Morphometric analysis of the cerebral cortex in the developing baboon

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Summary

Morphometric analysis of the wall of the cerebral cortex in 49- and 50-day old baboon foetuses was carried out using formalin-fixed stratified serial sections of the heads. Four zones -medial, superior, lateral and inferolateral- in the cortical wall of the left cerebral hemisphere were observed from frontal to occipital regions. In each zone, the thickness of the wall and laminae was measured with a calibrated integrating graticule using a light microscope. The relative volume of the cortical wall was 36-40% of the left cerebral hemisphere. The cortical wall increased progressively in thickness from the medial to superior, lateral and inferolateral zones. Five laminae of varying thicknesses composed of light- and dark-stained cells were observed in the cortex. No distinct neuronal types were identified in the lamina representing the vertical and horizontal migration of neuroblasts during development. The outermost lamina (a) was thin and uniform throughout, while lamina (b), (c) and (d-e) were alternately dark- and light-stained, being thickest in the inferolateral wall and in the parieto-temporal region. Morphometric variations in the cortical wall in 49-50-day old baboon foetuses are similar to those seen in the adult animals and may reflect the later functionally specialized regions of the cerebral cortex.

Key words: Developing baboon – Cerebal cortex – Wall – Laminae – Analysis

Introduction

As the development of the prosencephalon proceeds, the anterior part of the telencephalon forms the cerebral hemispheres, enclosing the lateral ventricles by stage 15, 30-33 days, in the monkey and 35-38 days in humans (Gribnau and Geijsbert, 1985). The neuroepithelial cells from the ependymal region migrate to the outer surface of the hemisphere to form the cerebral cortex. The laminar organization of the cortex develops as the germinal epithelium undergoes mitosis, and neuroblasts migrate vertically and horizontally as they mature (Gilbert, 1997).

In the developing brain it is not possible to histologically identify cell types within the laminar organization of the cortex (Korr, 1980). Increases in the size and thickness of the cerebrum in the developing human cortex occur from premature ages up to six years of age. Three cell layers eventually form six layers and adult cell types form post-natally (Rabinowicz, 1974).

Two cerebral hemispheres are formed by stage 16, 32-34 days in the monkey and 37-42 days in humans. Differentiation in the thickness along the cortical wall is evident from stage 18, 36-38 days in the monkey, and 46-49 days in humans, the lateral wall being thicker than the medial wall. The dorsal, lateral and caudal expansion of the hemisphere in stages 16-18 is followed by frontal and basal outgrowths, frontal and temporal lobes being formed by 36-42 days in the monkey and at 48-51 days in humans (Gribnau and Geijsbert, 1985).

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In the different regions of the telencephalon, the thickening of the wall of the ventricles occurs at different times and rates, resulting in morphogenetic transformation of the cerebrum during development (Fitzgerald, 1996). Expansion of the cerebral hemispheres is not uniform; one region on the lateral surface, the insula, is relatively quiescent and forms the pivot around which the expanding hemisphere rotates (Sanides, 1970).

As the organization of the cerebral lamina takes place, the width of the cortical wall is proportional to the size of the cells and the density of their distribution (Korr, 1980; Kaas, 2000). Comparison of the morphogenetic events during brain development in primates has shown that both the sequence and timing of such events are similar in the monkey and humans (Gribnau and Geijsbert, 1985).

The aim of the present study was to carry out qualitative and quantitative analyses of the developing cerebral cortex wall in 49- and 50-day old baboon foetus heads to provide baseline data on the regional thickness of the wall and laminae in the cerebral hemisphere.

Materials and Methods

The heads of one 49- and one 50-day old developing baboon foetuses were obtained from the Institute of Primate Research (IPR) during reproduction studies underway in 1985. The heads were fixed by immersion in 10% formalin and processed for paraffin wax embedding. Serial sections, (7 µm) in the coronal plane, from rostral to caudal, were made. All sections were collected and stained with cresyl/violet and neutral red dyes. The serial sections were sub-divided into frontal, parietal, temporal, and occipital regions of the brain in rostral to caudal direction.

Since the plane of section was slightly oblique for both heads, the left cerebral hemisphere was studied.

Qualitative and quantitative analyses of the wall of the cerebral hemisphere were carried out on every 20th section. A total of 16 sections from the 50-day and 15 sections from the 49-day foetuses were analyzed. In each of these sections, 4 zones were selected in the wall of the cerebral cortex Zone 1- middle of medial wall; zone 2- superior pole; zone 3- middle of lateral wall, and zone 4- inferolateral wall (Fig. 1).

A Leitz binocular light microscope was used to observe the laminae. The thicknesses of the wall and laminae in the four zones were measured using a calibrated integrating graticule (0.01 mm) in a single eyepiece.

Relative volume proportion

The method of point counting was used to determine the relative volume proportion of the cerebral wall, meninges and ventricles. The same stratified selected sections were projected onto a screen using a Leitz demonstration microscope (x 2.5). A grid with 300 points was superimposed on the image. The points falling on the cerebral wall, meninges, and ventricle were counted field-by-field to cover the entire left cerebral hemisphere.

Results

Qualitative analysis of the cortical wall of the left ventricle

In the selected stratified sections spaced every 20th section, the frontal region was defined from sections 1-4, the parieto-temporal region in sections 5-11 and the occipital region in sections 12-15(16). The temporal wall was observed laterally in sections 7-11.

In each of the regions, in the 4 different zones, the wall of the cerebral hemisphere was thinnest in the medial wall, becoming progressively thicker superiorly, laterally and inferolaterally (Fig. 1).

Microscopically, the outermost layer of the cerebral wall was the pia mater, with cerebral blood vessels. Five distinct laminae were identified as a, b, c, d and e from the pia mater inwards to the ependymal layer (Fig. 2).

Lamina a was a thin layer, lightly stained, with a few dispersed cells, and was of uniform thickness throughout the cerebral wall in all regions of the cerebral hemisphere.

Lamina b was darkly stained and was composed of cells with large nuclei, which were densely packed. It increased slightly in thickness from the medial, superior, lateral to inferolateral zones.

Lamina c was lightly stained, with a few widely dispersed cell nuclei showing many cell processes. It became thicker inferolaterally, along the rostro-caudal extension, being thickest in the parieto-temporal regions.

Lamina d was darkly stained, and it had densely packed cells with distinct nuclei. It increased progressively in thickness from medial, superior, lateral to inferolateral, being thickest in the parieto-temporal regions.

Lamina e was darkly stained, displaying very densely packed cells with large nuclei, which became rather sparse nearer the ependyma. The pattern of thickness along the wall, in all regions, was similar to lamina d.

Quantitative analysis of the cortical wall

The range of cortical wall thickness from frontal to occipital regions in zone 1 was 0.24-0.29 mm; zone 2 was 0.24-0.48 mm; zone 3 was 0.37-0.79 mm and zone 4 was 0.24-1.05 mm. The

thickness of the lateral wall of temporal lobe ranged from 0.44 to 0.54 mm.

Figure 3a,b shows the mean thicknesses of the laminae (a-e) in the cortical wall in the 49- and 50-day old baboon foetuses respectively along the medial, superior, lateral and inferolateral zones.

Figure 4a,b shows the mean thicknesses of the cortical laminae (a-e) in the selected sections 1-15 (16) in the 49- and 50-day old baboon foetuses respectively from frontal to occipital regions.

Figure 5a,b shows the relative volume proportions in the meninges, cerebral wall, and ventricles in the 49- and 50-day old baboon foetuses, respectively. The cortical wall comprised of 36.6% in the 49-day old and 40.4% of the left cerebral hemisphere in the 50-day old baboon foetuses.

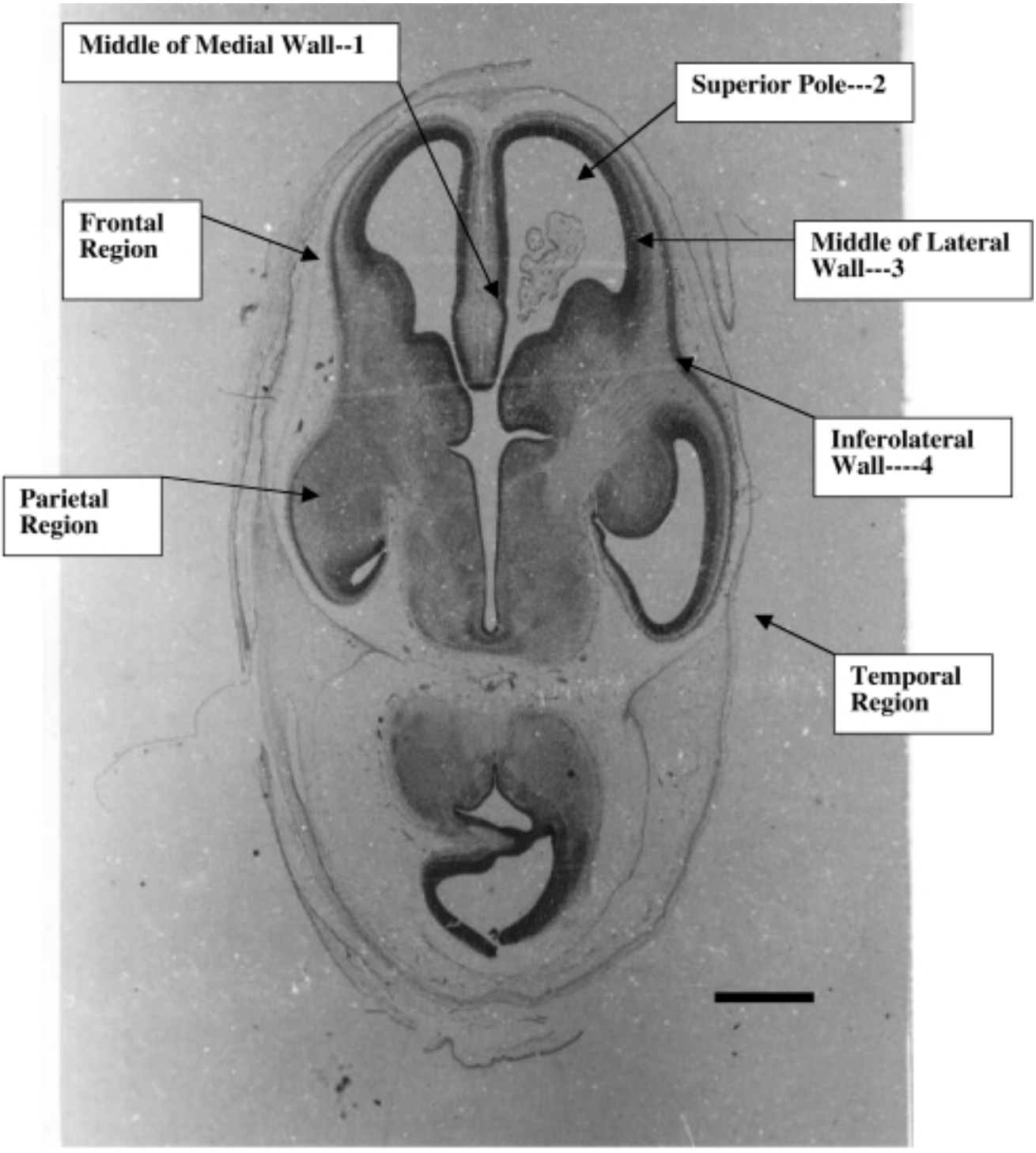

Fig. 1. Coronal section of the 50-day old developing baboon cerebral hemispheres showing the 4 zones and cortical regions. Scale bar = 0.32 mm.

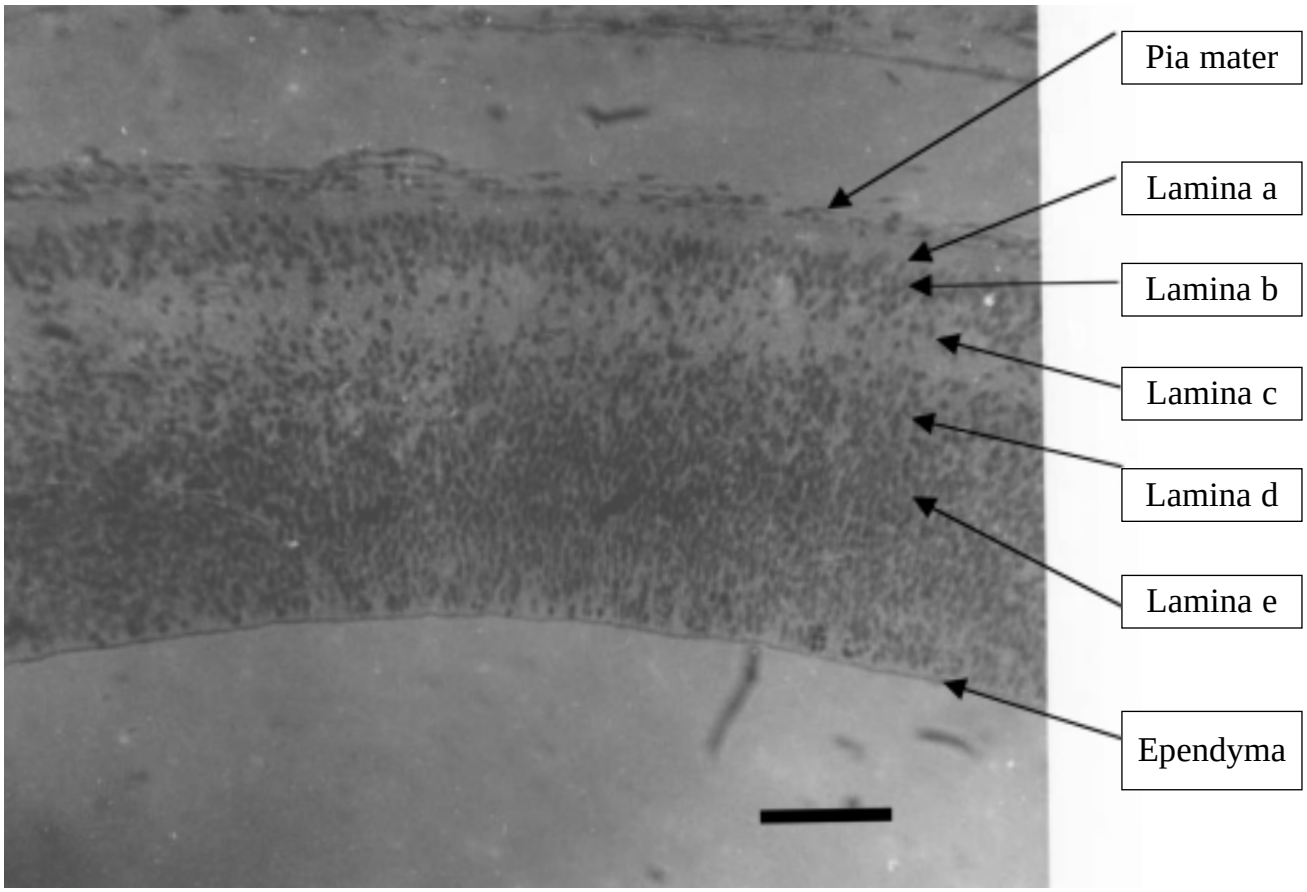

Fig. 2. Photomicrograph showing the microscopic structure of the lateral cortical wall of left ventricle in the 50-day old baboon foetus (x 10). Scale bar = 0.016 mm.

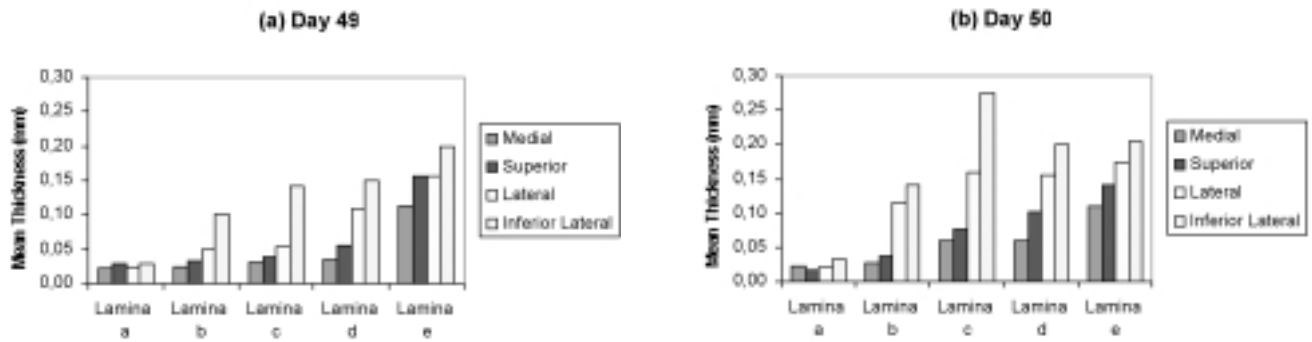

Fig. 3. Mean thickness of the laminae (a-e) in the cortical wall in (**a**) the 49- and (**b**) 50-day old baboon foetuses in the 4 zones.

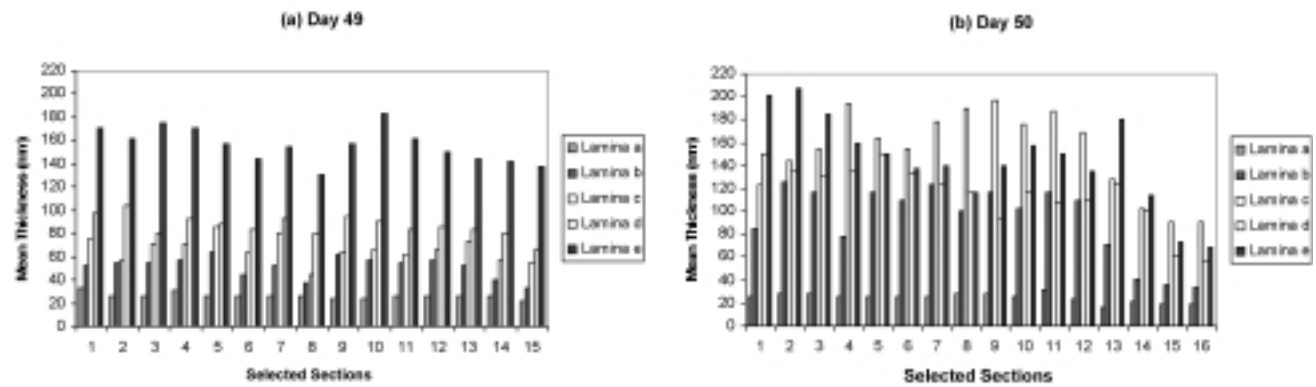

Fig. 4. Mean thickness of the cortical laminae (a-e) from frontal to occipital regions in selected sections 1-15(16) in (**a**) the 49-day and (**b**) 50-day old baboon foetuses.

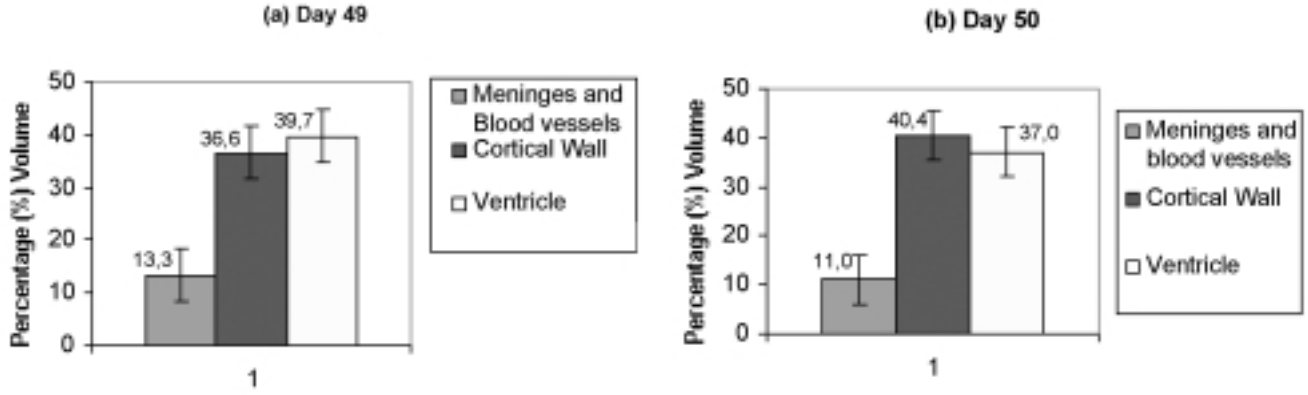

Fig. 5. Relative volume proportions of the cortical wall, meninges and ventricle in (**a**) the 49-day and (**b**) 50-day old baboon foetuses.

Discussion

In the 49- and 50-day old baboon foetus brains, equivalent to about human foetuses at day 57, the wall of the cerebral hemisphere was thinnest medially, becoming progressively thicker along the superior to lateral and inferolateral walls from the frontal to occipital regions. This pattern is similar to that seen in the adult human cerebral cortex (Sanides, 1970; Rabinowicz, 1974).

The thin, lightly stained lamina (a), with sparse cells adjacent to the pia mater, was of uniform thickness in all the zones. The progressive increase in the thickness of laminae (b) to (e) from medial to inferolateral zones may be related to later functional specializations of the cerebral cortex. (Sanides, 1970; Rabinowicz, 1974).

In the frontal and temporo-parietal regions, the laminae were of the greatest thickness and this may reflect their predominance in primates. In humans, the fronto-pariental region surface area accounts for 47% of the entire surface of the cerebral hemisphere (Blinkov and Glezer, 1968).

In the human isocortex in the 5th to 8th month foetus, the topographic changes of the cortical areas are caused by unequal growth of the hemispheres and the heterochromatic differentiation of cortical regions. The lateral and inferolateral zones have the greatest mean thickness, which in the neonate and adult would correspond to the area around the pre-central gyrus and temporal lobe, respectively (Kahle, 1966). Lamina (c) in the baboon cerebrum tends to be thick in the temporo-parietal region inferolaterally and may contribute to the formation of the corpus striatum (Rabinowicz, 1974).

The frontal lobes mature gradually along the cortical wall from lateral to basal, and the stratification of the isocortex begins frontally by the 4th month of foetal life (Kahle, 1966). The formation of sulci and gyri occurs later (Sanides, 1970; Rabinowicz, 1974).

Determination of the width of the cerebral cortex has shown that in a comparative anatomical series there is a tendency for the width of cortex to be thicker and more highly organized in primates (Blinkov and Glezer, 1968, Kaas, 2000).

A constant migration of neuroblasts occurs during development. The cerebral cells migrate horizontally and vertically from the ependymal layer. Of the young neurons, 80% migrate vertically from the ventricular zone into the cortex, while 12% migrate laterally from one area to another. The cells "know" in which area to settle. The cortical areas in the adult brain represent an organization that is already present in the ventricular zone of the foetal telencephalon. Each lamina in the cortex differs from others in functional properties, neuron types, and connections. The development of the cerebral cortex continues at foetal rate even after birth (Gilbert, 1997).

The relative volume proportion in 49- to 50-day old cerebral hemispheres shows a 4% increase in cortical wall as compared to the situation in a 49 day old foetus with a 3% decrease in ventricular volume. This difference may be a daily growth change or as a result of variance in the plane of sectioning in a limited sample. The volume proportion of the left cerebral hemisphere wall in the 50-day old baboon foetus is 40% as compared to 44% in the adult human (Blinkov and Glezer, 1968).

The laminar arrangement of neurons was apparent in the 50-day old developing cerebral cortex, with zonal variations in thickness. Phylogenetically, old elements, including the limbic cortex, are tri-laminar whereas six laminae are seen in the neo-cortex (Gilbert, 1997). In the developing baboon brain, the preliminary pattern for later cortical cytoarchitecture is seen to be established by day 50 in the walls.

Morphometric analysis of the developing baboon cerebral cortex has shown that the four zones and laminae in the walls differ from each other in the different regions and the pattern is similar to that seen in the adult. These zones may correspond to the later functionally specia-

lized regions of the cerebrum in the adult and may reflect the evolutionary, functional and phylogenetic aspects of the species (Kass, 2000).

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