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Growth patterns in the lateral wall of the mouse telencephalon. II. Histological changes during and subsequent to the period of isocortical neuron production

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INTRODUCTION

In this paper the growth of the lateral telencephalic wall of the mouse brain is traced from the first appearance of the telencephalic vesicle on the tenth day of embryonic life to the establishment of the main subdivisions of the adult forebrain. Our concern is to (1) identify the boundaries of the isocortex as they emerge during development from the background tissues, (2) identify the site of origin of isocortical neurons from the ventricular layer, (3) monitor the proliferative activity of the segment of ventricular layer from which isocortical neurons arise and (4) assess the growth of the ventricular and intermediate layers before, during and after the establishment of the cortical plate. The degree of correlation between these changes and the final distributions of successive generations of neurons as established by autoradiography in the first paper of this series (Smart & Smart, 1982) was then assessed.

Objectives one, three and four were investigated by making observations and various histometric measurements in an extensive library of serial sections of embryonic and postnatal mouse brains. The second objective was approached by means of a study of Golgi impregnated material in which periventricular cells and their processes had been preferentially stained. We utilised the procedure of Nieuwenhuys (1972) in which the processes of these cells are used as a 'natural' system of co-ordinates along which neuron populations have moved from their sites of origin in the periventricular region to their destinations at the periphery. Thus, by establishing the course run by the processes of periventricular cells, neuron populations may be referred back to the part of the stem cell compartment from which they originated. Using this procedure to correlate isocortical neurons with their site of origin, the concept of a radial unit of cortex, as utilised in the previous investigation (Smart & Smart, 1982), was extended to include the precursor cells associated with its production. This proved a dynamic analytic unit for use in working out the histogenetic sequences leading to the adult distributions. The paper concludes with a consideration of some of the implications arising from this approach.

TERMINOLOGY

In previous papers (Smart, 1972*a*, *b*, 1976) the terminology used for the layers of the neural epithelium differed from that recommended by the Boulder Committee (1970). In the present paper, for the sake of uniformity, the Boulder Committee

terminology has been adopted. We preface the present account of the changing structure of the lateral telencephalic wall by recapitulating the Boulder Committee terminology in order to add to the original static definitions our understanding of what is implied by these terms in relation to the dynamics of a growing tissue.

Initially, the wall of the telencephalic vesicle is formed by a *pseudostratified columnar epithelium*, a type of tissue organisation in which the majority of cells have cytoplasmic processes extending from the basement membrane to the apical network of terminal bars. In the neural epithelium, nuclei lie towards the apical or ventricular pole of the cell and this polarity is used to subdivide the early neural epithelium into a *ventricular layer* formed by pseudostratified nuclei and a *marginal layer* formed by the basal processes of the same cells (Fig. 1*a*). These two primary layers are not separate entities but different parts of the same cells. In our usage, *ventricular layer* refers to the location of the nuclei and *ventricular cell* to the complete cell including its processes. Ventricular cells initially form the primary precursor pool for other cell types. Increase in ventricular cell number results in an increase in the area of the neural tube wall and/or an increase in the degree of its pseudostratification. Conversely, regions of the neural tube undergoing such increases are in the process of enlarging their precursor pools. The size of the precursor pool prior to the commencement of major differentiation is an important antecedent factor in determining the number of neurons eventually produced (Smart, 1972*a*). It is, therefore, important in assessing the proliferative capacity of a segment of the neural tube to determine the size of the precursor pool and how it has been accommodated within the context of an epithelial organization by pseudostratification or area increase. Under progressive pseudostratification, ventricular cells may attain an almost 'hypercolumnar' form and yet retain the columnar cell characteristic of *interkinetic nuclear migration* (Sauer, 1935, 1936; Sauer & Walker, 1959). That is during the G2 phase of the cell cycle the nucleus migrates to the cell apex where cleavage occurs in a plane at right angles to the ventricular surface, the two daughter nuclei returning thereafter to the outer aspect of the ventricular layer. If the degree of pseudostratification increases or the ventricular cell cycle time decreases beyond a certain value, congestion of the system occurs due to too many mitotic figures competing for space at the ventricular surface (Smart, 1965, 1972*a*). The presence of *subsurface prophases* immediately deep to a ventricular surface occupied by mitotic figures is an indication that the degree of pseudostratification is not compatible with the prevailing cycle time of the ventricular cells. '*Ventricular choke*' of this nature may not occur if increasing cell numbers are accommodated by an appropriate increase in area of the ventricular layer. However, in this dimension, too, the prevailing geometry eventually imposes an upper limit to the permissible ballooning of the ventricle or to the infolding of its surface.

At some stage in development, ventricular cells differentiate into immature neurons and neuroglia and are released from the ventricular layer to form an *intermediate layer* (Fig. 1*b*). As the former cells have lost the power of further cell division the term neuroblast is inappropriate; we, therefore, use the circumlocution 'immature neuron', or Rickmann, Chronwall & Wolff's (1977) term 'preneuron', to denote young neurons which have yet to establish themselves in a permanent site. The advent of such cells transforms the neural epithelium into a 'compound epithelium' comprising a pseudostratified columnar element of ventricular cells between whose basal processes a stratified element of neurons is accommodated (Fig. 1*b*). The columnar element persists in most areas only until neuron production has been

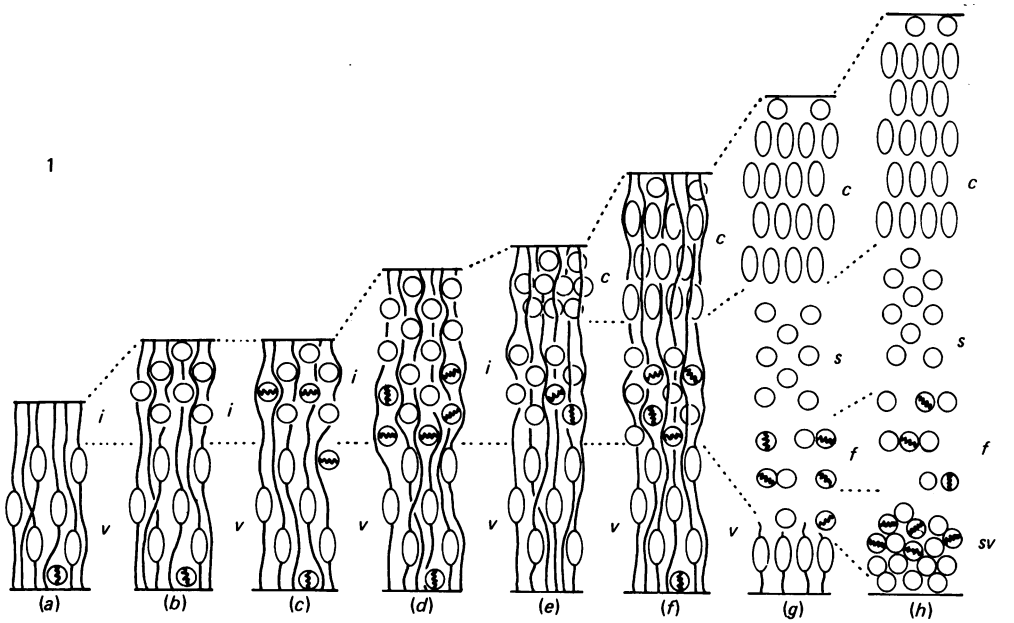


Fig. 1. Diagrams depicting main compartments of neural epithelium of isocorticogenic segment of lateral telencephalic wall. (*a*) Pseudostratified stage showing ventricular layer, *v*, and a mitotic figure indicated by divided circle at the ventricular surface. (*b*) 'Compound' epithelial stage showing pseudostratified ventricular cells and a 'stratified' element of preneurons among their basal processes forming the intermediate layer, *i*. (*c*) As (*b*) but illustrating non-surface mitotic figures among the preneurons. (*d*) As (*c*) but with increased population of intermediate layer cells. (*e*) Shows initial appearance of cortical plate, *c*, as an area of preneuron crowding. (*f*) As in (*e*) but with increased crowding of cortical plate nuclei producing radial elongation of nuclei. (*g*) Shows decline of ventricular cell processes and appearance of subplate, *s*, deep to which is a cell population, *f*, increasingly split up by cortico-petal and -fugal tracts which will be the future site of the corpus callosum. (*h*) As in (*g*) but depicting the appearance of a compact population of mitotically active cells, *sv*, deep to the diminished ventricular layer. This is the first appearance of a circumscribed population to which the name subventricular layer can be given.

completed, after which it declines (Fig. 1*g*). During the phase of neuron production, the basal processes of ventricular cells elongate progressively to accommodate immature neurons liberated from the ventricular layer (Fig. 1*b–f*). In areas where large numbers of neurons are produced the elongation may be substantial. It is the basis of Nieuwenhuys' (1972, 1977) hypothesis of a natural system of coordinates that neurons remain related to the basal processes among which they are released. Thus, when basal processes are deflected from their original radial orientation by differential growth the neurons they embrace are carried with them.

In our usage, it should be noted, the term ventricular cell refers to a morphological type and encompasses the entire columnar cell population. Initially, all such cells may be members of a homogeneous precursor pool concerned with reproducing their kind, but subsequent differentiation inevitably produces a widening penumbra to the original definition. The advent of the intermediate layer, for example, indicates that some ventricular cells have turned over to the production of neurons and neuroglia destined for the stratified element. There are as yet no nuclear criteria for identifying ventricular cells concerned with generating specific cell lineages. However, evidence exists for two ventricular cell populations proliferating at different rates (Waechter & Jaensch, 1972). Morphologically, also, there is ultrastructural and histochemical

evidence of a separate population of guide glia (Rakic, 1972; Levitt & Rakic, 1980) among the columnar element which provide the physical basis for a natural system of coordinates.

In terms of cell dynamics, the appearance of a separate intermediate layer and of specialised cells within the columnar element are signs that cells are being lost from the precursor pool. From observation it can be confirmed that differentiation, once commenced, leads in most areas to the release of increasing numbers of neurons and, therefore, eventually to extinction of the primary precursor pool. The rate of passage through this sequence can be followed by measurements of the changing dimensions of the ventricular cell compartment and the related segment of intermediate layer. The pattern of neuron release from the ventricular layer and, therefore, the way the intermediate layer becomes manifest, also show regional differences. A common pattern, for example, is for neuron release to commence at the ventral and to spread to the dorsal aspect of the neural tube, for example, from basal to alar plates as in the spinal cord. The identification of the pattern of neuron release is an additional important datum in assessing the status of histogenesis of an area.

Initially, the immature neurons of the intermediate layer are located next to their site of origin. Between this state and the regional diversifications of the adult, immature neurons undergo a sequence of intermediate distributions peculiar to their area and consequently requiring their own terminology. In the isocortical segment of the telencephalic wall, a conspicuous, though transient, feature is the appearance of a lamina of more closely opposed nuclei in the outer part of the intermediate layer. This is referred to as the *cortical plate* (Fig. 1*e–h*). It is a cell population whose differential growth and decay provides another useful quantitative guide to events in the transition of intermediate layer to adult cortex.

The Boulder Committee used the term 'medial and lateral ganglionic eminences' for the two convexities of the lateral wall of the lateral ventricle which are conspicuous during early telencephalic development. Because the term 'basal ganglia' for the adult striatum is now obsolete, we have adopted instead Lammers' (1976) term *ventricular elevations* for these Lammers' features. It is important to stress that elevations are not the primordia of any adult structure but represent transient localised enlargements of the precursor pool resulting from a localised increase in ventricular layer area which encompasses a burgeoning and eventually dominant subventricular layer (Smart, 1976). The term *caudatopallial angle* denotes the flexure of the ventricular wall marking the dorsal extremity of the lateral ventricular elevation and is retained for embryological use although, strictly speaking, it is only applicable after the lateral ventricular elevation has declined and been replaced by caudato-putamen neurons. In the term *subventricular layer*, the Boulder Committee recognised the presence of precursor cells which do not migrate to the ventricular surface to undergo mitosis. The phenomenon of *non-surface mitosis* may happen anywhere in the ventricular layer as a sporadic event but in certain regions it represents a major modification of the cell production mechanism resulting in the formation of a proliferative compartment freed from the constraints imposed on the ventricular layer by the various geometrical traps which have been described above. Secondary precursor cell populations which do not undergo interkinetic nuclear migration evolve in different ways in different parts of the neural tube. The status of these populations can be assessed for each area by determining their mode of origin, proliferative capacity and whether or not they are associated with a decline in the ventricular layer or are spatially separated from it. The term subventricular layer, if

not subject to further qualification, is usually taken to refer to populations of mitotic cells developing between the ventricular and intermediate layers in various areas of the telencephalon. It is part of the purpose of this paper to describe the mode of origin of such a population in relation to the isocorticogenic part of the ventricular layer.

We would like to recognize an additional site of cell division in the term *dispersed mitotic compartment*. This refers to the development of precursor cells, mostly of the neuroglia series, which are not restricted to the periventricular region or other circumscribed areas but are found singly or in small groups throughout the fibre tracts and grey matter of the telencephalic wall.

The operational criteria for identifying the Boulder Committee layers in routine haematoxylin and eosin-stained sections are based on the shape, orientation and pattern of distribution of nuclei since intranuclear detail is insufficiently differentiated and cytoplasmic characteristics are, for the most part, poorly revealed. The nuclei of the ventricular layer lie close to the ventricle, are elongated in proportion to the degree of pseudostratification and are usually orientated with their long axes at right angles to the ventricular surface. It is assumed that these nuclei belong to cells with processes extending to each surface of the epithelium, as depicted in successful Golgi impregnated material or by the scanning electron microscope.

Nuclei of the intermediate layer are rounded or irregular in shape but, as in the cortical plate, can become elongated in proportion to the degree of crowding. Intranuclear detail does not at first differ greatly from that of ventricular nuclei. The majority of the early-formed nuclei of the intermediate layer and, later, those in the cortical plate, we assume to belong to immature neurons, although some belong to members of the neuroglia series even at the very early stages of intermediate layer history (Rickmann *et al.* 1977). Later, the intermediate layer harbours a heterogeneous collection of nuclear types reflecting the move towards adult complexity.

As the Boulder Committee point out in their preamble, terminologies tend to become obsolete and to require re-definition as increasing knowledge renders established definitions inadequate. The amplification we seek to give relates the standard terms to cell populations proliferating and differentiating within the constraints of an epithelial format. From this standpoint we view neural histogenesis and phylogenesis as processes in which different parts of the neural tube produce different local compromises to the prevailing epithelial geometry. With respect to the isocortical segment of the ventricular and intermediate layers, the spatial geometry remains relatively simple, consisting of preneurons being fed into the intermediate layer where they accumulate between the parallel processes of the ventricular cells. The presence of parallel processes introduces the concept of 'columns' and this can lead to confusion with the same term in another branch of neurohistology. Functional columns are adult structures consisting of radially arranged neuron assemblies synapsing with each other along the length of the column but not with neurons of adjacent columns (Mountcastle, 1957; Hubel & Wiesel, 1962). *Developmental columns* are the supposed precursors of adult columns, composed of immature neurons in the cortical plate and subplate. There is some indirect evidence to suggest that developmental columns may exist as entities within the intermediate layer (Todd & Smart, 1982). Finally, the local use of the column concept in this and the previous paper (Smart & Smart, 1982) occurs in the term *radial unit* which refers to a radially orientated strip of cortex of some conveniently small breadth to serve as a sample of cortex at a particular location. In the present paper, the concept is extended

to include the ventricular and subventricular cells associated with each unit of cortex during the period of neuron production. Radial units would consist of many adult functional columns or embryonic developmental columns.

MATERIALS AND METHODS

General histological material

The study utilized a series of mouse brains taken at daily intervals from E10 to E19 and at frequent postnatal intervals. The brains were fixed by immersion in specimens killed prior to E13 and, in older specimens, by intracardiac perfusion with Bouin's solution. They were embedded in paraffin wax, sectioned at 6 μm in the coronal plane, and stained with haematoxylin and eosin. The sets used had been selected as the best and most similarly orientated specimens from a large library of serially sectioned brains which had been accumulated over the years. Although nominally taken at daily intervals of gestation, the variations in development between litters were sufficiently great, particularly in the early stages, to provide sets of slides covering the intermediate steps in the histogenesis of most telencephalic structures.

Histological descriptions

Detailed histological descriptions are reported for a coronal plane passing through the interventricular foramen. This is slightly caudal to the rostral commissure used in the antecedent autoradiographic study (Smart & Smart, 1982) which this histological investigation is designed to amplify but, because no anterior commissure is present until about E15 and little of the telencephalon exists rostral to the interventricular foramen until after E13, it was decided to use the level of the foramen as the rostrocaudal marker for this study.

Although only one coronal level in one set of sections had been chosen for description, the changes at this level have been interpreted against a knowledge of the events occurring at other levels and in other brain specimens. A rostrocaudal panorama was obtained from a comprehensive series of atlases and three dimensional models. The former consisted of photographs of every twentieth section of each set of serial sections from E11 to E19, printed at an enlargement of $\times 100$, from which the diagrams in Figure 2 were traced; the latter consisted of a matching set of polystyrene or plywood reconstructions. A set of reconstructed casts of the lateral ventricles provided a particularly useful surface for plotting the boundaries of areas concerned with the histogenesis of different populations of forebrain neurons (Fig. 14).

Mitotic counts

The location of mitotic figures at the ventricular surface was recorded by marking their positions on a drawing made through a drawing tube at a magnification of $\times 500$. Locations of mitotic figures were recorded in 10 alternate serial sections at the level of the interventricular foramen at each day from E12 to E16, inclusive. The extent of ventricular surface covered ran from the middle of the area of maximum curvature of the ventricular roof to the caudatopallial angle. The stages of mitosis recorded were restricted to the late prophase (i.e. thread stage and later), metaphase, anaphase, and early telophase (i.e. before obvious separation into two nuclei) only were recorded. The limitation to the recognition of prophase and telophase was

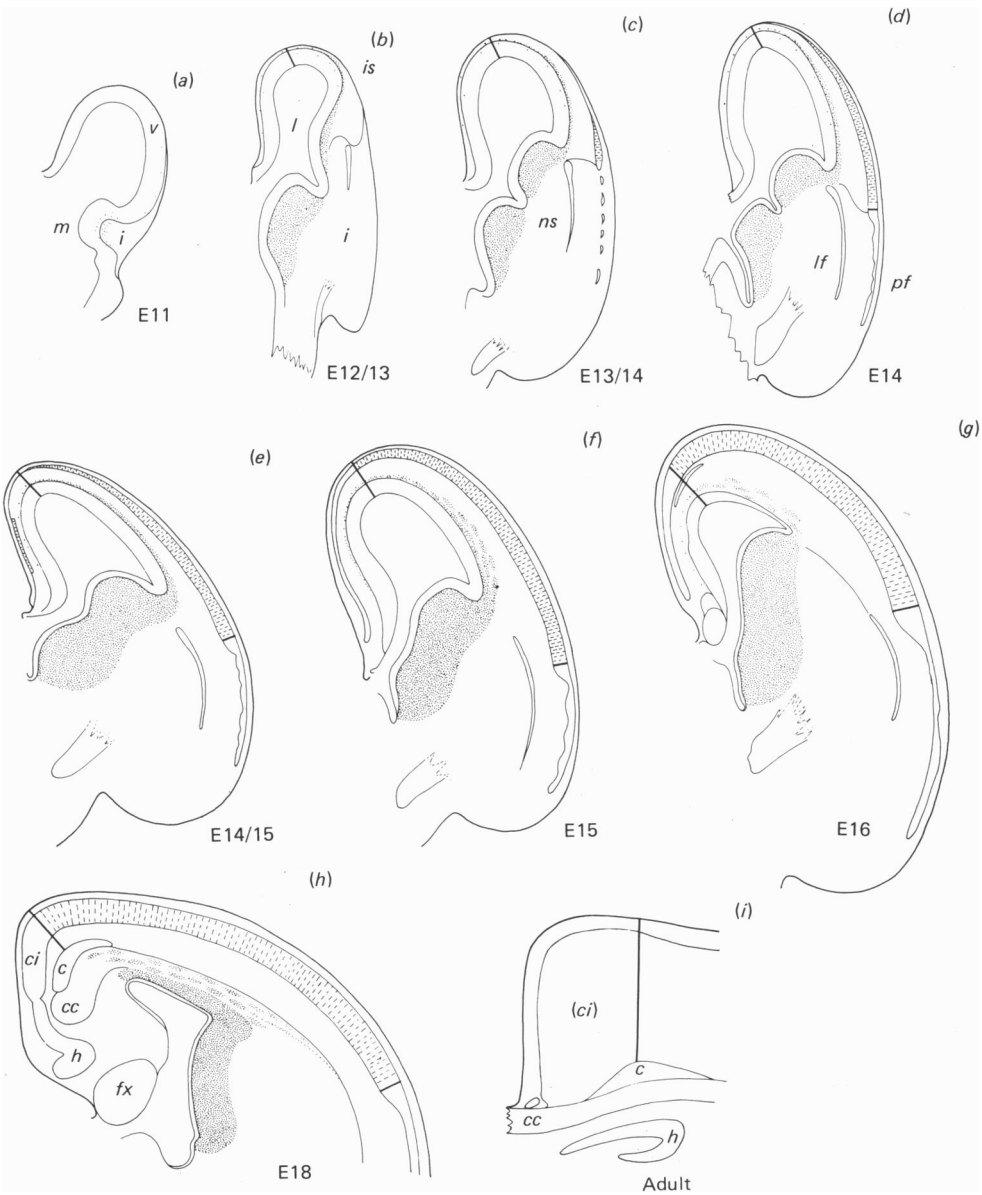


Fig. 2. Drawings of coronal sections through developing telencephalon at level of interventricular foramen at stated ages. Features are, for the most part, labelled at their first appearance only. *v*, ventricular layer; *i*, intermediate layer; *m*, medial ventricular elevation; *l*, lateral elevation; *lf*, linear feature described in text; *is*, initial site of isocortex; *pf*, pyriform cortex anlage; *ns*, neostriatum; *c*, cingulum; *cc*, corpus callosum; *ci*, cingulate cortex; *fx*, fornix; *h*, hippocampus. Subventricular layer is indicated by stippling and the isocortical part of the cortical plate by cross hatching.

imposed because, with experience, these stages can be detected progressively earlier and later, respectively, in their development so that it is difficult to maintain the boundary criteria for them if all recognisable figures are included.

The ventricular outline in the drawings was then marked off in centimetre divisions, starting at the caudatopallial angle. The number of mitotic figures at the

ventricular surface adjacent to each centimetre division was counted and expressed as a histogram (Fig. 15).

Estimating change in compartment size

Considerable differential growth takes place in the telencephalic wall during development so that it is difficult to measure comparable segments of the wall at successive developmental stages. The difficulty was resolved in two ways:

(1) It was known from a parallel Golgi study that in an area about two thirds of the way from the caudatopallial angle to the medial extremity of the ventricular roof the ventricular cell processes run directly to the pial surface, i.e. they show neither dorsal nor ventral deflections (Figs. 10–13). This area could be localised at different stages of development and provided a site where the intermediate layer and, eventually, the cortical plate lay opposite the putative site of origin of their component cells. Drawings were made at this area through the entire thickness of the telencephalic wall. This was done by following a strip of tissue (designated Site 1 in Figs. 10–13) under high power magnification and, using a drawing tube, marking the outlines of cell nuclei lying in one plane of focus.

(2) The ventral boundary of the isocortical plate (as defined in the next section of this paper) was distinguishable as the place where the plate tapered from deep to superficial (Figs. 11–13). The segments of the cortical plate immediately dorsal to this taper provided an area readily identifiable at each age and was designated Site 2. Drawings were made here, as at Site 1, of nuclear perimeters showing sharply on one plane of focus in a strip of cortical plate and in the immediate subplate area.

By restricting the drawings to the plane of sharp focus, a manageable sample of nuclei was extracted from the crowded superimposed nuclear profiles present in the 6 μm sections.

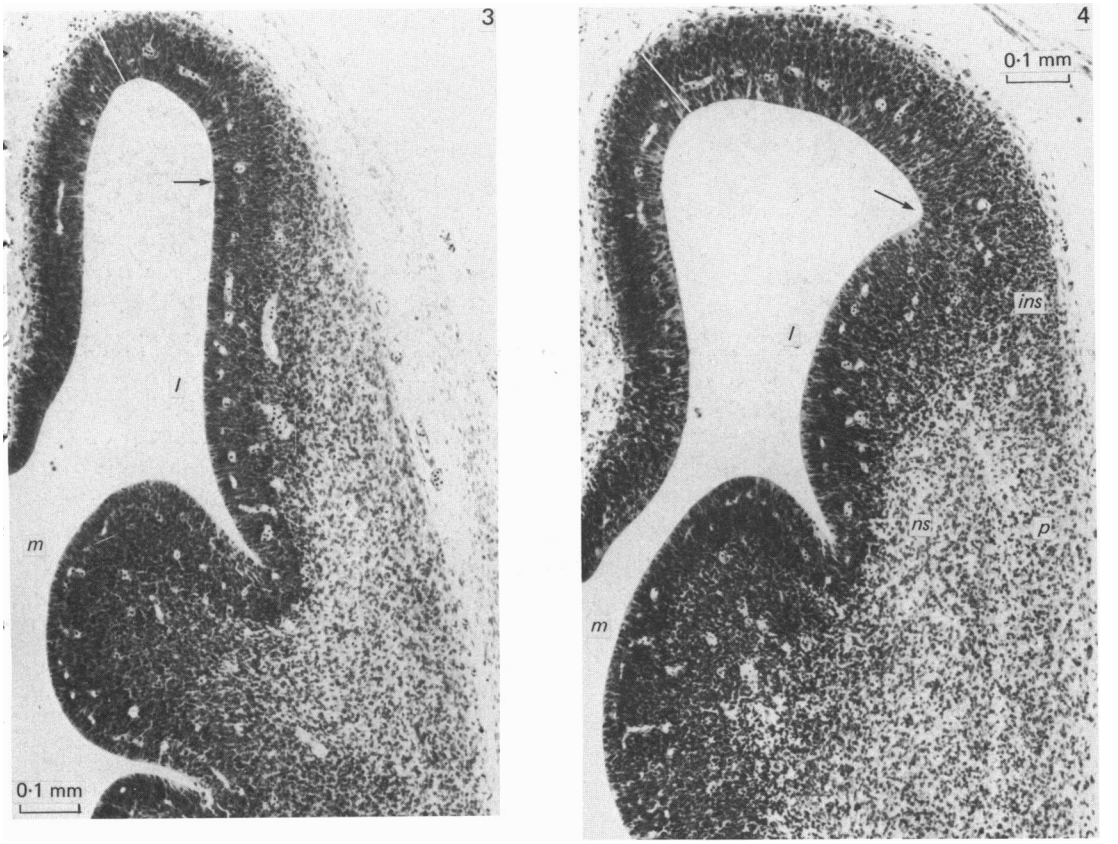
The apparent thickness of the layers may be affected by the changing curvature of the brain surface at the chosen level during growth and/or by chance variations in the plane of section. Both are unavoidable and make it difficult to claim significance for minor variations or for an apparent constant depth. Assertions are, therefore, made only about relatively major changes.

Golgi material

A large collection of mouse brains, taken at daily intervals from E11 to E19 and at frequent postnatal intervals, was treated by the rapid Golgi method as described by Stensaas (1967). The brains were cut in the coronal plane at thicknesses varying from 40–80 μm. The sections were searched for examples of ventricular or subventricular cells with processes extending towards the periphery. The outlines of sections with this feature were drawn with the aid of a drawing tube and the location of the silver-impregnated cells and processes drawn in. The accumulated drawings were then used to reconstruct the arrangement of this system of processes at the coronal level of the interventricular foramen and, in particular, to define the origin and course of those periventricular cell processes associated with the isocortical neuron population (Fig. 14).

FINDINGS

The findings are presented in the same sequence as the four main headings listed in the Introduction. The isocortical boundaries were first defined, the segment of the ventricular layer sending processes to the isocortical population was then determined,



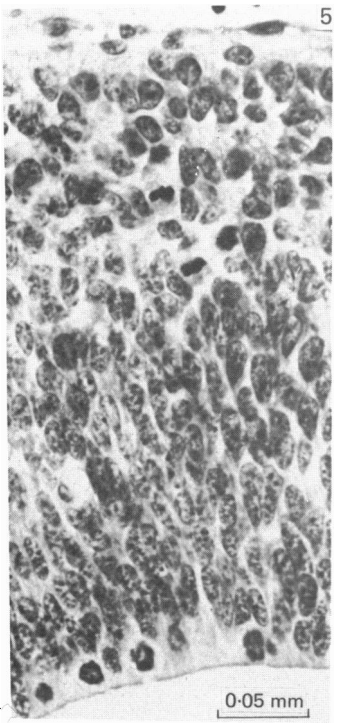
Figs. 3–4. Coronal sections through mouse brains at E12 and E13, respectively, taken at level of interventricular foramen. The line indicates the location of the supposed dorsal boundary of the isocorticogenic system at this age. *Ins*, insular cortex area; *p*, pyriform cortex area; *ns*, neostriatum. 'Linear feature' referred to in text intervenes between *ns* and *p*; *m* and *l*, medial and lateral ventricular elevations; arrows indicate caudatopallial angle in Fig. 4 and its supposed future site in Fig. 3. All sections in this and other photomicrographs are 6 μm thick and stained with haematoxylin and eosin.

its proliferative activity was assessed and finally the growth of the ventricular and intermediate layers was estimated.

(1) *Development of isocortical boundaries*

(*a*) *Prior to appearance of cortical plate*

The intermediate layer appeared at E11 as a thin layer of rounded nuclei located subpially in the lateral ventricular wall which, by E12, had extended over the ventricular roof and medial wall as far as the interventricular foramen (Fig. 2*a–b*). The layer was composed of a population of more or less evenly dispersed, relatively uncrowded nuclei, most numerous ventrally and decreasing dorsally (Fig. 3). By E13 a general increase in depth of the intermediate layer had also occurred, within which an area of more closely opposed nuclei had appeared opposite the dorsal half of the lateral ventricular elevation. The area was wedge-shaped with its base curving from the lateral ventricular elevation to the pial surface (Figs. 2*b*, 4). Those nuclei forming the dorsal part of the wedge were considered to belong to presumptive isocortical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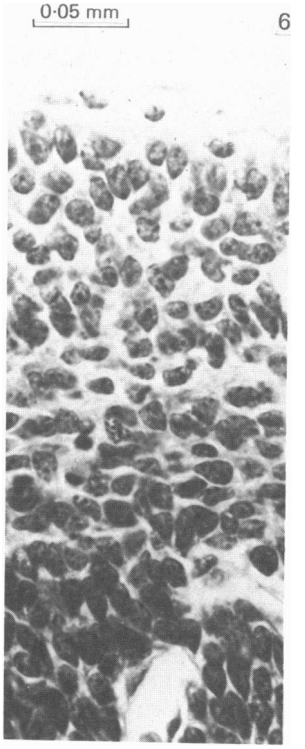
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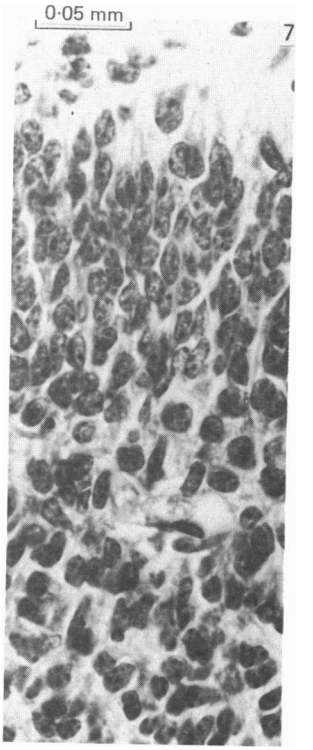
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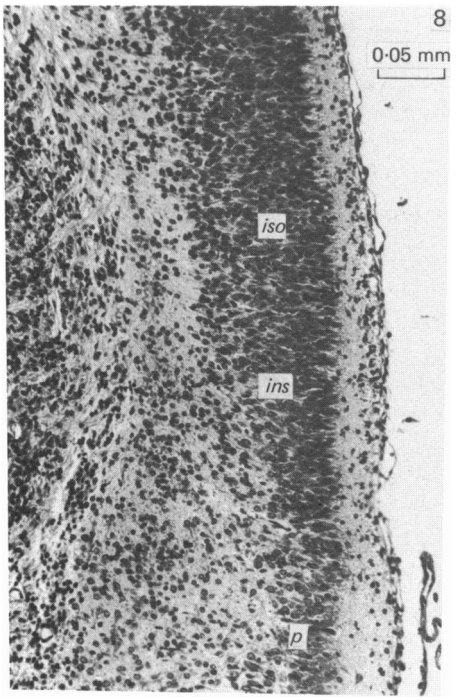
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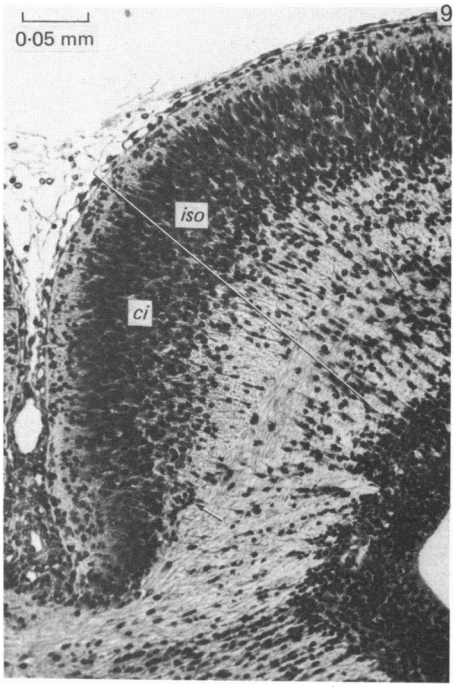
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preneurons (Fig. 4). The cell population at the base of the wedge was considered to be destined for the future insular cortex. Ventral to the base of the wedge of closely opposed nuclei, a useful landmark appeared in a line of looser tissue following a ventral course (Figs. 2*b*, 4). This linear feature separated the neostriatal anlage from the overlying presumptive claustrum/pyriform area (Fig. 4). The line probably represented an interface between differently organized types of tissue which had been artefactually exaggerated by sectioning. It persisted throughout development and marked the plane followed by a set of fibres and cells radiating from the caudato-pallial angle round the circumference of the striatum. This plane was also followed by developing axons emanating from cortical neurons and eventually became occupied by the corpus callosum.

(*b*) *After appearance of cortical plate*

In the E13–E14 period, the isocortical plate appeared. It was first evident as an area of rounded, more closely opposed nuclei in the outer part of the intermediate layer, extending dorsally from the lateral part of the boundary area, where it was deepest, to taper rapidly to a single line of nuclei (Fig. 2*c*). Slightly later in the E13–E14 period, the line of more closely packed nuclei forming the plate extended as far as the ventricular roof, where it again disappeared as the nuclei assumed the same spacing as in the rest of the dorsal region (Figs. 2*d*, 5). As the leading edge of the plate advanced dorsally, its ventral part deepened as the number of its nuclear layers increased (Figs. 6, 7). The plate thus took up the same wedge-shaped profile as the surrounding intermediate layer. Ventrally, the isocortical plate was continuous with the presumptive pyriform plate (Figs. 2*c–h*, 8), which presented as a lamina of less closely packed, less elongated and more 'mature' looking nuclei, i.e. paler staining with finer, more dispersed chromatin. Under low power magnification, the difference in structure of the pyriform plate was apparent in its looser appearance and less sharply demarcated and more irregular deep boundary. In the later part of the E13–E14 period, nuclear accumulation had increased the number of layers in each plate. A narrow transition zone between the two was now evident; as the isocortical plate was traced ventrally, the elongated radially arranged nuclei decreased from deep to superficial and were replaced *pari passu* by rounder, more loosely packed nuclei

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Figs. 5–9. Photomicrographs of different locations in the same cortical plate showing progressive increase in its thickness from dorsomedial to inferolateral. Fig. 5. Section through intermediate and ventricular layers of E14 mouse telencephalon at its most dorsal part, showing the slight increase in nuclear crowding in the outer intermediate layer which is indicative of the incipient cortical plate. Also shown are three mitotic figures in the intermediate layer. Note the 'granular' appearance of the nuclei in the deeper ventricular layer compared to those in the outer part. This is due to the chromatin clumping characteristic of early prophase. The appearance is consistent with a proliferative system encountering an incompatibility between the cycle time of interkinetically migrating cells and the availability of space at the ventricular surface.

Fig. 6. Section through the intermediate and the outer part of the ventricular layers at a more ventrolateral location than Fig. 5. Cortical plate crowding is more obvious. Also note presence of a nucleus in parallel-cleaving telophase in the deep intermediate layer.

Fig. 7. Section through the intermediate layer towards the ventral boundary of the cortical plate which is now composed of several layers of crowded and elongated nuclei.

Fig. 8. Ventrolateral part of cortical plate of E18 mouse. Line indicates boundary region between isocortex (*iso*) and insular cortex (*ins*).

Fig. 9. Dorsomedial part of cortical plate of E18 mouse. Arrows indicate extent of fibres of cingulum, line indicates the boundary region between isocortex (*iso*) and cingulate cortex (*ci*).

(Fig. 8). This transition area was tentatively identified as the presumptive insular cortex, and the ventral isocortical boundary was fixed at the point where the reduction in radially arranged nuclei commenced (Figs. 2*d–h*, 8).

The dorsal boundary of the isocortical plate was defined by reference to the cingulum, the landmark used for this purpose in the adult (Smart & Smart, 1982). The adult cingulum is a rostrocaudal running tract of fibres, triangular in cross section, lying on the dorsal surface of the corpus callosum (Fig. 2*i*). A line from its apex to the dorsal surface of the brain coincides (in the rostral forebrain, at least) with the most medial extension of the conspicuous isocortical layer V (see illustration in Fig. 1 of Smart & Smart, 1977). Prenatally, the cingulum can first be distinguished about E16 as a thin band of cross sectioned fibres lying immediately deep to the cortical plate. The band spans the region of maximum curvature of the dorsal telencephalon (Fig. 2*g*) where it is traversed by the processes of ventricular cells and migrating neuroblasts. The fibre bundles of the cingulum are thus fixed in relation to a set of radial processes emanating from a specific site in the ventricular layer to a corresponding site in the cortical plate and so provide a marker in this debatable land. A line running directly from the middle of the cingulum to the pial surface along the course of the ventricular cell processes was, therefore, used from E16 onwards to demarcate the isocingulate transition zone (Figs. 2*g*, 2*h*, 9). Prior to E16, the boundary was taken to lie on a similarly orientated line commencing at the middle of the area of maximum curvature of the ventricular roof (Fig. 2*b–f*).

(2) *Demarcation of the isocortical segment of ventricular layers*

(*a*) *Dorsomedial boundary*

The locations of the ventricular cell populations sending processes to each subdivision of the cortical plate were determined by reference to Golgi impregnated material. The description of these connections is most conveniently started at the E15–E16 period, by which time positive criteria for demarcating both boundaries of the cortical plate had developed. At E16, when the cingulum was first evident, the ventricular cell processes passing through its mid-region emanated from cells at the middle of the area of greatest curvature of the ventricular surface, that is from the area where the ventricular roof inclined ventrally to become the medial wall. This line, therefore, was taken to mark the dorsomedial boundary of the isocortical segment of the ventricular layer (Fig. 2*g*). Prior to E16, the middle of the area of greatest curvature of the roof was assumed to correspond to the future site of the middle of the cingulum, and ventricular cells sending fibres through this area were considered to lie at the dorsomedial boundary of the isocorticogenic segment of the ventricular layer.

(*b*) *Ventrolateral boundary*

The ventrolateral boundary of the isocorticogenic area was taken to lie at the caudatopallial angle because processes from ventricular cells adjacent to the angle on its dorsal limb ran to the ventral region of the cortical plate (Figs. 14–16), whereas fibres emanating from cells at the ventral limb of the angle (and, therefore, topographically within the dorsal ventricular elevation) ran to the area of the anlage of the insular cortex. Within these boundaries, ventricular cell processes radiated through the preneuron populations of the isocortical arc (Figs. 14–16) until E15. By E16, however, an important change had occurred in the arrangement of the ventricular

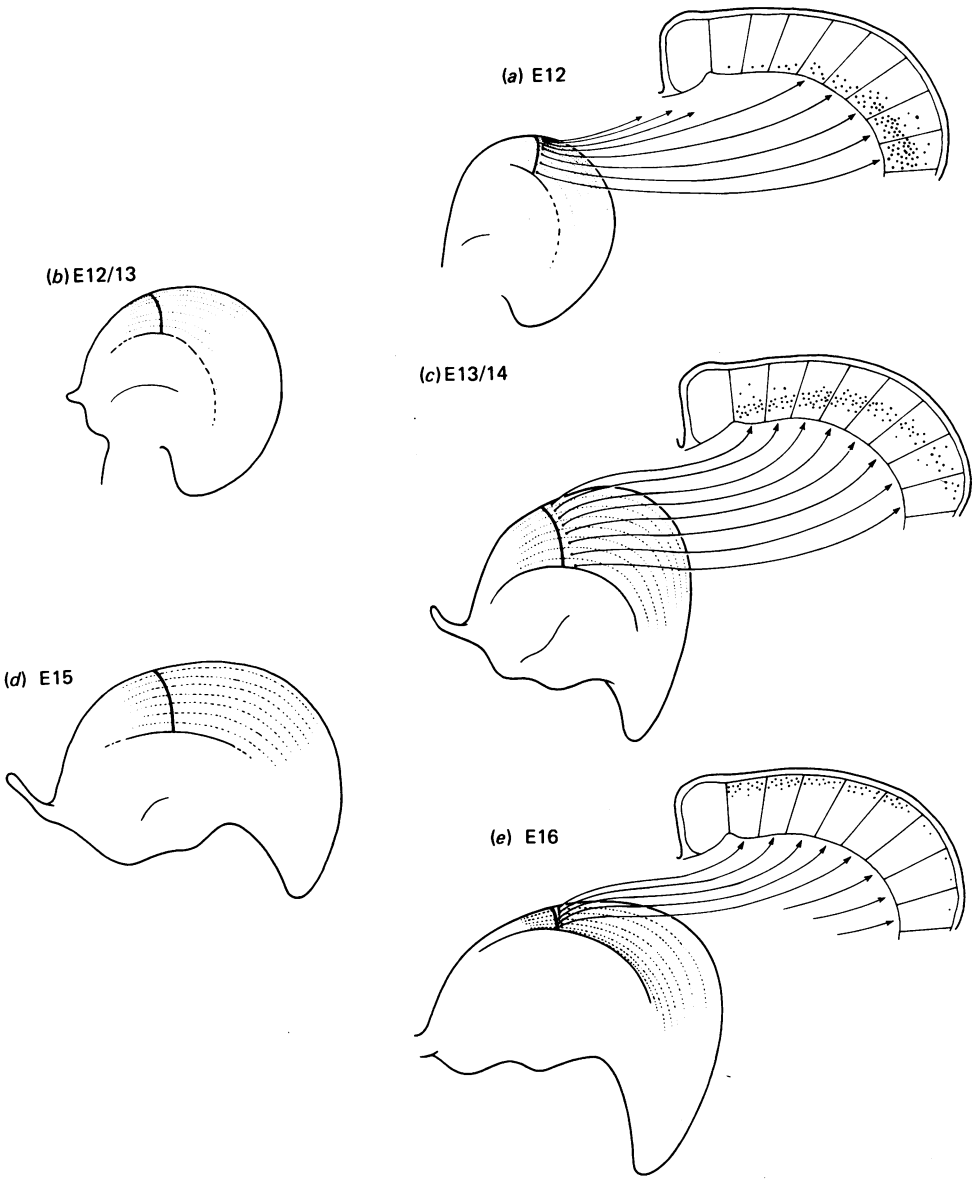

Fig. 10(*a–e*). Drawings of solid 'casts' of lumen of left lateral ventricle of prenatal mice brains at the stated ages. Casts were reconstructed in plywood from serial sections and are viewed from their lateral aspect. The upper part of each cast indicated by the peaked lines is related to the isocorticogenic surface. The line running in the coronal plane across the rostral part of the isocorticogenic surface marks the coronal level of the sections used in this investigation. In Fig. 10(*a*), (*c*) and (*e*) an attempt is made to correlate the sites of production of isocortical neurons with a diagram depicting their final location in the adult, as determined autoradiographically in the first paper of this series (Smart & Smart, 1982).

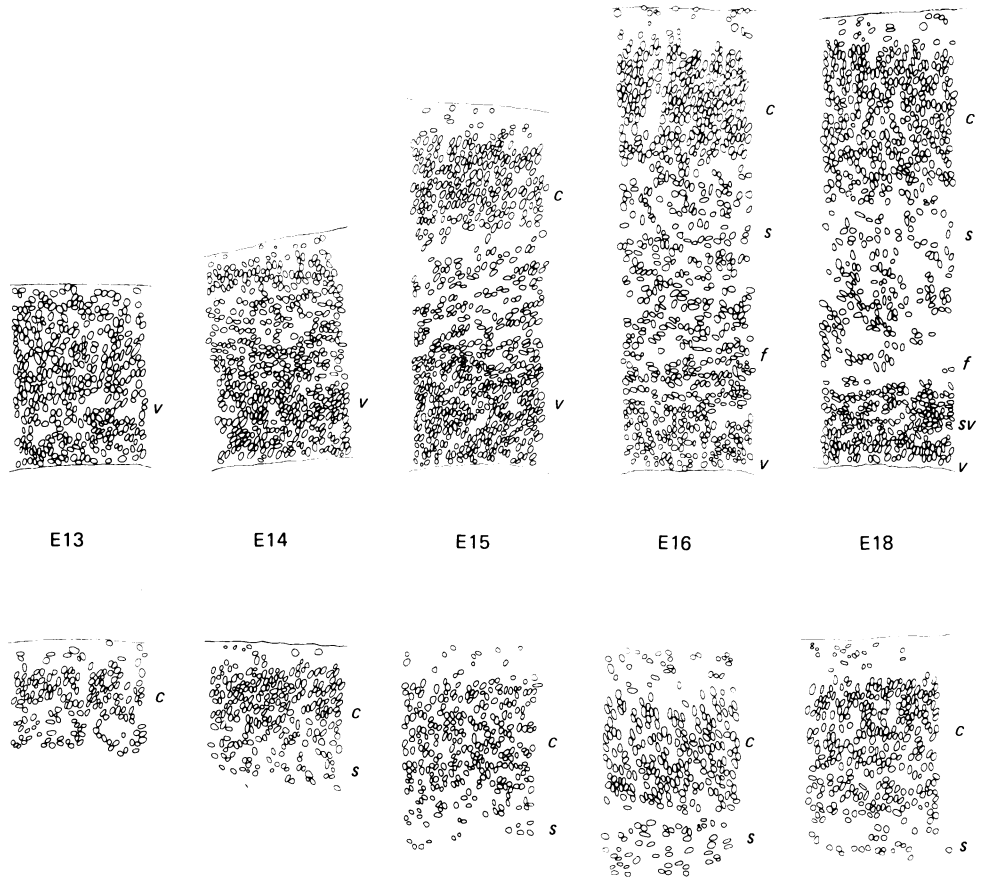

Fig. 11. Samples of nuclear populations from Site 1 (top row) and Site 2 (bottom row) at stated embryonic ages. Abbreviations as in Fig. 1.

cells adjacent to the dorsal limb of the caudatopallial angle. Until E15, the bodies of these cells were obliquely orientated with respect to the ventricular surface (Fig. 16) but, at E16 and subsequently, they stood at right angles to the ventricular surface and their processes radiated to the middle of the arc of the cortical plate instead of to its ventrolateral boundary (Fig. 17).

(3) *Changes in the isocortical segment of the ventricular layer*

(*a*) *Change in form and area of the ventricular surface*

The cerebral vesicle appeared, at E10, as an outpocketing of the neural tube wall produced by localized increase in ventricular cell number. By E11, continuing ventricular cell proliferation had led to ballooning of the vesicle, as in Figure 2*a*. Between E11 and E12, further increase in area of the vesicle occurred mainly in the form of two incurvings of the inner surface of its lateral wall, the first (the medial ventricular elevation) appearing at E11 (Fig. 2*a*) and the second (the lateral ventricular elevation) at E12 (Fig. 2*b*). Growth of the lateral elevation led to the appearance of the caudatopallial angle, the marker for the medial extent of the isocorticogenic segment of the ventricular layer. At E12, the lateral elevation sloped gradually into

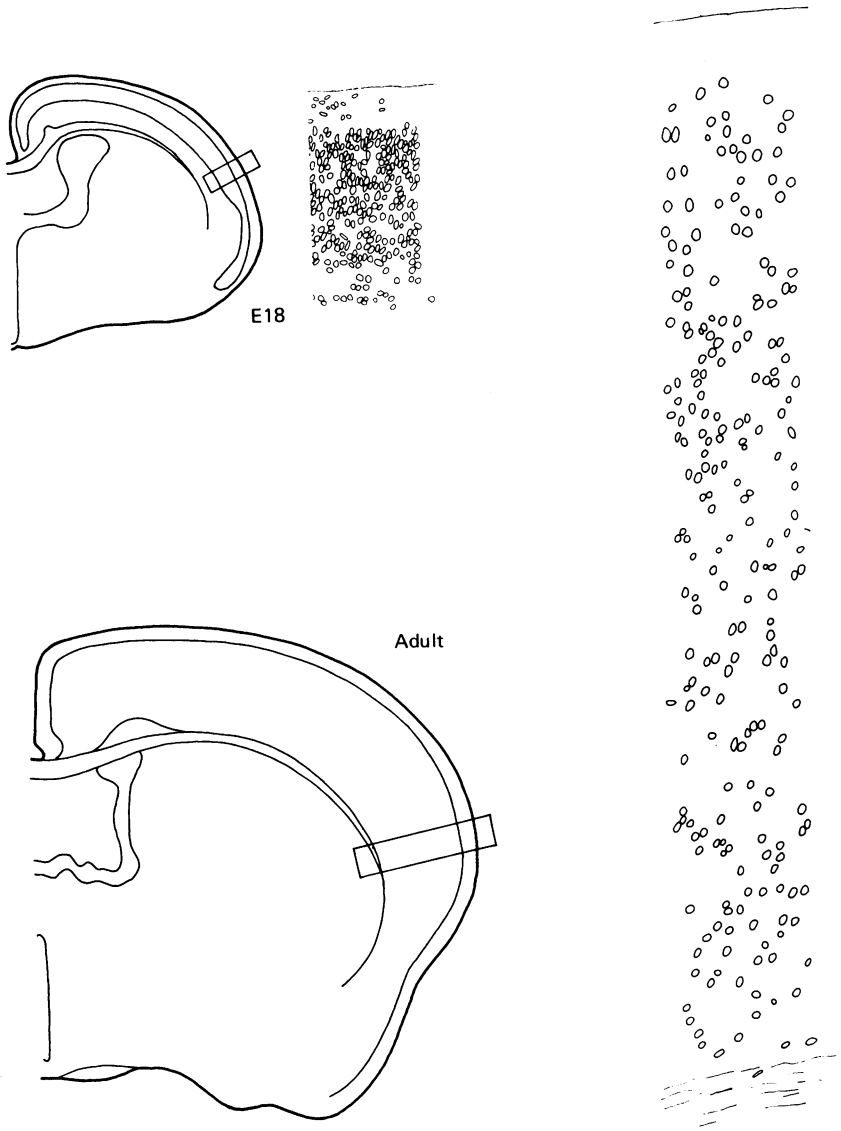

Fig. 12. Coronal sections through the anterior forebrain at E18 and in the adult are portrayed diagrammatically. Alongside each cross section is a sample of nuclei taken from Site 2, i.e. the area of cortex indicated by the box. Between E18 and the adult the depth of the area occupied by isocortical cells increases about four times and the length of the cortical arc approximately doubles.

the lateral wall, so that the site of the angle could not be precisely localised (Fig.3). At E13, the angle was evident (Fig. 4) and thereafter became progressively more acute as, under differential growth, the lateral wall of the ventricle flexed medially to become the *de facto* roof (Fig. 2*d–g*).

From inspection of Figure 2, the area of the ventricular surface associated with isocortical cell production can be seen to increase from E12 to E13–E14, to remain roughly the same between E14 and E15 and to decrease by E16. These changes, as seen in coronal sections at one level, were consistent with the three dimensional form of the ventricle, as illustrated by the drawings in Figure 10*a–e* portraying casts of the

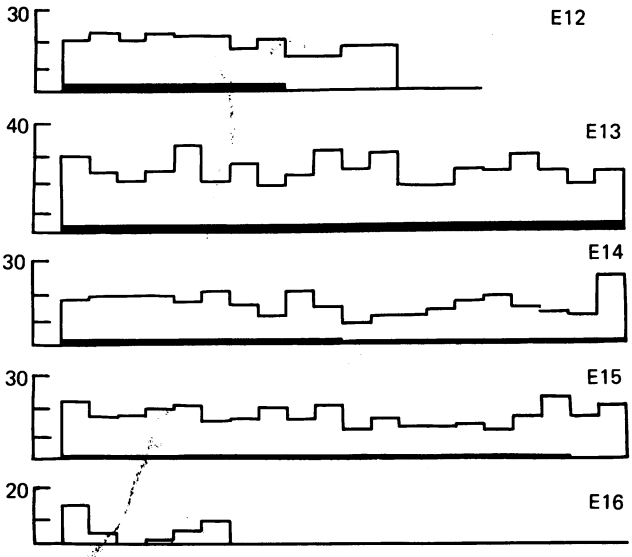

Fig. 13. Histograms showing the number of mitotic figures at equal intervals along the part of the lateral ventricular surface which gives rise to isocortical cells, as indicated in Fig. 2(*b–g*). The bar along the base of the columns is an estimate of the prevalence of subsurface prophases.

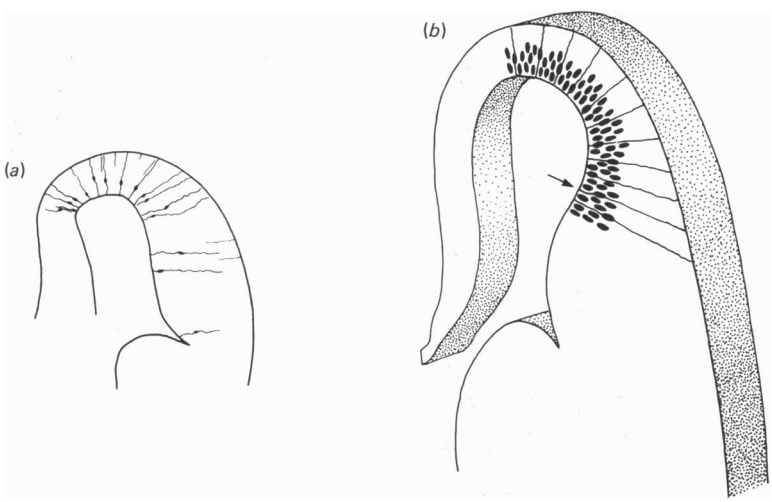

Fig. 14. (*a*) Drawing of ventricular cells and their processes in one Golgi section of E12 mouse brain cut in coronal plane at level of interventricular foramen. (*b*) Diagram synthesised from appearances in many Golgi sections showing ventricular cells associated with isocortical segment of lateral telencephalic wall. Arrow indicates probable location of future site of caudato-pallial angle.

lateral ventricles reconstructed from the same set of serial sections used to draw Figure 2*a*, *d*, *f* and *h*.

(*b*) *Ventricular layer mitosis*

Mitotic figures per unit area of the ventricular surface increased in number from E12 to E13, underwent a decrease from E14 to E15 and a further, more substantial decrease at E16 (Fig. 13). Subsurface prophases (Fig. 5) were present from E12 to

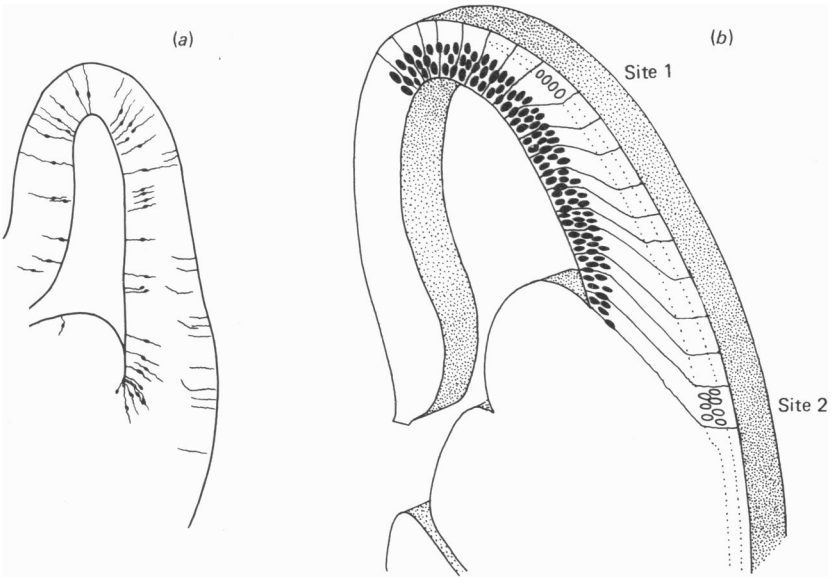

Fig. 15 (*a–b*). As in Fig. 14 (*a*) and (*b*), but representing status at E14. Sites of sampling of nuclear population in Fig. 11 are indicated.

E15, the estimated density decreasing after E13. At E16, no subsurface prophases were seen.

(*c*) *Subventricular layer*

Mitotic figures were present in the outer part of the ventricular layer and in the intermediate layer at E12 and E13 (Fig. 5). Where the latter was thin, dividing cells were seen, even subpially. As the cortical plate advanced dorsally through the intermediate layer, non-surface mitotic figures became localized to the area beneath the plate (Fig. 6). As the intermediate layer subsequently increased in depth from E14 to E16 and became infiltrated by circumferentially running fibres, mitotic figures were found to be more frequent in the area adjacent to the ventricular layer. After E16, a population of closely packed, mitotically active nuclei appeared over the ventricular roof between the declining ventricular layer and the deepest of the circumferentially running fibre bundles of the incipient corpus callosum (Fig. 2*g*, *h*). This population declined soon after birth.

(4) *Estimated changes in cell populations*

The cell populations of two sites of the lateral telencephalic wall were sampled by outlining the nuclear perimeters in one plane of focus. The first site traversed the entire thickness of the lateral wall at a point where the ventricular fibres followed a direct line from their origin to the pia mater (Figs. 15–17). The second site lay towards the ventrolateral boundary (Figs. 15–17) and included the cortical plate and subplate only. The resulting diagrams are reproduced in Figures 11 and 12.

(*a*) *Ventricular layer at Site* 1

The ventricular layer from E11 to E15 was formed by a population of crowded, elongated nuclei approximately 12 deep. By E16, the ventricular layer had decreased

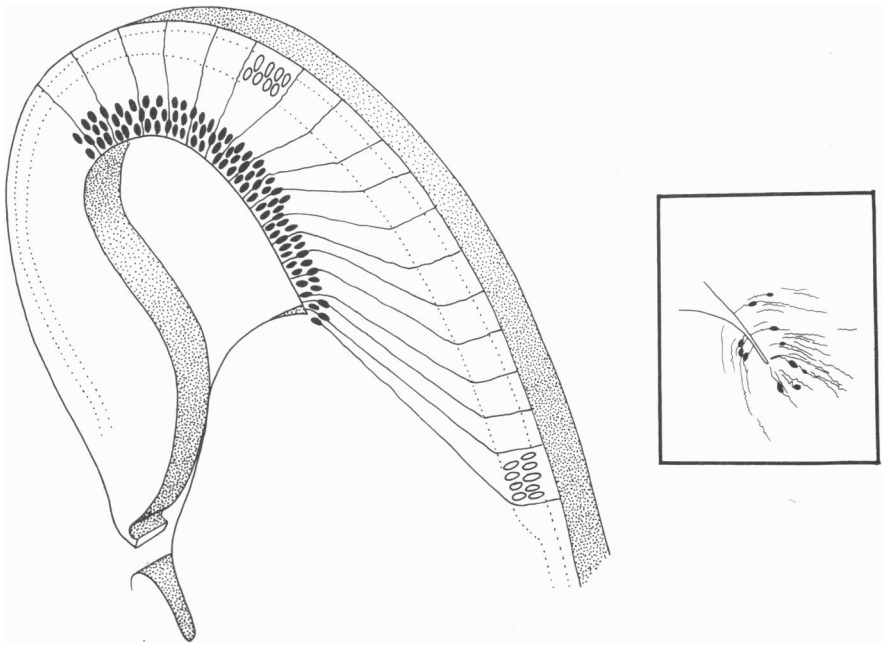

Fig. 16. As in Fig. 14(*b*). Inset shows drawings of Golgi impregnated cells at caudatopallial angle. Note obliquity of nuclei and emanating processes.

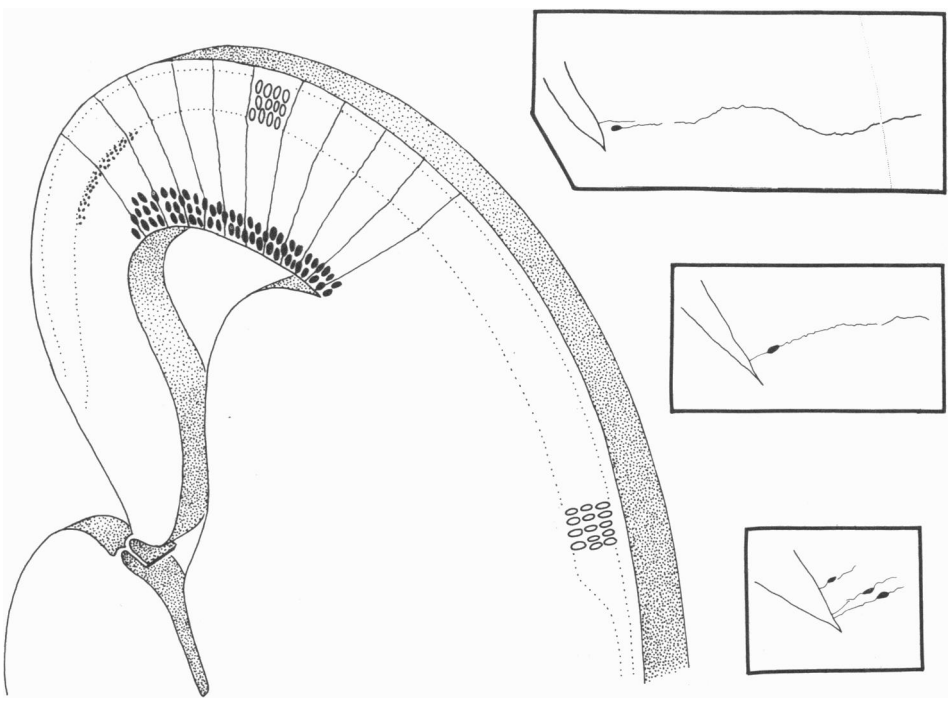

Fig. 17. As in Fig. 14(*b*). Insets show drawings of Golgi impregnated cells at caudatopallial angle. Note bodies of ventricular cells standing at right angles to the ventricular surface and their processes extending directly to cortical plate.

to about half its previous depth and, by E18, a further decline of similar magnitude had occurred (Fig. 11).

(*b*) *Intermediate layer at Site* 1

Prior to E13, there were a few rounded nuclei present between the pia mater and the ventricular layer boundary. The intermediate layer became more conspicuous at E13 (amounting to about half the depth of the ventricular layer) and, by E14, the two layers were of equal depth and the incipient cortical plate had appeared. By E15, a major increase in the depth of the intermediate layer had occurred and three main zones were apparent: a densely populated cortical plate, a more sparsely populated subplate and a deeper zone characterized by tangentially running fibre tracts running through a substantial population of nuclei.

(*c*) *Cortical plate at Site* 1

The plate was first evident here at the E14 period, as an area of more closely opposed nuclei two to three deep. By E15, a large increase in depth of the layer had occurred and was accompanied by nuclear crowding. After E15, the plate continued to increase in depth and cell number up to E18 (Fig. 11).

(*d*) *Cortical plate at Site* 2

In this location, the plate was first evident at the E13 to E14 period before it was apparent at Site 1. It underwent a considerable increase in depth and cell number between E14 and E15, and a lesser increase up to E16 (Fig. 11). In Figure 12, the nuclear density of this area of cortical plate is shown at E18 and in the adult, and the magnitude of the dispersal undergone by cortical plate nuclei to take up their adult locations is demonstrated.

DISCUSSION

The natural system of coordinates

The use of ventricular cell processes as a means of relating a peripheral neuron population to its putative site of origin from the periventricular germinal layer is an application of Nieuwenhuys' (1972, 1977) concept of a 'natural' system of co-ordinates. Using ventricular cell processes as one set of coordinates and proportional distances along them as another allows differential growth to be portrayed as the deformation of an originally simple coordinate net. The method has been used successfully in submammals where periventricular cells with processes running to the pial surface persist into adult life as conspicuous members of the neuroglia population which retain the deflections of their processes produced by differential growth. The converse procedure of reversing the sequence is also informative; this involves shrinking the adult coordinate system back to its original simplicity and is useful in determining the sites of origin of peripheral cell populations from the ventricular layer and their primal relationship to each other. The maps of the floor of the fourth ventricle in different species, produced by Nieuwenhuys, portray various brain stem structures projected back along their associated ependymoglia fibres to the ventricular surface. By this procedure, it was possible to compare neuron populations which had originated from homologous areas of the ventricular layer but had undergone different degrees and directions of migration in different species. With such maps, it was possible to examine more carefully the concept that different vertebrate brain

stems present variations of a common ground plan, e.g. Opdam, Kemali & Nieuwenhuys (1976).

In mammals, radial fibres emanating from ventricular layer cells are conspicuously present during embryonic life but, except in a few restricted locations, decline and disappear before birth. There is considerable circumstantial evidence to suggest that, while present, these cells are in some way implicated in conducting immature neurons from their site of origin to their peripheral destination. The migration may be accomplished either by a neuroblast following a process externally (Rakic, 1972) or by the nucleus of a future neuroblast migrating within the cytoplasm of a process to bud off as a separate cell at its peripheral destination (Berry & Rogers, 1965; Morest, 1970). With either mechanism, it appears that ventricular cell processes map specific sites of neuron production at the ventricular surface to correspondingly specific locations at the periphery (Nieuwenhuys, 1972, 1977; Rakic, 1972; Mountcastle, 1979).

The boundaries of the isocortical system

Applying the foregoing principles to the isocortical area requires the development of reliable criteria for defining the isocortical segment of the intermediate layer. Due to the lack of landmarks in the first stages of development, firm boundaries cannot be established until after the appearance of both the cortical plate and the cingulum. The ventrolateral boundary coincides with a narrowing plate at a level consistent with the transition to insular cortex and is first evident at the E13–E14 period. The cingulum provides a reliable landmark for the dorsomedial boundary, but this is not evident until E16. The equivalent sites at earlier stages of development therefore had to be estimated. The area of ventricular layer radiating processes to the arc thus determined ran from the middle of the area of greatest curvature of the ventricular roof to the caudatopallial angle, as set out diagramatically in Figures 10–13. We believe that these boundaries define the area of isocortical cell production, migration and accumulation closely enough to be useful for our present purposes.

Evidence for a gradient of histogenesis

Prior to the appearance of the cortical plate, the part of the intermediate layer harbouring isocortical preneurons can be taken to extend from the apex of the roof to a ventral boundary within the wedge of more densely packed cells illustrated in Figures 3 and 4. The isocortical intermediate layer is, therefore, also wedge-shaped in cross section with its base directed ventrally (Figs. 3, 4). In terms of the radial units alluded to in the section on Terminology, the wedge shape indicates that the radial units traversing the base of the wedge have commenced neuron acquisition before those at the apex where, self-evidently, fewer preneurons are present (Figs. 3, 4). At late E13, the cortical plate appears as a linear area of more densely packed nuclei at the outer, ventral part of the isocortical intermediate layer (Fig. 2*c*). The number of densely packed nuclei is greater ventrally than dorsally, giving the plate the outline of a thin wedge (Figs. 2*c–e*, 5, 6, 7). At E14, the leading edge of the plate moves dorsomedially within the established intermediate layer to pass the putative boundary of the isocortex at the point of greatest curvature of the ventricular roof (Fig. 2*e–f*). The nuclear crowding characterising the cortical plate is consistent with cell input into the intermediate layer occurring at a rate greater than its areal expansion. The increased input evidently commences in the ventral columns and progresses dorsally. The isocortical plate then undergoes general deepening as cell

crowding continues, indicating that columns across the entire isocortical arc are receiving cells. During the final stage of its growth, completed about E18, the plate reaches a more or less uniform depth across its arc, which is consistent with cells entering the dorsomedial isocortex after further additions to the ventromedial cortex have ceased. This last phase accords with the evidence that after E15 the ventricular area decreases (Figs. 2*f–g*, 10*d–e*) by the loss from its lateral aspect of the ventricular cells with processes extending to the ventral isocortical plate (Figs. 16, 17) while the medial ventricular layer is still active (Figs. 2*g*, 10, 13). These changes are consistent with three phases of neuron production: (1) a period when differentiation spreads across the ventricular layer from lateral to medial, progressively switching over ventricular cells to neuron production and preneurons start accumulating in the ventrolateral columns (Fig. 10*a*); (2) a period when neuron production is taking place over the entire isocortical pool and neurons are entering columns across the entire isocortical arc (Fig. 10*c*); (3) a final period of decline when neuron production ceases first for the lateral and lastly for the medial columns (Fig. 10*e*). The above sequence also matches the autoradiographic evidence presented in the first paper of this series (Smart & Smart, 1982). Also in Figure 10, the sequence is sketched in three dimensions. At each age, the boundaries on the ventricular surface of corresponding radial units have been joined up at different coronal levels to give crescentic areas which depict the spread and decline of neuron production as wave fronts proceed across the generative layers from the curvature followed by the caudatopallial angle.

Evidence for peaks in cell production

The ventricular layer reaches an apparent maximum depth as early as E11. Its area, however, continues to increase until the E13–E14 period, during which time the number of mitotic figures per unit area of ventricular surface increases and subsurface prophases appear. This represents an expanding precursor pool proliferating at the maximum rate permitted by the prevailing cycle time and degree of pseudostratification. Between E13 and E14, the depth of the ventricular layer remains about the same, but the number of mitotic figures at the surface decreases and ventricular choke is less evident. After E15, there is a rapid decrease in the area and depth of the ventricular layer and in the number of mitoses present, indicating a major release of cells and termination of this phase of neuron production. The E13 period, therefore, seems to mark a peak in the growth of the ventricular layer at the chosen coronal level and the period just subsequent to E15 marks the final demise of the layer as a major neuron producer. Inspection of the samples of the nuclear populations of the telencephalic wall in Figure 16 shows that between E14 and E15 there is a substantial increase in the depth of the cortical plate. This is consistent with the arrival in the outer intermediate layer of cells released from the precursor pool about two days previously, using the estimates of the migration rate of isocortical preneurons given by Hicks & d'Amato (1968) in the rat and Rakic (1974) in the monkey. From the results of the autoradiographic study reported in the first paper of this series (Smart & Smart, 1982) it was concluded that increasing numbers of neurons were born at successive generations from E11 to E13 and that this was followed by a decline in neuron production. In keeping with the presence of a gradient, the autoradiographically determined peak seemed to reach the lateral cortex at E12 and the dorsal cortex at E13. There was also some autoradiographic evidence of a second increase in neuron production at E15–E16 (Smart & Smart, 1982) which could coincide with the terminal release of neurons

from the periventricular layers. The histological evidence of a period of more rapid increase in the number of cortical plate neurons at E17 and E18 was, however, equivocal. The sum of the evidence is thus that (1) up to E13, the precursor pool is expanding at a maximum proliferative rate and releasing increasing numbers of neurons, (2) about E13, there is a decrease in rate of neuron production from a less active proliferative layer, (3) the peak in production moves from lateral to medial across the ventricular layer between E12 and E13 and (4) there may be a final rapid phase of neuron release about E15 and E16, caused by the remaining precursor cells differentiating into neurons. It is interesting to note that two peaks of neuron production have been reported in human material by Poliakov (1935–38) and that apart from a short account by Sidman & Rakic (1973) no other mention of the phenomenon appears to have been made outside the Russian literature.

Non-surface mitosis and the origin of the subventricular layer

Numerous mitotic figures are present in the outer part of the ventricular layer and in the adjacent intermediate layer very early in the phase of neuron production (Fig. 5). In a previous study (Smart, 1973) such non-surface mitotic figures were found to be particularly numerous in advance of and under the leading edge of the isocortical plate; the majority also appeared to be partitioning tangentially to give deep and superficial daughter cells. The location of these figures in time and space is such that they would be admirably placed to augment the supply of neurons and contribute to the peak of neuron production that precedes the formation and rapid increase in depth of the cortical plate. Our tentative hypothesis, therefore, is that these early non-surface figures are producing mainly preneurons. Later in development, the nuclear populations between the ventricular layer and subplate are ncreasingly split up and dispersed into cords of cells by traversing fibre bundles and there is no impression of a circumscribed subventricular 'layer'. Mitotic figures are still numerous in this population and we suppose that these cells are increasingly concerned with the formation of neuroglia and give rise to the scattered mitosis-capable neuroglia precursor cells observed in the grey matter and tracts in various parts of the telencephalon (Sturrock, 1979) and which are referred to in the Terminology section of this paper as the dispersed mitotic compartment. Between E16 and E17, however, after major neuron production is over, there is the somewhat sudden appearance of a densely packed population of nuclei between the diminished ventricular layer and the deepest of the growing number of tangentially running fibre bundles. The floreat of this population is brief, for it declines and all but disappears in the immediate postnatal period. We assume that its appearance is concerned with sending neuroglia precursors into the massive fibre bundles of the corpus callosum which become such a dominating feature of the inner surface of the cortical arc during the perinatal period.

The isocorticogenic system in terms of radial units

The history of an individual unit

A radial unit is a strip of ventricular and intermediate layer of some conveniently small breadth containing ventricular cells and their progeny of neurons. The components of the system are few and subject to so many geometrical constraints that the possible sequences of cell movement are limited. The following pattern is consistent

with the available observational and experimental evidence. The pseudostratification of ventricular layer nuclei indicates that a unit is under some form of constraint and is freer to expand radially than laterally. Under such constraints the number of ventricular cells reaches a certain maximum when ventricular choke appears. If cells are then lost from the ventricular layer at an average rate of one daughter cell per cell division, the ventricular layer remains in equilibrium. Increasing output per unit of time from a system in equilibrium can occur by decreasing the cycle time. The experimental evidence, however, is unanimous in suggesting that the average cycle time of ventricular cells increases during the corticogenic period (Korr, 1980). Alternatively, increased cell output from a unit can be achieved by losing, on average, more than one daughter cell per division. This would tie increasing neuron production to a progressive reduction in ventricular cell number. This was not observed to occur, except as a terminal event (Fig. 2*f–g*), and, therefore, cannot be used to account for the autoradiographic evidence that between E11 and E13 there is a progressive increase in the number of neurons produced (Smart & Smart, 1982), although the possibility remains that the peak of neuron production observed at E13 could result from a fall and rapid restitution of the number of ventricular cells. To account for the progressive increase in neuron production between E11 and E13, a contribution from the daughter cells of non-surface mitotic figures (Fig. 5) can be invoked.

Preneurons released from the ventricular layer enter an intermediate layer subject to similar lateral constraints and are also required to migrate through the neuron populations already formed to gain the pial end of the unit. The visual history of the intermediate layer is one of increasing nuclear crowding as released neurons accumulate between the processes of ventricular cells to produce a replication, at the pial end of the unit, of the nuclear pseudostratification of the ventricular layer at the opposite end. The probable steps in the development of the intermediate layer were examined with the help of the simple diagrams set out in Figure 18. In these diagrams, three generations of neurons (each of increasing number) are represented by different symbols and are pictured migrating through each other to reach the top of a radial unit where they are subjected to crowding. The earliest formed neurons are seen to be submerged in later arrivals and, finally, to re-emerge as the first sign of a subplate as the ascending later-born cells pass them by. The subsequent history shows the system being relieved of lateral constraints as the cortex matures and the distance between nuclei increases. It is interesting to note that, during this process of lateral expansion, crowding of nuclei in the diminishing cortical plate is observed to continue. This can only occur if nuclei originally lying more deeply in the plate are taken up into more superficial layers, which suggests that there is some delay in the arrival of the last neuron generations in completing their radial migration. The sequence also illustrates a few of the first formed nuclei remaining above the plate to represent those few that do so and contribute to the molecular layer as described by Marin-Padilla (1971, 1978).

The final stage of the model demonstrates the magnitude of the dispersal required to be undergone during the transition of the crowded nuclear populations of the cortical plate into the adult cortex with its wider average internuclear distances.

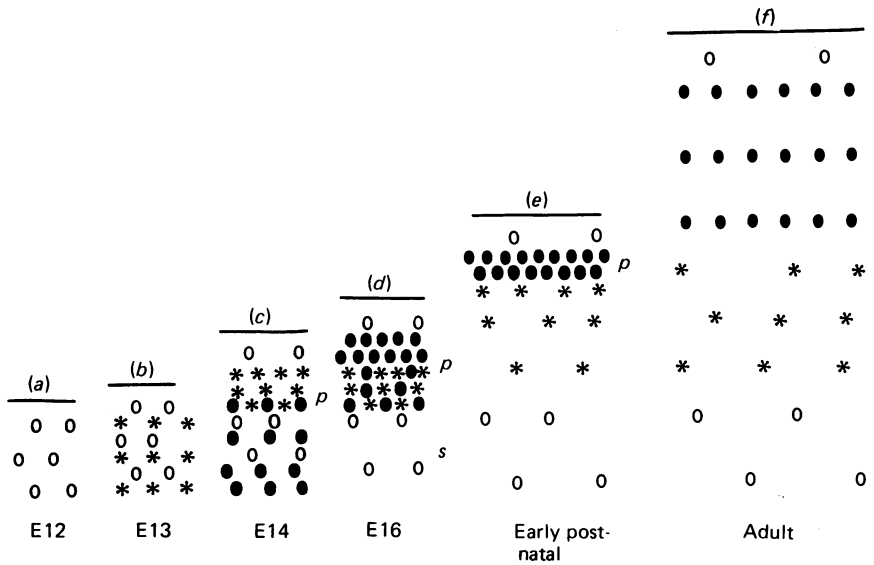

Fig. 18. Diagrams of movement of three stages of a continuum of cell generations entering intermediate layer from ventricular layer. (*a*) First generations, represented by open ovals, forming intermediate layer equivalent to that of, say, E12 (Fig. 3). (*b*) Middle generations, represented by asterisks, migrating through first generations, a state equivalent to, say, E13 (Fig. 4). (*c*) Final generations, represented by solid ovals, entering intermediate layer and beginning to overtake second generations. The latter are represented as beginning to crowd together below pia mater (represented by transverse line) to form cortical plate (*p*). (*d*) Final generations completing their migration through first and middle generations; first generations now emerging as subplate. (*e*) First stages of cortical maturation; neuron nuclei becoming separated by neuropil; cortical plate thinned by cell loss to subplate and obligation to extend over increasing area. (*f*) Adult dispersal.

The isocortex as the sum of its radial units.

The concept of a radial unit comprised of some conveniently small sample of ventricular cells and the neurons they have produced is a useful dynamic unit from which to reconstruct the general properties of the total neuron production–accumulation system giving rise to the isocortex. In previous studies (Smart, 1972*a*, *b*, 1973) it has been found to be heuristically useful to examine the responses open to different cell production mechanisms when required to produce the maximum number of cells. In the case of the isocortex, increase in the number of radial units will result in an increase in the area of the isocortical segment of the lateral ventricle. Therefore, ventricular area will be an index of the magnitude of the population of ventricular cells available for subsequent neuron production. There are, presumably, some limits to the size of this population set by the degree of permissible ballooning of the ventricles. The number of neurons produced by each unit will correspond to the total number of cell cycles completed by neuron-producing cells within the unit and, therefore, duration of neuron production modified by the cell cycle time will be an important component in the total productivity of a unit. Continuing neuron production will lead to accumulation of neurons and eventually to the establishment of the cortical plate. Increasing depth of a crowded cortical plate incurs a future obligation to undergo a proportional increase in area as its constituent cells undergo dispersal to achieve their adult separation. The phylogenetic development of the isocortex by this hypothesis can, therefore, be traced as a transition from a segment of telencephalic

wall composed of relatively few radial units producing a modest number of neurons as, for example, in reptiles (Kirsche, 1972) to one composed of many units of high productivity leading to the establishment of a cortical plate of a depth incompatible with future lissencephaly.

Control of neuron production and differentiation in a radial unit

Two main theoretical approaches to the important topic of the control of differentiation in a cell system are currently being developed. The first relates the developmental sequences to changing concentration gradients of organising chemicals (Wolpert, 1969). In this type of model, a cell knows its relative position in the system by the concentration of one or more chemicals reaching it from one or more foci in the tissue and behaves according to the ambient concentration. The chemicals may diffuse extracellularly or be transmitted more specifically from cell to cell by specialised intercellular junctions (Furshpan & Potter, 1968). In the case of the isocortex, this would suppose (1) a graded signal spreading across the ventricular layer from, say, the caudotopallial angle, and initiating differentiation in successive radial units (its advancing front corresponding to the successive positions of the pecked lines in Fig. 10) and (2) another graded signal creating an axis of differentiation from deep to superficial within each radial unit. A second model, not necessarily incompatible with the first, is based on a theory of cell lineages which states that, after a certain number of cell cycles, a stem cell becomes determined, that is, the number of its future divisions and the succession of cell types among its progeny is invariant under normal conditions of development. An excellent summary of the work supporting this model has been presented by Ehrenstein & Schierenberg (1980). The evidence, as in most basic cell differentiation studies, is derived from experiments performed on simpler animals than mammals. In the nematode model discussed by Ehrenstein & Schierenberg, the lineage of each of the 322 neurons composing the nervous system has been followed through from the initial stem cell pool. The function of a neuron was found to be correlated with its position in its family tree, that is, lineally equivalent progeny differentiated into functionally equivalent cells. In our present isocortical studies, there is also a correlation between the birth date of a neuron and its future location (and, therefore, with its functional type) which is compatible with a lineage model. Also of interest from the invertebrate work is the observation that each precursor assigned to the production of the ventral nerve cord generates the family of neurons for one of the repeating units of which the cord is formed. The parallel between this and the repeating radial units used for our description of isocortical histogenesis is obviously worth closer examination.

The role of the cortical plate

The cortical plate is a conspicuous, though transient, feature in the history of isocortical development. Its formation may be a response to the paradoxical time relationship between cortical maturation and cell production. Maturation requires the establishment of complex circuitry and takes a relatively long time; full cortical maturity in the mouse is not reached until some weeks after birth. This is to be contrasted with the odd fact that the time allotted to isocortical neuron production is short, occurs very early in development and is completed almost before there is any neuronal circuitry to which the name 'isocortex' can reasonably be given. The isocortical plate is therefore a bank of preneurons established against future need by the 'prescient' activity of the periventricular layers. The appearance of the plate can

be seen as arising out of the normal tendency of the neurogenic process to 'overproduce' neurons and for the surplus to be removed by cell death (Jacobson, 1978). The two processes are conceived by him as a means of ensuring that all available synaptic targets are provided with a presynaptic neuron, selective cell death being the complementary means of removing those neurons which are least capable of competing for the limited number of postsynaptic targets. This may be so in the generally cited case of anterior horn cells competing for synaptic sites on muscle cells. However, within the central nervous system a slight adjustment to this interpretation provides a more rewarding hypothesis, namely, that because neuron 'overproduction' is of such common occurrence as to be normal, it is likely that mechanisms will arise permitting the survival of increasing numbers of preneurons until later developing synaptic sites become available. The evolution of mechanisms for maintaining preneurons would allow networks to increase in connectivity by serial addition of new cells over a long period. The nature of the proposed maintenance mechanisms can only be speculated about. They are undoubtedly complex and are likely to involve metabolic, vascular and neuroglial changes. Adaptations of the latter would represent a very 'epithelial' response to the situation (Smart, 1961). Some modification in the programming of cell production must also be an integral part of the process. The apparent peak in neuron production prior to the establishment of the cortical plate implies the birth of an increased cell population with the 'foreknowledge' that it will survive until required. The establishment of the cortical plate, however, does not eliminate cell death.

We have observed necrotic cells in the mouse isocortical region to be particularly numerous towards the end of neuron production at E16, which suggests (if the dying cells are indeed neurons) that the cortical plate attains its full complement of cells within the productive capacity of the system. However, recent studies of the development of the invertebrate nervous system summarized by Ehrenstein & Schierenberg (1980) indicate that cell death may be more than a failure in a crude competitive struggle for a peripheral niche. In the species studied, cell death was found to occur in predictable members of certain lineages. Cell death here is clearly not a random event. The implication of these studies is that the number of cells is regulated by programmed cell death. The death of a cell, thus, may represent the 'planned' truncation of an 'unrequired' sub-branch of a particular lineage.

SUMMARY

The histogenesis of the isocortical segment of the lateral telencephalic wall at the coronal level of the interventricular foramen was studied in mice between the ages of E10 and the adult. The proliferative activity of the periventricular germinal layers was correlated with changes in cell distributions in the intermediate layer. The appearances were consistent with a wave of differentiation moving across the ventricular layer from lateral to medial and a peak of neuron production occurring about E13. The sequence of changes was analysed using the concept of a radial unit composed of ventricular cells and their related progeny of neurons. The observed histological changes were interpreted as the result of radial units of similar productive history entering and completing the histogenetic sequence at successively later times along a lateromedial gradient. Some of the implications of this approach were examined and discussed in relation to the general evolutionary properties of such a system of histogenesis.

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