

Gyrus formation in the cerebral cortex of the ferret.

II. Description of the internal histological changes

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INTRODUCTION

In the preceding paper (Smart & McSherry, 1986) the external features of the folding sequence in the telencephalic cortex of the ferret were described. The observations made in that work indicated (i) that during the folding of the surface of the hemisphere, the floors of the sulci remain relatively fixed both in relation to the deep surface of the cortex and to each other and (ii) that a gyrus is produced by the expansion of the inter-sulcal tissue. The purpose of the present paper is to analyse the structural changes within the cortical compartment during this transformation.

MATERIALS AND METHODS

The study was based on postnatal ferret brains which had been fixed by intracardiac perfusion with Bouin's fluid after the vascular system had been flushed out with normal saline (Smart & McSherry, 1986). The brains were embedded in paraffin wax, serially sectioned at a thickness of 10 μm in the coronal plane, and stained with haematoxylin and eosin.

Internal changes in the cortex during folding were displayed by using the intrinsic structure of the tissue as a natural indicator of growth. The method is a development of that originated by Nieuwenhuys (1972, 1977) and applied by him in a series of comparative studies of brain stem development. The cortex is particularly suitable for this type of treatment as it has a well developed radial texture apparent even in material stained with haematoxylin and eosin. This is derived initially from the hypercolumnar ventricular cells which traverse the neural epithelium from ventricular to pial surface and, later, from the course run by axons entering and leaving the maturing cortical compartment. Such radial fibres are, in effect, protoplasmic strands tethering the cortex to its bed of white matter. For the present purpose, they also serve as built-in gauges whose change in length and direction indicate growth movements within the cortex.

Neuron nuclei released from the periventricular layers accumulate in rows paralleling the fibre systems. The varying amount of radial separation undergone by nuclei as the crowded cortical plate expands to become mature cortex is an indicator of local growth at different depths in the cortex. The tangential banding which gives the familiar cortical layers is, in a sense, a secondary effect produced by areas of equal nuclear separation on adjoining radial axes keeping in register as the cortex deepens. Fibres and their associated nuclei thus provide an abacus-like arrangement which displays considerable information about cortical growth.

The courses of the radial tissue lines and the position of the tangential banding were recorded at different stages of cortical development on drawings of sections made with a Leitz drawing tube at a magnification of $\times 25$. The procedure adopted was to draw first the outline of the pial surface and the junction of cortical layers I and II. This junction was chosen as the datum line and marked off in steps of 1 cm. The drawing was replaced under the drawing tube and the courses of the radial tissue lines which originated from the marked steps at the outer boundary of layer II were marked in. In areas where fibre bundles or nuclear rows were lacking or ill developed, an estimate of the grain of the tissue was made from the orientation of the cytoplasmic 'tails' issuing from the neuronal perikarya and from the direction of elongation of cell nuclei. It was only necessary to do this to any extent in the adult specimen where tissue lines were less conspicuous in haematoxylin and eosin sections. During the active stage of gyrogenesis, however, tissue lines for the most part were well defined.

Two sets of drawings were also made of transects of the cortical compartment, one set passing from the crown of the coronal gyrus and the other through the floor of the suprasylvian sulcus, the two sites where the tissue lines remained uncurved. These were made at high magnification using the Leitz drawing tube. The drawings recorded the shape, degree of crowding and certain characteristics of nuclear and cytoplasmic detail at different stages of cortical maturation. At the enlargement used, a drawing of a transect through the crown of a developed gyrus measured about a metre in length and so was unsuitable for reduction to page size. As a compromise, features from these drawings required for illustrating nuclear movement were extracted and are presented in diagrammatic form. Only the changes within the coronal gyrus and its bounding sulci are described in detail as these were deemed to be representative of the events occurring in other gyri.

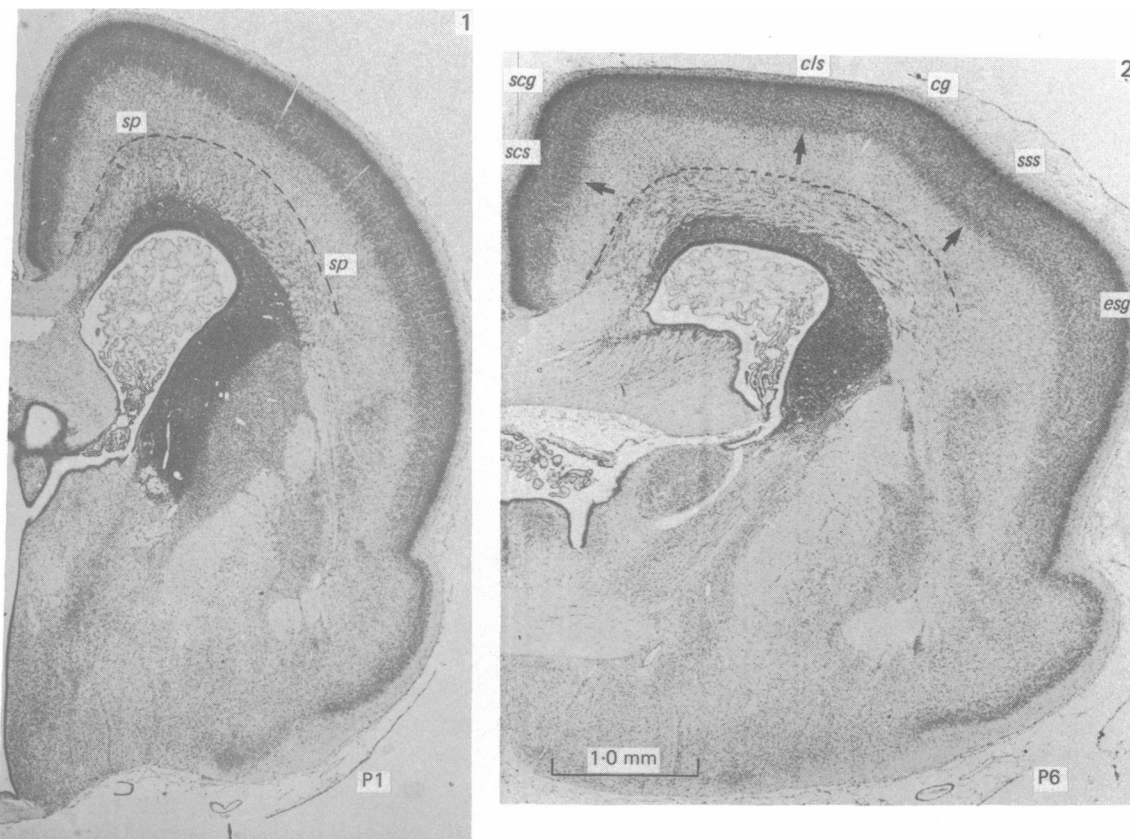
OBSERVATIONS

Changes in radial lines within gyral crowns

At birth the cortical surface showed little evidence of the location of future gyri (Fig. 1). A few days after birth, the sites of gyri and sulci were minimally evident; the gyral crowns were represented by areas of greater curvature of the cortical surface and of the underlying plate. At this early stage the segment of cortical plate subjacent to each sulcus had a greater depth than that related to the sulcal crowns, as can be seen by close examination of Figure 2.

The succession of changes undergone by the radial tissue lines within the coronal gyrus is depicted in Figure 3. The lines initially ran more or less straight radial courses to the pial surface. As the gyrus increased in height and surface area, the lines lengthened and fanned out from the gyral base towards the spreading gyral crowns to enclose elongated triangles of cortical tissue. On either side of the gyral crown the lines became progressively more curved as the sulcal floor was approached.

There was also a certain asymmetry in the development of the coronal gyrus. The ventral wall initially sloped steeply towards the floor of the suprasylvian sulcus while the dorsal wall shelved gently to the less conspicuous floor of the coronolateral sulcus (Fig. 3, P11 and Fig. 4). During later growth, the dorsal part also grew outwards and the external form of the gyrus became roughly symmetrical (Fig. 3, P14). Within the gyrus, however, a feature of the original asymmetry remained in that the radial lines



Figs. 1-2. Coronal sections of ferret brains taken at the level of the interventricular foramen at birth (Fig. 1) and 6 days postnatum (Fig. 2). The broken line indicates the deep surface of the subplate. The arrows in Figure 2 point to the incipient sulcal fundi where the cortical plate has a greater depth than in the intervening segment of future gyral crown. *scs*, site of incipient splenocruciate sulcus; *scg*, spleniocruciate gyrus; *cls*, coronolateral sulcus; *cg*, coronal gyrus; *sss*, suprasylvian sulcus; *esg*, ectosylvian gyrus.

still tended to cross the cortical layers more obliquely in the ventral than in the dorsal half of the gyrus (Fig. 3, P26 and Fig. 9).

Changes in radial lines in parasulcal area

In the cortex underlying the sulcal floors and adjacent segment of wall a different sequence was observed (Fig. 5*a-c*). Here the radial lines followed the pial surface as it hinged upwards to form the sulcal walls. On each side of the sulcal centre the lines became curved, running outwards from the subplate and then recurving towards the pial surface as if setting the sulcal centre in parenthesis. The distance between the lines was greatest on each side of the sulcal centre line (Fig. 5*b*). Further from the centre the distance between the lines remained more or less constant as they were traced from deep to superficial. Later in the development of the sulcal floors, the radial element diminished and became restricted to the outer cortex while the tissue elements of the deep cortex acquired a predominantly tangential trend (Figs. 5*b, c*, 6, 7). The total depth of the cortex in this area remained small and did not achieve a depth greater than that of the original cortical plate.

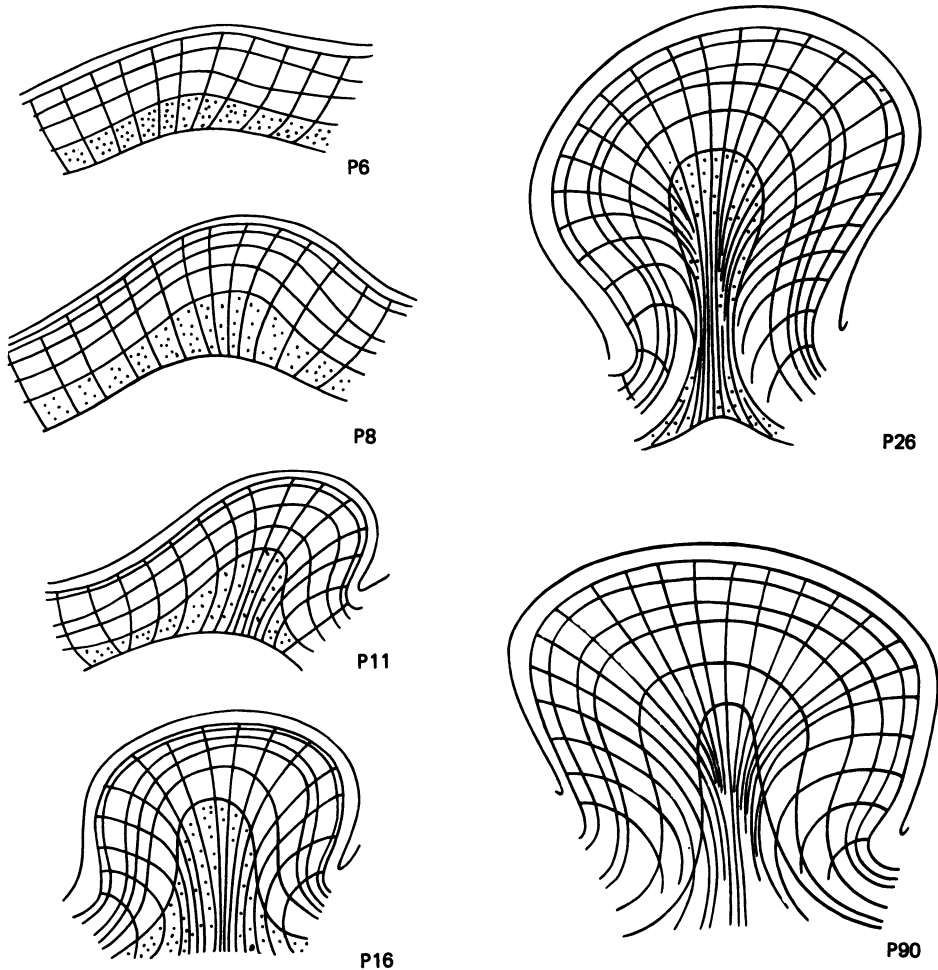


Fig. 3. Drawings made with a projection microscope of cross sections of coronal gyrus and its bonding sulci (suprasylvian to the right, coronolateral to the left) at level of interventricular foramen at stated postnatal ages (P6–P90; 6–90 days). The drawings show the changes undergone by the radial tissue lines and cortical layers as the gyrus develops. The stippling in the deepest layers represents the neurons of the subplate.

Changes in nuclear distribution within gyral crowns

Just prior to birth the cortical compartment consisted of a molecular layer, a cortical plate and a subplate. The diagrams in Figure 8 schematise the transformation of this simple arrangement into mature cortex by following the changes in nuclear spacing along a radial transect taken through the summit of the coronal gyrus. The changes in the pattern of nuclear dispersal at this site were typical of those occurring over the gyral crown.

The following selection of observations derived from these diagrams is of particular relevance to the present study. The maximum increase in depth of the developing cortex occurred in the subplate (Fig. 8, E40–P18). This less crowded compartment was initially populated by neurons with round nuclei. These became more widely separated as the subplate deepened (Figs. 10–13). Later in development the nuclei of subplate neurons became oval with conspicuous cytoplasmic tails (Fig. 14). Neurons

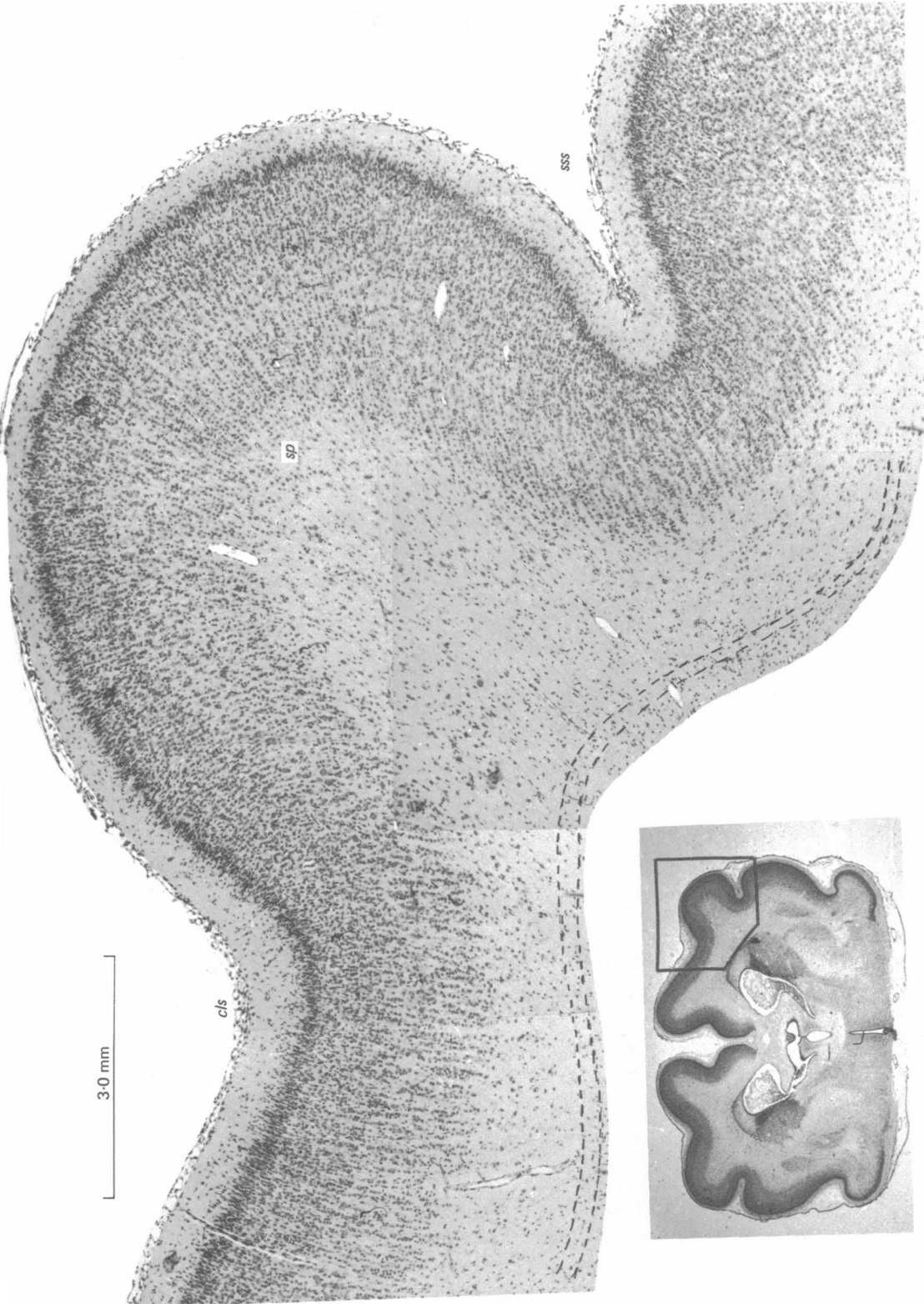


Fig. 4. Photocollage of coronal gyrus and its bounding sulci at eleven days postnatum. Inset shows approximate area from which enlargement has been taken. Note asymmetry of gyral development. The cortex (layers I-VI) of the ventral half (to the right) including the suprasylvian sulcus (sss) are at a more advanced stage of development than the cortex of the dorsal half and coronolateral sulcus (c/s) to the left. The double broken line delineates the boundary zone between the subplate (sp) and subcortical white matter.

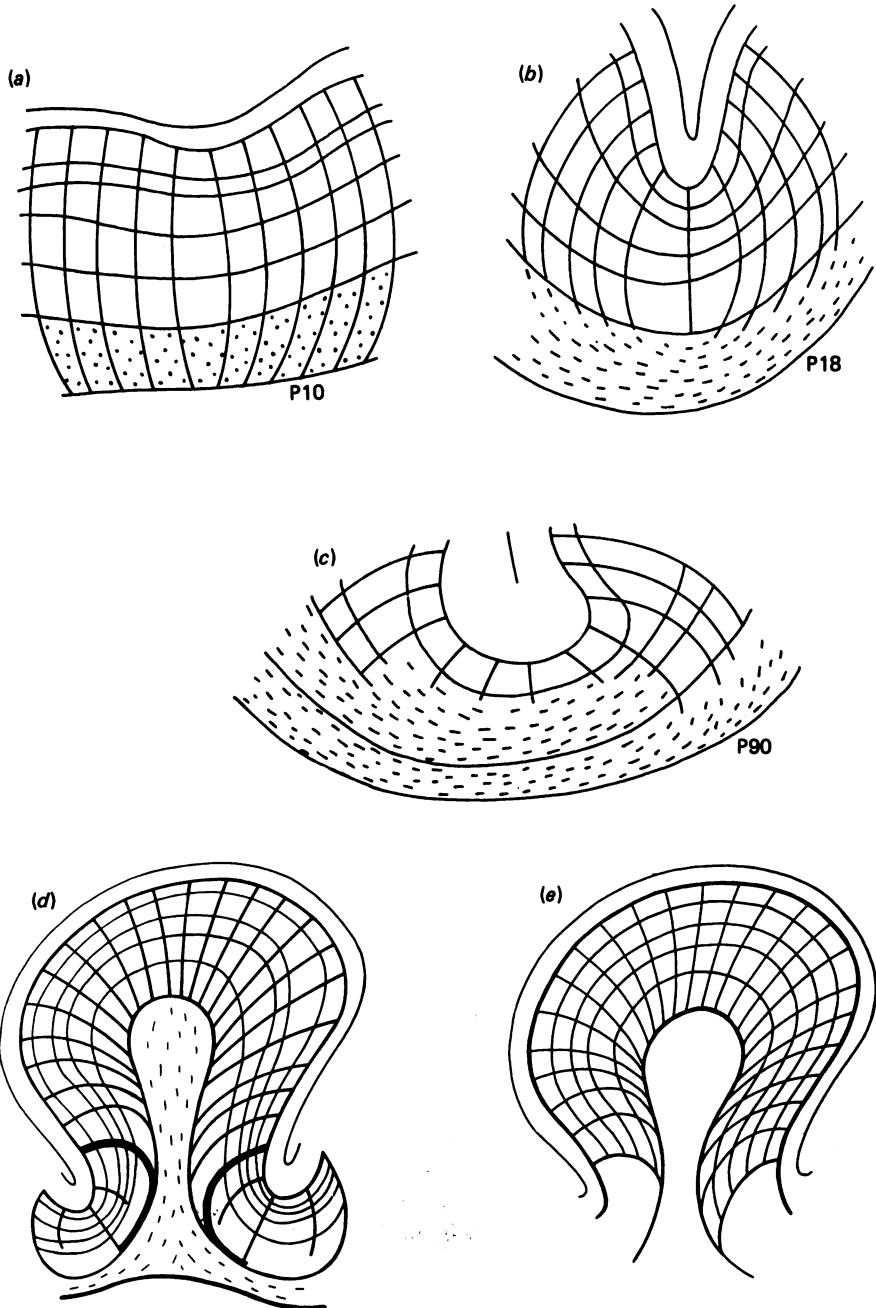


Fig. 5(a-e). For legend see opposite.

eventually disappeared from the subplate in a deep to superficial sequence which began about P25 when the gyrus was reaching its maximum height and was completed some time between 36 and 90 days after birth leaving the P90 gyrus with a core devoid of neurons.

Towards the end of the gestation period, the overlying cortical plate was composed of radially elongated nuclei which were more crowded in its outer third. In the inner two thirds, crowding was less and some round or oval nuclei were present among the dominant, radially elongated type. At the deep surface of the plate, round nuclei predominated and were more loosely packed than those above. By birth the cortical plate had increased in depth. The nuclei of its outer third were radially elongated and crowded. In the deeper plate, radially elongated nuclei were outnumbered by less crowded round or oval nuclei. At this early stage cortical layers V and VI could be distinguished, the former by the large nuclei of future pyramidal cells and the latter by radially orientated rows of smaller round nuclei. Cortical layer III appeared slightly later at P6 as the rounder, larger, nuclei of future small pyramidal neurons differentiated in the middle of the outer crowded part of the cortical plate to intervene between layers II and IV. Radially elongated nuclei which were originally found throughout the depth of the plate decreased in number in deep to superficial sequence during this period. The boundary with the subplate was not sharp and cortical plate nuclei appeared to grade into the subplate population (Fig. 12).

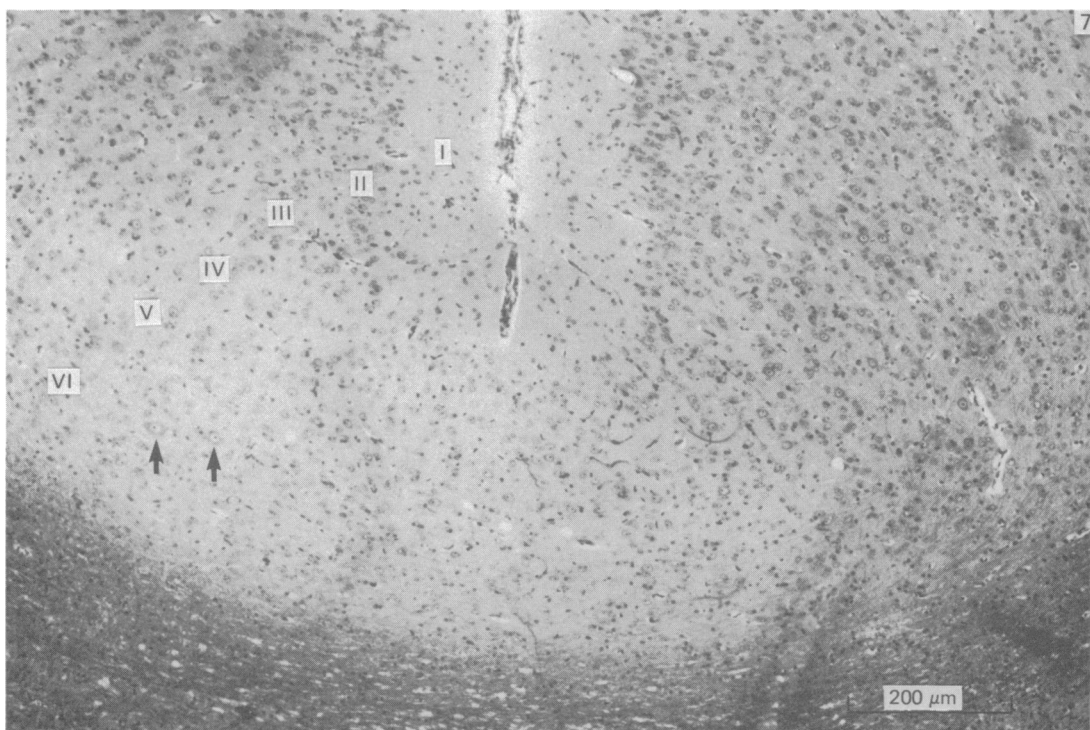
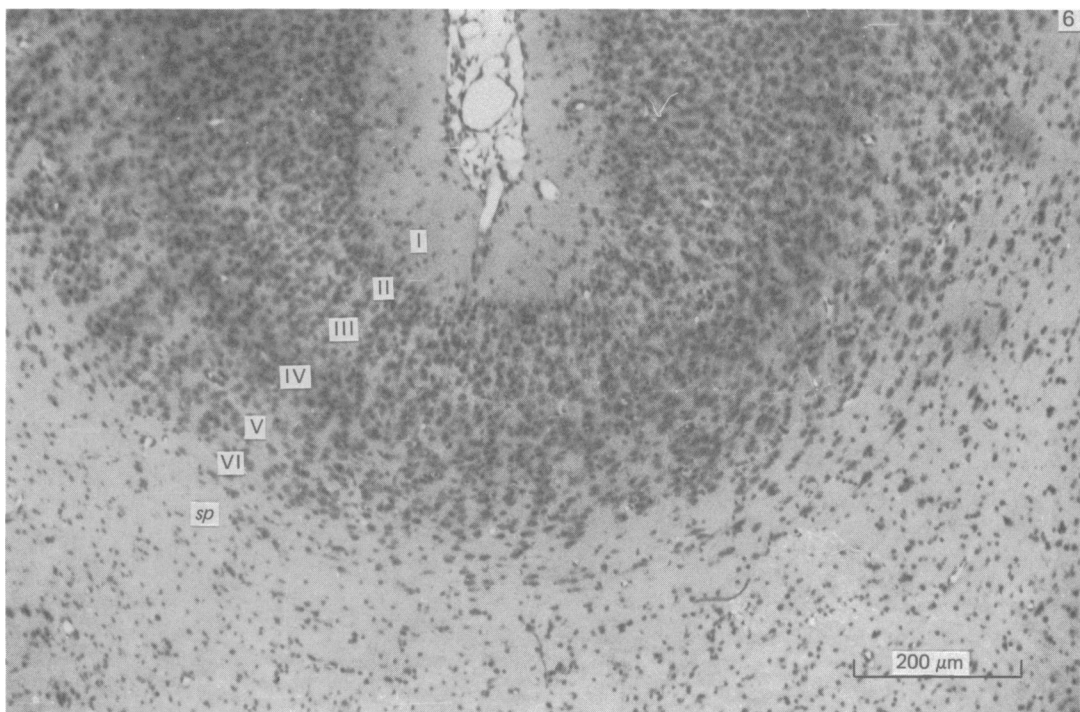
Changes in nuclear distribution in parasulcal area

The abacus diagrams in Figure 9 schematise the somewhat different changes in nuclear distribution in the cortex near the sulcal floors. Here the subplate deepened only minimally. The cortical layers emerged in the same sequences as at the gyral summits but during this process the total depth of the cortical plate did not change although nuclear separation increased. Later, all layers of the fundic cortex except layer I diminished in depth and the neurons of layers V and VI changed from a radial to a tangential orientation (Figs. 6, 7).

Other observations

Some further points of relevance can be distinguished in Figure 4 which shows an early stage of development of the coronal gyrus. The asymmetry of the picture is useful in providing a range of developmental stages. In the ventral half of the gyrus, maturation of the cortical plate was further advanced. Here the six cortical layers were broadest. They diminished in depth when traced further ventrally towards the suprasylvian sulcus but nevertheless could be distinguished traversing the thin sulcal segment of the plate. Traced dorsally from the crown, the layers converged on a cortical plate of undiminished depth lying under the shallower concavity of the

Fig. 5(a-e). (a-c) Drawings made with a projection microscope of the cortical region deep to the suprasylvian sulcus at the stated postnatal ages (10-90 days) The drawings show the change in the radial tissue lines and cortical layers as the sulcus develops. The stippling in the deepest layer represents the neurons of the subplate. Note the curving of the radial lines to form a 'parasulcal parenthesis'. (d) Tracing of a cross section through coronal gyrus prepared as in Figure 3. The heavy black lines in the parasulcal area indicate the position of the last column of standard length that can be accommodated entirely within an intersulcal segment. Standard length is defined as the length of a column at the gyral crown. (e) Tracing of pial surface outline and internal radial lines from Figure 5(d). The cortical layers in this case have been reconstructed by marking off on each radial line the depth of the cortical layers as measured in a standard column at the crown of the gyrus and then joining up corresponding points to form the layer boundaries. This construction conserves layer depth as the sulci are approached.



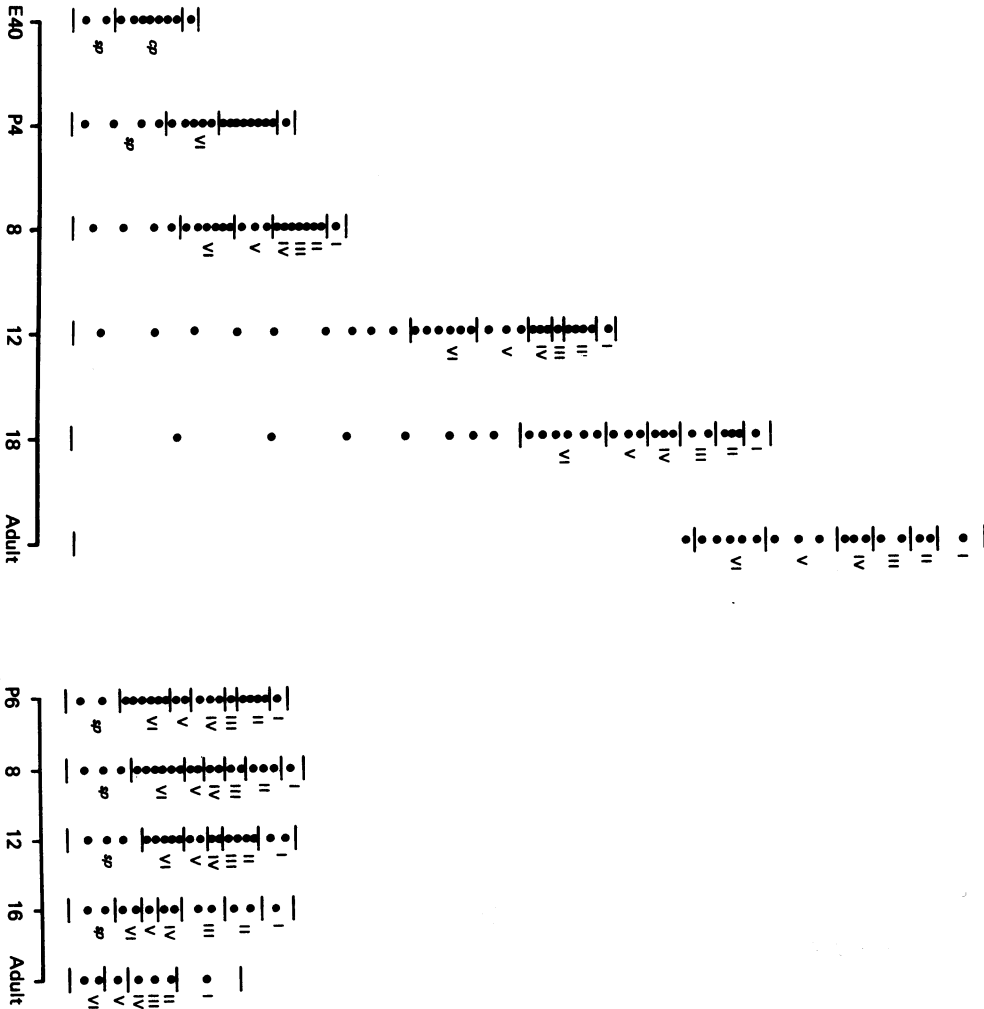
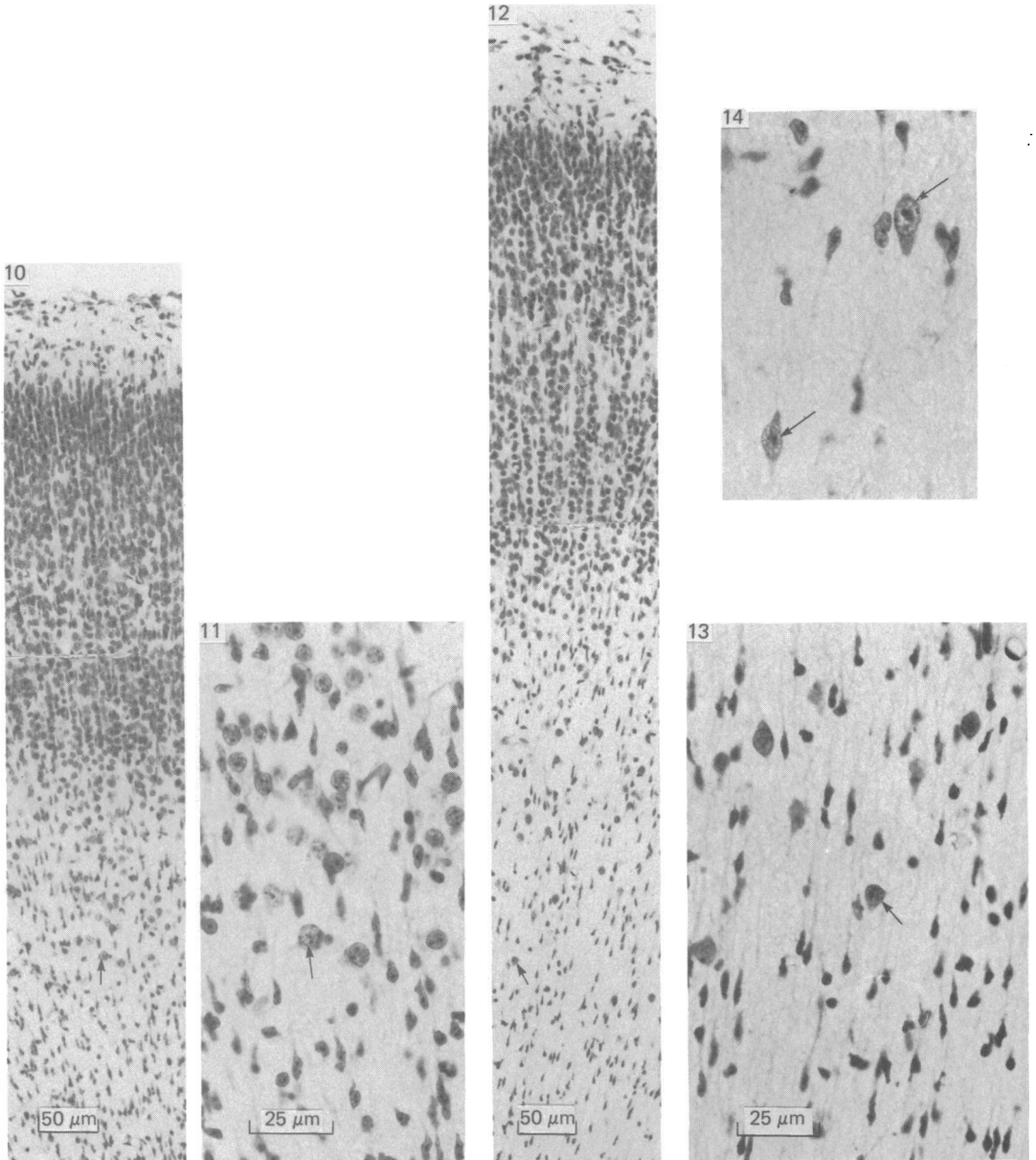


Fig. 8. Abacus model of nuclear movement along a radial line through the summit of the coronal gyrus extending from the pial surface (above) to the junction of the subplate with the white matter (below). The filled circles schematise the position of the nuclei of the emerging cortical layers at the stated ages. Roman numerals indicate cortical layers, *cp* denotes cortical plate, *sp* subplate. *P* refers to days postnatum; E 40, 40 days postconception.

Fig. 9. Abacus model of nuclear movement along a radial line passing through the midline of the floor of the suprasylvian sulcus at the stated postnatal ages. Conventions as in Figure 8.

Fig. 6. Section through fundus of coronal sulcus at 31 days postnatum. Note that all cortical layers (I–VI) can be distinguished although, with the exception of layer I, they are thinner than in the parasulcal cortex. The neurons of the subplate (*sp*) are tangentially rather than radially orientated.

Fig. 7. Section through fundus of coronal sulcus at 90 days postnatum showing the definitive adult state. Note that (i) the cortical layers (I–VI) are less well defined; (ii) the neurons of layer VI and even of layer V (arrows) are for the most part tangentially orientated; (iii) a separate layer VI and subplate cannot be distinguished; (iv) the depth of the fundic cortex is the same as at P21 (Fig. 6) but cell density is conspicuously less, indicating that there has been tangential spreading in the parasulcal area.



Figs. 10 and 12. Section through cortex of newborn and 6 days old ferret respectively. The arrows mark neurons lying at the boundary between the subplate and white matter.

Figs. 11 and 13. Enlargement of boundary area between subplate and subcortical white matter from Figures 10 and 12 respectively. Arrows denote the same reference neurons indicated by arrows in Figures 10 and 12.

Fig. 14. Photomicrograph of core of P26 ferret brain. Two large radially orientated subplate neurons are indicated by arrows.

coronal sulcus. As the sulcus was approached, layer III tended to be less well developed and at the sulcus itself layers III and IV merged and became difficult to distinguish as separate entities.

Figure 5*d-e* also displays some aspects of the folding geometry which may have functional relevance. Figure 5*d*, for example, shows the radial and tangential lines traced from a histological section of a developed coronal gyrus. Taking the length of a radial line at the summit of a gyrus as a standard column length, this distance was marked off on the other radial lines. The heavy radial lines in Figure 5*d* indicate the location of the last column of standard length that can be accommodated within the gyrus. Columns originating nearer the sulci would be required to shorten in order to remain within the bounding sulci. Figure 5*e* was prepared by tracing the radial lines from Figure 5*d*. The depths of the individual cortical layers from a standard column at the gyral crown of Figure 5*d* were then marked off on all the other radial lines and corresponding points joined up. The cortical layer boundaries thus created resembled the natural boundaries in Figure 5*d* which suggests that the internal structure of the gyrus reflects a tendency to conserve not only total column length but also the depth of the cortical layers. The other gyri displayed similar changes. The spleniocruciate, however, was a large gyral system which eventually developed into an extensive flap of tissue. It provided an arrangement with long flat surfaces which accommodated correspondingly large numbers of straight parallel columns with, except at its crown, none of the curving columns evident in smaller gyri.

DISCUSSION

The tissue lines used in this study undergo a series of changes which reflect the movements taking place in the maturing cortical compartment. The changes show that during gyrus formation the part of the cortical plate between the future sulcal floors moves radially on an increasing depth of subplate. This indicates that the radial processes in the subplate are increasing in length so that the cortical plate is, in effect, riding outwards on lengthening subplate fibres. Within the plate itself the radial fibres also begin to lengthen but initially remain more or less parallel to each other. As the gyrus develops, the radial lines fan out towards the surface of the spreading gyral crowns so that increasing amounts of cortical tissue are enclosed between the diverging lines. Thus, the cortical columns in much of the ferret cortex have the form of elongated wedges. Also, the true length of a cortical column is to be measured along the curvature of the tissue lines and not at right angles to the local cortical surface. Measured thus the columns have a much more even length (Fig. 5*d, e*). The tangential subdivision of the columns into layers also tends to remain more uniform if the depth of each layer is measured along the curvature of the lines rather than straight through the cortex at right angles to the gyral surface (Fig. 5*d, e*).

In interpreting the movement of nuclei in the intersulcal segment it is assumed that in the ferret cortex the final arrangement of neurons is for younger cells to lie superficial to older. This has been found in autoradiographic studies of the cortex of both lissencephalic (Angevine & Sidman, 1961; Fernandez & Bravo, 1974) and gyrencephalic brains (Rakic, 1974). Layer II, for example, is the last to be relieved of nuclear crowding even though, being on the outside of the gyral curvature, it is undergoing the greatest amount of tangential expansion. This indicates that additional space in the outer layer acquired by spreading of the cortical surface continues to be filled by the immigration of deeper nuclei. The disappearance of radially elongated nuclei from layers VI to III, in that order, may also indicate that these nuclei belong

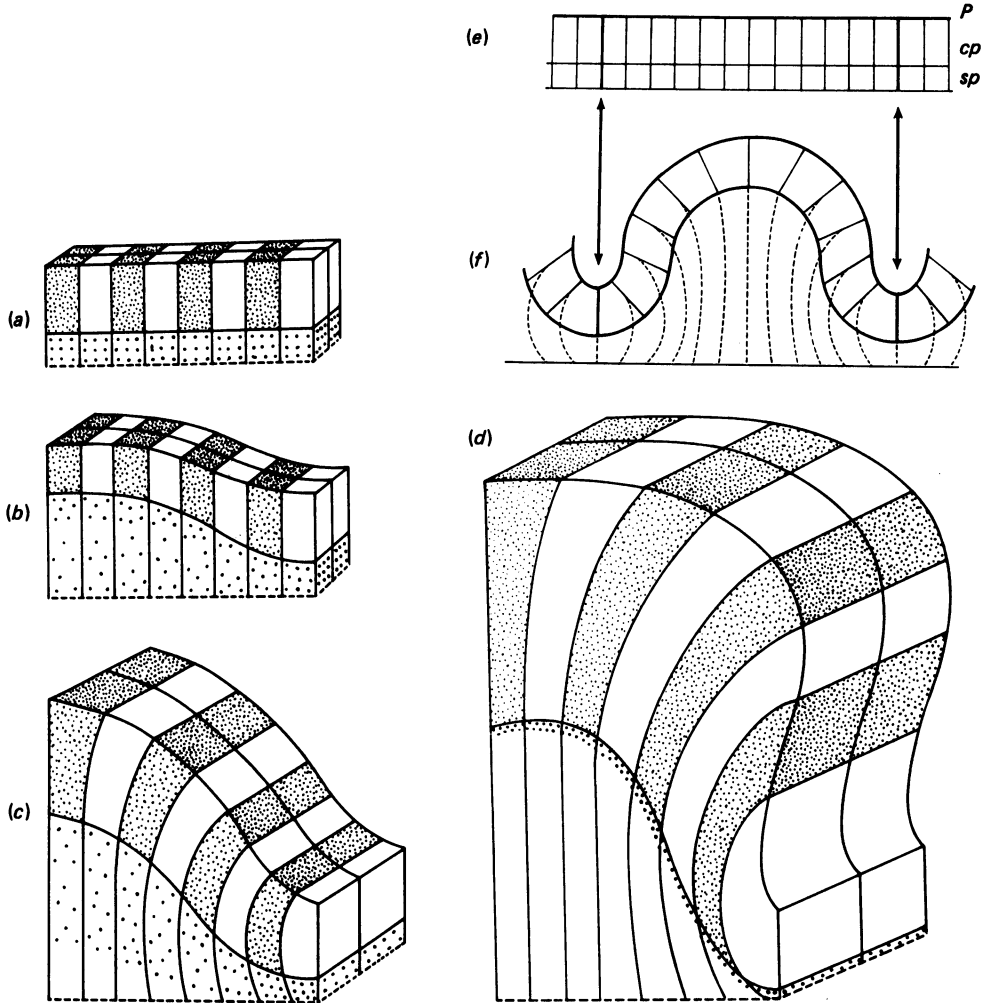


Fig. 15 (a-f). (a-d) Suggested model of differential growth during formation of coronal gyrus. The drawings show a three dimensional representation of a sample cross section taken through one half of a gyrus. The upper compartment represents the cortical plate, and the lower the subplate. (a) unfolded system; (b) the cortical plate rides outwards on the subplate; (c) the gyral crown develops with spreading of radial lines while sulcal area remains little changed; (d) the final stage in which there has been tangential spreading of parasulcal cortex. During these changes the rostrocaudal length of the segment increases progressively in length and the neurons of the subplate, indicated by stippling, become restricted to the deep surface of the cortical plate as in (d).

(e-f) Diagrams depicting a modified 'carpet pile' model of cortical folding. Figure 15(e) represents an unfolded two layered system composed of a cortical plate (cp) and a subplate (sp). The pial surface (P), representing the back of the carpet, is uppermost while the radial lines represent the pile. The centres of the future sulci are marked by heavier lines. Figure 15(f) represents the system after folding. The radial lines descending from the crown of the fold converge naturally on their sites of origin and undergo little deviation in the subplate. The lines emanating from the pial surface at the parasulcal region would diverge naturally if they were unconstrained; this is represented by the course of the unbroken lines. However, as the fibres these lines represent are not free but are entering or leaving the subcortical fibre systems they are in fact tethered and thus constrained to recurve to their sites of penetration of the white matter. The recurving course is represented by pecked lines and simulates the observed parenthesis effect on each side of the sulcus.

to the last born neurons still migrating through older, already maturing neurons to their destination in the outer cortex. However, it is not only the later generations that continue to move radially; the whole population of maturing cortical plate cells lying between sulci is also moving radially on the subplate. Within the plate, the radial spacing between nuclei in all the emerging layers is also increasing, indicating that each nucleus is moving radially at a faster rate than the nuclei deep to it; this must be the case if the distance between them is to increase (Fig. 8).

The changes in the parasulcal cortex are different. During development, the radial tissue lines curve but do not lengthen greatly and their nesting curvatures appear to bracket the sulcal centre line (Fig. 5*a, b*). The curving radial lines of the 'parasulcal parenthesis' keep much the same distance apart as they run from superficial to deep until in the deeper cortex they converge towards their earlier established points of penetration of the white matter. This course indicates that the parasulcal columns are more uniform in breadth, rather than triangular in cross section as in the gyrus proper.

The radial movement of nuclei in the parasulcal area is also minimal (Fig. 9) and the local columns are consequently shorter, yet candidate neurons for all cortical layers appear to be present (Figs. 6, 7). This implies that at the parasulcal area there is more tangential spreading of the maturing cortical plate giving rise to many short columns whereas at the sulcal crowns an equal transect of cortical plate would give rise to fewer but longer columns.

The overall changes in the radial lines in intersulcal and sulcal segments suggest that they have behaved like the pile of a folded carpet, i.e. they have converged from the concave internal surface of the gyral crown and diverged from the convex internal surface at the sulci. While convergence onto a fixed base is geometrically possible at the crowns, the full development of divergence from the parasulcal area is prevented because cell processes are required to curve back towards their fixed points of origin from the white matter, producing the parenthesis effect (Fig. 5*b*). The following steps in the formation of a gyrus are consistent with the observed changes in the tissue lines described above and set out in the diagrams in Figure 15*a-d*: (i) initially a segment of cortical plate moves radially on lengthening subplate fibres and the lines of the growing system remain more or less parallel (Fig. 15*a, b*); (ii) as growth continues, the radial lines increase in length in the intersulcal segment and also undergo tangential spreading to accommodate increasing cell volume (Fig. 15*c*): at this stage the cortical plate at the sulcal region retains much of its original depth and degree of nuclear crowding; (iii) during subsequent growth the cortical plate on each side of the sulcal centre line diminishes in both depth and nuclear packing density indicating that in this region there has now been considerable tangential expansion (Fig. 15*d*). The diagrams in Figure 15*a-d* also depict that in both gyri and sulci these events are accompanied by considerable longitudinal growth (Smart & McSherry, 1986).

The history of the subplate neurons is of contrasting interest. Initially, the subplate is thin and its neurons are fairly crowded (Fig. 10). As the subplate deepens its component neurons become more dispersed (Figs. 10, 12). At the boundary of the cortical plate, however, there is a transition zone where the different degrees of nuclear crowding grade into each other. It is difficult to decide whether this zone is inner cortical plate or outer subplate (Fig. 12). It is thus quite possible that some subplate neurons are derived from deeper nuclei of the cortical plate which are left behind as the plate moves radially. Such cell loss to the subplate could account for

the thinning of the intersulcal segment of the cortical plate observed in the early stages of gyrogenesis (Fig. 2). According to Marin-Padilla, preplate neurons are the first cells to be released and the only source of neurons for cortical layers I and VIb; the neural organisation in which these cells take part is interpreted as the expression of an evolutionarily ancient part of the original developmental program and is said to recall features of the premammal cortex (Marin-Padilla, 1971, 1978; Rickman, Chronwall & Wolff, 1979). By contrast, the cortical plate is held to be a later evolutionary innovation giving rise exclusively to cortical layers II-VIa. The possible acquisition of cortical plate neurons by the subplate in the ferret suggests that this putatively primeval organisation may also be colonised and modified by later generations of neurons. The eventual fate of the large subplate neurons so conspicuously evident in the case of ferret gyri (Fig. 14) remains to be determined. It is probable that they eventually migrate radially to form layer VIb as Marin-Padilla has suggested.

The mechanism that determines so predictably which parts of an initially more or less uniform cortical plate become gyri and which sulci is unknown. The present observations, however, are consistent with the ideas expressed by Welker & Campos (1963) in their study of carnivore brains. They have suggested that as the cortex develops "distinct groups of thalamic afferents project to adjacent cortical regions in the smooth foetal brain and [these regions] are separated by thin zones of athalamic cortex that are relatively barren of such connections". They also state that during subsequent development "the fundic cortex fails to grow and differentiate unlike the budding gyral crowns". We would modify the last statement to read merely 'fails to grow radially', for the present observations indicate that the fundic cortex does indeed grow tangentially and longitudinally. Also, while there is little change in depth of the cortex underlying the sulcal fundus, this segment appears to undergo a rather specialised differentiation during which the fibres and neurons of the deeper cortical layers become tangentially orientated (Figs. 6, 7). This arrangement is also consistent with Welker & Campos' (1963) neurophysiological mapping observations. Within the Sm1 region, for example, they found that most sulci developed between the representation of body parts that were anatomically separate at the periphery such as the tips of adjacent digits. The tangential organisation in the fundic cortex might function as an active commissure regulating by virtue of its neuronal content the transmission of impulses between gyri. Certain differences have also been observed in the structure of the cortex associated with other sulci. This suggests that corresponding differences exist in the functional properties of this specialised segment of cortex.

The present results can now be correlated with some earlier studies on the subject of cortical folding. A major work by Bok (1929), one of the few investigations based primarily on extensive observation and measurement, deals with folding of the human cortex. His approach was to consider the effect of folding a layered system with a radial structure which retained a constant volume during the folding process, the volume being that of the adult cortex. He catalogued the possible geometrical deformations that would emerge in the folding of such a tissue and compared these with the observed structure of adult gyri and sulci. However, the human cortex, like that of the ferret, commences to fold prior to achieving its final volume, that is during the cortical plate stage, and continues to fold while increasing substantially in both depth and area. The inclusion of a growth component is therefore an essential part of any model of gyrogenesis. Bok's deformations also tend to impose an idealised

geometrical structure on the cortex whereas in our treatment the tissue lines themselves are allowed to indicate the changes taking place in the system. Bok's approach and results, however, are similar to those presented here and have some fifty years' precedence. Interestingly Bok, too, in man describes the fundic cortex as thinner and cortical layer VI as tangentially organised.

Richman, Stewart, Hutchison & Caviness (1975) embarked on a theoretical approach to the folding process in normal and abnormal human cortex. Their mechanical model was based on differential growth occurring between inner and outer cortical laminae (composed of layers I–III and IV–VI respectively) and made no allowance for the influence exerted by the inherent radial structure of the growing cortex. This approach discounts the principal direction of differential growth and while their treatment is mathematically interesting it does not describe what is actually happening in the cortex.

The present observations are consistent with the results of Todd (1985) who was able to measure the changing curvature of the cortical surface of the early postnatal ferret brain by digitising the contours of Moiré fringe patterns produced by suitable illumination of a brain specimen. A mathematical analysis of the surface changes indicated that growth over the cortical crowns is greater than along the sulci. For operational reasons, Todd's study was limited to the stages of gyrogenesis prior to P6, that is, before the sulci become too deep and the contour lines consequently too crowded to digitise. During this early period, the fundic cortical plate does not decrease in depth nor do the parasulcal tissue lines show much distortion whereas, as seen here, there are signs of active growth in the sulcal crowns (Fig. 3). These early stages of gyrus formation are consistent with the results of Todd's study. During development after P6, however, the situation appears to reverse and growth in the parasulcal area becomes more active as the cortical plate spanning the sulcal fundi undergoes tangential expansion.

There is no evidence in the ferret that local compression by the surrounding calvarium exerts a need to fold; such a purely mechanical effect of the skull on the cerebrum occurs much later after gyrogenesis has been completed (Smart & McSherry, 1986). Nor is there any evidence in the ferret that local variations in the depth or intrinsic structure of the pre-folded cortical plate, as postulated by Clark (1945), are the primary mechanical base of folding. However, he also suggested that increase in cortical area between two fixed points causes the cortex to buckle and this may account for the hairpin form of the ectosylvian gyrus, as its ends are fixed to the underlying striatum and its limbs are free to follow the growth of the pallial vault.

Todd (1982) has suggested that the direction of the sulci is geometrically determined and follows the lines where the radius of curvature of the cortical surface is greatest. While the sulci may indeed follow such lines on the adult cortex, the local surface curvature of the unfolded immature cortex cannot be considered to be the primary determinant of the direction of cortical folding. The sulcal floors, as we have seen, represent specialised zones which initially are characterised by retardation of growth. It seems unlikely that the direction of these lines of retarded growth is determined by the local surface curvature.

Finally, it is worth noting that the ferret cerebrum lies at the lower end of the gyrencephalic size scale and ferret gyri are by mammalian standards 'microgyri' with, in the case of the coronal gyrus, a wavelength about the same as the depth of an average cortical column. At the scale of magnitude prevailing in the ferret, a gyrus may thus be a means of accommodating the maximum number of long cortical

columns into a base of minimal width, as seen in Figure 5*d*. It is possible that ferret gyri represent the smallest practicable gyral configuration. Consequently it would be interesting to know if carnivoran gyri first evolved at minimal size in small brains and were subsequently scaled up to the size found in larger carnivores or if they are miniaturisations of initially larger gyri. The illustrations in Radinsky's (1969) paper on the early evolution of canid and felid brains suggest the former as these early carnivores were of small size with correspondingly small brains. Further palaeontological evidence of carnivoran evolution may eventually answer this question.

It must also be considered, as gyri appear to have evolved independently in different mammalian lineages, that there may be no general model of gyrogenesis. Different mammalian lineages may have arrived at a gyral configuration in different ways and folding may have been related to different patterns of organisational change within the evolving nervous system. The methodology presented here, however, should be applicable to other systems and should provide a convenient descriptive procedure for comparing gyrogenesis in different evolutionary lineages.

SUMMARY

The internal changes within a developing gyrus of the ferret cerebral cortex were studied by recording (i) the changing length and direction of the radial tissue lines and (ii) the emergence of the tangential banding of the classical six cortical layers. Together these lines provided a coordinate net whose deformations during development gave an indication of the differential growth occurring within a gyrus. The changes in these features suggested that a gyrus was initiated by an area of local growth appearing in the subplate and then in the suprajacent segment of cortical plate. During subsequent growth there was tangential spreading of the more mature tissue at the gyral crown while at the site of the future sulci the cortical plate remained immature and growth was retarded. During later stages the majority of tangential growth occurred in the parasulcal area. At this site a very much thinner cortex was generated from a segment of cortical plate of the same depth and degree of nuclear crowding as elsewhere, implying that growth here was resolved into tangential spreading. The cells and fibres of the deeper cortical layers of the sulcal cortex eventually became tangentially orientated suggesting that they subserved a commissural function between the columnar systems of adjacent gyri. At the scale prevailing in the ferret, gyrus formation was seen as a configuration which tended to conserve both the total length of the cortical columns and the depth of the individual cortical layers.

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