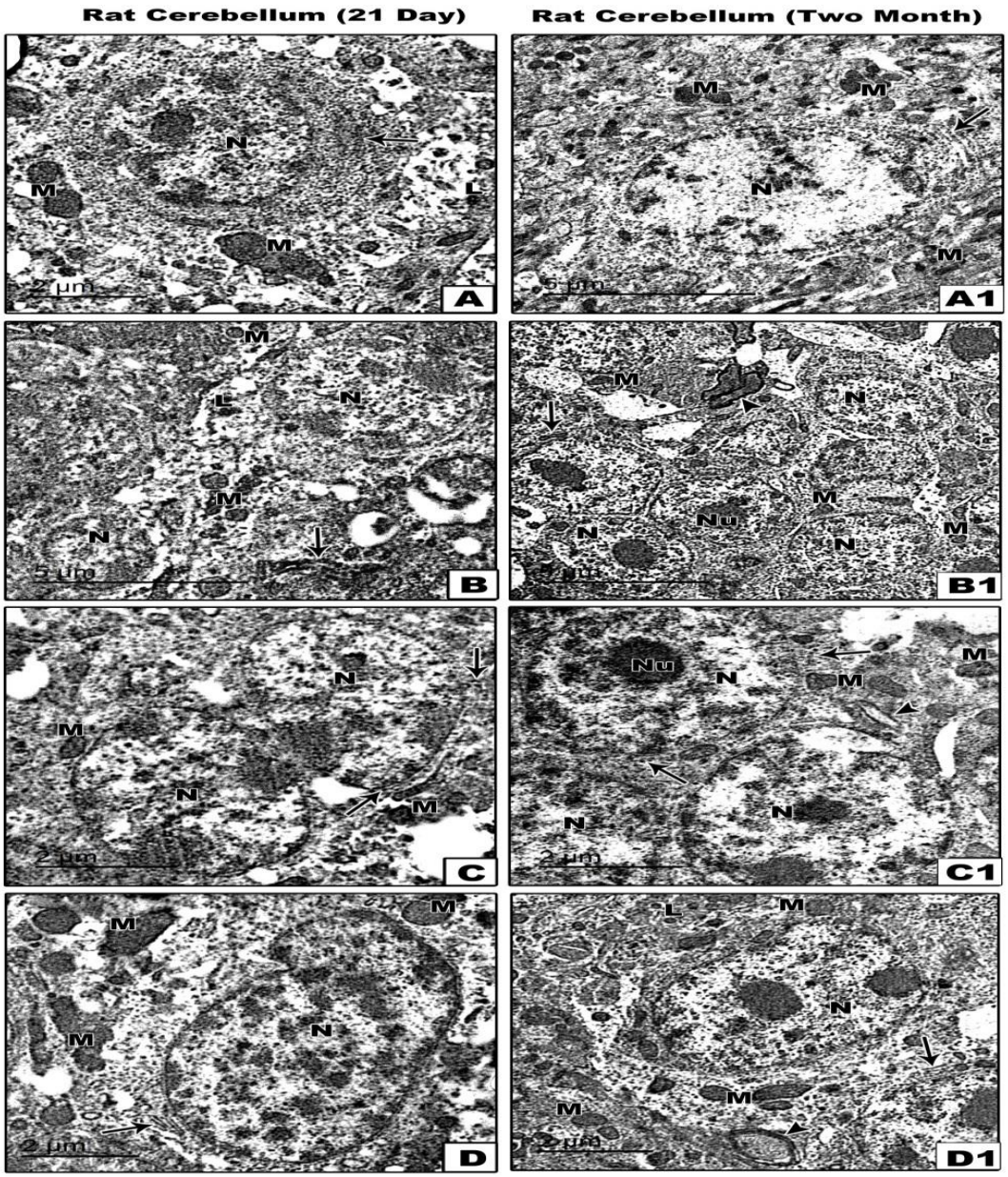

Fig 4. Transmission electron micrographs (TEM) through the cells of cerebellar cortex from albino rats at PND-21&2month. Panel (A, A1): basket cell, panel B, B1&C, C1: granular cells, and panel D, D1: Purkinje cell. N: nucleus, M: mitochondria, myelinated axons (arrowheads), and rough endoplasmic reticulum (arrows).

3-Immunohistochemical results for the determination of Caspase-3 in cerebellar tissue

As illustrated in Figure (5), the cerebellar cortex of Japanese quail and Albino rats displayed a gradual increase in the degree of caspase-3 immunoreactivity with the development of age. Comparatively among the two studied species, the quantitative evaluation of caspase-3 positively expressed cells (by using image analysis) appeared the same (0.455) at PHD 16 of Japanese quail and PND21 of Albino rats however, at PHD7 and 30 the caspase-3 activity appeared significantly higher in Japanese quail (0.179 and 1.93 respectively) if compared those of PND 7and two months (0.119 and 0.554 respectively) of Albino rat.

4 **Flow cytometric detection of proliferating cellular nuclear antigen (PCNA) in the cerebellum**

In quail and albino rats, the percentage of PCNA-positive cells in each studied age of the cerebellum increased significantly ($p < 0.05$) if compared with the previous age**.** In addition, in all studied ages, the percentage of PCNA-positive cells of albino rats is significantly higher ($p < 0.05$) than those cells in the comparable ages of quail(Fig 6).

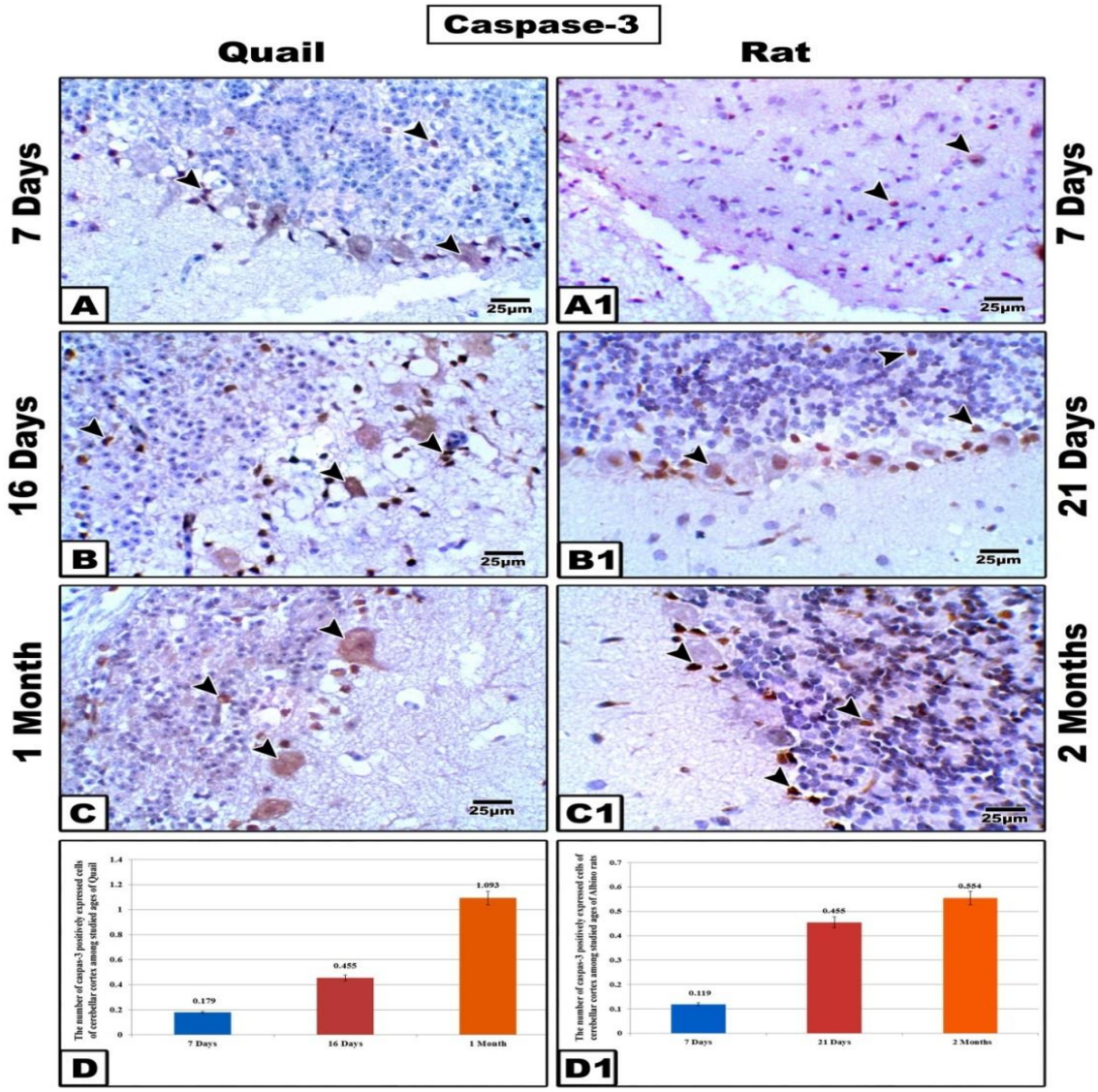

Fig5. Histological sections of the cerebellar cortex of the selected ages of Japanese quail (A-C) and Albino rat (A1-C1) stained with caspase-3 antibody. Panels D&D1 reveal the quantification of caspase-3 positively expressed cells of cerebellar cortex between selected ages of Quail and Albino rats. The arrowheads point to the cells stained with caspase-3 antibody.

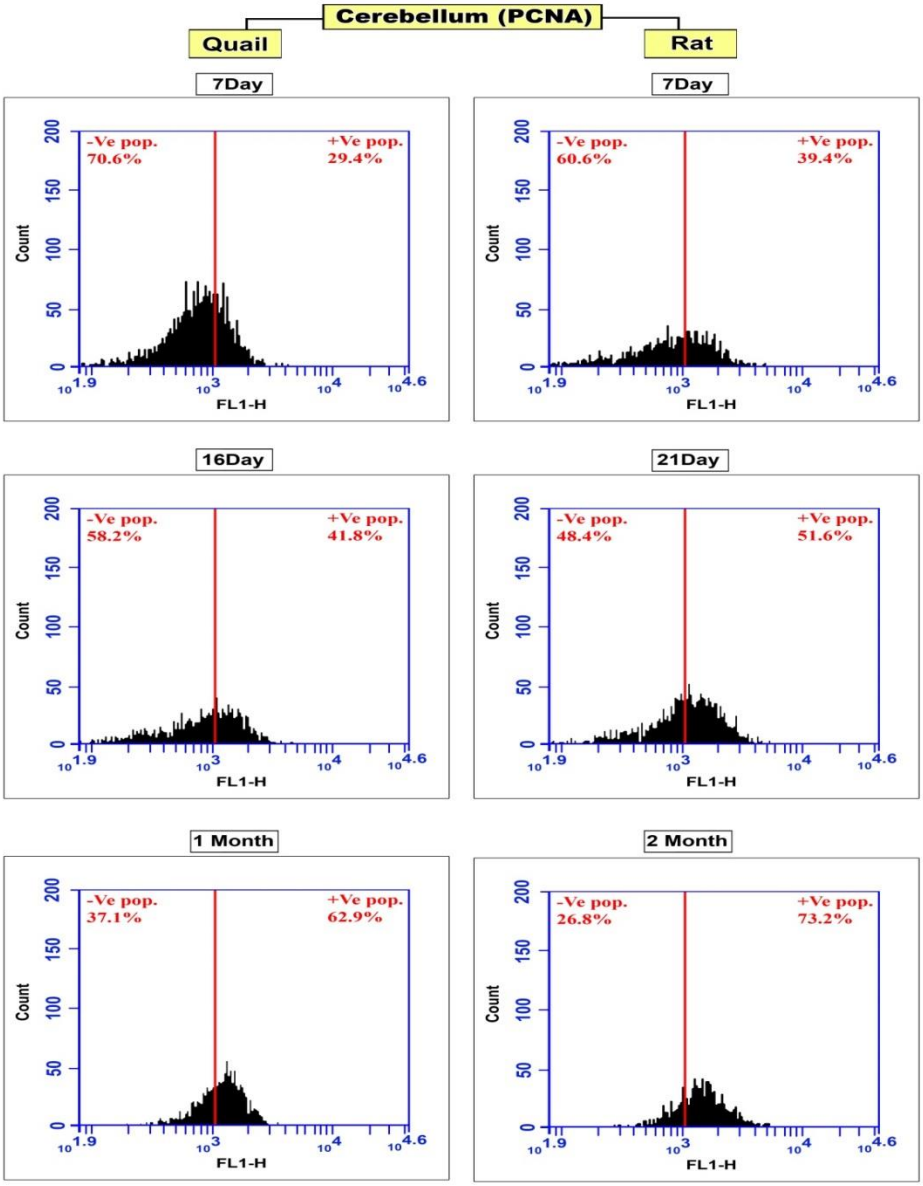

Fig 6. Flow cytometry chart of the cerebellum in different studied ages of quail and albino rat showing PCNA negative cells (-ve pop.) indicating the percentage of nonproliferating cells and PCNA positive cells (+ve pop.) indicating the percentage of proliferating cells.

DISCUSSION

The cerebellar cortex is an exceptionally well-studied example of how cortical structures are organized in distinct functional, biochemical, and morphological compartments [15, 16]. The cerebellum plays a vital role in maintaining equilibrium and is frequently studied concerning the motor system due to its strong association with motor disorders. The cells inside the cerebellum act as a control system that connects the sensory and motor pathways of the body [17]. Research has demonstrated that the cerebellum's function is altered by experience, and this structure plays a crucial part in the learning of motor skills [18]. The variations in the size, as well as morphological, and histological aspects of the cerebellum, seem to be associated with the performance of different kinds of tasks. While the cerebellum's morphologic and histological organization is generally comparable across all vertebrates, there are known variations based on the species' overall anatomical structure and behavioral characteristics [19, 20].

The most important cells of the cerebellar cortex include Purkinje cells, granular cells, and basket cells. A Purkinje cell is essential for the coordination of movement and has an essential role in different functions, including cognition and emotion **[20, 21].** The increasing number of Purkinje cells results in an increased rise in behavioral complexity, including activities such as flying and jumping, as well as an enhancement in cognitive ability **[22].** The smallest and most abundant neurons that play a role in a wide range of processes are the granule cells, involving the processing of visual and motor information, as well as learning and memory. Within the cerebellum, they are the only excitatory neurons and are responsible for transmitting information from the central nervous system to the cerebellar cortex **[23]**. Basket cells of the molecular layer of the cerebellum are a type of inhibitory interneurons that regulate the Purkinje cells' activity. The vital role of these interneurons in behavioral tasks, motor coordination, and motor learning has been uncovered recently through genetic investigations **[24].**

In this study, the size, number, and complexity of Purkinje cells appeared more prominent in the cerebellar cortex of quail ages compared with rats. This suggests the necessity of these cells in the maintenance of equilibrium during flying in quail rather than in rats which undergo movement behavior **[25,26].** On the other side, the density of baskets and granular cells appeared more prominent in rats compared with quail. This result reflects the more inhibitory effect on the conductivity circuit in the cerebellum of rats rather than quail **[27],** and while higher processing of visual and motor information to learning and memory **[28].** These data could be of particular interest to comparative biologists and physiologists in behavioral studies. Also, the result of the current investigation revealed that the cerebellar cortex of Japanese quail reaches the full histogenesis before PHD-7 while in rats this occurs after PND-7. The full histogenesis of the cerebellum in quail indicates the role of the cerebellum in motor balance and standing in the first week of hatching while in rats this may extend to the second week after birth **[29, 27].**

Iwaniuk et al **[20]** have been documented that both allometric and phylogenetic effects have the strongest effects on avian cerebellar foliation, while developmental mode showed a weak effect. The phylogenetic distribution of highly foliated cerebella also suggests that cognitive and/or behavioral differences play a role in the evolution of the cerebellum.

Heuer et al **[30]** Studies have shown that the folding of the cerebellum is believed to be crucial in the formation and arrangement of the mammalian cerebellum. Interestingly, smaller mammals tend to have a greater number of folds in their cerebellum compared to larger mammals. During the experiment, it was observed that the cerebellar cortex of rats exhibited noticeable branching of folia as they aged, in comparison to the cerebellar cortex of Japanese quail. The decreased number of cerebellar folia in quail, as compared to rats, results in a decreased surface area of the cerebellum. This adaptation is necessary to accommodate the smaller skull size of birds, which is a distinguishing feature from mammals. It is regarded as one of the structural adaptations that enable birds to Fly.

The result of the TEM investigation showed that the cerebellar cortex of Japanese quail ages had significant myelination and a high density of lysosomes. However, when compared to age-related rats, the quail showed a lower density of RER and mitochondria. Myelin enhances the transmission of nerve impulses in comparison to un-myelinated fibers with the same diameter, reduces the time it takes to respond to stimuli, and improves the ability to escape from sudden predatory attacks **[31, 32].**

Zalc et al **[33]** reported that birds have a larger density of myelinated nerve fibers in their cerebellar cortex compared to mammals. This allows them to respond quickly to escape from predators. The abundance of lysosomes in the cerebellum cells of quail, as compared to rats, may indicate an excessive autophagy response to foreign substances. This

response is mostly associated with frequent bird infections [34]. The endoplasmic reticulum (ER) occupies the whole neuron, from the cell body to the ends of the axon and dendritic spines. It plays a role in local calcium signaling, as well as the production and movement of proteins that result from synaptic activity [35]. The existence of an abundance of rough endoplasmic reticulum (RER) in the cerebellar cells of rats, if compared to quails, indicates the essential role of this organelle in protein synthesis and calcium regulation during the transmission of nerve impulses in mammals. Birds, on the other hand, compensate for this by having myelinated fibers [20,36]. The combinations of electrical and chemical signals are essential for the operation of the brain, such processes need high energy demands [37]. Neurons, which are the fundamental functional components of the brain, are the most energy-demanding cell type in the brain [38]. The findings of this study indicate that there was a higher number of mitochondria in the cells of the cerebellar cortex of rats compared to quail. This observation aligns with the research conducted by ***Faitg et al*** [39], who also observed a greater density of mitochondria in the brains of mammals compared to birds. This discovery indicates that the metabolic rate in the cerebellar cortex of rats is higher than that of quail.

The developmental process involves a complex balance between removal of the defective or excess cells and the increase in cell mass. Apoptosis happened subsequently to the initiation of nuclear and cytoskeletal changes by Caspase 3. Recently, it was evidenced that caspase 3 has been implicated in the cell cycle regulation and proliferation of the cerebellum [40]. The expression of caspase 3 especially in Bergman glia may be due to neuronal differentiation rather than cell death [41].

In the current work, the caspase 3 expression was observed in the cerebellar cortex Purkinje cells at three weeks old albino rat more than the expression at 7 days old. ***Marzban et al*** [42] explained that, in altricial mammals, a great part of the cerebellum develops within two to three weeks in rodents and rabbits and three months in humans [42]. Through these stages, the external granular layer disappeared upon completion of cerebellar differentiation. So, the high expression of caspase 3 in three-week-old rats may be due to the great developmental processes of the cerebellum during this stage.

The increased expression of caspase 3 at two months old of albino rats appeared significantly through the internal granular layer and can be explained by ***Lossi et al*** [41] who mentioned that there is evidence that the failure of establishing synaptic contacts with the target cells was associated with the location of caspase 3 in Purkinje and granule cells of the cerebellum [41]. They explained that in altricial species including mice, rats, rabbits, and humans, naturally occurring neuronal death (NOND) follows two important postnatal apoptotic processes. The first one (primary apoptosis) attacks the pre-migratory cerebellar granule cells (CGCs) in the external granular layer, while the second process occurs through mature CGCs in the internal granular layer. Primary apoptosis may be closely correlated with cell cycle dysregulation. It is also independent of the synaptic target connections and is very possible to be completed without the intervention of caspase-3. Secondary apoptosis occurred after a failure to connect to the Purkinje neurons' dendritic tree that are the main synaptic target of the mature cerebellar granular cell axons (the parallel fibers) and is closely correlated with caspase-3 activation.

Amelio et al [43] reported that much recent research carried out on birds, rats, and snails revealed that caspase 3 was implicated in the regulation of synaptic plasticity more than just its role in the neuronal apoptotic process [43]. This plasticity is based on two implications; Firstly, proteins that play an essential role in neuronal plasticity are substrates for caspase 3 [44]. Secondly, findings in both *vivo* and *vitro* detected the implications of caspase 3 in memory and learning molecular mechanisms [45]. In quail cerebellum, the present immunohistochemical caspase 3 study revealed increasing expression during 16 days and one month old which may reflect

a high level of synaptic plasticity during these ages. ***Nkomozepi et al*** [11] reported that in quail, neuronal production during the post-hatch period seems to be restricted to telencephalic and olfactory ventricles while neuronal incorporation is confined to the cerebellum and telencephalon. Furthermore, both processes persist into adulthood and appear to decrease with post-hatching age beginning from the sixth week. Neurogenesis in the postnatal and adult periods is considered an important marker for determining plasticity in specific brain regions in both birds and mammals. There is no systematic study evaluating the adult neurogenesis of birds through their lifespan [46]. However, adult neurogenesis in birds is more widespread if compared to mammals, the generated cells appeared not only in the lateral and medial (parolfactory lobe) striatum, the hippocampus, and the olfactory lobe but also in different regions of the forebrain and various song control nuclei [5, 2].

The observed increased PCNA-positive cells of cerebellar tissues with increasing age in both quail and albino rats may indicate that cerebellar neurogenesis is more active in adult animals. These results partially agreed with the results of ***Rosmanah*** [47] . They indicated an increase in PCNA immune expression with increasing age in the brain's cortical regions of long-tailed macaques while in the hippocampus region PCNA gene expression was higher in the adult group than elderly groups. They explained that differences in proliferation responses could be found in different regions of the brain. ***Amrein et al*** [48] reported that adult neurogenesis of homing pigeons has a lifespan course that follows a similar pattern to many mammalian species [48].

Meskenaite et al [46] recorded that the number of neurons in the hippocampus of the oldest birds was about twice the number of neurons in adult and young birds. This increase could refer to the expanded spatial memory and accumulated experience necessary for survival, reproduction, and navigation. In addition, the observed PCNA-positive cells in some old birds suggest that proliferation was also going on [46].

3. Conclusion

The authors demonstrated that the different selected developmental modes (Altricial and precocial aspects) of both rats and quail have consequences in various aspects involving structural and functional changes of the cerebellum and other physiological mechanisms. Such evolutionary differences displayed an intriguing contrast that needs more research in the future.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

Funding: NIL

DATA AVAILABILITY STATEMENT

The manuscript has involved all the data.

References

[1] Alunni A and Bally-Cuif L (2016). "A comparative view of regenerative neurogenesis in vertebrates." Development. 143(5): 741-753.

[2] Barnea A and Pravosudov V (2011). "Birds as a model to study adult neurogenesis: bridging evolutionary, comparative and neuroethological approaches." European Journal of Neuroscience. 34(6): 884-907.

[3] Kaslin J, Ganz J and Brand M (2008). "Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain." Philosophical Transactions of the Royal Society B: Biological Sciences. 363(1489): 101-122.

[4] Olaleye O O and Ihunwo A O (2014). "Adult neurogenesis in the four-striped mice (Rhabdomys pumilio)." Neural Regeneration Research. 9(21): 1907-1911.

[5] Vellema M, Van der Linden A and Gahr M (2010). "Area-specific migration and recruitment of new neurons in the adult songbird brain." Journal of Comparative Neurology. 518(9): 1442-1459.

[6] Hall Z J, Bauchinger U, Gerson A R, Price E R, Langlois L A, Boyles M, Pierce B, McWilliams S R, Sherry D F and MacDougall Shackleton S A (2014). "Site-specific regulation of adult neurogenesis by dietary fatty acid content, vitamin E and flight exercise in European starlings." European Journal of Neuroscience. 39(6): 875-882.

[7] Reiner A (2005). "Avian brains." Encyclopedia of Life Sciences.

[8] Versace E (2017). Precocial. Encyclopedia of animal cognition and behavior, Springer, Cham: 1-3.

[9] Bienboire-Frosini C, Marcet-Rius M, Orihuela A, Domínguez-Oliva A, Mora-Medina P, Olmos-Hernández A, Casas-Alvarado A and Mota-Rojas D (2023). "Mother–young bonding: Neurobiological aspects and maternal biochemical signaling in altricial domesticated mammals." Animals. 13(3): 532.

[10] Huss D, Poynter G and Lansford R (2008). "Japanese quail (Coturnix japonica) as a laboratory animal model." Lab animal. 37(11): 513-519.

[11] Nkomozepi P, Mazengenya P and Ihunwo A (2018). "Changes in neurogenesis with posthatching age in the male Japanese quail (Cortunix japonica) brain." Acta neurobiologiae experimentalis. 78(2): 173-186.

[12] Sekulic S, Jovanovic A, Zivanovic Z, Simic S, Kesic S, Petkovic B, Capo I and van Loon J J (2022). "Which precocial rodent species is more suitable as the experimental model of microgravity influence on prenatal musculoskeletal development on international space station?" Life Sciences in Space Research. 33: 48-57.

[13] Abdel-Kader D H and Sayed S S E (2018). "Immunolocalization of laminin during postnatal development of the testis, epididymis and vas deferens of albino rat." The Egyptian Journal of Hospital Medicine. 50(1): 34-59.

[14] Bancroft D, Layton C and Suvarna S K (2012). Bancroft's Theory and Practice of Histological Techniques: Expert consult: Online and print,7e, UK: Churchill Livingstone.

[15] Hawkes R (1997). An anatomical model of cerebellar modules. Progress in brain research. 114: 39–52.

[16] [Oberdick J, Baader S L and Schilling K (1998). From zebra stripes to postal zones: deciphering patterns of gene expression in the cerebellum. Trends in neurosciences. 21(9): 383–390.

[17] Masabanda J S, Burt D W, O'Brien P C, Vignal A, Fillon V, Walsh P S, Cox H, Tempest H G, Smith J, Habermann F, Schmid M, Matsuda Y, Ferguson-Smith M A, Crooijmans R P, Groenen M A and Griffin D K (2004). Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. Genetics. 166(3):1367–1373.

[18] Pal B, Chowdhury S and Ghosh R K (2003). Comparative anatomical study of the cerebellum of man and fowl. Journal of the Anatomical Society of India. 52: 32–37.

[19] Miall R C and Reckess G Z (2002). The cerebellum and the timing of coordinated eye and hand tracking. Brain and cognition. 48(1): 212–226.

[20] Iwaniuk A N, Hurd P L and Wylie D R (2006). The comparative morphology of the cerebellum in caprimulgiform birds: evolutionary and functional implications. Brain, behavior and evolution. 67(1): 53–68.

[21] van Welie I, Smith I T and Watt A J (2011). The metamorphosis of the developing cerebellar microcircuit. Current opinion in neurobiology. 21(2): 245–253.

[22] Kaneko M, Yamaguchi K, Eiraku M, Sato M, Takata N, Kiyohara Y, Mishina M, Hirase H, Hashikawa T and Kengaku M (2011). Remodeling of monoplanar Purkinje cell dendrites during cerebellar circuit formation. PloS one.6(5), e20108.

[23] Llinas R R, Walton K D and Lang E J (2004). "Ch. 7 Cerebellum". In Shepherd GM (ed.). The Synaptic Organization of the Brain. New York: Oxford University Press.

[24] Zhou J, Brown A M, Lackey E P, Arancillo M, Lin T and Sillitoe R V (2020). Purkinje cell neurotransmission patterns cerebellar basket cells into zonal modules defined by distinct pinceau sizes. eLife. 9: e55569.

[25] Koziol L F, Budding D E and Chidekel D (2010). Adaptation, expertise, and giftedness: towards an understanding of cortical, subcortical, and cerebellar network contributions. Cerebellum (London, England).9(4): 499–529.

[26] Koziol L F, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu, H, Ito M, Manto M, Marvel C, Parker K, Pezzulo G, Ramnani N, Riva D, Schmahmann J, Vandervert L and Yamazaki T (2014). Consensus paper: the cerebellum's role in movement and cognition. Cerebellum (London, England).13(1): 151–177.

[27] Sepp M, Leiss K, Murat F, Okonechnikov K, Joshi P, Leushkin E, Spänig L, Mbengue N, Schneider C, Schmidt J, Trost N, Schauer M, Khaitovich P, Lisgo S, Palkovits M, Giere P, Kutscher L M, Anders S, Cardoso-Moreira M, Sarropoulos I and Kaessmann H (2024). Cellular development and evolution of the mammalian cerebellum. Nature. 625(7996): 788–796.

[28] Lackey E P, Heck D H and Sillitoe R V (2018). Recent advances in understanding the mechanisms of cerebellar granule cell development and function and their contribution to behavior. F1000Research. 7, F1000 Faculty Rev-1142.

[29] Iwaniuk A N, Hurd P L and Wylie D R (2007). Comparative morphology of the avian cerebellum: II. Size of folia. Brain, behavior and evolution. 69(3): 196–219.

[30] Heuer K, Traut N, de Sousa A A, Talk S L, Clavel J and Toro R (2023). Diversity and evolution of cerebellar folding in mammals. eLife.12: e85907.

[31] Giuliodori M J and DiCarlo S E (2004). Myelinated vs. unmyelinated nerve conduction: a novel way of understanding the mechanisms. Advances in physiology education. 28(1-4): 80–81.

[32] Díez-Revuelta N, Higuero A M, Velasco S, Peñas-de-la-Iglesia M, Gabius H J and Abad-Rodríguez J (2017). Neurons define non-myelinated axon segments by the regulation of galectin-4-containing axon membrane domains. Scientific reports. 7(1): 12246.

[33] Zalc B, Goujet D and Colman D (2008). The origin of the myelination program in vertebrates. Current biology: CB. 18(12): 511–512.

[

M. (2018). Lysosomes and Brain Health. Annual review of neuroscience.41: 255–276.

[35] Kennedy M J and Hanus C (2019). Architecture and Dynamics of the Neuronal Secretory Network. Annual review of cell and developmental biology.35: 543–566.

[36] Nowrozi M and Goodarzi N (2022). Microanatomy of the cerebellum in Persian squirrel (Sciurus anomalus), a transmission electron microscopy, and immunohistochemical study. Microscopy research and technique. 85(12): 3850–3859.

[37] Pereda A E (2014). Electrical synapses and their functional interactions with chemical synapses. Nature reviews. Neuroscience.15(4): 250–263.

[38] Briscoe J and Marín O (2020). Looking at neurodevelopment through a big data lens. Science (New York, N.Y.), 369(6510), eaaz8627.

[39] Faitg J, Lacefield C, Davey T, White K, Laws R, Kosmidis S, Reeve A K, Kandel E R, Vincent A E and Picard M (2021). 3D neuronal mitochondrial morphology in axons, dendrites,

and somata of the aging mouse hippocampus. Cell reports. 36(6): 109509.

[40] Oomman S, Finckbone V, Dertien J, Attridge J, Henne W, Medina M, Mansouri B, Singh H, Strahlendorf H and Strahlendorf J(2004). "Active caspase-3 expression during postnatal development of rat cerebellum is not systematically or consistently associated with apoptosis." Journal of Comparative Neurology .476(2): 154-173.

[41] Lossi L, Castagna C and Merighi A(2018). "Caspase-3 mediated cell death in the normal development of the mammalian cerebellum." International journal of molecular sciences. 19(12): 3999.

[42] Marzban H, Del Bigio M R, Alizadeh J, Ghavami S, Zachariah R M and Rastegar M (2015). "Cellular commitment in the developing cerebellum." Frontiers in cellular neuroscience. 8: 450.

[43] Amelio D, Lorentini S, Schwarz M and Amichetti M (2010). "Intensity-modulated radiation therapy in newly diagnosed glioblastoma: a systematic review on clinical and technical issues." Radiotherapy and Oncology. 97(3): 361-369.

[44] Chan S L and Mattson M P (1999). "Caspase and calpain substrates: roles in synaptic plasticity and cell death." Journal of neuroscience research. 58(1): 167-190.

[45] Dash P K, Blum S and Moore A N (2000). "Caspase activity plays an essential role in long-term memory." Neuroreport. 11(12): 2811-2816.

[46] Meskenaite V, Krackow S and Lipp H P (2016). "Age-dependent neurogenesis and neuron numbers within the olfactory bulb and hippocampus of homing pigeons." Frontiers in behavioral neuroscience. 10: 126.

[47] Rosmanah L (2023). "The PCNA GENE EXPRESSION AS A MARKER OF ALZHEIMER'S DISEASE IN THE BRAIN OF LONG-TAILED MACAQUES (Macaca fascicularis)." Indonesian Journal of Primatology. 2(2): 68-75.

[48] Amrein I, Isler K, and Lipp H P (2011). "Comparing adult hippocampal neurogenesis in mammalian species and orders: influence of chronological age and life history stage." European Journal of Neuroscience. 34(6): 978-987.