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Structural Organization of the Human Cerebral Cortex Prior to the Appearance of the Cortical Plate*

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Summary. The early development and the structural organization of the human cerebral cortex, prior to the appearanc of the cortical plate (Carnegie stage 22, ca. 54 days), was studied in two embryos: 43 (stage 18) and 50 day old (stage 20), respectively. It has been shown that the human cerebral cortex begins its ontogenetic development around the sixth rather than around the eighth week of gestation as it has been previously assumed. The human cerebral cortex starts to develop soon after the cerebral vesicles have been formed (stage 15) and a primitive internal capsule has been established (stage 17, ca. 41 days). By stage 18 of human development fibres from this primitive internal capsule have reached and probably have penetrated into the developing cerebral vesicle, through its more superficial zone. Fibres from this primitive internal capsule have been traced backward through the ventral thalamus to the mesencephalic tegmentum. The possible existence of primitive ascending fibres from the mid-brain which terminate in the superficial zone of the developing cerebral cortex (tegmento-thalamo-striato-cortical tract) is suggested. The arrival of these primitive corticipetal fibres establishes in the outer zone of the cerebral cortex a primordial plexiform lamina or an external white matter. Horizontal-bipolar cells (embryonic Cajal-Retzius neurons) begin to differentiate by stage 18 of human development (43 days in our case). By stage 20 (50 days in our case), the primordial plexiform lamina is well established, extends throughout the entire surface of the developing cerebral cortex, and is considered to be functionally active. It is, by this age, a superficial, 40 μm thick, complex fibrillar neuronal organization composed of numerous horizontal corticipetal fibres (demonstrable with silver methods), horizontal-bipolar Cajal-Retzius neurons and a few other, less defined,

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cellular elements. This primordial plexiform lamina is considered to represent a primitive "premammalian" cortical organization. The next event in cortical ontogenesis is the appearance of the cortical plate or the mammalian neocortical grey at stage 22 (ca. 54 days). Migrating neuroblasts attracted toward the preexisting primordial plexiform lamina and guided by glial fibres start to accumulate within it. The appearance of the mammalian neocortical grey divides the primordial plexiform lamina into a superficial plexiform or layer I (external white matter) and a deep plexiform or layer VII (subplate zone). Layer I is considered to play a significant role in the overall structural organization of the cerebral cortex by controlling the migration of all its pyramidal neurons. In cortical ontogenesis the mammalian neocortical grey (cortical plate) will only give rise to layers VI, V, IV, III and II of the adult cerebral cortex. These observations further corroborate the concept of the dual origin, composition and nature of the mammalian cerebral cortex including that of man. They also demonstrate that the human cerebral cortex starts to develop much earlier than was previously thought.

Key words: Origin – Organization – Cerebral cortex – Human

Introduction

Since classical times (His 1904), the appearance of the cortical plate (the primordial cortical grey) has been considered to mark the beginning of mammalian cortical neurogenesis, including that of man (Bartelmez and Dekaban 1962). This classical concept, which is acknowledged by most textbooks of embryology and neurohistology and is still favored by some investigators (Boulder Committee 1970; Rakic 1972, 1974, 1982), has been challenged by Marin-Padilla (1971, 1978). He has pointed out that the first change observed in the undifferentiated neuroepithelium of the mammalian cerebral vesicles is the formation of a superficial plexiform lamina (external white matter) composed of both corticipetal fibres and horizontal-bipolar neurons. The formation of this primordial plexiform layer, which corresponds anatomically to the "marginal zone" of classical terminology, marks the actual beginning of cortical ontogenesis. The second change is the appearance of the so-called cortical plate *within* the preexisting primordial plexiform lamina, dividing it into a superficial plexiform or layer I (external white matter) and a deep plexiform or layer VII (subplate zone). This new concept also proposes that layer I plays a significant role in controlling the progressive "inside-out" formation of the cortical plate and consequently in the overall organization of the pyramidal neurons of the mammalian neocortex (Marin-Padilla and Marin-Padilla 1982).

The presence of both horizontal-bipolar neurons (Cajal-Retzius neurons) and corticipetal fibers in the superficial lamina of the cerebral cortex prior to the appearance of the cortical plate (which was originally described in

cat embryos) has been corroborated in mouse (Goffinet and Lyon 1979), rat (König et al. 1975, 1977, 1981; König and Marthy 1981; Raedler and Sievers 1976; Raedler and Raedler 1978; Raedler et al. 1980; Rickmann et al. 1977; Rickmann and Wolf 1981; Sievers and Raedler 1981) and recently in human embryos (Larroche 1981; Larroche and Houcine 1982).

However, the early events in the human cerebral cortex leading to the establishment of the primordial plexiform lamina have not been adequately investigated. Neither has the "embryonic timing" for these early fundamental events been determined in human cortical neurogenesis. This lack of knowledge is undoubtedly due to the fact that the early developmental stages of the human cerebral cortex, prior to the appearance of the cortical plate, have not been adequately studied, perhaps because there was no need to study them since the appearance of the cortical plate has been generally assumed to mark the beginning of cortical neurogenesis. Also, the "marginal zone" of the developing cerebral cortex has been considered to lack neuronal elements early in its development (His 1904; Boulder 1970; Rakic 1982).

The present communication will analyze the structural organization of the human cerebral cortex in two embryonic stages (Carnegie stages 18 and 20) prior to the appearance of the cortical plate. The cortical plate is first recognized in the human cerebral cortex at stage 22 of embryonic development (His 1904; Bartelmez and Dekaban 1962; O'Rahilly and Gardner 1971, 1977). However, Molliver et al. (1973) have pointed out that it might begin to form at stage 21. Our observations concerning the development of the human cerebral cortex at stages 18 and 20 will be analyzed and correlated with data from stages 17 and 22, respectively, gathered form the literature (His 1904; Hochstetter 1919; Hines 1922; Gilbert 1935; Streeter 1948; Hewitt 1961; Bartelmez and Dekaban 1962; Yokoh 1968; O'Rahilly and Gardner 1971, 1977).

Material and Methods

Two human embryos were studied. The younger one was obtained from a tubal pregnancy which was diagnosed by ultrasound and surgically removed unruptured. This embryo measured 15 mm in crown-rump length and was ca. 43 days old (Fig. 1A). The older embryo was obtained from an hysterectomy specimen and measures 22 mm in crown-rump length and was ca. 50 days old (Fig. 1B). The age, size, external configuration, and development of the extremities and head place these embryos at Carnegie embryonic stages 18 and 20, respectively (Bartelmez and Dekaban 1962; O'Rahilly and Gardner 1971, 1977). They represent therefore four and two embryonic stages prior to that of the appearance of the cortical plate, respectively.

The cephalic region of the younger embryo was fixed in buffered formalin and that of the older one in Bouin's solution. After fixation, they were serially sectioned (10 μm sections) in their entirety. The cephalic region of the younger embryo was sectioned in the coronal plane, while that of the older one was sectioned in the sagittal plane as recommended by Gilbert (1935) for a better visualization of longitudinal fiber tracts. Most sections were stained with hematoxylin and eosin and mounted consecutively on glass slides. Selected sections from both cases were also silver stained according to the Bielschowsky and Holmes methods. The best results were obtained with a modification of the Holmes method used in our laboratory. The basic modificatons consisted of using stronger impregnating solution (5 ml 1% $AgNO_3$ and 10 ml 10% aqueous solution of pyridine), keeping the slides in this solution for 24 h

at 37° centrigrade in the incubator, keeping the slides later in the procedure for 10 min in the 2% oxalic acid solution, and using thicker (10 micrometer) preparations.

Observations

In addition to the analysis of the structural organization of the human cerebral cortex at embryonic stages 18 and 20, presented herein for the first time, pertinent information on stages 17 and 22 will also be analyzed in this study. Our objective is to analyze and to correlate the progressive differentiation of the human cerebral cortex through these four consecutive stages of embryonic development. Each one is characterized by a significant event in the development of the cerebral cortex. At stage 17 the human cerebral cortex still is undifferentiated; at stage 18 the superficial plexiform layer (external white matter) starts to form; at stage 20 the fibrillar neuronal organization of the primordial plexofirm layer is fully developed; and at stage 22 the cortical plate starts to form *within* the preexisting primordial plexiform layer. Each one of these embryonic stages will be analyzed separately and in greater detail.

Stage 17 (ca. 41 days, 11–14 mm)

This stage of human development corresponds to that of 19 day cat embryos (Marin-Padilla 1978) and 12 day mouse embryos (Theiler 1972; Goffinet and Lyon 1979). At this stage the telencephalon is characterized by clearly outlined cerebral vesicles, which have expanded slightly beyond the lamina terminalis. The growth of the neopallium, during this stage, has led to the formation of distinct lateral cerebral vesicles which were barely outlined at the previous developmental stage. As far as can be gathered from the literature, the neuroepithelium of the cerebral cortex at this stage still is undifferentiated. The corpus striatum, first recognized at the previous stage, has undergone some degree of growth and differentiation. Its outer region (mantle zone) is broad and has started to differentiate.

Undoubtedly, the most important event which occurs during this stage is the arrival of the first fibres of the future internal capsule at the telencephalon. These fibres are found passing through the di-telencephalic sulcus which has widened considerably during this embryonic stage. Fibres from this primitive internal capsule extend into the outer zone of the striatum and "a strand of fibers is also present extending from the mantle zone of the striatum dorsally to the lateral wall of the cerebral vesicle" (Bartelmez and Dekaban 1962). Fibres from this primitive internal capsule have been traced backward to the developing ventral thalamus and lower down in the neuraxis to the mesencephalic tegmentum (Gilbert 1935; Bartelmez and Dekaban 1962; Windle 1970).

Other important events which characterize this embryonic stage include the following. The site of origin of the cerebral choroid plexus is marked by increased vascularity. The ventral thalamus shows a greater degree of differentiation than the dorsal one and is already separated from it. The

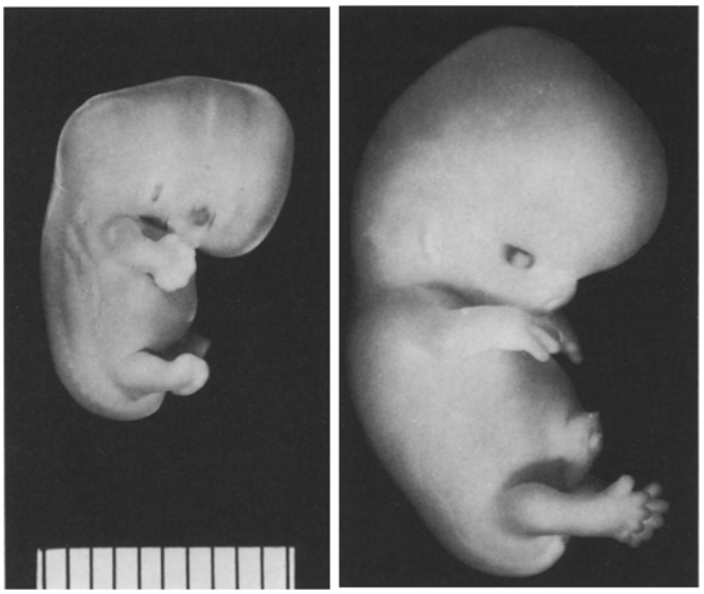


Fig. 1. Size, external appearance and degree of maturation of the two embryos studied: a 43 day, 15 mm, human embryo (left) recovered from an unruptured tubal pregnancy; and a 50 day, 22 mm, human embryo (right) recovered from an hysterectomy specimen. They represent examples of stages 18 and 20 of human development, respectively. Scale: mm

mesencephalic tegmentum shows an advanced degree of differentiation and structural organization and is crossed by prominent longitudinal fibre tracts. Mammillotegmental and mammillothalamic tracts are also recognized at this age.

Stage 18 (ca. 44 days, 13–17 mm)

The structural organization and degree of differentiation of the human cerebral cortex which characterize this embryonic stage was analyzed in a ca. 43 day (15 mm) embryo. The size, external configuration and degree of maturation as well as the size and position of the head of this embryo are depicted in Fig. 1. This stage of human embryonic development corresponds to that of 20 day cat embryos (Marin-Padilla 1978) and $12^1/_2$ day mouse embryos (Theiler 1972; Goffinet and Lyon 1979).

The cerebral vesicles have doubled their size from that of the previous stage (Fig. 2A). Although wide open and communicating, the lateral ventricles are recognized at this age as well as a wide third ventricle (Fig. 2A). The primordia of the hippocampus and that of the choroid plexus are also recognizable as well as the ventral and dorsal regions of the corpus striatum (Fig. 2A). The di-telencephalic sulcus has broadened considerably and its cellular elements are more separated from each other there suggesting an increase in the number of fibres passing through its outer zone into the telencephalon.

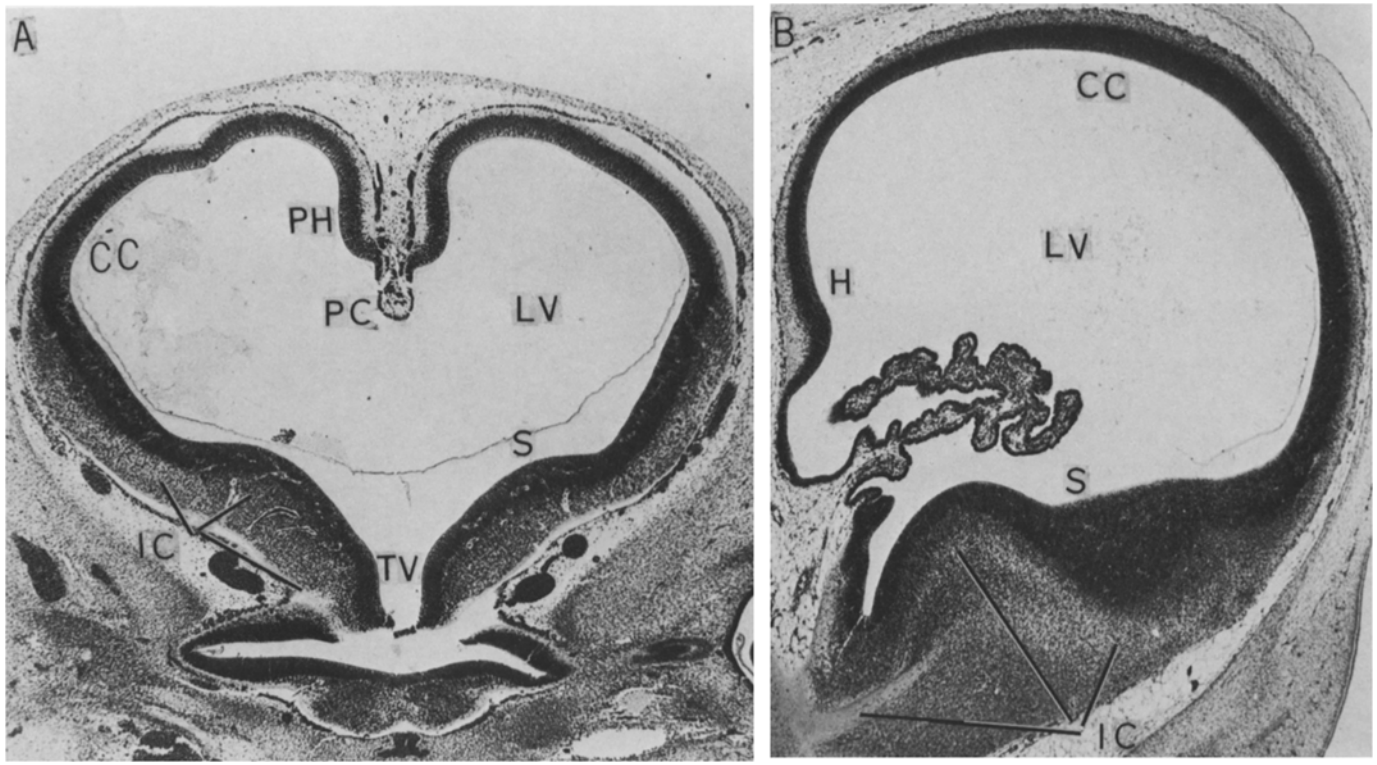


Fig. 2. A Coronal section of the cephalic region of the younger embryo illustrating the early development of the cerebral vesicles (*CC*) and the primordial of the hippocampus (*PH*), choroid plexus (*C*) and striatum (*S*). The lateral ventricles (*LV*) and the third ventricle (*TV*) are still widely connected. The di-telencephalic sulcus is broad and is crossed by numerous fibres which form a primitive ill-defined internal capsule (*IC*). The developing cerebral cortex is a thin membrane with a prominent matrix zone and a distinct narrow superficial plexiform lamina (marginal zone) with few horizontal-bipolar cells scattered throughout. **B** Parasagittal section of a telencephalic vesicle of the older embryo illustrating the considerable enlargement and differentiation of the cerebral cortex (*CC*), hippocampus (*H*), choroid plexus (*C*), corpus striatum (*S*) and internal capsule (*IC*) observed at this age. The internal capsule is a prominent structure composed of three branches (*arrows*) which extend into the ventral (upper) and dorsal (lower) regions of the striatal prominence and an intermediate one which extends toward the cerebral cortex. Its fibres penetrate through the most superficial zone of the developing cerebral cortex under the pial membrane, establishing a prominent primordial plexiform lamina (external white matter) which extends through its entire surface. H&E, approx. × 35

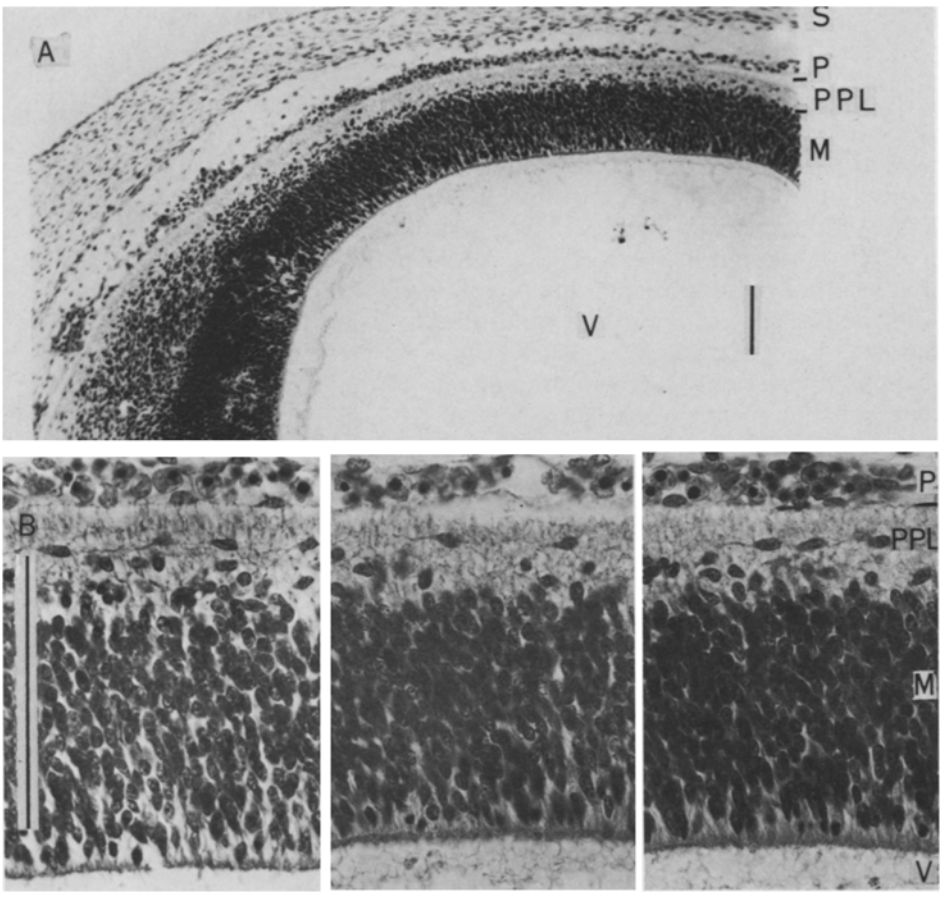


Fig. 3. A Detail of the developing cerebral cortex of the younger embryo illustrating the narrow but distinct superficial plexiform lamina (marginal zone) under the pial membrane. **B** High power views of the developing cerebral cortex of this embryo to illustrate the few but distinct horizontal-bipolar cells of its primordial plexiform lamina. These cells are considered to represent embryonic Cajal-Retzius neurons. This case represents the youngest human embryo in which these neurons have been described. Key: Scalp (*S*), pial membrane (*P*) below the arachnoidal capillaries, primordial plexiform layer (*PPL*), matrix (*M*), ventricle (*V*). H&E, scale 100 μm

This outer zone (mantle) of low cellular density of the telencephalon is considered to be caused by the presence of a primitive internal capsule. At this age, this zone of low cellular density extends toward the lateral wall of the cerebral vesicle (Figs. 2A, 3B). Perhaps, corticipetal fibres from this primitive internal capsule are advancing cephalad and some have begun to penetrate into the superficial lamina of the cerebral cortex which is recognized at this age (Fig. 3B). At this age, this superficial lamina has a distinct plexiform appearance, few horizontal-bipolar neurons, extends throughout the entire surface of the cerebral vesicle, corresponds anatomically with

the so-called "marginal zone" of classic terminology, and measures between 20 and 22 micrometers in thickness (Figs. 2A, 3A–B).

Histologic study of this superficial plexiform lamina demonstrated the presence of a few but distinct horizontal-bipolar cells "embryonic Cajal-Retzius neurons" scattered throughout its entire length (Fig. 3B). The horizontal-bipolar shape of these cellular elements contrasts sharply with the predominant vertical or perpendicular orientation of the remaining neuroepithelial elements of the developing cerebral cortex (Fig. 3B).

The horizontal-bipolar configuration of these cells is considered to be the result of their differentiation among preexisting horizontal fibres. Without the presence of these horizontal fibres, within the superficial plexiform lamina, the morphology of these cells will be difficult to explain since supposedly the only fibrillar components of the marginal zone at this age will be the perpendicular terminations of the radial glial. However, repeated attempts to confirm the presence of horizontal corticipetal fibres in the superficial plexiform lamina of the cerebral cortex of this embryo, by using various silver methods, have failed. Perhaps, because they are too primitive at this age to be stained, as with the same methods they have been clearly demonstrated in the next embryonic stage (stage 20) studied.

Stage 20 (ca. 51 days, 18–22 mm)

The structural organization and degree of differentiation of the human cerebral cortex which characterize this developmental age were studied in a ca. 50 day (22 mm) embryo. The size, external configuration and degree of maturation as well as the size and position of the head of this human embryo are illustrated in Fig. 1. This embryonic stage of human development corrsponds to that of 22 day cat embryos (Marin-Padilla 1978) and 13 day mouse embryos (Theiler 1962; Goffinet and Lyon 1979).

This developmental stage is characterized by an extraordinary growth of all components of the central nervous system, particularly of the cerebral vesicles (compare A and B of Figs. 1, 2). At this age the cerebral vesicles have more than doubled their size from that of the previous stage, and they overlap part of the diencephalon. The outstanding development of the cerebral vesicles has resulted in a significant enlargement of the head and, particularly, of the frontal region of these embryos (Fig. 1B). Both the large head and particularly the prominent frontal enlargement of this embryo (Fig. 1B) are unquestionably human attributes as no other mammalian embryo undergoes such an extraordinary cephalic development at this early embryonic age.

Serial parasagittal sections of the head of this embryo including all regions of the developing central nervous system have been prepared and studied (Fig. 4). This type of section is necessary for comparative studies on the degree of differentiation among the various components of the developing central nervous system as well as for the study of their interrelationships. They are also excellent for the study of the longitudinal fibre tracts which characterize the central nervous system at this age (Fig. 4). The study

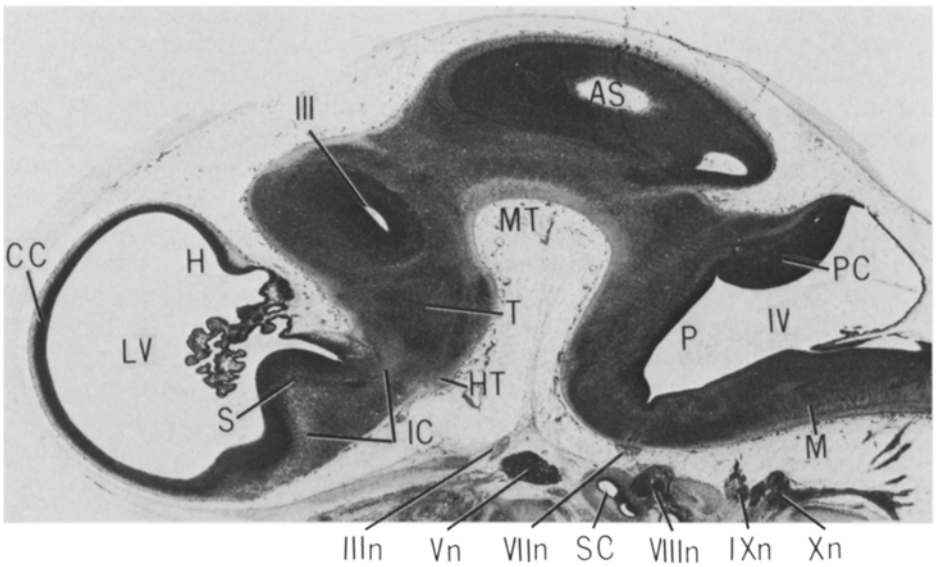


Fig. 4. Parasagittal section of the entire head of the oldest embryo illustrating the degree of differentiation and anatomical relationships of its various regions and the prominent longitudinal fibre tracts which connect them. The following structures are recognized: the myelencephalon (*M*), fourth ventricle (*IV*), cerebellar primordium (*PC*), pons (*P*), mesencephalic tegmentum (*MT*), aqueduct of Sylvius (*AS*), diencephalon with the third ventricle (*III*), ventral thalamus (*T*), hypothalamic region (*HT*), and the telencephalon with the developing cerebral cortex (*C*), hippocampus (*H*), striatum (*S*) and the prominent internal capsule (*IC*). Also illustrated are the prominent longitudinal fibre tracts which characterize this development age. Fibres from the internal capsule could be traced backward through the ventral thalamus and hypothalamic zone into the mesencephalic tegmentun and rostral region of the pons. The possible existence of an ascending tegmento-thalamostriato-cortical fibre tract has been suggested in this study. Also illusrated are various cranial nerves and their ganglia as well as the semicircular canals (*SC*) of the internal ear. H&E, approx. ×20

of these sections demonstrated that the myelencephalon, the metencephalic pons and the mesencephalic tegmentum below the aqueduct of Sylvius are by far the most differentiated regions of the CNS at this age (Fig. 4). The fourth ventricle still is a large cavity with a membranous roof, small developing choroid plexus and a cerebellar primordium which still is mostly undifferentiated. The diencephalic third ventricle is narrow and the ventral thalamus is more differentiated and developed than the dorsal thalamus. The hypothalamus is well differentiatd and composed of many fibrillar elements (Fig. 4). The cerebral vesicle has enlarged considerably and has a recognizable hippocampus and prominent choroid plexus. The corpus striatum has also enlarged considerably and its dorsolateral and ventromedial regions are well formed at this age (Fig. 4). The outer region of the striatum is more developed than its paraventricular zone. It should also be emphasized that the cerebral cortex of this embryo lacks a recognizable cortical plate (Fig. 2B).

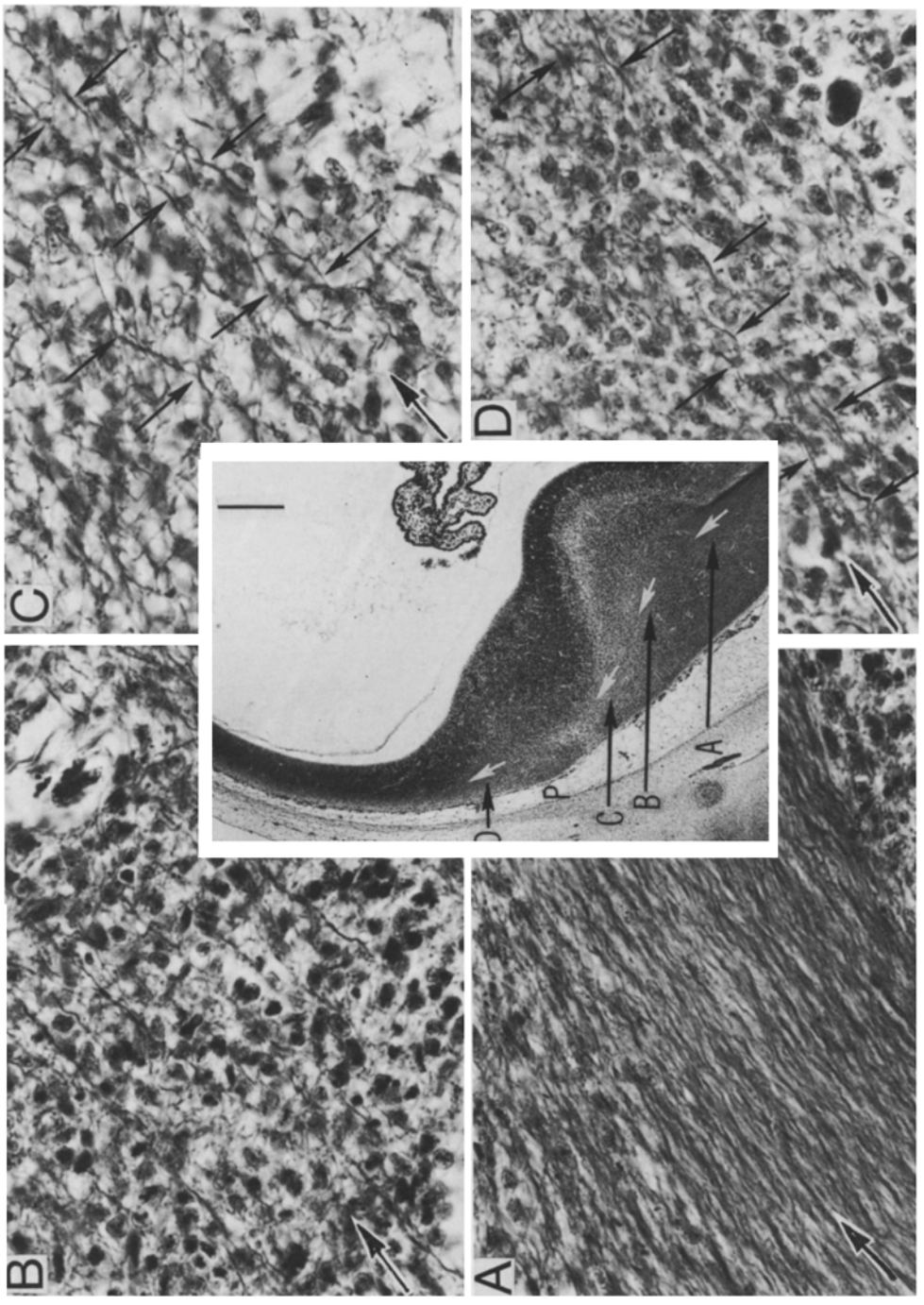
Prominent longitudinal fibre tracts are also clearly visible in these parasagittal sections (Fig. 4). The most prominent tracts are those passing in an

anteroposterior direction through islands of developing neurons in the mesencephalic tegmentum. Some of these tegmental fibre tracts become continuous with others which extend in both caudal and cephalad directions connecting, in a manner of speaking, all major regions of the CNS at this embryonic age (Fig. 4).

Caudally, some tegmental fibre tracts become continuous with others that, crossing between islands of developing neurons, penetrate into the outer ventrolateral region of the pons and extend into the myelencephalon (Fig. 4). Other tegmental tracts seem to extend posteriorly into the outer dorsal region of the cerebellar prominence. From this cerebellar region fibre tracts are also seen extending into, or arriving from, the pons (Fig. 4). Many of these developmental tracts were studied in great detail by Windle (1970) in human embryos from four to seven weeks of gestation. Since our main objective is the early differentiation of the human cerebral cortex and its relationship to the arrival of primitive corticipetal fibres to it, the reader is referred to the above classic account of the early developmental fibre tracts of the human brain.

Some longitudinal tegmental fibre tracts also extend cephalad and could be followed as far as the telencephalic vesicle (Fig. 4). Through the ditelencephalic sulcus they penetrate into the developing telencephalon, establishing in it a distinct and broad internal capsule (Figs. 2B, 4, 5). In man, the internal capsule is already well established at this embryonic age, and its is composed of many fibres which branch into three main tracts confirming previous observations in much older human embryos (Hewitt 1961). Fibres from its upper tract extend toward the paraventricular zone of the striatum, which will evolve into the caudate nucleus. Fibres from its lower tract extend toward the outer lateral zone of the striatal mass which will evolve into the lenticular nucleus. The intermediate tract, wich is quite prominent at this embryonic age, extends toward the lateral wall of the cerebral vesicle and its fibres penetrate into it (Figs. 2B, 4, 5). The anatomical location of this intermediate tract, dividing the striatal prominence into ventral (caudate nucleus) and dorsal (lenticular nucleus) regions already corresponds to the actual position of the internal capsule in the adult brain (Figs. 2B, 4, 5).

Fig. 5. Montage of fotomicrographs to illustrate the internal capsule and the general cephalad direction of its corticipetal fibres of the 50-day-old human embryo. The *insert* illustrates, at a low magnification, the internal capsule and the general direction (*white arrows*) of the corticipetal fibres at four ascending consecutive levels marked by *long arrows* (**A, B, C, D**). Also illustrated in the insert is a small cellular mass (*P*) representing part of the future piriform cortex. Corticipetal fibres are seen passing between this piriform cortex and the undifferentiated cellular mass of the dorsolateral region of the striatum (scale 2 mm). The outside fotomicrographs illustrate, at a much higher resolution, the number, the cephalad direction (from lower left to upper right) marked by *large arrows*, and the different degree of silver impregnation of the corticipetal fibres at the four consecutive levels (**A, B, C, D**) of the *insert*. The number of corticipetal fibres diminish progressively as well as the degree of silver impregnation from proximal to distal regions or from **A** to **D**. Because the lighter silver impregnation of the corticipetal fibres passing through levels **C** and **D** makes their recognition, in these reproductions, more difficult, they are marked by *arrows* for easier identification. From modified Holme's silver preparations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After several attempts, we succedded in staining the fibres of this primitive internal capsule with a slightly modified version of the Holme's method (Fig. 5). The main body or proximal portion of the internal capsule is a broad bundle composed of numerous and closely packed fibres (Fig. 5A). As this bundle approaches the level of the striatal mass, its fibres fan out in at least three main directions and become less conspicuos and numerous (Fig. 5, insert). Fibres from the intermediate tract of this primitive internal capsule are seen advancing cephalad toward the cerebral cortex (Fig. 5B). These advancing corticipetal fibres are seen bordering externally the large paraventricular, mostly undifferentiated, cellular mass of the dorsolateral region of the striatum (Fig. 5C). Some continue to advance cephalad, passing between the undifferentiated paraventricular region and a small cellular prominence which represents a portion of the future piriform cortex (Fig. 5P). Finally, they reach the cerebral cortex and penetrate into it superficially, between the pial surface and the matrix zone (Fig. 5D). These corticipetal fibres extend throughout the entire surface of the cerebral cortex at this age, establishing in it a prominent superficial plexiform lamina layer or external white matter (Fig. 6).

As the corticipetal fibres of this primitive internal capsule advance toward the cerebral cortex, they become less numerous and their staining properties change progressively. The fibres closer to the internal capsule (Fig. 5B) are more impregnated with silver and hence are darker than those further away from it (Fig. 5D) which are lighter and less impregnated. These staining differences are believed to reflect the age and degree of maturation of the advancing corticipetal fibres. Fibres closer to the internal capsule are older and more mature than those further away from it, which are still growing or advancing.

Detailed histologic study of the crebral cortex of this embryo (Fig. 6) has demonstrated, first of all, that the cortical plate has not yet appeared in it; and, secondly, that the superficial plexiform lamina has increased in both thickness and structural complexity when compared with that of the previous stage (compare Figs. 3, 5, 6). While the matrix zone has increased in thickness only slightly since the previous stage, the superficial plexiform layer has nearly doubled in thickness. This enlargement is believed to be caused by an increase in both the number of horizontal fibres and of horizontal-bipolar cells (Cajal-Retzius neurons), and of the differentiation of other, less well defined cellular elements.

Silver preparations of the primordial plexiform lamina of the cerebral cortex and this infant (Fig. 6) confirmed the presence of horizontal fibres (external white matter) as well as of horizontal-bipolar Cajal-Retzius neurons throughout its entire surface. Some of these primitive horizontal fibres are quite fine and somewhat beaded. Their photographic reproduction is difficult. Some examples of these horizontal fibres, the best, are reproduced in Fig. 6 and marked by arrows. Both the presence of horizontal Cajal-Retzius neurons (Fig. 6A, B) and of horizontal fibres (Fig. 6C–F) explain the plexiform appearance as well as the composition of this primitive superficial cortical lamina. This basic plexiform composition of layer I will remain

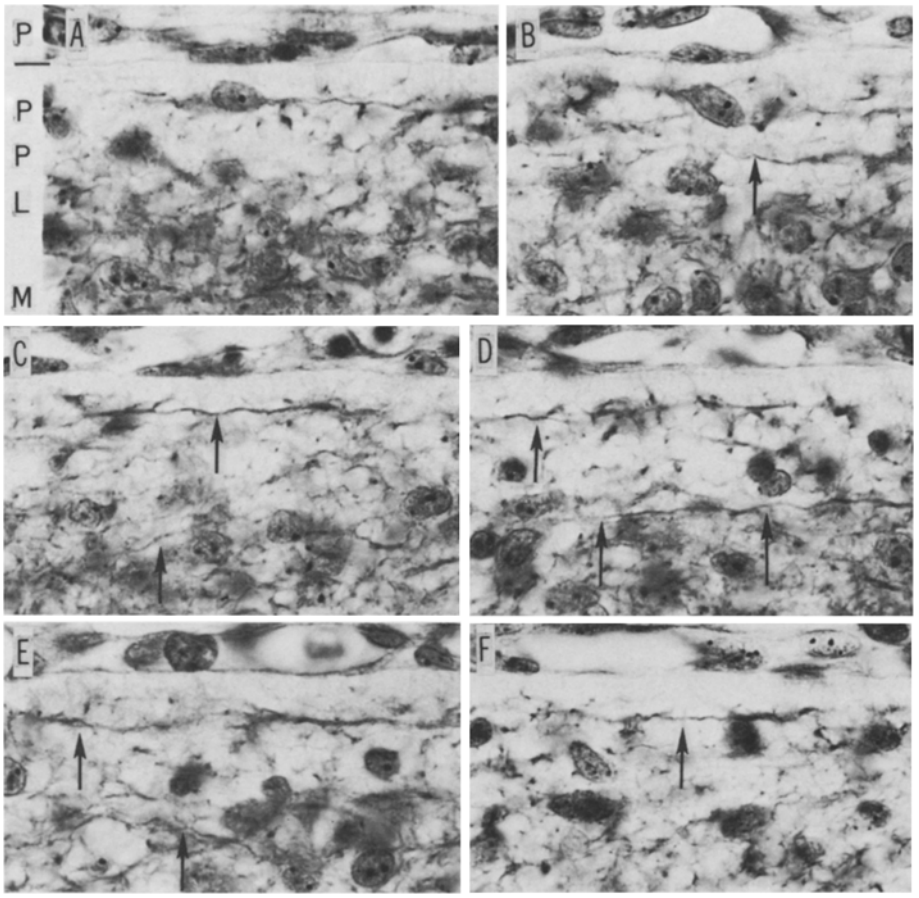

Fig. 6. High power views of the primordial plexiform lamina of the developing cerebral cortex of the older embryo illustrating horizontal-bipolar Cajal-Retzius neurons (**A, B**) and several horizonal coticipetal fibres (**C–F**) under the pial membrane (*P*). The horizontal fibres believed to represented corticipetal fibres are marked by arrows. In some of the illustrations (**A, B**) the closely packed cellular elements of the matrix zone (*M*) are visible and in all of them the arachnoidal capillaries are clearly outlined above the pial membrane. The illustrations represent an area of the pirmordial plexiform layer approximately 35 μm thick. From modified Holme's silver preparations

essentially unchanged throughout the course of prenatal cortical ontogenesis. The horizontal fibres as well as the Cajal-Retzius neurons depict already a tendency to assume an antero-posterior orientation which is similar to the preferential orientation of these elements of layer I in the adult cerebral cortex (Fleischhauer et al. 1977; Marin-Padilla and Marin-Padilla 1982).

While the presence of horizontal Cajal-Retzius neurons within this primitive superficial plexiform lamina can be established with certainty, the origin and nature of the horizontal fibres observed in the silver preparation may be more difficult to ascertain. They could represent the long horizontal processes which characterized the Cajal-Retzius neurons as well as the hori-

zontal corticipetal fibres arrived from the internal capsule. The thin sections studied and the various staining procedures are inadequate to establish with certainty the continuity between these horizontal fibres and those of the internal capsule. However, the study of these fibres through several sections following their progressive advancement from the internal capsule into the cerebral cortex have convinced me that they are indeed afferent in nature. Also, some of them could represent corticofugal fibres for the primordial plexiform lamina. Further studies are obviously needed to elucidate more accurately the nature of these horizontal fibres.

Stage 22 (ca. 54 days, 23–28 mm)

This stage stage of human development is characterized by the appearance of the cortical plate in the cerbral cortex, which according to the most prevalent hypothesis marks the beginning of cortical neurogenesis. However, this event marks only the appearance of the mammalian neocortex or the primordial neocortical grey since it is preceded, in cortical ontogenesis, by a much older and primitive cortical organization (the primordial plexiform lamina). Its appearance divides this primitive lamina into a superficial plexiform or layer I and a deep plexiform or layer VII (subplate zone). Therefore, the primordial cortical grey will give rise only to layers VI, V, IV, III, and II of the future cerebral cortex. This embryonic stage of human development corresponds with that of 25 day cat embryos (Marin-Padilla 1978) and 14 day mouse embryos (Theiler 1970; Goffinet and Lyon 1979).

Discussion

The human cerebral cortex begins its ontogenetic development by a series of transformations which culminate with the establishment of a superficial primordial plexiform lamina followed by the appearance and formation of the cortical plate *within it*. These transformations begin soon after the formation or recognizable cerebral vesicles in the telencephalon and therefore much earlier than previously thought or suspected (Fig. 7).

The first transformation observed in the telencephalon following the formation of cerebral vesicles (stage 15) is the appearance of a primitive internal capsule in it. This primitive internal capsule is first recognized by stage 17 (ca. 41 days) of human development (Gilbert 1935; Hewitt 1961; Bartelmez and Dekaban 1962; Windle 1970). A few fibres from this primitive internal capsule have been described advancing toward the lateral wall of the newly formed cerebral vesicle as early as the stage 17 of human development (Bartelmez and Dekaban 1962). By stage 18 (ca. 44 days), they have reached the cerebral vesicle and possibly some of them have already penetrated into it since horizontal-bipolar Cajal-Retzius neurons have begun to differentiate at this age. These primitive corticipetal fibres penetrate into the cerebral cortex through its external or more superficial region between the pial surface and the undifferentiated matrix zone. Their arrival coincides with the establishment of a distinct superfical plexiform lamina in the cerebral cortex which coincides anatomically with the "mariginal zone", first

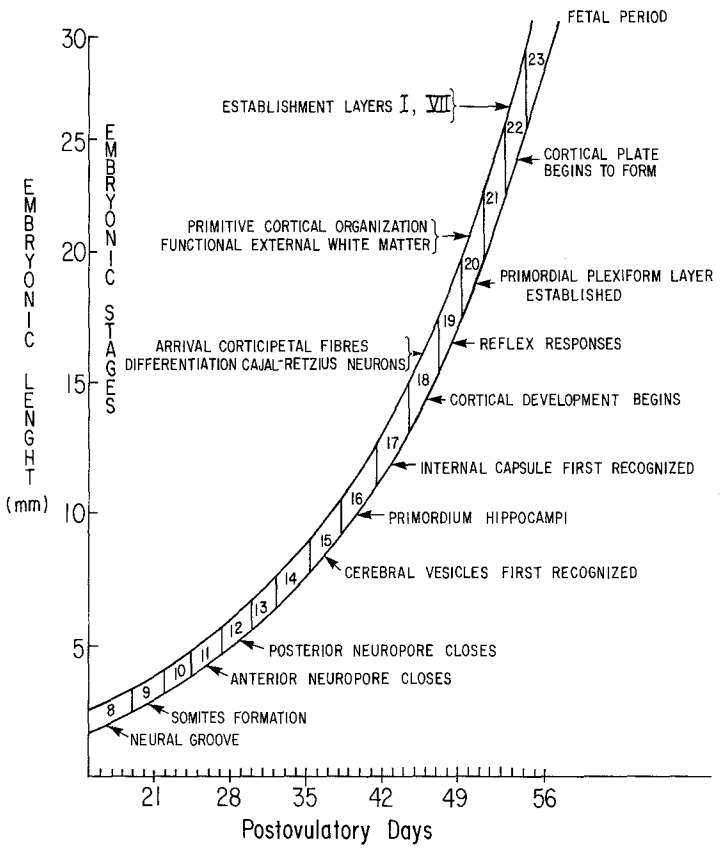

Fig. 7. Schematic representation of the major events observed during the early ontogenesis of the human cerebral cortex correlated with embryonic age, size and developmental stage. The major events following the appearance of cerebral vesicles (stage 15) are: the appearance of the internal capsule (stage 17); the possible arrival of corticipetal fibres to the cortex with differentiation of horizontal Cajal-Retzius neurons (stage 18); the establishment of the primordial plexiform lamina as a primitive functional cortical organization (stage 20); the appearance of the cortical plate within the primordial plexifom lamina (stage 22); and the establishment of layers I and VII of the future cerebral cortex (stage 22). The cortical plate will, therefore, only give rise to layers VI, V, IV, III, and II of the future cerebral cortex. The basic format used illustrating the evolution of the different embryonic stages has been obtained from the work of O'Rahilly and Gardner (1977)

recognized by stage 18 of human development. The appearance of this primordial plexiform lamina represents the second major transformation observed in cortical ontogenesis (Fig. 7). It also marks the actual beginning of human cortical ontogenesis. The 43-day-old human embryo studied represents the youngest one in which Cajal-Retzius neurons have been described marking the beginning of cortical ontogenesis (Larroche 1981; Larroche and Houcine 1982; Marin-Padilla and Marin-Padilla 1982).

The differentiation of horizontal Cajal-Retzius neurons in the primordial plexiform lamina of the cerebral cortex at this embryonic age is considered to be induced by the presence of corticipetal fibres which have arrived

earlier to it. The early arrival of primitive corticipetal fibres to the cerebral cortex has been already suggested as the possible stimulus for the differentiation of these neurons (Marin-Padilla 1971, 1978). However, it shold be pointed out that it has not been possible to confirm their presence, within the superficial plexiform lamina of the cerebral cortex of this embryo, with a variety of silver methods. Perhaps at this age (stage 18) these corticipetal fibres are still too primitive to be impregnated by silver. This idea is supported by the fact that the presence of many corticipetal fibres has been demonstrated with similar silver methods in the next embryonic stage (stage 20) studied. Furthermore, it has been noticed that the degree of silver impregnation among the corticipetal fibres of the internal capsule, at that age varies considerably. The silver impregnation is heavier among the proximal, older and hence more mature fibres of the internal capsule, while it is lighter among the more distal, still growing, younger and hence more immature ones. These observations suggest that the amount of silver impregnatin of a given primitive fibre may depend on its degree of maturation.

By stage 20 (ca. 51 days), the human cerebral cortex is characterized by a fully developed primordial plexiform lamina composed of numerous horizontal Cajal-Retzius neurons, of a few other, less defined, cellular elements, and of numerous horizontal corticipetal fibres. The presence of all of these components has been confirmed with a modified version of the Holme's method corroborating previous electronmicroscopic observations (Laroche 1981; Larroche and Houcine 1982). By this age the primordial plexiform lamina is considered to be a functionally active (external functional white matter) cortical organization. Primitive types of synaptic contacts have been demonstrated in this lamina in a recent electronmicroscopic study of the cerebral cortex of a seven-week-old human embryo (Larroche 1981). The primordial plexiform lamina is considered to represent a primitive "premammalian" cortical organization which not only precedes the appearance of the "cortical plate", but is essential for its subsequent "inside-out" formation.

The cortical plate (the actual mammalian neocortical grey) starts to form within the preexisting primordial plexiform lamina by stage 22 (ca. 54 days) of human development. Its appearance represents the third major transformation observed in cortical ontogenesis (Fig. 7). Migrating neuroblasts, guided by the glial fibres (Rakic 1972, 1974, 1982) and attracted toward the primordial plexiform layer (Marin-Padilla and Marin-Padilla 1982), began to accumulate within it in an "inside-out" fashion (Agenvine and Sidman 1961). The arrival of these migrating neuroblasts and their accumulation with the primordial plexiform lamina divides it into a superficial plexiform or layer I and a deep plexiform or layer VII (subplate zone). The establishment of layers I and VII of the future cerebral cortex represents the fourth major transformation observed in cortical ontogenesis (Fig. 7). Therefore, the mammalian neocortical grey (cortical plate) will give rise only to layers VI, V, IV, III, and II of the future cerebral cortex.

As the migrating neuroblast forming the cortical plate come in contact with layer I, they develop apical dendritic bouquets, establishing structuro-

functional relationships with its elements (Berry and Rogers 1965; Morest 1970; Marin-Padilla 1972; Peters and Feldman 1973; König et al. 1975; Marin-Padilla and Marin-Padilla 1982). It seems that as the migrating neuroblasts establish contacts with layer I, they are progressively transformed into young pyramidal neurons. Since it seems that all future pyramidal neurons of the cerebral cortex must reach and establish connections with layer I the cortical plate must necessarily grow in an "inside-out" fashion. Any migrating neuroblast, representing a future pyramidal neuron, must bypass all preceding ones in order to establish contact with layer I, develop an apical dendritic bouquet and become transformed into a young pyramidal neuron. These observations imply that layer I (part of a primitive premammalian cortical organization) by controlling the progressive migration of all future pyramidal neurons of the cerebral cortex must play a significant role in its overall structural and laminal organization. Studies should be developed to further elucidate this function. Perhaps by inducing timed disruptions of the effect of layer I upon the migrating neuroblast, it will be possible to observe abnormalities in the specific laminations of the cerebral cortex and in the morphology of pyramidal neurons which fail to reach layer I.

These observations further imply that all pyramidal neurons regardless of size, cortical location or functional role, become anchored to layer I, early in their development, by their apical dendritic bouquet and remain so attached throughout the course of cortial ontogenesis. This could explain the universal radial orientation of the apical dendrite of all pyramidal neurons (regardless of size, location or functional role) to layer I; their morphologic uniformity among different mammalian species and different cortical regions; and perhaps their abundance throughout the cerebral cortex.

Some final comments should be made concerning the possible nature, composition and origin of the fibres of the primitive internal capsule of the human embryos studied herein. The human internal capsule, by stage 20, already occupies its future location and is a broad and prominent bundle composed of numerous and closely packed fibres. Available information (Hines 1922; Hewitt 1961; Bartelmez and Dekaban 1962; Windle 1970) indicates that tegmento-striatal and thalamo-striatal fibres are already present in the internal capsule at this age (stage 20). It is possible that it could also carry descending striatofugal fibres. But more important is the fact that the human internal capsule by this age also carries ascending corticipetal fibres which reach and penetrate into the developing cerebral cortex. Their presence, reported herein for the first time, has been demonstrated with a modified version of the Holme's method. The origin of these corticipetal fibres remains unknown. However, it should be pointed out that some of them could be traced backward as they become continuous with fibre tracts that bypassing the ventral thalamus extend into the mesencephalic tegmentum and the rostral region of the pons. The possible mid-brain origin of these corticipetal fibres corroborates current concepts concerning their possible catecholaminergic nature (Morrison et al. 1978; Lidov et al. 1978; Molliver 1982; Molliver et al. 1982; Levitt and Rakic 1982) as well as their

possible origin from the reticular activating system of that region of the CNS recently suggested (Marin-Padilla and Marin-Padilla 1982). Obviously, further studies are needed to elucidate more accurately the nature, composition and source of this primitive corticipetal fibres.

The observations presented in this study further corroborate the concept of the dual origin, composition, and nature of the mammalian cerebral cortex. They demonstrate that the human cerebral cortex begins its ontogenetic development much earlier than previously thought, namely around the sixth rather than the eighth week of gestation. It is believed that the human cerebral cortex starts to develop with the arrival of primitive corticipetal fibres, followed by the differentiation of horizontal Cajal-Retzius neurons and the establishment of a superficial primordial plexiform lamina. This primordial (premammalian) plexiform lamina precedes the appearance of, and may be necessary for the subsequent inside-out formation of the cortical plate which represents the actual mammalian neocortical grey. It is suggested that layer I (which is part of the primordial plexiform lamina) plays a significant role in the overall structural organization of the mammalian cerebral cortex by controlling the migration of all its future pyramidal neurons regardless of size, cortical location or functional role.

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