Pre-postnatal morphogenesis of rat hippocampal pyramidal cells

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ABSTRACT

Objectives: To follow-up the morphological changes of the neuroblasts as they develop to pyramidal cell in the hippocampal cerebral cortex beginning from 15 days prenatal and ending 14 days postnatal. Particular metric and numerical estimations of the dendrites developed from these cells were carried out.

Methods: Fifty pregnant rats classified into 5 groups were used to obtain 15 and 18 day embryos; birth, 7 day and 14 day pups. Brains of these animals were processed for histological examination using iron hematoxylin and golgi mixed methods of staining from paraffin sections. Multiplicity and length of the dendrites were assessed. The practical work was carried out in the Anatomy Department of the School of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia during the year 2002.

Results: At 15 days prenatal, a few pyramidal cells appeared which greatly increased at 18 days prenatal with the development of their beaded apical dendrites. At birth, the dendrite stem became shorter but had more branches and beads. Postnatal one and 2 weeks consecutively witnessed the arise of axons and basal dendrites.

Conclusion: These results showed the very early postnatal full morphological development of the hippocampus cortex pyramidal cells. The progressive increase in length of the apical dendrites during the period from 18 days prenatal when they first appeared to 14 days postnatal was highly significant ($P < 0.00$).

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The development of the cerebral cortex is evidently a reflection of the gradual establishment of the higher and also the basic life support function of the brain.[1] The ultimate control of the neuroblasts around the neural tube should undergo a complex anatomical endeavor to accomplish the entire neurophysiological requirements for the familiar picture of survival.[2,3] Differentiation of the cerebral cortical pyramidal cells has been investigated by many authors.[4-7] These authors were able to establish that the neuroblasts differentiate to pyramidal cells through acquiring the well known characteristics. This begins as early as the relatively late prenatal period. Indeed, the full morphological view of these cells as seen in the adult hippocampus cerebral cortex can be observed at a very early postnatal time.[8-13] Further understanding of how these morphological changes takes place is still unfortunately almost totally unknown. Therefore, the present work was primarily directed to histologically examine changes in the shape of the hippocampus (Ammon's horn) pyramidal cells at frequent short intervals during the late prenatal and early postnatal periods. Quantitative assessment of the dendrites of the developing cortical neurons was conducted.

Methods.

Experimental animals and procedures. A total of 50 rats (D-strain albino rat, inbred at the animal house of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia) at the age of 4-8 months were individually selected for estimation of the pregnancy period and vaginal smear method was used to

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diagnose pregnancy. Pregnant rats were classified into 5 groups (each group comprised 10 rats) in relation to pre-postnatal time. On 5 occasions, days 15, 18 prenatal, birth and days 7, 14 postnatal, the pregnant rats or their pups were sacrificed using deep ether anesthesia for examination of fetuses and pups in each of the above mother groups. During pregnancy and in the postnatal period the animals were kept in the animal house at room temperature and supplied with water and standard food pellets. Fetuses were obtained by cesarean section. The fetuses and pups were fixed in 4% paraformaldehyde for 3 days and then their brains were immediately postfixed in Bouin's solution for 2 hours.[14] The hippocampi were dissected from the brains and directly processed for light microscope examination using iron hematoxylin and golgi mixed staining techniques after 7 micron thick paraffin sections had been prepared.[15] The iron hematoxylin was used to stain the cell body of neurons to show the nucleus and cytoplasm. The golgi mixed method was carried out to illustrate the neuron as a whole in an almost uniformly distributed relatively black color. Therefore, the last staining was essential for conducting the morphometric study on the neuronal dendrites. This practical work was carried out in the Anatomy Department of the School of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia during the year 2002.

Morphometric studies. The growth in length of the apical dendrites was employed as a factor for assessment of the morphological changes in the pyramidal cells.[16] The dendrites are kinky, therefore, the total length of an apical dendrite was calculated as the sum of the individual measurements of the segments in between the kinks. A calibrated ocular scale grid was used to conduct this estimation. The mean ± SD was then calculated from 50 cells taken from 5 cross sections (10 cells randomly collected from each section) for each fetus or rat pup involved in all of the 5 occasions of this study.

Statistical study. Statistical analysis of the data was based on frequent measurements of the dendrites of the developing pyramidal cells. The statistical significance of this data was assessed through the linear model obtained versus a linear regression relating age to the total length and number of segments of the cell dendrite. The coefficient of determination and the P values were then calculated.

Results. A 15-day-old fetus, presented a well defined layer of germinal cells, currently described as the subependymal (germinal) layer. These cells were abundant, their cytoplasm was faintly stained and the nuclei appeared large and dark. Mitotic figures were numerous (**Figure 1**). A few cells which were nearly triangular in shape with short apical dendrites had been found between the germinal layer and the ependymal layer, these cells might be interpreted as the earliest form of the so called pyramidal cells (**Figure 2**). Eighteen day old rat fetuses, had an increase in the number of the developing pyramidal cells in the region inner to the ependymal layer but these cells were widely separated among themselves. This last finding is a well-known behavior of golgi stain that it does not stain all cells. The apical dendrites of the proposed pyramidal cells had 2-4 beaded branches sprouting from the relatively long stem of each (**Figure 3)**. At birth, the stems of apical dendrites were much shorter or even unseen but they had more branches with much numerous beading. In addition, the spaces between the growing pyramidal cells became narrower than the previous stage (**Figure 4**). One week old pups showed the first appearance of axons coming out of the lower pole of the pyramidal cells and the presence of spines on the branches of the apical dendrites. A few (almost 1-3) beads remained on each of the branches of the apical dendrites (**Figure 5**). Two week old pups revealed that development of the pyramidal cells was probably complete as the basal dendrites had been seen (**Figure 6**).

Numerical study of the dendrites. The length of the dendrites during the prenatal and postnatal examined periods was considerably increasing (**Table 1**). The number of segments of the apical dendrites was increased progressively in all groups. The values of increase in the above 2 parameters were highly significant ($P<0.00$).

Discussion. The present study introduced 2 important findings. The first was the direct proportion between age and growth of both the total length and number of segments of the apical dendrites of the pyramidal cells. The second was the full development of these cells, 2 weeks after birth. The age related appearance of the hippocampus pyramidal cells processes in the present study was a reproduction of similar results obtained by other authors.[17-23] The authors of these previous investigations postulated the presence of a correlative relationship between the functional advancement and the sequential springing of the pyramidal cells processes. Obviously, this relationship is established through the gradually increasing synaptic connections. In support of the above postulation, it was incidentally found that behavior abnormalities in Reeler mice had been associated with abnormally reduced number of hippocampal pyramidal cells.[24]

Interpretation of the present results may be simply viewed in the interest of reconfirmation of the well established understanding of the early and rapid development of the hippocampus cerebral cortex. Unfortunately, the exact nature of this development is still greatly obscure. However, in the context of the present subject many questions should be imposed in imagining the behavior of the neuroblasts along their course in giving rise to pyramidal cells. These questions may be useful in exploring this course: 1. How long do they keep their mitotic activity before they finally differentiate into the known cortical pyramidal neurons? 2. Do all of them then completely and finally disappear? 3. Do they immediately differentiate into the

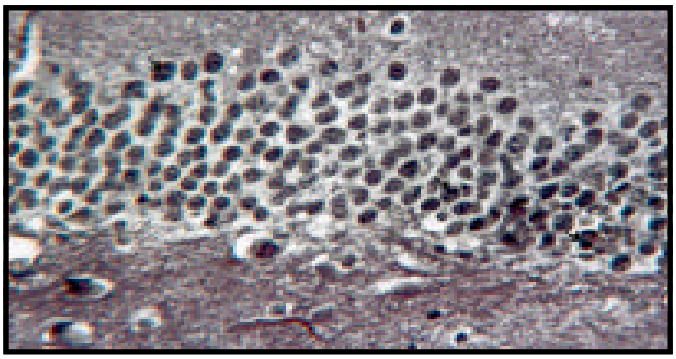

Figure 1 - A photomicrograph of the cerebral cortex in a 15-day-old rat embryo. The cross sections represented a relatively dense cell population which were arranged in a clearly distinct subependymal (germinal) layer. The cells cytoplasm was faintly stained while the nuclei looked dark and contained multiple small bodies. Many mitotic figures (see the arrows) were seen. Iron hematoxylin X 100.

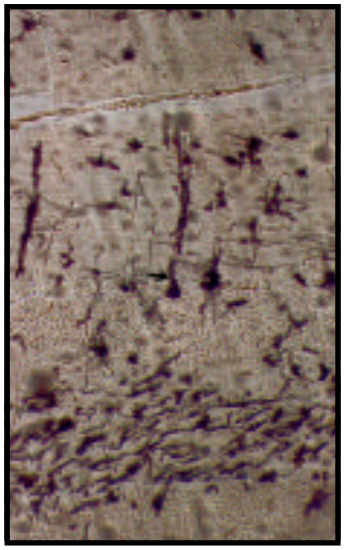

Figure 2 - A photomicrograph of the cerebral cortex in a 15-day-old rat embryo. The cells of the germinal layer appeared dark with irregular short multipolar processes. Cells of different shapes were observed in the transition zone between the germinal layer and the ependymal layer. Some of these cells seemed morphologically as developing pyramidal cells with a triangular shape and an elongated apical process (see the arrows). Golgi mixed stain X 100.

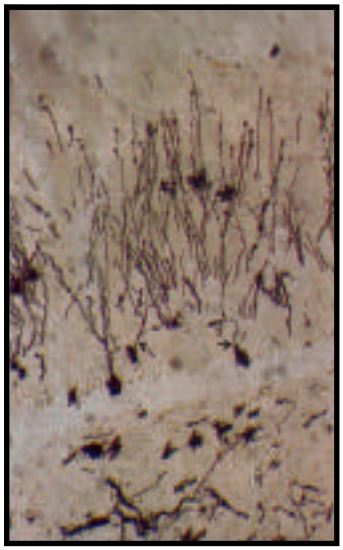

Figure 3 - A photomicrograph of the cerebral cortex of an 18-day-old rat embryo, the growing pyramidal cells presented long apical dendrites. Each apical dendrite exhibited a relatively long stem (see the arrows) with 2-4 beaded branches. Golgi mixed stain X 100

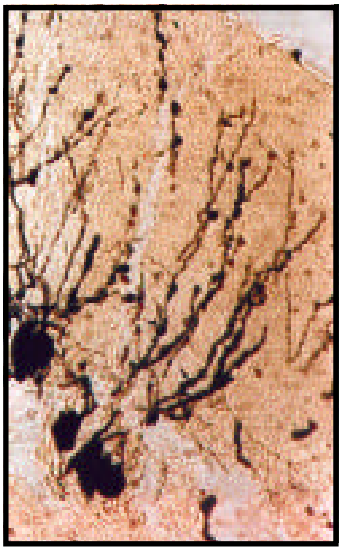

Figure 4 - A photomicrograph of the cerebral cortex of birth rat. The stems of the apical dendrites of the pyramidal cells became shorter and beading of the dendrites (see the arrows) was more numerous than seen at earlier ages as described in the above figures. Golgi mixed stain X 250.

Figure 5 - A photomicrograph of the cerebral cortex of a one-week-old rat. The apical dendrites and their branches developed fine spines which were perpendicularly situated on their axes. Beading of these dendrites was scarce and for the first time a display of the pyramidal cells axons (see the arrows) had been detected. Golgi mixed stain X 250.

Figure 6 - A photomicrograph of the cerebral cortex of a 2-week-old rat, the pyramidal cells maintained their apical dendrites in a much similar condition as the already described preceding stage of development. There was an elongated well defined axon for each pyramidal cell. The initial appearance of 2 or more basal dendrites (see the arrows) was the main feature at this stage of development of the pyramidal cells. Golgi mixed stain X 250.

Table 1 - The means of length and number of apical dendritic segments through pre-postnatal stages.

Successive pre-postnatal ages	Mean total length of a dendrite	Mean number of segments in a dendrite
15 days	50.135±5.45	3.45±2.40
18 days	159.375±3.75	9.935±1.27
Day of birth	311.20±4.32	17.65±2.50
One week	535.02±3.42	24.55±3.25
2 weeks	793.86±3.17	37.33±3.40

morphologically familiar 4 fields of pyramidal neurons or primarily accomplish this through making descendants of several kinds namely the first differential form or do forms pursue different sequences to ultimately produce the permanent neurons? 4. Do they differentiate in the stratum germinosum (subependymal layer) and then migrate to the stratum pyramidale or migrate first to differentiate later? 5. Do they migrate at all or it is just simply an expanding cell population as a result of the original germ cells having a replication multipotent capacity and later differentiation in situ? 6. Does the variable morphological (size and shape) differentiation of these cells randomly take place as a mechanical bouncing of a crowd of cells where each one competes for a place to grow and establishes its connections with other neurons in a rather limited space or could there be a determined pregenetical physiological significance behind this specific display of morphological varieties of cells?

Answers for the above questions can only be speculative at the moment and for as long as the clear evidence is absent. However, the true answers may be achieved through development of further techniques that can give a better illustration of events involved in development of the cerebral cortex.

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