Gyrus formation in the cerebral cortex in the ferret.
I. Description of the external changes

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INTRODUCTION

The ferret is the smallest laboratory animal with a folded cerebral cortex and thus provides the most readily available source of developmental material on which to base a description of the development of cortical folding. The phenomenon of gyrus formation has been a topic of recurrent interest over the years and considerable published information on the subject already exists (Schaffer, 1923; Bok, 1929; Clark, 1945; Barron, 1950; Richman, Stewart, Hutchison & Caviness, 1975; Todd, 1982). There is, however, no detailed study correlating the stages in the growth of a gyrus as seen from the outside with the histological changes taking place internally. The purpose of this study is to provide such a correlation. This paper describes the external features of the pallium during the folding process and the succeeding paper (Smart & McSherry, 1986) deals with the histological changes occurring within a gyrus as it develops and grows.

MATERIALS AND METHODS

Ferrets were allowed to litter and two offspring were killed at intervals of 2 days from birth up to 14 days and at weekly intervals or less up to 36 days; adults over one year old were also killed. The animals were anaesthetised with Sagatal and the brains were fixed by intracardiac perfusion with Bouin's fluid after flushing the vascular system with normal saline. Photographs were taken of the medial, lateral and dorsal surfaces of the cerebral hemispheres of the brain at each age. Tracings were made from the photographs of the outlines of the perimeter of the hemisphere and of the sulcal patterns at each age. By superimposing these outlines and keeping certain landmarks in register the differential growth of the whole hemisphere and its gross folding pattern were identified.

The other brain of each pair was embedded in paraffin wax, serially sectioned at a thickness of 10 μm in the coronal plane and stained with haematoxylin and eosin. These sections were used to correlate the cross sectional appearance of the gyri with their course in the intact brain. Three dimensional representations of the folding sequence were also made by modelling in plasticine the gyral pattern at different stages of development.

The names of the sulci and gyri are based on the terminology used by Radinsky (1971, 1973, 1975) for the carnivore brain in general. The sulcal pattern of the mustelids is among the simpler carnivoran configurations, that of the ferret being one of the least complex of the group, so a correspondingly reduced terminology
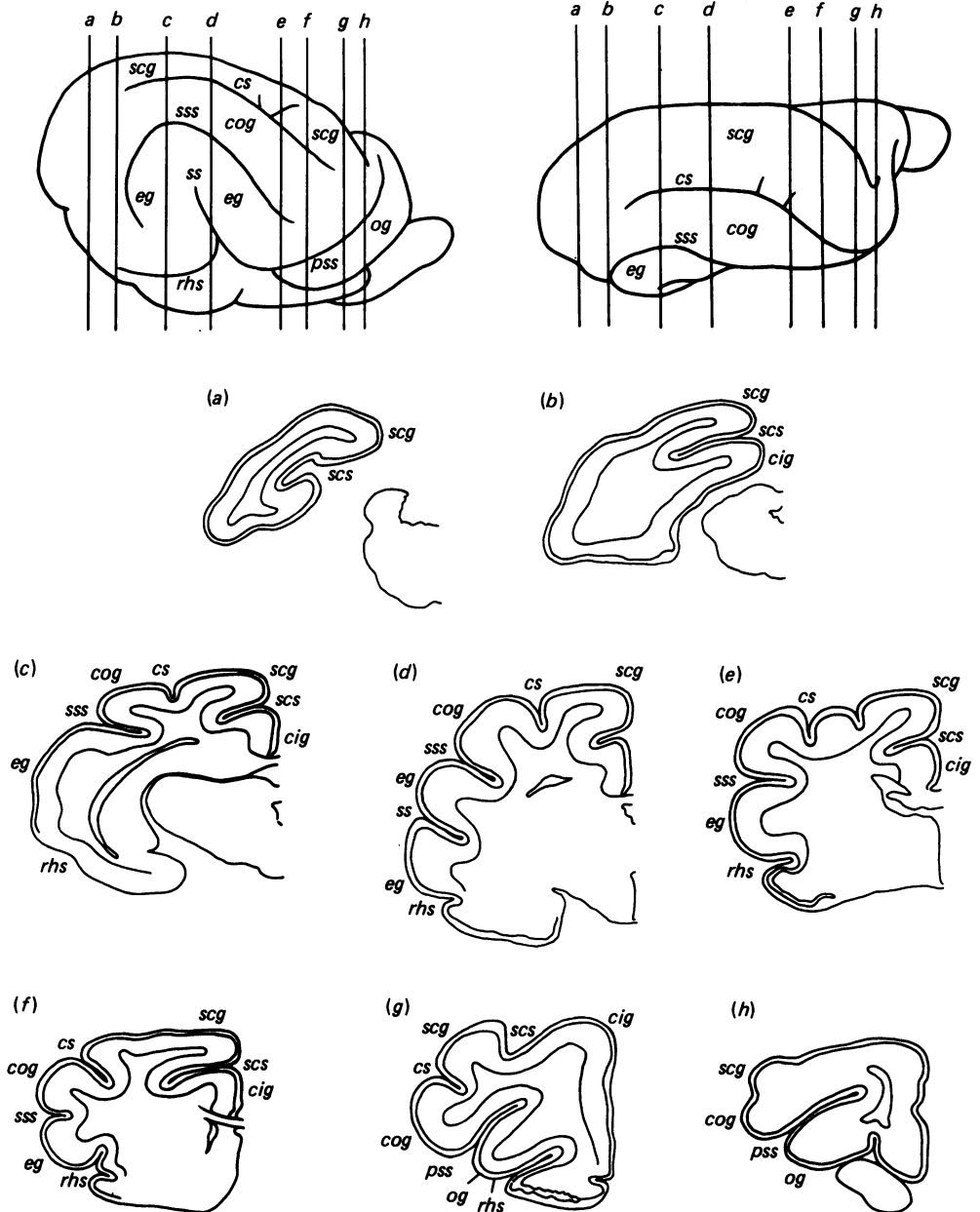
Fig. 1 (*a–h*). The upper drawings show lateral and dorsal views of the right cerebral hemisphere of a 36 days old ferret. The coronal levels labelled *a–h* in these drawings correspond to the similarly labelled coronal sections depicted in the lower part of the Figure. *cig*, cingulate gyrus; *cs*, coronolateral sulcus; *cog*, coronal gyrus; *eg*, ectosylvian gyrus; *og*, orbital gyrus; *pss*, presylvian sulcus; *rhs*, rhinal sulcus; *scg*, spleniocruciate gyrus; *scs*, spleniocruciate sulcus; *ss*, sylvian sulcus; *sss*, suprasylvian sulcus. The two compression folds running medially from the rostral end of the coronolateral sulcus have been dubbed ansate sulcus and central sulcus by Radinsky (1975). The ansate lies more caudally.
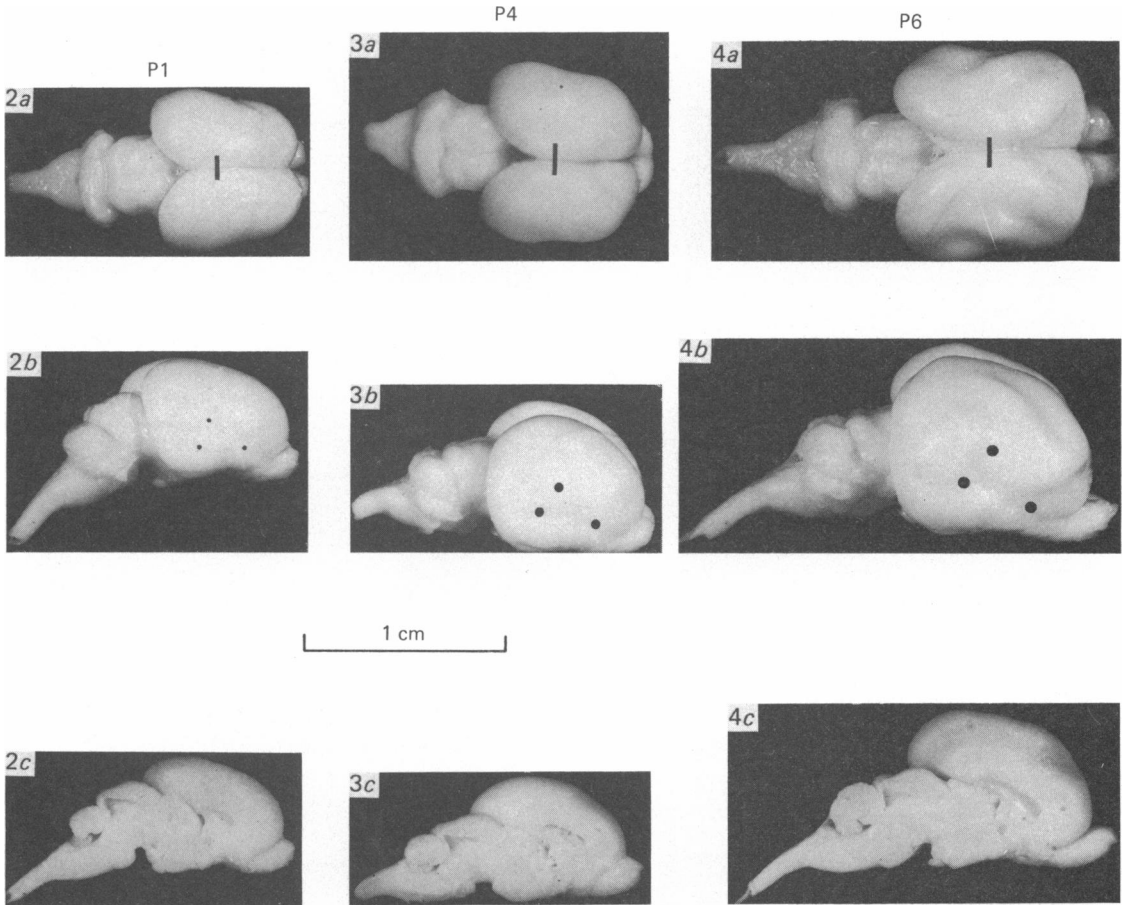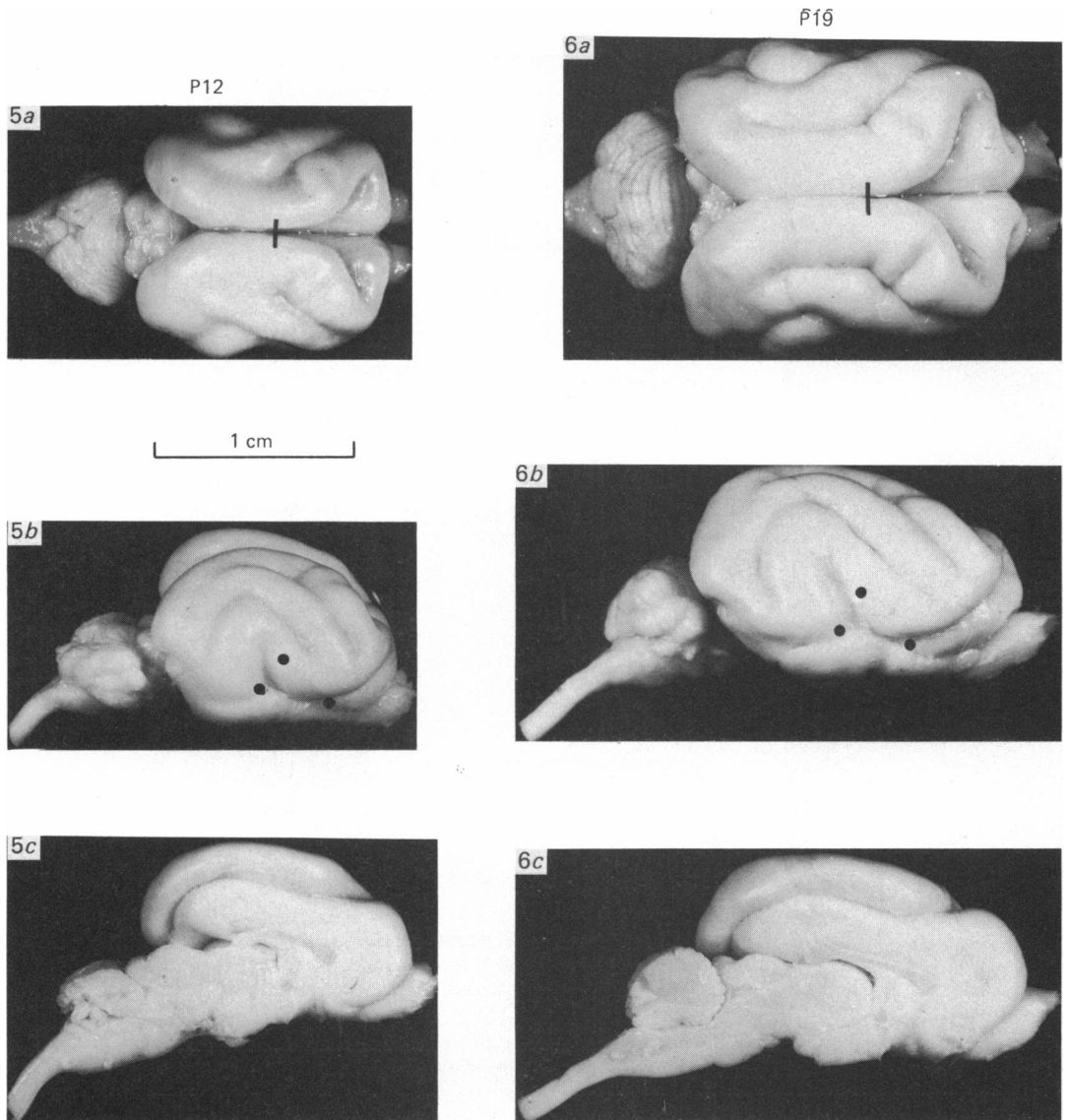
Fig. 2 (*a–c*). Dorsal, lateral and medial views respectively of a 1 day postnatal ferret brain. In Figure 2*a* the bar marks the level of the interventricular foramen. In Figure 2*b* the rostral dot marks the end of the orbital gyrus, the most caudal dot the end of the ectosylvian gyrus and the upper dot the location of the interventricular foramen projected onto the lateral surface.

Figs. 3(*a–c*) and 4(*a–c*). Dorsal, lateral and medial views of 4 and 6 days postnatal ferret brains respectively. Landmarks indicated are explained in the legend to Figure 2*a* and *b*. In Figure 4 the sulci and gyri have started to emerge and their nomenclature can be derived from Figure 1.

was required. This is portrayed in Figure 1. Only the general folding pattern was under consideration and so the names of sulci and gyri in continuity have been combined. For example, the cruciate and splenial sulci are the rostral and caudal ends of the same fissure which, when referred to in its entirety, is termed the spleniocruciate sulcus. Similarly the ectosylvian gyri circumscribing the sylvian sulcus for the purposes of this study required no separate identification as anterior or posterior and so the whole elevation is termed 'ectosylvian'.


OBSERVATIONS

At birth and for the subsequent few days the pallium presented a convex lateral surface, with a very slight transverse waisting about midway along its rostrocaudal axis, and a more flattened medial surface (Figs. 2*a–c*, 3*a–c*). Within the first week of postnatal life, all the sulci and gyri of the adult brain were present in incipient form.

Figs. 5(a–c) and 6(a–c). Dorsal, lateral and medial views of 12 and 19 days postnatal ferret brains respectively. Landmarks indicated are explained in legend to Figure 2a and b.

These are visible in the six days postnatal specimen illustrated in Figure 4a–c. On the medial side of the hemisphere at this age, a sulcus extended along the length of the brain. It was shallow in its middle part and deepened rostrally and caudally. The rostral end represented the incipient cruciate sulcus and the caudal end the splenial sulcus. The cingulate gyrus lying below the spleniocruciate sulcus was broad, with its medial surface flattened against its fellow of the opposite side (Fig. 8). The medial border of the spleniocruciate gyrus lying above that sulcus was rounded and the gyrus had the form of a flap or flange of pallial tissue (Figs. 4a, 5a, 8). Rostrally, a conspicuous transverse presylvian sulcus was present. The short orbital gyrus ventral to the presylvian sulcus was continuous medially with the rostral end of the
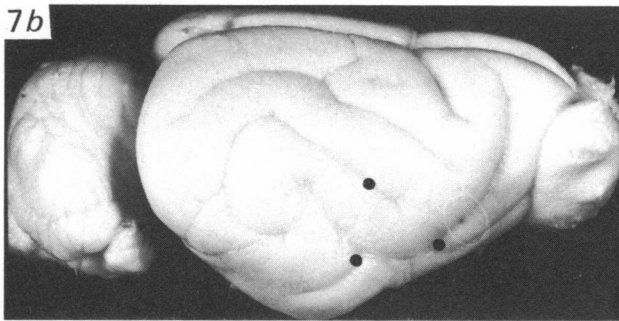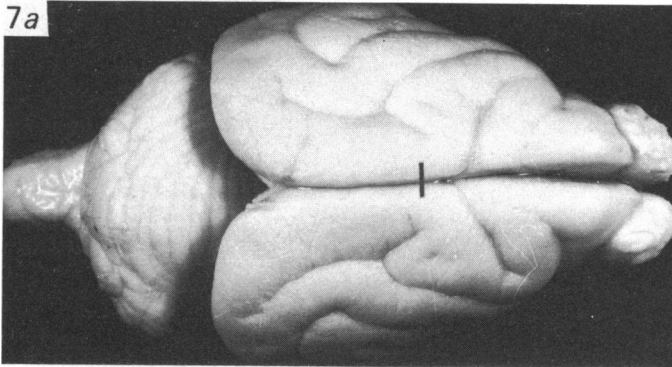
Fig. 7(*a–c*). Dorsal, lateral and medial views respectively of six months postnatal ferret brain. Indicated landmarks are explained in legend to Figure 2*a* and *b*.

cingulate gyrus and, when followed laterally, curved on to the lateral surface to terminate just rostral to the sylvian fissure. More caudally on the lateral surface, a rounded boss of tissue was present producing above it a shallow, curved, suprasylvian sulcus. Rostrally the bulge abutted a depression marking the incipient sylvian sulcus. On the more dorsal aspect of the lateral surface, a linear longitudinally running depression, deepest rostrally, marked the location of the coronolateral sulcus, the final major fissure of the ferret brain.

In progressively younger specimens of the hemisphere, the incipient gyri between the sulci receded into the hemisphere. At birth, the future gyral crowns were evident as areas of slightly increased surface curvature while the flatter areas between them marked the future walls and floor of the intervening sulci (Fig. 8). Prior to birth even this feature was lost. In older specimens the features present at 6 days postconception became more pronounced as the gyri grew in height and length (Fig. 5). By 26 days postnatum, the hemispheres had achieved the shape and dimensions illustrated in Figure 6. The gyri were rounded with open sulci and the profiles of the whole hemisphere when seen from the dorsal and lateral aspects were roughly rectangular. This shape persisted until the time of weaning, after which the hemispheres were remoulded to give brain profiles with a more pointed rostral pole (Fig. 7). During this remoulding the walls of the sulci became closely opposed and the summits of the gyri more flattened. These features can be seen clearly in the photomicrographs of coronal sections of a series of developing brains from birth to adulthood set out in Figure 8.

Assessment of relative growth

It was found by trial that, when seen in lateral view, the following three points changed little in relation to each other during the considerable growth of the cerebral hemisphere between the neonatal period and the adult: (*a*) the projection of the location of the interventricular foramen on to the lateral surface; (*b*) the tip of the caudal limb of the ectosylvian gyrus; (*c*) the caudal extremity of the orbital gyrus.

The positions of these points are marked by dots in Figures 2*b* to 7*b*. The reason for the relative immobility of points *a* and *b* probably resides in the fact that they lie in the middle of the ventral boundary of the cortex which overlies and is adherent to the striatum, whereas the pallium proper is not attached to the subjacent grey matter. In Figures 9, 10, 14 and 16, the lateral profiles of the hemisphere are superimposed while keeping the three reference points in approximate register and, in Figures 11 to 13, the dorsal profiles are similarly superimposed, in this case keeping the level of the interventricular foramen in register. Growth around these points seemed to be fairly symmetrical in the pre-weaning brain. Between weaning and the adult, however, the hemisphere increased in height and length (Figs. 6*b*, 7*b*, 15) but diminished in breadth particularly rostrally (Figs 6*a*, 7*a*, 12).

In Figures 13 to 15 the changes in position and length of the main sulci during growth are shown. The floor of the cruciate sulcus, where visible rostrally, migrated progressively away from the midline (Fig. 13). The coronolateral sulcus increased in length at both its rostral and caudal ends (Fig. 13). The sylvian sulcus lengthened dorsocaudally (Fig. 14). The curve of the suprasylvian sulcus also moved dorsocaudally as its limbs lengthened from their relatively fixed extremities (Fig. 16).
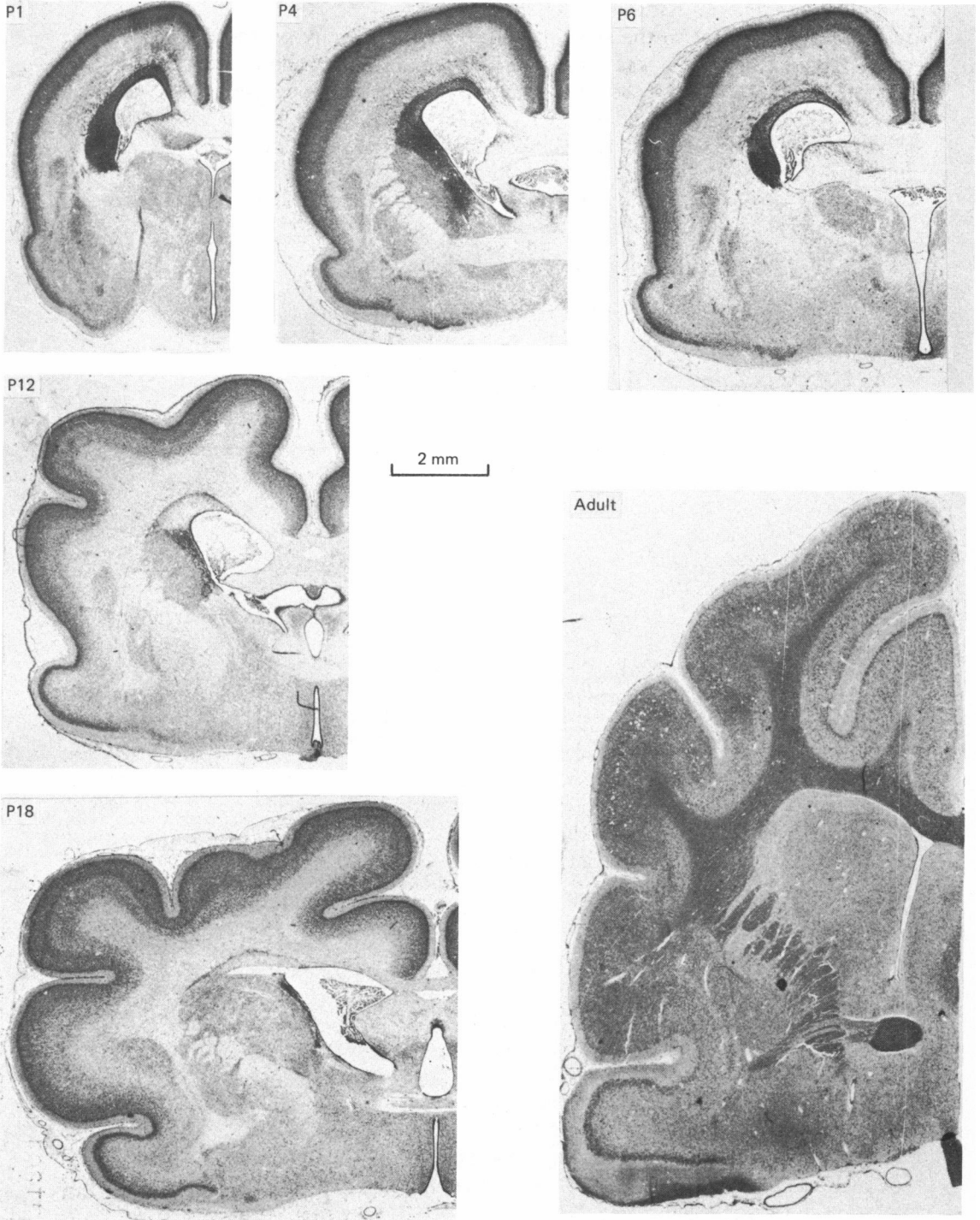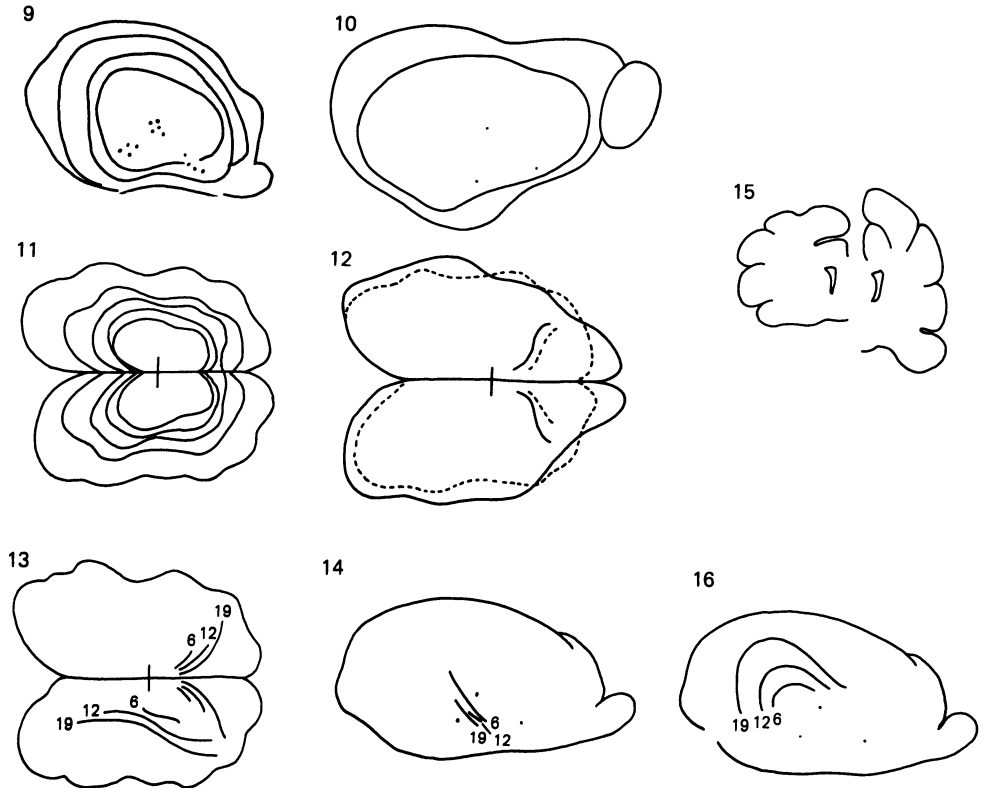
Fig. 8. Photographs of coronal sections of ferret brains at stated postnatal ages. The level of section is at or near to the interventricular foramen.

Figs. 9–16. Diagrams showing brain outlines at different ages superimposed with the landmarks *a, b, c* (defined in Fig. 2*b*) in register. Fig. 9. Superimposed lateral views of 1, 6, 12 and 19 days postnatal brains. Fig. 10. Superimposed lateral views of 19 days postnatal and adult brains. Fig. 11. Superimposed dorsal views of 1, 4, 6, 12 and 19 days postnatal brains with level of interventricular foramen (indicated by bar) in register. Fig. 12. Superimposed dorsal views of 19 days postnatal brain (indicated by broken outline) and adult brain. Bar, level of interventricular foramen. Fig. 13. Dorsal outline of 19 days brain showing locations of cruciate and coronolateral sulci at 6, 12 and 19 days. Fig. 14. Lateral view of 19 days postnatal brain showing location of sylvian sulcus at 6, 12 and 19 days. Fig. 15. Outline of 19 days postnatal brain showing location of suprasylvian sulcus at 6, 12 and 19 days. Fig. 15. Cross sections of 19 days postnatal (on left) and adult (on right) brains at level of interventricular foramen showing change in transverse and vertical proportions.

Gross features of internal changes

In Figure 17*a–d*, diagrams are shown of coronal sections at the level of the interventricular foramen at four different stages of development. These demonstrate that during postnatal growth the whole cortical compartment is displaced laterally by the increase in volume of the striatum and diencephalon which form the core of the hemisphere. In Figure 17*e*, the outlines of sections at the beginning and end of the folding processes are superimposed with the deep surfaces of the cortex in register in each case. This demonstrates that the floors of the sulci do not move much in relation to the inner boundary of the cortex or to each other whereas the intervening segments of cortical tissue expand outwards.

The gyri on the convex surface of the hemisphere were free to grow outwards in this manner. On the mutually opposed medial surfaces, however, 'outward' expansion, i.e. towards the midline, was not possible. In the spleniocruciate sulcus, there-
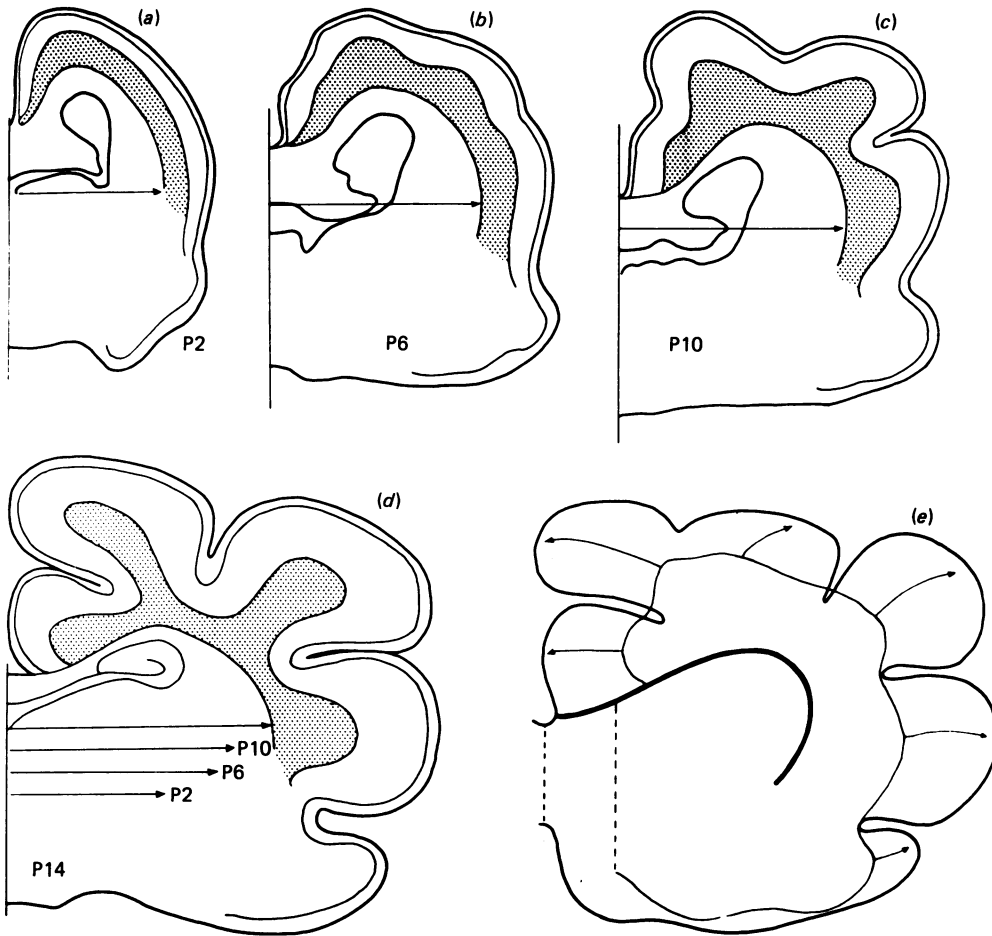
Fig. 17 (*a–e*). (*a–c*) Tracings of coronal section of postnatal ferret brains aged 2, 6 and 10 days respectively taken at the level of the interventricular foramen. The shaded area indicates the extent of the subplate. The arrows indicate the distance from the midline to the deep surface of the subplate. (*d*) Coronal section of a 14 days postnatal ferret brain. The arrows indicate the distance of the deep surface of the subplate from the midline at the stated postnatal ages, showing that the cortex moves laterally as the core of the hemisphere enlarges. (*e*) The outline of a 14 days postnatal brain superimposed on that of a 6 days brain with the deep surfaces of their subplates in register. This shows that the sulcal floors do not move much relative to the deep surface of the subplate whereas the sulcal crowns move centrifugally (arrows) from the datum line of the boundary between the subplate and the white matter indicated by heavy line.

fore, the summits of the gyri remained adjacent to the median plane while the floor of the sulcus followed the movement of the whole cortex as it was carried laterally by the growing core of the forebrain (Figs. 8, 13, 19). It is also relevant to note for the purposes of future interpretation that during cortical growth the lateral expansion of the basal telencephalon was considerably greater caudally than rostrally (Fig. 18).

DISCUSSION

The basic form of the gyral arrangement in the adult ferret pallium can be resolved into two systems of folds. The first, more extensive, system is represented by a broad flange of tissue corresponding for most of its course to the spleniocruciate gyrus.
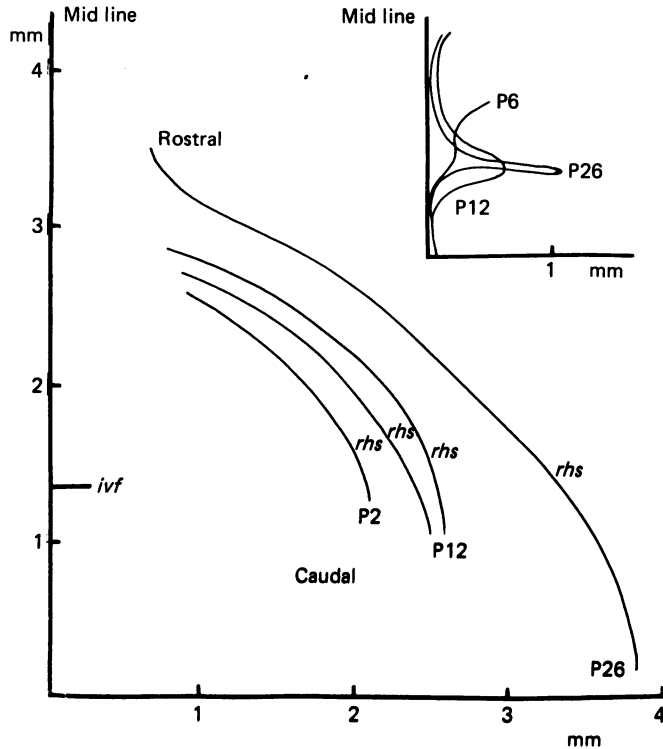
Fig. 18. This Figure plots the distance of the rhinal sulcus from the midline at the stated post-natal ages. It shows that the lateral expansion of the basal telencephalon is considerably greater in the caudal regions. *ivf*, level of interventricular foramen; *rhs*, rhinal sulcus.

Fig. 19. The inset graph records the location of the fundus of the spleniocruciate gyrus relative to the midline as seen in coronal section at the stated postnatal ages. The fundus moves progressively laterally away from the midline.

At its rostral end, the flange is double folded to give this end of the spleniocruciate system a sigmoid appearance (Fig. 6a). The lower limb of the sigmoid corresponds to the orbital gyrus (Fig. 1). The coronal and ectosylvian gyri can be regarded as a second set of two separate folds arising from the dorsolateral surface of the cortical convexity.

The formation of a gyrus results from expansion of the tissue lying between sulci (Fig. 17e). Expansion of a gyrus along its long axis is substantial and is accommodated by increase in the rostrocaudal length of the hemisphere (Figs. 9–16). In the case of the spleniocruciate and coronolateral gyri, their extremities are free to follow the growth of the hemisphere and the longitudinal extension of these gyri is more or less symmetrical about the line of the interventricular foramen (Fig. 13). In the suprasylvian gyrus, by contrast, both ends of the system are fixed to the underlying striatum. In this case, increase in gyral length produces a 'hairpin' bending effect and the increase in length of the two limbs is accommodated by the bend of the hairpin following the caudal growth of the pallial wall (Fig. 15). The sylvian sulcus between the two limbs correspondingly increases in length only at its dorsocaudal extremity (Fig. 14). Increase in breadth of a gyrus is limited to the segment above the fixed floors of the bounding sulci and if sufficiently extensive leads to mild pedunculation (Fig. 8). Increase in gyral height occurs by the segment of cortex between

sulcal floors growing outwards. In the case of the spleniocruciate sulcus, however, the lips of the sulcus cannot move much before meeting their contralateral neighbour at the midline. The sulcus in this case deepens as its floor is carried away from the midline as growth of the core tissue of the hemisphere displaces the whole cortex laterally (Figs. 8, 13, 19). Thus, the tissue expansion of the cingulate and spleniocruciate gyri is accommodated in the space provided by the floor of the intervening sulcus moving laterally. A similar process occurs during the formation of the folded Ammon's horn in the mouse hippocampus (Smart, 1982). The rostral edge of the combined cruciate and orbital gyri gives a conspicuous sigmoid appearance to the frontal pole of the cortex while the occipital pole is graced by a single fold around the splenial sulcus. This configuration may represent a response to the growth of the underlying basal telencephalon upon which the cortex rests. The distance of the rhinal fissure from the median plane at successive postnatal ages is plotted in Figure 18 which demonstrates that the occipital pole of the overlying cortex is carried further laterally than the frontal. The sigmoid folding of the rostral edge of the gyral system may therefore result from the increasing area of the frontal cortex accommodating itself by double folding on to a relatively narrow base. The occipital cortex, in addition to lying on a broader foundation, also has a greater opportunity to expand caudally into the less architecturally constrained cranial vault, in which direction it progressively overrides the mesencephalon (Figs. 4*a*–7*a*). The frontal cortex is also subject to further major constraints by the growth of the surrounding skull which, for intrinsic functional reasons, is destined to acquire a pointed facial skeleton and impresses this form on the post-weaning brain (Fig. 7*a, b*).

According to Radinsky, the first sulci to appear in the evolutionary history of carnivores are the coronolateral and suprasylvian. These are already evident in primitive carnivore brains which have still a relatively undeveloped pallium, that is, in brains with a high rhinal fissure, small frontal lobes and minimal caudal extension of the pallium over the mesencephalon (Radinsky, 1969). The later appearance of a spleniocruciate sulcus in the evolutionary history of the carnivore lineage is associated with relatively greater vertical growth above the rhinal fissure and caudal extension of the pallium to overlap the midbrain and cerebellum (Radinsky, 1971). The position of the 'newer' spleniocruciate system within the cortex is consistent with the underlying pattern of histogenesis of cortical neuron populations. It is known that neuron release begins at a ventral site in the lateral telencephalic wall and spreads from there across the pallial vault. This has been described in the mouse by Smart (1973, 1983), in the cat by Marin-Padilla (1978), in man by Sidman & Rakic (1982) and specifically in the ferret by McSherry (1984). In this type of growth, the cortical plate increases in area by increments received at its perimeter. The creation of phylogenetically 'new' pallial territory can therefore be expected to occur ontogenetically around the edge of a previously established cortex. The spleniocruciate sulcus appears in such a peripheral area with respect to the histogenetic gradient of the cortex and its location is thus consistent with the subdivision of a later acquired peripheral increment in cortical territory. The fact that a spleniocruciate system has evolved independently in at least five different carnivoran families (Radinsky, 1971) suggests that growth of the carnivore pallium is channelled by some common constraints into the subdivision of new territory in a similar way.

SUMMARY

The external features of gyrus formation in the postnatal ferret cerebral cortex are described and correlated with certain internal changes. The observations indicate that gyri are formed by longitudinal and radial expansion of the cortical compartment occurring between relatively fixed areas which form the sulcal floors. The gyri were initially rounded with open sulci and the cerebrum had a rectangular outline when seen in lateral and dorsal view. By adult life the hemisphere had been subjected to considerable moulding by the growing skull, so that the frontal pole of the cerebrum became pointed while the sulcal walls became closely opposed and the gyral crowns flattened.

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