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Histomorphological studies on the cerebellar cortex of pig (*Sus scrofa*)

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Abstract

The histomorphological aspects of cerebellar cortex were studied in 18 Large White Yorkshire pigs at different age groups. The cerebellar cortex revealed three layers viz., the molecular layer, the Purkinje cell layer and the granular layer. Five neuronal cell types were observed in the cerebellar cortex viz., the granule cells, Purkinje cells, stellate cells, Basket cells and Golgi cells. There was an increase in the dendritic arborization from preweaner to grower age group, whereas there was discontinuation in dendritic branching in finisher age group. Significant difference was noticed in width of the layers between preweaner and grower age groups, whereas no significant difference was observed between grower and finisher age group. Lipofuscin pigmentation was seen only in Purkinje cells of the cerebellar cortex and it was increased from grower to finisher age groups. There was a slight decrease in the neuronal cell number, size of cell bodies, amount of Nissl substance and an increase in neuropil, glial cell number was noticed with advancement of age.

Keywords: Histomorphology, cerebellar cortex, Pig (*Sus scrofa*), Purkinje cell

Introduction

Pigs are highly social and intelligent animals. Pig husbandry is one of the best and profitable enterprises especially for small and marginal farmers as they possess high prolificacy, better feed conversion efficiency, faster growth rate, high dressing percentage etc. The cerebellar cortex has become the focus of intense research because it is presumed to be responsible for planning movement and adapting to special conditions and involved in storing memories over various time-periods (Attwell *et al.*, 2002) ^[1]. Very little information is available on histological aspects and age related changes of cerebellar cortex in pig. Hence the present study was undertaken to provide detailed histological changes during normal aging in the cerebellar cortex at different age groups of pigs.

Materials and Methods

The present study was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati. The tissue samples were collected from 18 pigs which were obtained from the slaughter house of AICRP on pigs, College of Veterinary Science, Tirupati. The pigs were categorized into three groups based on their age, i.e., Preweaners (1 to 42 days), Growers (43 days to 8 months) and Finishers (9 months and above) with six in each group. For Histomorphological study, fresh tissue samples were collected from the cerebellar cortex, fixed in 10% Neutral Buffered Formalin and processed for routine paraffin sectioning as per methods described by Singh and Sulochana (1997) ^[2]. The sections were subjected to routine and special histomorphological staining methods i.e., Haematoxylin and Eosin staining method for routine histological study, Beilschowsky method for nerve fibres (Singh and Sulochana, 1997) ^[2], Phosphotungstic acid Haematoxylin method for glial cells (Bancroft and Gamble, 2008) ^[3], Zeihl-Neelson modified stain for lipofuscin (Martindale, 2009) ^[4].

Results and Discussion

The cerebellar cortex was composed of three distinct layers i.e., an outer molecular layer, a middle layer of Purkinje cells and an inner granular layer from above downwards as reported in different domestic animals (Eurell and Frappier, 2006; Zhang *et al.*, 2006; Salankar, 2017) ^[5-7]. Five distinct cell types were observed in cerebellum viz., the granule cells, Golgi cells, Purkinje cells, stellate cells and basket cells. Similar observations was reported in human (Patestas and Gartner, 2006) ^[8].

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The molecular layer was the outermost layer of the cerebellar cortex, which consisted of few neuronal cells with spindle or stellate shaped neuronal cell bodies, glial cells and more of neuropil. The cell bodies of the basket cells were found in the deep molecular layer lying close to the Purkinje cell bodies (Fig. 2). This was also noticed by Eurell and Frappier (2006)^[5] in domestic animals and Salankar (2017)^[7] in goats. There was an increase in the size of the neuronal cells and the amount of neuropil and decrease in the amount of Nissl substance in neuronal cells from preweaner to finisher age group. Perineuronal vacuolation was seen around some cells of finisher age group (Fig. 2). The neuropil consisted of compact arborization of dendrites that arise from the Purkinje cells. The dendritic arborization of Purkinje cells was found to be short, less branched and did not reach the outer surface of molecular layer in preweaner age group (Fig. 3). However, in grower age group it was tall, more prominent, highly branched and reached the outer surface of the molecular layer (Fig. 4). Reduction and discontinuation in the dendritic branching was observed in the finisher age group. Hadj-Sahraoui *et al.* (2001)^[9] also observed that the dendritic arborizations of Purkinje cells were obviously atrophied in senile mouse. The width of the molecular layer was significantly increased from preweaner to grower age groups, while from grower to finisher age groups, it was decreased gradually. The loss of thickness in molecular layer was caused largely by progressive defoliation of dendrites in the ageing Purkinje cells.

The middle Purkinje cell layer was a very prominent and thinnest layer and consisted of a layer of large Purkinje cells as reported by Eurell and Frappier (2006)^[5]; Salankar (2017)^[7] in domestic animals. Purkinje cells were very prominent and found in round or flask or pyramidal shaped with heterochromatic nuclei and prominent nucleoli. Kuehnel (2003)^[10] also reported rounded Purkinje cell bodies in human. The dendrites of the Purkinje cells were thick, highly branched and emerged from their apices and extended into the molecular layer. Decrease in the amount of Nissl substance was observed in Purkinje cells from preweaner to finisher age group (Figs. 1, 2). An increase in the width of this layer was noticed from preweaner to grower age group. However there was no significant difference in the width of this layer was observed from grower to finisher age group. The size of the soma of Purkinje cells was increased from preweaner to grower age groups, however no significant change was noticed in the size of the soma from grower to finisher age groups. Jelsing *et al.* (2006)^[11] also reported an increase in the perikaryon volume of Purkinje cells from neonates to the adults in the cerebellum of Gottingen minipig. However, the perikaryon of Purkinje cells was reported to be decreased with age in human by Andersen *et al.* (2003)^[12].

The Purkinje cells were found to be distinctly pyramidal in preweaner and grower age groups, whereas in finisher age group the cells appeared in elongated pyramidal shape. Significant reduction in number of Purkinje cells was observed in the cerebellar cortex from preweaner to finisher age group. Similar observation was noted with advancement of age in cat (Zhang *et al.*, 2006)^[6] and human (Yesmin *et al.*, 2011)^[13]. In contrary to this Jelsing *et al.* (2006)^[11] reported

an increase in the number of Purkinje cells from neonates to the adults of Gottingen minipig. Decreased number of Purkinje cells may be attributed to the reduction in the dendritic branching which may interfere with the information exchange. There was an increase in the lipofuscin pigmentation in the Purkinje cells with advancement of age as reported by Nandy (1981)^[14] in *Macaca nemestrina* and Salankar (2017)^[7] in goat.

The granular layer was the innermost thickest layer of the cerebellar cortex and made up of many granular cells, few glial cells and neuropil. The granular layer was thick at the upper part of gyri regions, while thinner in grooves of sulci regions. The granule cells were densely packed, small, spherical shaped with a very thin rim of cytoplasm and large central heterochromatic nuclei as reported in domestic animals and human (Banks, 1993; Viswasom *et al.*, 2013)^[15-16]. Few spindle shaped Golgi cells were also present in the superficial part of the granular layer with centrally located nuclei as noticed in goat by Salankar (2017)^[7].

The granule cells were closely packed in preweaner age group. The number of granule cells was decreased, whereas neuropil was increased with age from preweaner to finisher age group. Similarly, Viswasom *et al.* (2013)^[16] also identified that the granule cells were closely packed in younger age group and the fibre elements become prominent with age in human. However, Nandy (1981)^[14] stated the number of granule cells could not undergo any changes with age in *Macaca nemestrina*. The loss of granular cells indicate that it may directly lesser the excitatory input to the Purkinje cells and may greatly affect motor learning in aged animals. The width of this layer was significantly increased from preweaner to grower age, whereas slight decrease was observed from grower to finisher age group. The total cortical width of cerebellum was decreased with age from grower to finisher age group in agreement with the findings of Yesmin *et al.* (2011)^[13] in human, Zhang *et al.* (2006)^[6] in cat.

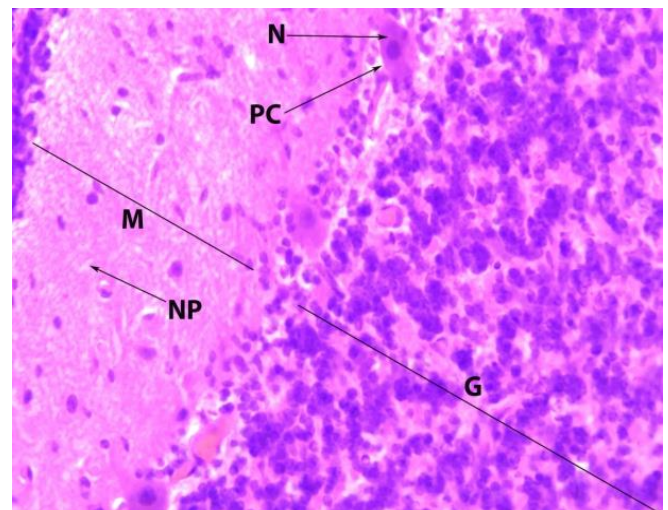


Fig 1: Photomicrograph of the cerebellar cortex of preweaner pig showing thin molecular layer (M) with less neuropil (NP), Purkinje cell (PC) with more nissl substance (N) and granular layer (G).
Haematoxylin and Eosin ×400

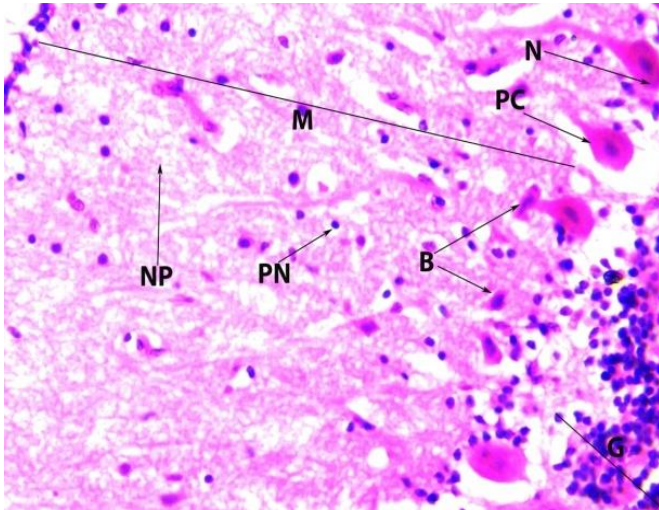


Fig 2: Photomicrograph of the cerebellar cortex of finisher pig showing thick molecular layer (M) with more neuropil (NP), Purkinje cell (PC) with less Nissl substance (N) and granular layer (G). PN- Perineuronal Vacuolation, B- Basket cell. Haematoxylin and Eosin $\times 400$

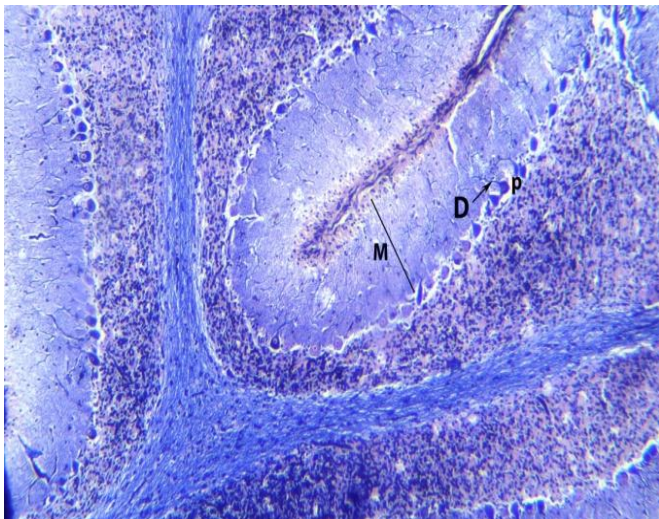


Fig 3: Photomicrograph of the cerebellar cortex of preweaner pig showing short, dendritic branches (D) of Purkinje cell (P) in the molecular layer (M). Phosphotungstic Acid Haematoxylin $\times 100$

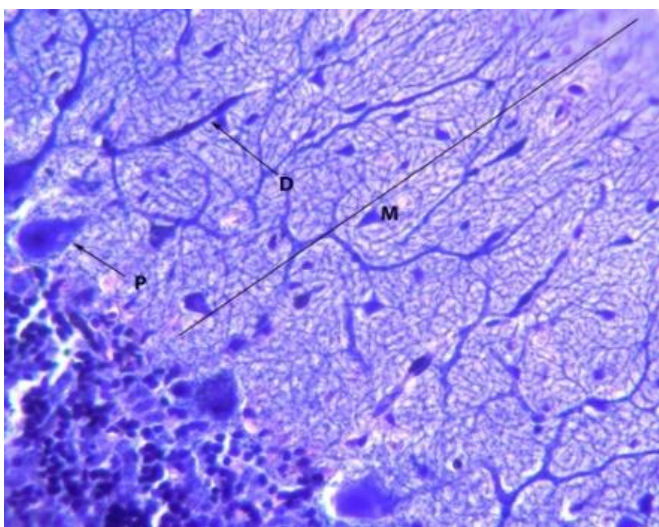


Fig 4: Photomicrograph of the cerebellar cortex of grower pig showing thick, prominent, highly branched dendrites (D) of Purkinje cells (P) in the molecular layer (M). Phosphotungstic Acid Haematoxylin $\times 400$

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