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The Marginal Layer in the Neocortex of a 7 Week-Old Human Embryo

A Light and Electron Microscopic Study

Jeanne-Claudie Larroche

Maître de Recherches au C.N.R.S., Laboratoire d'Anatomie Pathologique, INSERM U 29, Hôpital Port-Royal, 123 Bd. de Port-Royal, Paris 75674 Cedex 14, France

Summary. Ultrastructural study of the molecular layer of the neocortex of a 7 week-old human embryo confirms recent observations on various laboratory animals that call for revision of some classical concepts of corticogenesis.

1. At 7 weeks, the subpial, marginal or molecular layer is the first layer to differentiate from the ventricular layer and represents almost half the thickness of the telencephalic vesicle.

2. The first cells that have already migrated from the ventricular zone, even before any cortical plate is visible, are to be found in this marginal layer. These large cells are well differentiated and most probably represent the so called Cajal Retzius cells.

3. The earliest synapses ever seen in human embryol are found in the marginal or plexiform layer; this indicates the presence of a precocious set-up for an elaborate neuronal circuitry at this level.

Key words: Neocorticogenesis – Marginal layer – Human embryo

Introduction

For many years the formation of the molecular or plexiform layer has intrigued histologists. Some have ignored it when describing the process of cortical plate formation (Sidman and Rakic 1973); the Boulder committee (1970) stated: "this zone contains no primary cell type of its own and the nuclei of the ventricular cells do not enter it"; others asserted that the layer appears after the cortical plate (Meller et al. 1968, Takashima 1980), or simultaneously (Poliakov 1961). However, the cellular components of this layer were already described a century ago by our illustrious predecessors, Cajal (1980) and Retzius (1891), hence their designation as Cajal-Retzius cells. The present electron microscopic study of the wall of the hemisphere in a 7 week-old embryo provides information regarding the time-course of neocorticogenesis, and morphological details of the plexiform layer and Cajal-Retzius cells.

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Material and Methods

A 7 week-old human embryo (20 mm C.R. length) became available from a surgical hysterectomy. The brain was fixed in toto by immersion in a solution made up of 3% glutaraldehyde in cacodylate buffer 0, I.M. A week later, the telencephalic vesicles or cerebral hemispheres were dissected out and cut on coronal planes. Slices of about 1 mm thick were made at the level of the anterior and superior one-third of the external wall of the vesicle, including the entire thickness of the wall. Blocks of tissue were postfixed in a 1,25% osmium tetroxide solution for 2 h and subsequently dehydrated in increasing concentrations of ethanol, then embedded in araldite. Semithin sections were stained with toluidine blue. Ultrathin sections were stained on the grids with uranyl acetate and lead citrate, and studied with a Philips 300 E.M.

Results

Semithin Section (Fig. 1). In the human embryo of 7 weeks (9 weeks amenorrhea or gestational age), the external wall of the mid anterior part of the brain is about 1 mm thick and is composed of two distinct layers:

a) The *inner layer,* the matrix or germinative zone or ventricular layer, which looks like a pseudo-stratified epithelium. Many mitoses are visible at various levels near the ependyma; in the outer part, the nuclei of the immature cells are lighter and rounder than in the inner part of the matrix; the cell density is somewhat higher and the columnar arrangement of the outermost immature cells is readily visible.

At this stage of development there is no cortical plate.

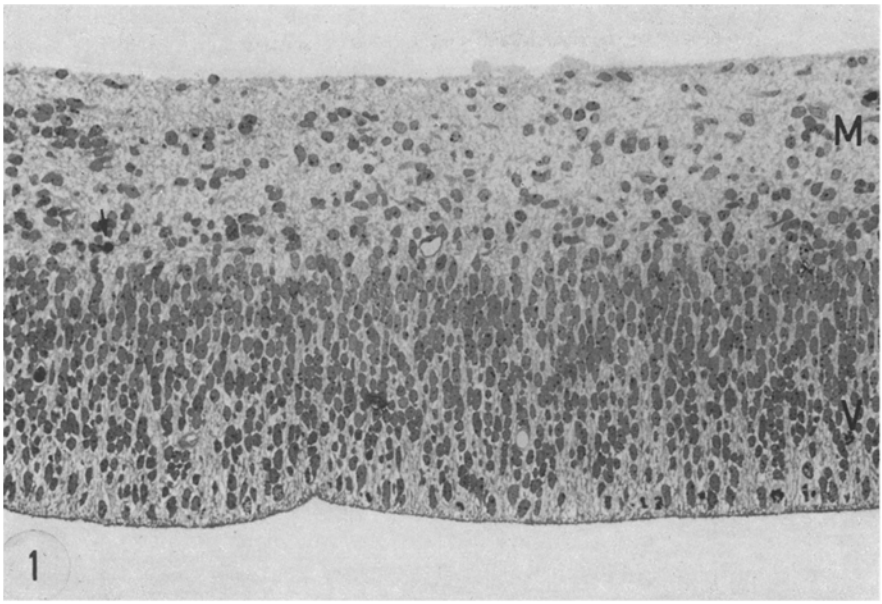
b) *The outer layer* or molecular, marginal, plexiform layer or layer I, comprises the external half of the ventricular wall. The cell density is much higher than that of a newborn and that of an adult. All the nuclei are lighter than those of the ventricular layer; at times, a large cytoplasmic extension is found parallel to the surface.

Ultrathin sections. We focused our attention on the outer layer or plexiform layer. The pia mater has been slightly torn and the external limiting membrane is occasionally disrupted. The general plexiform structure of the entire layer is remarkable. However, at this early stage the loose aspect of the tissue is particularly striking; it is due to the wide extracellular space (Fig. 2) already recognizable in animal embryos (Johnson and Armstrong 1970). The osmolarity of the fixative solutions does not seem responsible for this peculiar appearance since other specimens, two weeks older, and fixed with the same method, present less "extracellular space", and since the intracellular structures in the case under study are well preserved.

The Cells

By contrast with the low cell density of the molecular layer in a 36 weeks (Rabinowicz 1964), or 40 weeks newborn (Conel 1930) we found many cells, different from those of the ventricular zone and bearing characteristic features of Cajal Retzius cells, (C.R.c)[1]. The cells are dispersed in the molecular layer,

[1] Although Retzius was the first to describe these cells in man, we adopted, after König (1978), the terminology of Cajal-Retzius cells



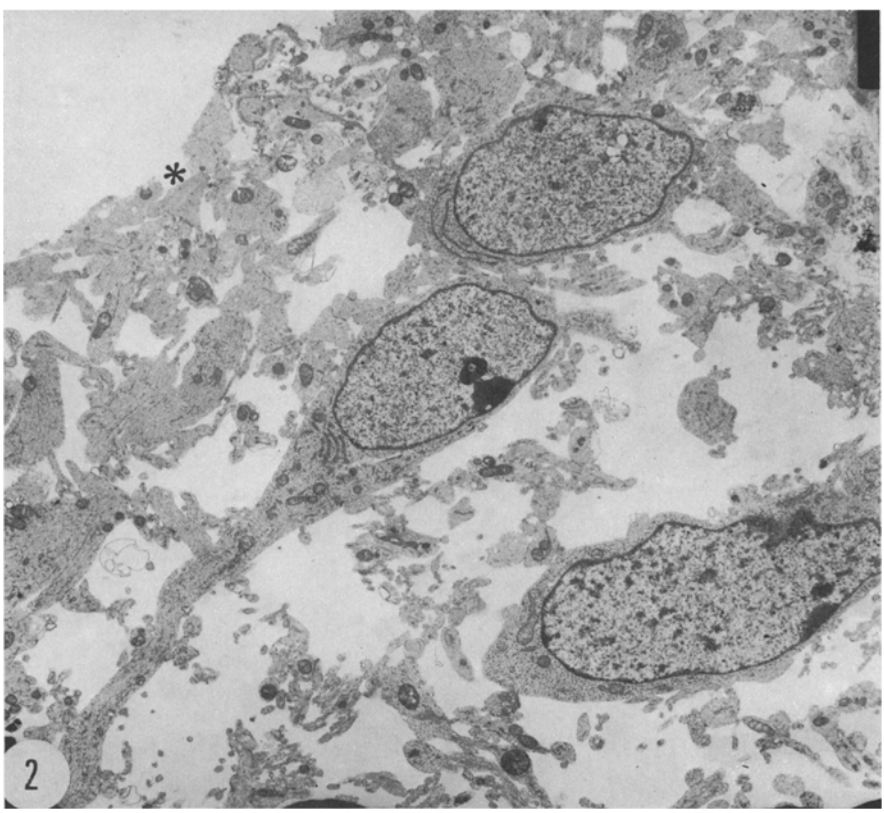


Fig. 1. Semithin section of the entire telencephalic wall. Note the thick, cellular marginal layer (*M*), and the one mitosis (*arrow*) at the junction with the ventricular zone (*V*) × 600

Fig. 2. Ultrathin section of the plexiform layer. Wide extracellular space. Three Cajal-Retzius cells and glial feet (*asterisk*). × 5,000

but even the most superficial ones are separated from the meninges by interposed glial processes (Fig. 3). The nuclei are ovoid, often irregular; the chromatin is fine and evenly distributed, with some clumps on the nuclear membrane which presents a three-layered structure. One or several nucleoli may be found.

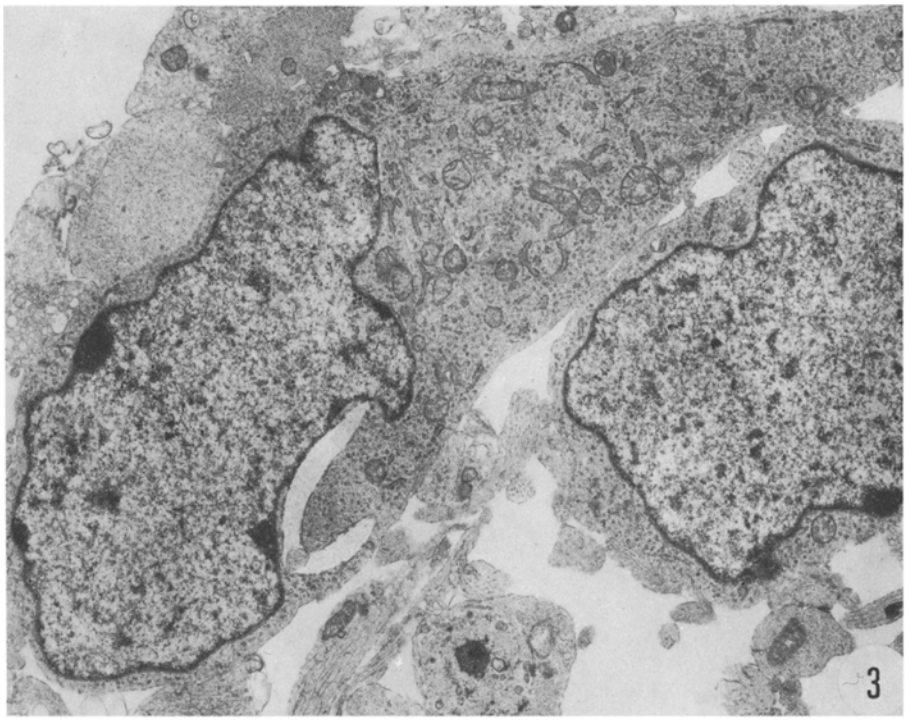
The cells exhibit a voluminous, horizontally oriented cytoplasm, containing accumulations of organelles (Fig. 3) such as mitochondria, granular endoplasmic reticulum (Fig. 4) and dispersed ribosomes, dense bodies or lysosomes, smooth reticulum with Golgi complexes and vesicles (Fig. 5). Both cilia and centrioles are found near the nucleus. Many cells have thick protoplasmic processes resembling extensions of the perikaryon; in the dense matrix, typical cell organelles are recognizable such as large, elongated mitochondria, numbers of microtubules in parallel rows or intermingled with rosettes of ribosomes and vesicles. At times, the tubules turn at right angles in order to enter into another large process branching off the main shaft (Fig. 6).

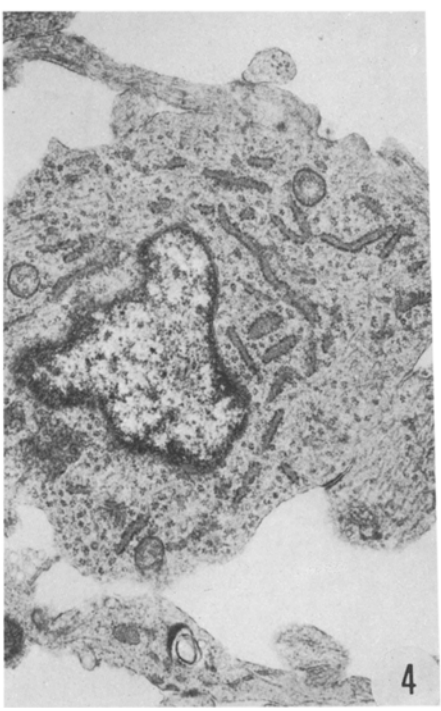
A few other nuclei, slightly different, are found in the marginal zone but cannot be identified with certainty. In view of the dense chromatin of the nucleus they could be young C.R.c., and since their cytoplasm contains large, dense vacuoles they may be degenerated cells. None of them seems to pertain to glial cells. Surprisingly, one mitosis was found in the deep part of the plexiform layer (Fig. 7).

The Processes

The plexiform character of layer I is due to the many processes oriented in various planes (Fig. 2); at times it may be difficult to identify them as axons, dendrites or glial fibers; on cross section the diameter of the processes varies from that of tiny filopodia of less than 100 nm. to that of large vesicular formations of several microns. Some contain few tubules, rosettes of ribosomes, mitochondria, smooth endoplasmic reticulum, many vesicles and multivesicular bodies; others look like axons although the double-lined axonal membrane cannot be identified; they contain neurotubules, occasional mitochondria, vesicles and very scarce ribosomes. Other processes contain almost no profile structures but have a fine electron lucent matrix and a few vesicles. Coated vesicles and coated invaginations of the plasma membrane occur with considerable frequency (Fig. 8). The most striking feature of this layer is the multitude of large varicosities or growth cones, either dendritic or axonal, (Povlishock 1976). These enlargements sprout from the end processes or even from a point very close to the soma of a C.R.c.. They are filled with multiple spherical vacuoles (Fig. 9).

The radial glial fibers are not identifiable for a long distance, since the plane of section was not exactly perpendicular to the pial surface. Instead of forming closely apposed end feet on the basal lamina as in an 8–9 week old fetus, (Larroche et al. 1981), they form a sort of cobble-stone arangement; in their fine, electron lucent matrix, various structures such as mitochondria and smooth endoplasmic reticulum are recognized; glycogen is scarce and glial filaments could not be found.





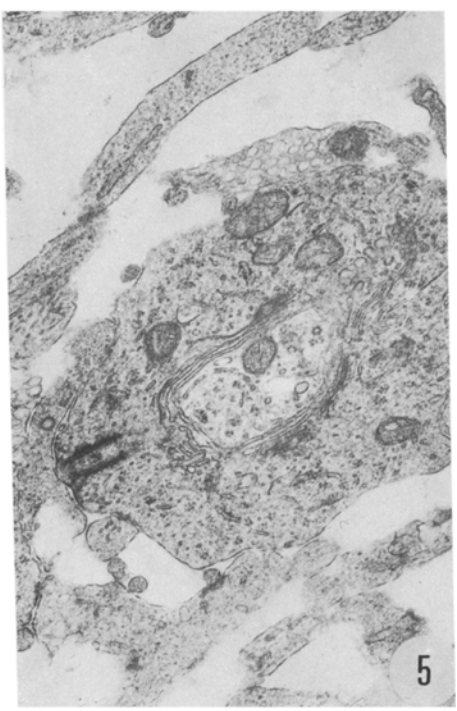
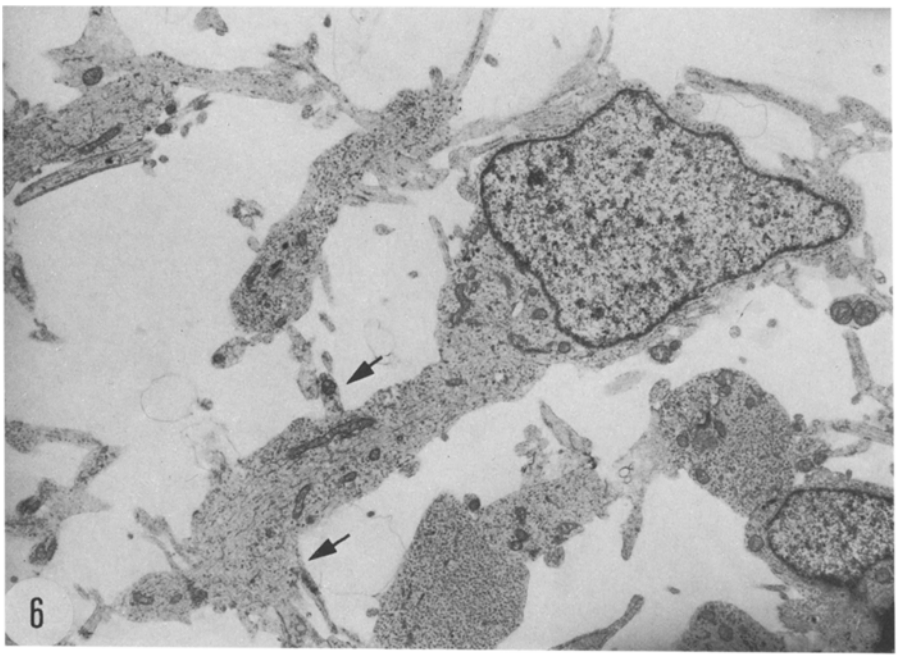


Fig. 3. Details of the perikaryon of a Cajal-Retzius cell. Note the glial feet interposed between the pia and the C.R.c. ×8,800

Fig. 4. Fragment of nucleus and abundant rough endoplasmic reticulum in the perikaryon. ×12,000

Fig. 5. Fragment of the perikaryon, with Golgi apparatus and basal body of a cilium, ×20,000



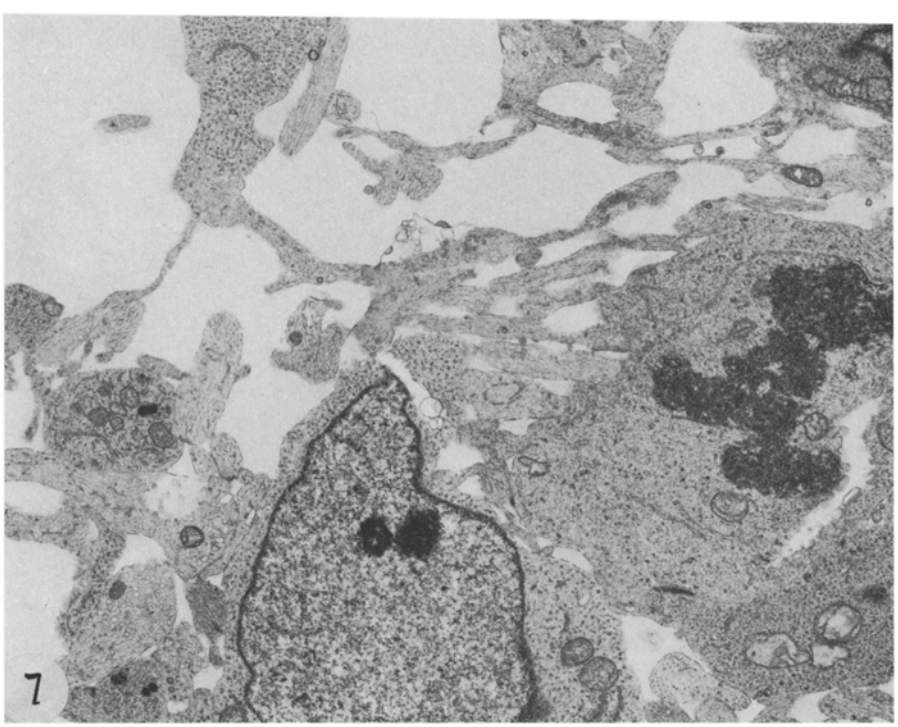


Fig. 6. Horizontal process of a C.R.c., containing elongated mitochondria and neurotubules. Perpendicular branchings of various sizes (*arrows*). × 5,500

Fig. 7. Cajal-Retzius cell and a mitosis in the deep plexiform layer. × 7,200

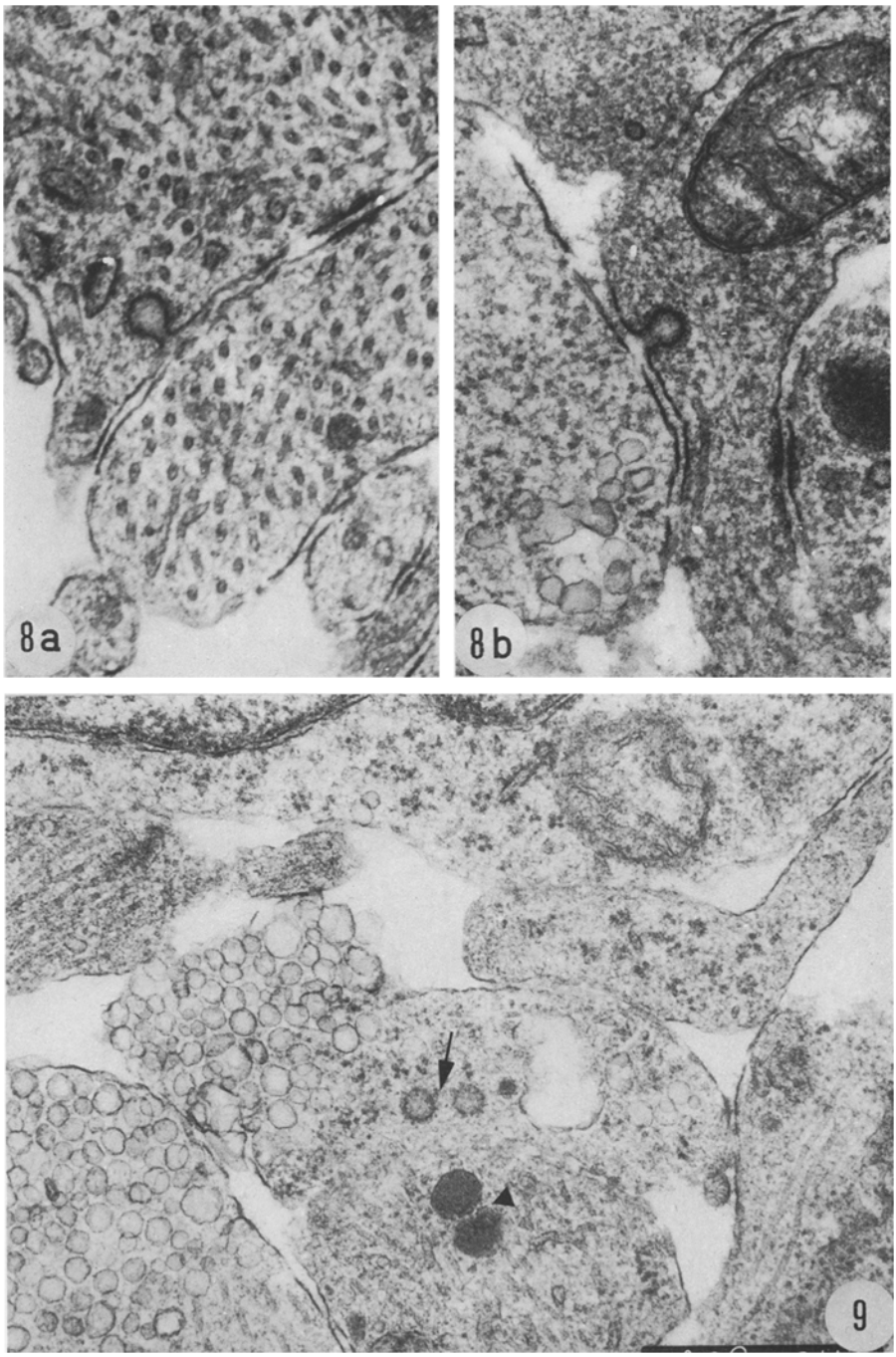


Fig.8a, b. Adhesion plaques and coated invaginations. × 60,000

Fig. 9. Details of growth cones filled with multiple vesicles, ribosomes, lysosomes (*arrow head*) and coated vesicles (*arrow*). × 39,000

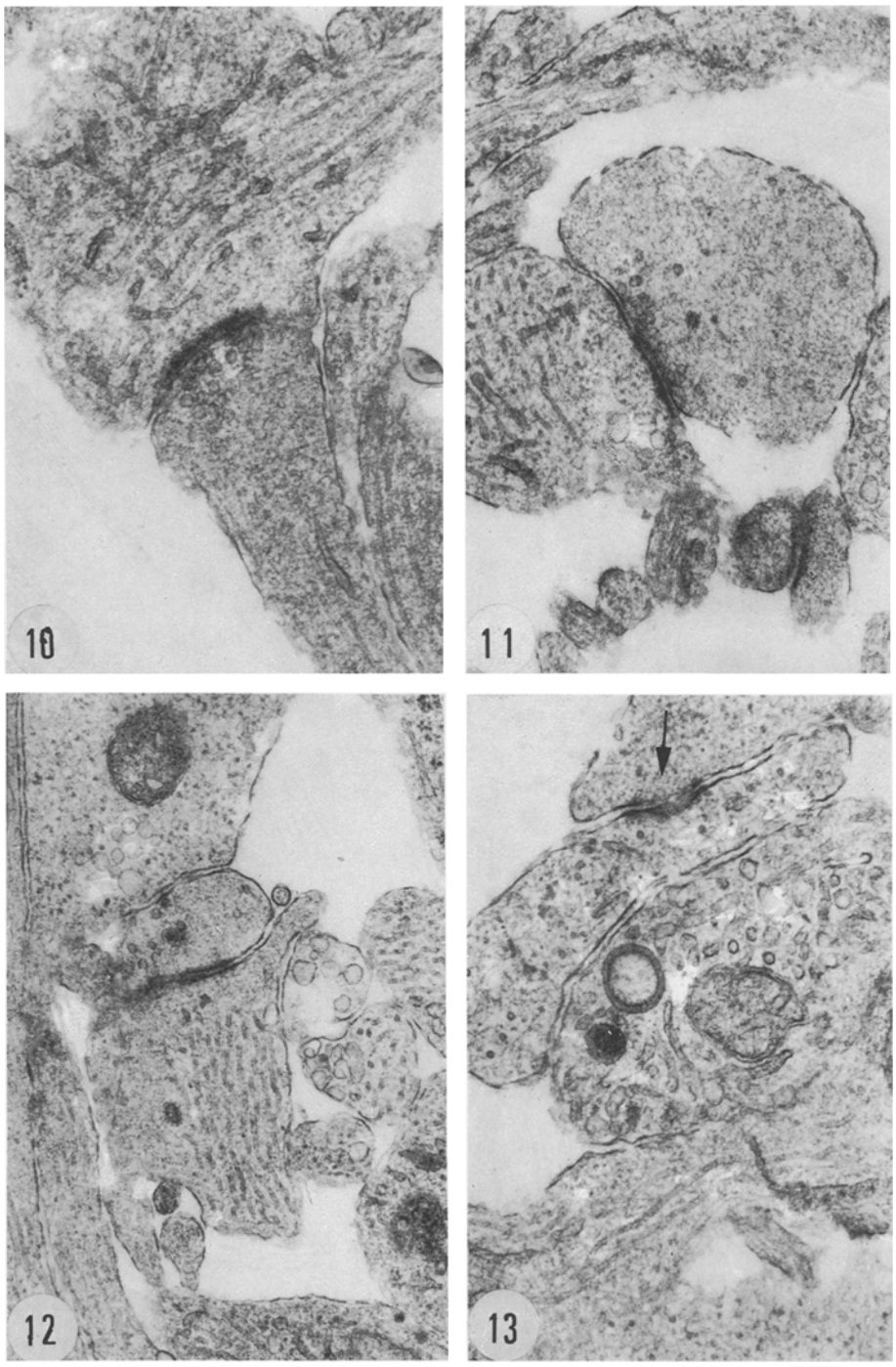


Fig. 10. Axo-dendritic synapse with markedly asymmetrical densities. ×42,000

Fig. 11. Axo-dendritic synapse with slightly asymmetrical densities. ×30,000

Fig. 12. Synapse "en passant" with central cored vesicle. ×30,000

Fig. 13. Adhesion plaque (*arrow*) and various profiles in a nearby growth cone. ×42,000

Intercellular Junctions

Synapses are rare (Figs. 10–11); the few that are identifiable are axodendritic and present the classical morphological features. However, the asymmetry of the membrane densities is not always evident and the vesicles are relatively fewer than in older fetuses (Larroche et al. 1981). In a synapse "en passant", central cored vesicles are associated with clear vesicles (Fig. 12); they resemble those described by Johnson and Armstrong (1970) in a 1 day-old rat. Cell junctions of other types are abundant. Various names have been given to these formations such as "desmosome-like junctions" "adhesion plaques" "maculae adherentes diminutae" "punctate intermediate junctions" (Fig. 13). They are found between homologous elements such as two dendrites, or between a dendrite and a soma, less frequently between an axon and a soma or an axon and a dendrite. The reciprocal membrane densities are symmetrical and there are no associated vesicles.

Discussion

In the seven week-old human embryo, the neopallium comprises two layers only: the ventricular zone or matrix, and the superficial plexiform layer. This is also the case, for example, in mice embryos of 11/12 days (Derer 1977), in rat embryos of 12/13 days (Readler and Sievers, 1975–76; König et al. 1977; Wolff 1978) or even of 13/14 days for (Rickmann et al. 1977), in cat embryos of 20/25 days (Marin-Padilla 1971) and in sheep embryos of 20/25 days (Aström 1967). The neopallium at this stage of development resembles the telencephalon of the chick embryo of 3/5 days incubation (Wechsler and Meller 1967).

The plexiform layer contains large neurons or Cajal Retzius cells whose morphological attributes resemble those described in older human embryos (Larroche et al. 1981) and in laboratory animal embryos (Sousa Pinto et al. 1975; Raedler and Sievers 1975–76; Bradford et al. 1977; Rickmann et al. 1977; Shoukimas and Hinds 1978). These neurons form a special class whose development and maturation proceeds in advance of those of the rest of the brain. Duckett and Pearse (1968) showed that in young human fetuses they are the first site of cholinesterase activity, which indicates potential excitability; thus, presumably, they are the earliest functional neurons in the developing human brain. Purpura (1961) opined that they could be responsible for the predominance of surface negative potentials.

Moreover, the first synapses to develop are observed in this layer. This confirms the precocity of the structures of this area and the early organization of an elaborated circuitry at this level. In laboratory animals, synapses were also first seen in the molecular layer by Voeller et al. (1963); König et al. (1975) and Rickman et al. (1977).

These observations do not correspond to the concept of cortical neurogenesis and differentiation characterized by the inside-out process, proposed by Angevine and Sidman in 1961 and since generally accepted. Meanwhile, Marin-Padilla (1970–71–72–78), who described the primitive neocortical organization and the primordial plexiform layer in cat embryos, offered an alternative theory, i.e. "The dual origin of the mammalian neocortex". The primordial layer of the

mid anterior hemispheres composed at first of corticopetal fibers from the thalamus (Bartelmez and Dekaban 1962) induces the maturation of the first neurons, including the Cajal-Retzius cells, that have migrated out of the ventricular layer. This external layer of white matter would be a reminiscence of the amphibian brain. Sanides and Sanides (1974) also consider the subpial layer to be the oldest one, phylogenetically as well as ontogenetically.

In human embryos about two weeks older we found (Larroche et al. 1981) synapses not only in the marginal layer but also below the newly-formed cortical plate. This double site for early synaptogenesis was also noticed in man by Molliver et al. (1973) and Povlishock (1976), and by Wolff in the rat (1978).

In animal experiments a few authors have paid passing attention to the labelled neurons above and below the cortical plate (Angevine 1965; Aström 1967). Others (Derer et al. 1977 and Raedler et al. 1980) have emphasized this unique event in the tempo of migration and partition of early-formed neurons. Our observations of primitive neurons and early synapses corroborate the concept of Marin-Padilla of a primordial plexiform layer being secondarily split by the forming cortical plate into a superficial layer I and a deep layer VII. Although Rickmann et al. (1977) recognize, from their experiment, the separation of the molecular layer by the cortical plate into a superficial and a deep portion, they do not agree with Marin-Padilla's interpretation of this evolution (layer I and VII) from the reptilian cortex.

A comment should be made regarding the various types of junctions which we found in the plexiform layer. These intercellular junctions have been described in various animal embryos and called "maculae adherentes diminutae" "adhesion plaques" or "attachment plates", "punctate intermediate junctions" (Rickmann et al. 1977; Derer et al. 1977). In human fetuses they were first described by Gruner in 1970. Their functions are still debated and their fate unknown. However, the high frequency of occurrence of such temporary junctions in developing brains suggests a relationship with true synapses and a possible role in facilitating development of more elaborate junctions (Gruner 1970). Other authors (Privat 1974) opine that such membrane densities may be continuous with invaginations and may be removed actively by a process of sequestration into coated vesicles. Hence, the large number of desmosome-like junctions may not necessarily be associated with the process of synaptogenesis.

This puzzling phenomena of multiple coated vesicles in early human neurogenesis is under study. Their considerable frequency in young rats was already noticed by Johnson and Armstrong-James (1970), who suggested that the coated invaginations were involved in micropinocytosis.

The last point of discussion concerns the fate of the Cajal-Retzius cells. The remarkable density of the C.R.c. population in the plexiform layer of young human embryos should be emphasized. Does the number of Cajal-Retzius cells increase even after the cortical plate has been formed? Can they be produced locally, as Rickmann et al. (1977) questioned? The mitosis that we, unexpectedly, observed in the plexiform layer is interesting in this context. Does this number represent the definitive quota of cells already estabished by 7 weeks, as the mature aspect of the marginal layer would suggest? If so, the small number of cells, or even their "absence", in infant and adult brains would imply a

"dilution" by virtue of a much greater augmentation of the superficies, i.e. of the molecular layer (Fox and Inman, 1966 and Raedler and Sievers, 1976).

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