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Mapping of cortical histogenesis in the ferret

By G. M. McSHERRY

Department of Anatomy, Medical Sciences Institute, The University, Dundee, DD1 4HN, U.K.

SUMMARY

The columnar organization of forebrain cortical neuron production allows the neuroepithelium to be analysed in terms of a simple growth model. The pattern of neuron release within each column is repeated with great regularity across the surface of the developing cerebral hemisphere, resulting in the accumulation of cortical neurons in radial stacks above the proliferative ventricular epithelium. The time course of recruitment of adjacent tissue into neuron production accounts for the observed rostrocaudal and laterodorsal gradients of cortical neuron release. The present study plots the history of this front of neuron production across the surface of the developing cerebral vesicle in the ferret and interprets the resulting maps of cortical development stages in terms of a unified growth model that links the radial and tangential aspects of neuron production. These observations are discussed in relation to other studies of gradients in developing nervous tissue and their implications for the study of experimentally induced abnormalities of brain development.

INTRODUCTION

The epithelium forming the neural tube is initially a system of pseudostratified columnar cells. As development proceeds, nerve cells are released from the apical surface of this epithelium and accumulate among the basal processes of the columnar cells. In most parts of the mammalian central nervous system the columnar ventricular cells decline after the neuron production phase is over and their product of neurons is reorganized so that little remains of the original columnar structure. In the region of the forebrain concerned with generating the cerebral cortex, however, the radial structure is particularly dominant during neuron production, so that released nerve cells accumulate above their site of origin to form stacks or columns running parallel to the processes of the ventricular cells. The radial stacking of neurons is retained in the final adult organization and the more obvious horizontal layering of the cerebral cortex arises from corresponding levels in adjacent radial columns coming into register.

Neuron release in the cortex does not begin simultaneously over the entire surface of the cerebral vesicle devoted to cortical cell production, but starts at a focus on the lateral wall of the hemisphere and spreads from there to the limits of the cortical area. Fig. 1 shows three stages of cerebral hemisphere growth which illustrate the progressive increase in cortical plate depth by cell accumulation. The initial stages of cortical development thus provide a system in which a wave of cell release is propagated across the forebrain neuroepithelium. The

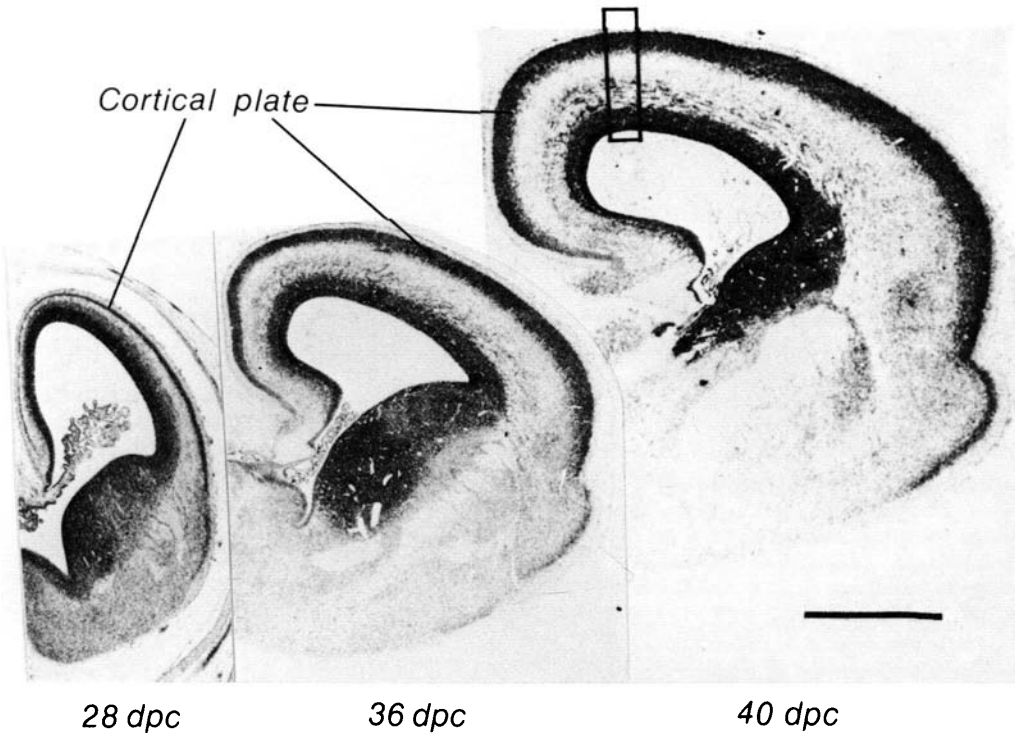

Fig. 1. Coronal sections through ferret cerebral hemispheres at 28, 36 and 40 days postconception (dpc). Cortical plate appears initially as a thin wedge of cells which gains in depth as more migratory neurons accumulate. The outlined area shows the location of the radial columnar samples used to determine developmental stages (see Fig. 4).

progress of this wave of cell production in the developing ferret pallium is the subject of the present investigation.

The ferret (*Mustela putorius*), is a small carnivore, which is the cheapest, easily maintained laboratory animal with a relatively large brain and a long gestation period. With regard to the early proliferative phase of forebrain development, the size of the ferret brain and the duration of neuron production permit the resolution of events and stages of growth which are compressed in smaller, faster developing brains such as the mouse. The procedure adopted was to portray the cortical segment of the cerebral vesicle as a flattened map and to plot on these maps the locations of developmental stages which marked the progression of cell release from the ventricular layers.

MATERIALS AND METHODS

1. *Tissue preparation*

A colony of ferrets was maintained on a small carnivore diet on a 14:10 h light/dark schedule. Females were housed in a group cage until mating, then

individually during pregnancy. Mating was kept to a reasonably short duration (1 day) as a compromise between the need for accurately timed gestation and minimal risk of false pregnancy. The gestation period for the ferret is 42 days, and gestations were timed from the day after mating.

It was known from previous pilot studies that 24 days postconception (days pc) preceded the appearance of the cortical plate, while the bulk of forebrain cortical neuron production was complete by 36 days pc. The ages selected for study were 24, 28, 32, 36 and 40 days pc.

On the required day of gestation, pregnant females were anaesthetized with Sagatal and the embryos removed surgically and perfused intracardially with physiological saline, followed by Bouin's solution. The embryos were left in Bouin's for 4–6 h, after which the brains were removed and left in Bouin's overnight. After dehydration the brains were blocked in paraffin wax, serially sectioned in the coronal plane at 6 μm and stained in haemotoxylin and eosin.

2. *Preparation of outline drawings*

Sections were drawn using a Leitz drawing tube. Every 40th section was drawn in outline at a magnification at ×100, and relevant tissue details (such as medial

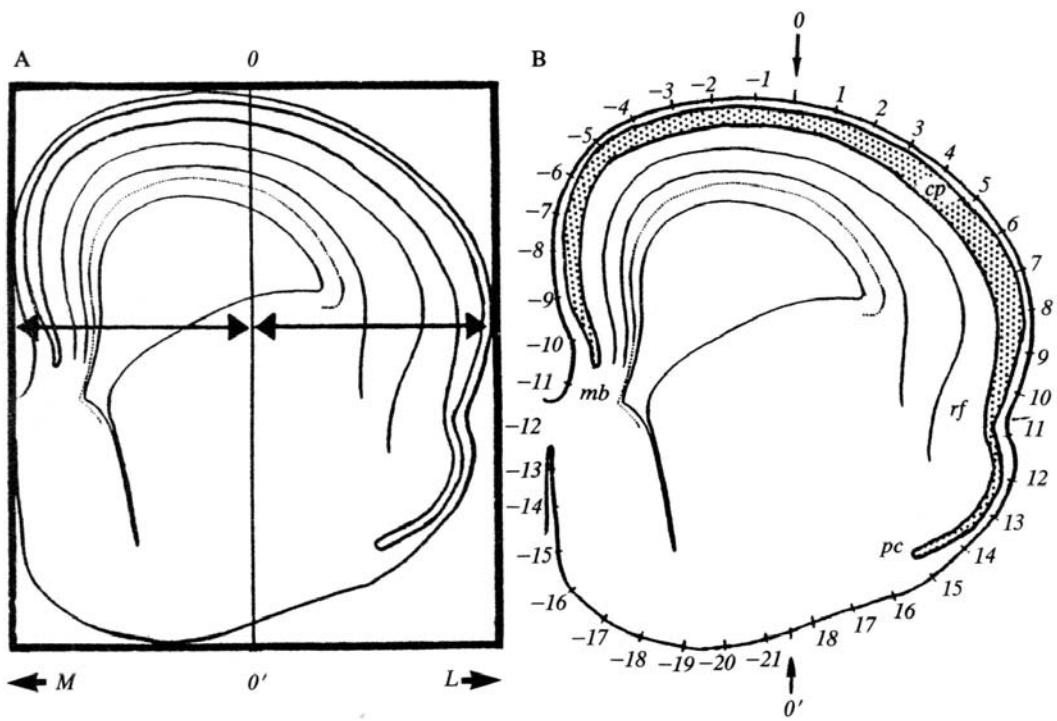

Fig. 2. A) Alignment of outline drawing of coronal section within a measuring frame used to determine the mid-points, 0, 0′ (M: medial; L: lateral). B) Circumferential coordinate system used in plotting variations in cortical plate depth (cp: cortical plate; mb: medial boundary of cortical plate; pc: ventral boundary of piriform cortex; rf: rhinal fissure).

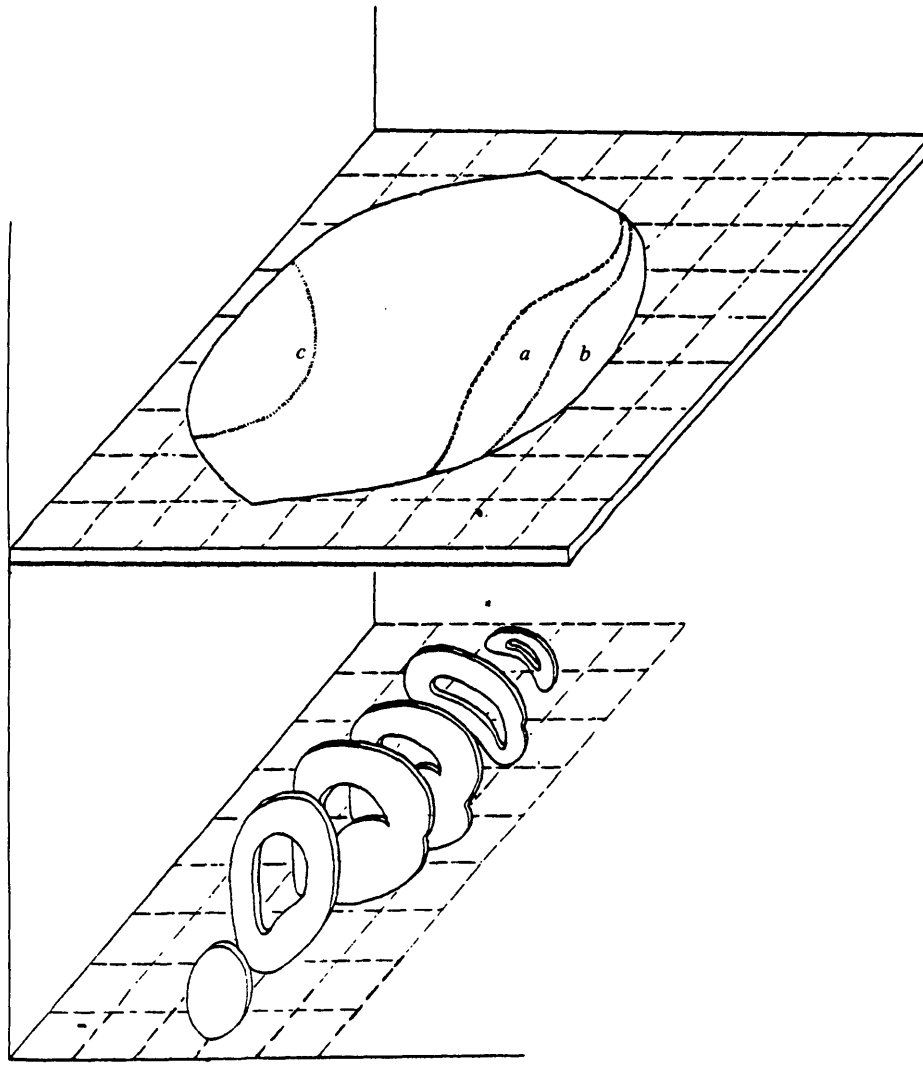

Fig. 3. The graduated circumference of each section flattened onto a line projection. Each section contributes a single line; successive sections projected in this manner constitute a flattened map of the hemisphere surface (a: projection of rhinal fissure; *c*: projection of medial boundary of cortical plate; *b*: projection of ventral boundary of piriform cortex).

boundary of cortical plate, ventral boundary of piriform cortex, depth of ventricular layer and location of rhinal fissure) were noted. This survey yielded a set of large-scale drawings of tissue sections at known intervals for each of the selected ages.

Each drawing was then oriented within a measuring frame that was aligned with the observed medial plane of the cerebral hemisphere, and the points 0, 0′ marked as the mid-width (Fig. 2A). The perimeter of each drawing was marked in equal intervals from the point 0 (positive in clockwise, negative in

anti-clockwise direction). This provided a circumferential coordinate system along which cortical developmental stages could be plotted (Fig. 2B).

Outline maps were prepared by 'straightening' each circumference onto a line projection. Line projections from successive sections were aligned along the mid-axis defined by the 0, 0′ coordinates. This results in a flattened representation of the cerebral hemisphere. Fig. 3 shows in diagrammatic form the projection of six serial sections onto such a flat map. Lines a, b and c show how anatomical features, such as the rhinal fissure, the medial boundary of the cortical plate and the ventral boundary of the piriform cortex are represented in this projection.

3. *Survey of developmental stages*

Sections from brains at each of the selected ages were examined to determine the progression of cortical plate development. A radial columnar sample from the forebrain wall, at a fixed location close to the dorsal apex of the cerebral vesicle, was examined from each brain (Fig. 4A). This served to provide fixed developmental steps connected with known ages of gestation. Sections were scanned to find interpolated stages, and the complete progression, (from the least mature site in 24 days pc to the most mature site in 40 days pc) was then divided into 10 stages as convenient steps with which to assay the progress of development (Fig. 4B). The developmental stages were:

0 Ventricular layer (vl) with scattered neurons.
1 Additional subventricular layer (svl) present, with an increased number of scattered neurons released above these layers.
2 vl, svl and free neurons present, with first appearance of nuclear crowding, indicating commencement of cortical plate formation.
3–5 Distinct cortical plate, migration layer, and svl and vl. Cortical plate increasing in depth with successive stages.
6–9 Increasing growth in depth of column samples, comprising accumulation of cells in cortical plate, increasing sub-plate and appearance of tangentially running fibres between svl and migration layers.

4. *Assay of developmental stages within sections*

Drawings were then surveyed and the distribution of these developmental stages marked around the circumferential coordinate system for each section. Starting at the lateral and proceeding to the medial boundary, the varying thickness of the cortical plate was logged onto the graduated outline drawings, using the 10 developmental stages as steps in the assay (Fig. 4A). These accumulated surveys were then transferred to the flattened maps as described above, to yield contour intervals that marked the boundaries between developmental stages. Detailed maps were prepared from one set of serial sections at each age, and these were checked against additional reference sets. Repeating the process for each brain surveyed yielded a series of developmental maps showing the progress

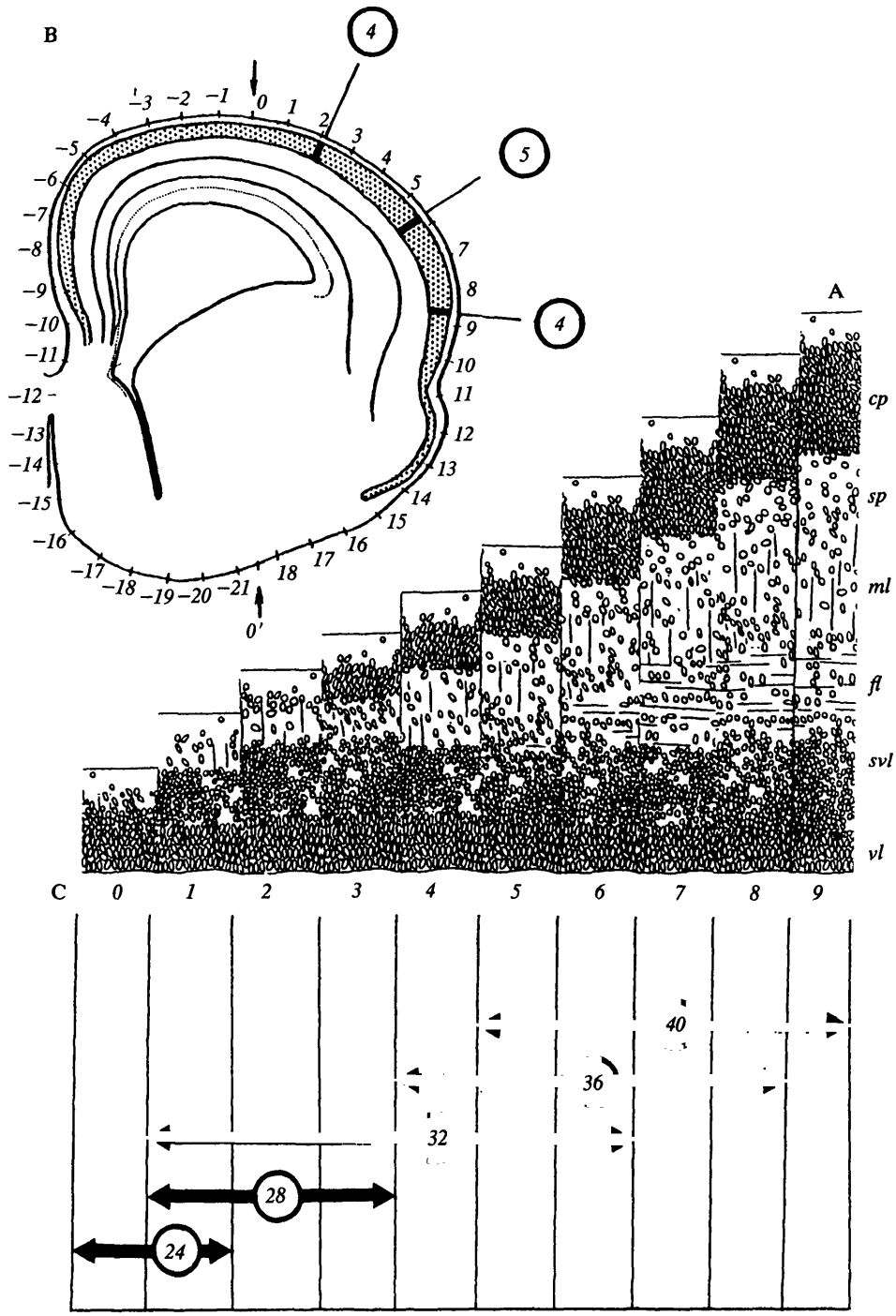

Fig. 4. (A) Full series of developmental stages used in preparing the maps of cortical plate depth (*vl*: ventricular layer; *svl*: sub-ventricular layer; *fl*: fibre layer; *ml*: migration layer; *sp*: sub-plate; *cp*: cortical plate). (B) Sampling of cortical plate to assign developmental stages within the circumferential coordinate system. (C) Spread of developmental stages. The numbers in circles refer to days postconception.

of cortical plate production across the rostrocaudal and laterodorsal axes of the forebrain. Fig. 9 depicts the relationship between such a two-dimensional map and the unsectioned brain.

FINDINGS

Findings are presented in two sections: 1) chronological and developmental comparison and 2) maps of cortical development.

1. *Chronological and developmental comparison*

Fig. 4C shows developmental stages arranged with respect to chronological age of the specimens. 24 days pc contains stages 0–1; 28 days pc contains stages 1–2; 32 days pc spans stages 1–6; 36 days pc contains stages 4–8 and 40 days pc contains stages 5–9. Thus some chronological ages contain a substantial spread of developmental stages. Defining maturity in terms of degree of cell production, surveys of the entire serially sectioned brain at each chronological age reveal that within each brain the more mature stages are found rostrolaterally while more immature stages are found caudomedially.

2. *Maps of cortical development*

Figs 5–8 show maps of cortical plate depth prepared according to the method outlined above. Boundaries enclose regions containing the same developmental

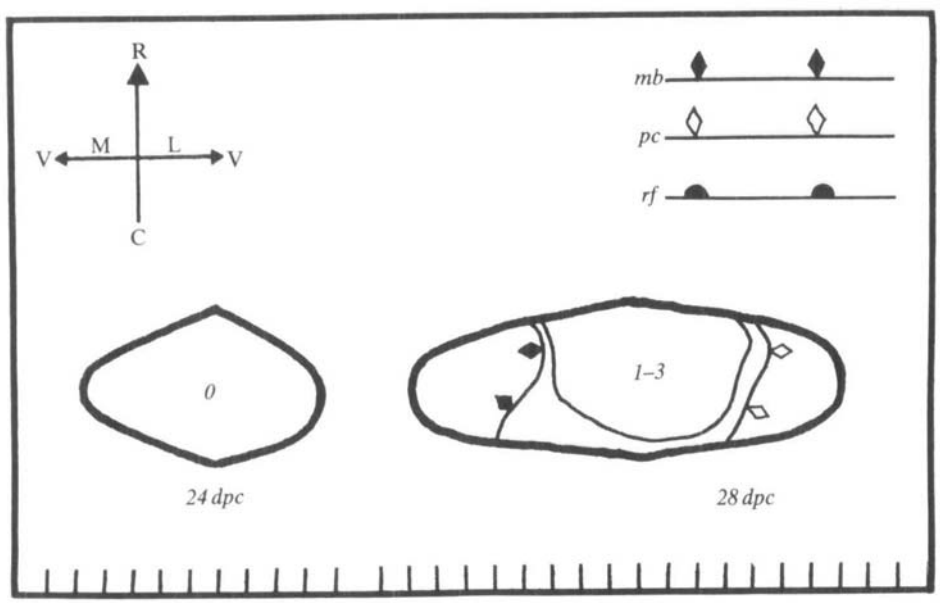

Fig. 5. Contour map of cortical plate indicating depth at 24 and 28 days pc. Each graduation equals 0·5 mm. Abbreviations are: R: rostral; C: caudal; V: ventral; M: medial; L: lateral; *mb*: medial boundary of cortical plate; *pc*: ventral boundary of piriform cortex; *rf*: rhinal fissure.

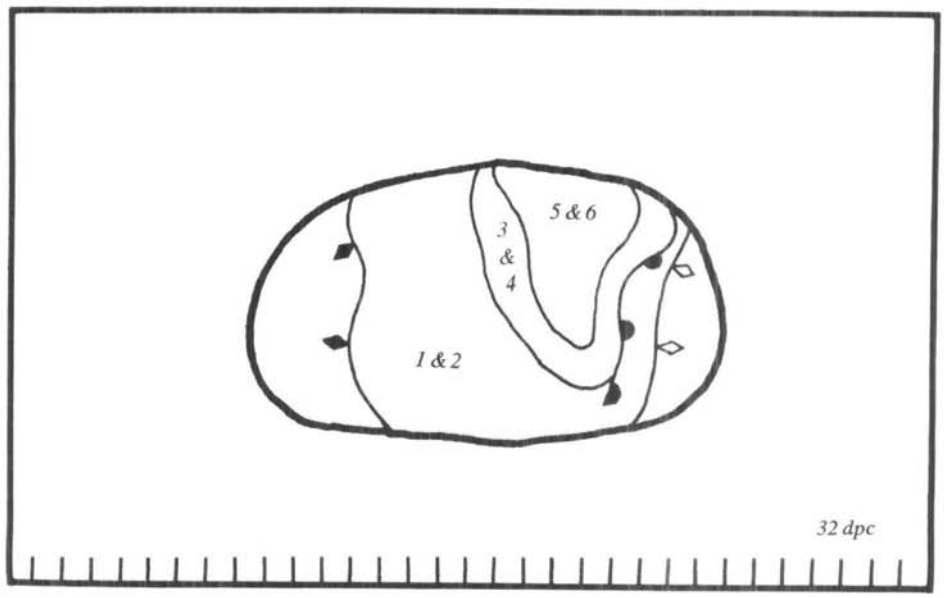

Fig. 6. Contour map indicating cortical plate depth at 32 days pc.

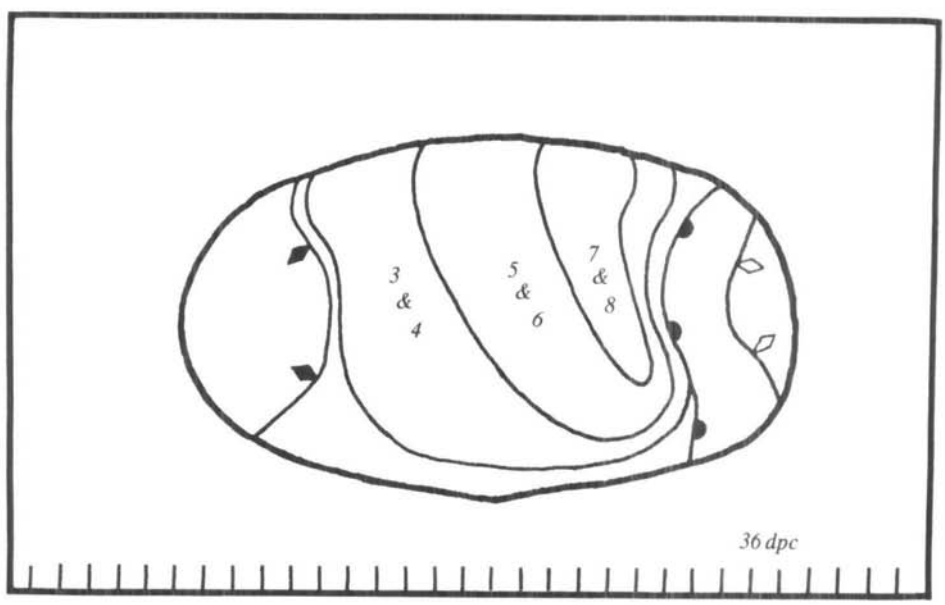

Fig. 7. Contour map indicating cortical plate depth at 36 days pc.

stages. Medial and lateral boundaries of cortical plate, and the course of the rhinal fissure are included on these maps to provide anatomical landmarks. The maps provide information on 1) the location of the presumed initial site of cortical neuron production; 2) the distribution of developmental stages within one chronological age and 3) the progressive advance of these stages across the

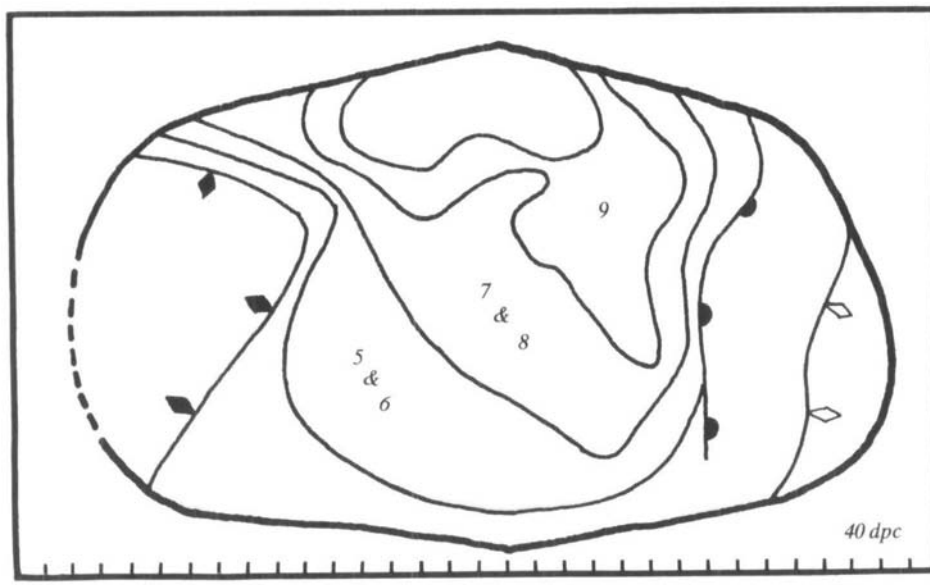

Fig. 8. Contour map indicating cortical plate depth at 40 days pc.

surface of the brain by comparison of successive ages. For example, the region containing stages 5 and 6 is found close to the focus of cortical plate production at 32 days pc; as an oblique band straddling the dorsomedial area of the brain (mainly in the rostral half of the cerebral hemisphere) at 36 days pc, and as an oblique band predominantly in the caudal region at 40 days pc. It can also be seen that the region containing stages 7 and 8, while not present at earlier ages, is focally distributed at 36 days pc, and found more medially and caudally at 40 days pc.

DISCUSSION

The pattern of cell production in the mammalian forebrain presents a system of interest to the developmental biologist. Fundamentally epithelial in character, the forebrain tissue initially grows as a sheet of cells forming hollow cerebral vesicles, and later achieves an increase of depth as neuron migration commences. Thus the mature cerebral cortex, with its intricate assembly of cell types and interconnections, has a developmental history of a much simpler character – a proliferative regime constrained within a sheet of periventricular cells.

1. *Summary of development*

The results presented here show that cortical plate growth may be resolved into two components: increase in depth by accumulation of migratory neurons from the subjacent periventricular germinal compartment and increase in area by germinal cells at the periphery of the plate turning over to neuron production.

This progressive recruitment into neuron release produces a gradient of cortical plate depth which is greatest at the rostrolateral focus and declines with increasing distance across the vesicle surface. The radial glial fibre system which maintains the producing and accumulating surfaces in register is thought to guide neuron migration from the periventricular layers (Rakic, 1972). The rate of growth of the radial columns of neurons within the cortical plate, and the eventual maximum depth attained are features that reveal the cell production regime of the periventricular germinal layers.

2. *Interpretation of cortical development maps*

Angevine & Sidman (1961) demonstrated that early born neurons lie predominantly in deep cortex while later born cells are found in the outermost regions. This classic 'inside-out' pattern of cell accumulation in the cortical plate constitutes a temporal gradient that relates radial depth within the cortex with order of birth. Hicks & D'Amato (1968) in rat and Fernandez & Bravo (1974) in the rabbit noted that cells of a particular generation were found at deep locations in dorsal cortex and more superficially in lateral cortex. In the light of the general 'inside-out' pattern of cortical formation, lateral cortex is therefore developmentally more advanced than medial.

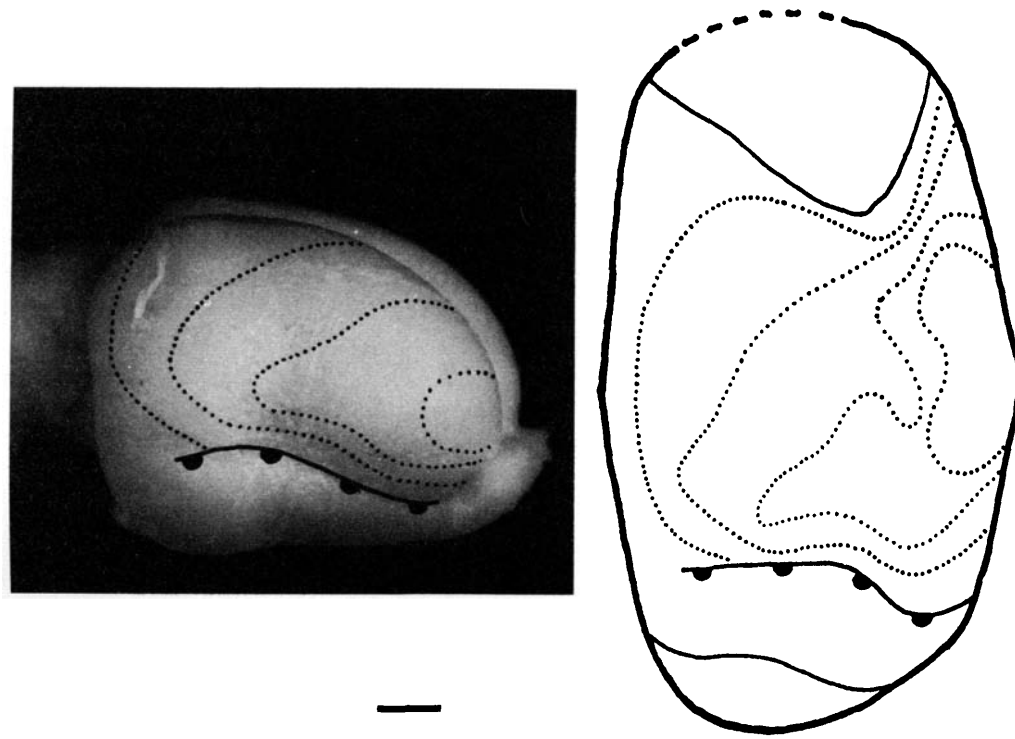

Fig. 9. On the right is a contour map of cortical plate indicating depth at 40 days postconception. Rhinal fissure is depicted as in earlier maps. On the left is a lateral view of the whole brain at 40 days showing position of cortical plate depth contours.

Marin-Padilla's (1978) study in the cat, and Smart's (1973) in the mouse, describe the progressive growth of the neocortex, from an initial site opposite the internal capsule. In the cat the progress of cortical plate across the surface of the cerebral vesicle is accomplished in about 5 days. Measuring both cortical plate depth and distribution of autoradiographically labelled cells in embryonic mouse forebrain, Smart & Smart (1982) and Smart & McSherry (1982) identified a wave of increased neuron birth, consistent with a travelling 'pulse' of neuron production which traversed the cerebral hemisphere in less than 2 days. Schmahl (1983) has demonstrated a laterodorsal gradient of cell cycle duration within the ventricular epithelium in the developing mouse cerebral vesicle suggesting that this variable is part of the cell production pattern.

The present study confirms and extends these findings regarding gradients of cortical neuron production. By analysing the entire ferret forebrain cortical surface in terms of standard developmental stages, it has been possible to produce a global map which unifies rostrocaudal and laterodorsal gradients. The progressive development of cortical plate is represented by a series of wavefronts which mark the boundaries of developmentally equivalent regions. Fig. 9 shows the map of cortical plate depth from Fig. 8 visualized on the 40 days pc forebrain surface. Since we are concerned with the cell production phase of brain growth, the propagation and control of this front of neuron birth across the surface of the neuroepithelium is a matter of considerable interest.

The local behaviour of the forebrain neuroepithelium may be interpreted in terms of control processes regulating initiation and duration of neuron production. Initiation switches the neuroepithelium from precursor pool growth to neuron release. Since these neurons are non-mitotic, the timing of this change has implications for the kinetics of brain growth. Duration controls, by and large, the quantity of neurons produced at each site. Since the processes of migration and aggregation in the cortical plate result in radial stacks of neurons, this quantity is effectively converted into a depth of plate.

3. *Mechanisms of tissue gradients*

The progress of ferret cortical plate production as demonstrated in Figs 5–8 suggests a wave of neuron production passing across the vesicle surface (Fig. 9). In analysing any developmental problem involving the appearance of a wavefront of tissue behaviour, it is necessary to consider the possibility of a genuine propagatory wave, involving local cell–cell interactions with transmission of some signal, and a 'kinematic' wave which is the expression of some relatively autonomous local tissue activity controlled by a timing gradient which exists as a stable prepattern in the tissue. With respect to neuron release in the sheet of forebrain neuroepithelium, the alternatives are a travelling wave of some controlling signal, moving across the tissue and regulating the field of neuron-producing cells, and an intrinsic growth programme, regulating the cell production regime at each site coupled with a maturation signal distributed

across the entire tissue. The nature and properties of a possible travelling wave involved in the control of neuroepithelial behaviour must await experimental manipulation of mammalian forebrain development. However, the role of developmental timing in neuron production has been investigated in a variety of organisms and these approaches are summarized here.

The radial columnar arrangement implies that the cells within each cortical column are clonally related to each other (Levitt & Rakic, 1980). Thus a cell at the 'outer' (pial) margin of the embryonic cortical plate differs from a 'deeper' cell in that it was produced later in the series of divisions undergone by ventricular germinal cells connected to that region of the cortical plate.

Two lines of evidence suggest that developmental timing, rather than positional information, may be an important factor in the early growth and differentiation of the brain.

1) Studies on the development of the cerebral cortex in neurological mutants have indicated that neuron type is largely determined by order of birth (Caviness & Rakic, 1978).

2) Maturation and differentiation can occur in dissociated cultures of foetal brain tissue (Sensenbrenner, Wittendorp, Barakat & Rechenmann, 1980; Rioux, Derbin, Margules, Joubert & Bisconte, 1980). *In vitro* studies of differentiation in embryonic rat brain tissue have demonstrated that the appearance of the main classes of glial cells during the period of neuron production follows the same schedule as in the intact animal (Abney, Bartlett & Raff, 1981). Thus, evidence from studies of brain development in neurological mutants (where cell position is abnormal), and from *in vitro* studies of dissociated brain tissue (where positional information is disrupted), point to the importance of developmental timing in the control of differentiation. In addition, studies of neurogenesis in invertebrates reveal a precise lineage mechanism in which sequences of cell division are of crucial importance in determining the functional type and also the survival of particular neurons (Sulston & Horvitz, 1977; Ehrenstein & Schierenberg, 1980).

We might visualize the sheet of neuroepithelium as a field of alarm clocks, timed to ring progressively later as we move from 'centre' to 'periphery'. As each clock rings, the local neuroepithelium enters an autonomous programme of neuron production, characterized by a definite sequence of cell divisions that release neurons whose functional type and competence to form connections are largely determined by order of birth. Thus, the depth of the cortical plate becomes a spatial map of the original time course of neuron birth. As the proliferative clocks cease ringing at each site, a 'wave' of precursor depletion traverses the neuroepithelium.

Presenting forebrain cortical neurogenesis in this manner suggests problem areas for further study: what is the nature of the maturation gradient prepattern; is its formation in the tissue correlated with the morphological growth of the forebrain, or imposed at a later stage? In particular, what is the nature of the

growth programme expressed locally at each site within the neuroepithelium; is the full range of cortical neuron types expressed by the progeny of each ventricular germinal cell or is the neuroepithelium floor a micromosaic of different precursor types?

4. *Conclusions*

This paper presents a preliminary study of the pattern of cortical neurogenesis in the ferret forebrain showing the common origin of the rostrocaudal and laterodorsal gradients of cortical neuron production. The variety of developmental stages present at any one chronological age has implications for experimental studies of brain development involving the use of cytotoxic agents (Haddad & Rabe, 1980) as there may be spatiotemporal variations in sensitivity within the forebrain. Both cell labelling and cell deletion studies need to be interpreted within the context of the developmental pattern outlined here.

The forebrain neuroepithelium comprises a field of cells exhibiting behaviour of interest to experimental morphologists as well as developmental neurobiologists. A graded pattern of cell production coupled with a tightly controlled migration regime places certain constraints on tissue growth. The factors controlling neuron release and cortical plate formation are thus expressed within an epithelial format. In contrast to the lineage organization which seems to control the radial dimension of neuron production, the position-related timing of the onset of neuron release along the plane of the neuroepithelium exemplifies the role of positional information in forebrain cortical neurogenesis. Experimental manipulation of the tissue involving localized mechanical displacement within the field of neuron-producing cells and selective interference with sequences of cell divisions is required to analyse the relationship between the cell-production gradients revealed here and local lineages.

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