Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer

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Summary
We have used a retroviral vector that codes for the bacterial enzyme β-galactosidase to study cell lineage in the rat cerebral cortex. This vector has been used to label progenitor cells in the cerebral cortices of rat embryos during the period of neurogenesis. When these embryos are allowed to develop to adulthood, the clones of cells derived from the marked progenitor cells can be identified histochemically. In this way, we can ask what are the lineage relationships between different neural cell types. From these studies, we conclude that there are two distinct types of progenitor cells in the developing cortex. One generates only grey matter astrocytes, whereas the second gives rise to neurones – both pyramidal and nonpyramidal – and to another class of cells that we have tentatively identified as glial cells of the white matter. We have also been able to address the question of how neurones are dispersed in the cortex during histogenesis. It had been previously hypothesized that clonally related neurones migrated radially to form columns in the mature cortex. However, we find that clones of neurones do not form radial columns; rather, they tend to occupy the same or neighbouring cortical laminae and to be spread over several hundreds of micrometers of cortex in the horizontal dimension. This spread occurs in both mediolateral and rostrocaudal directions.

Key words: cell lineage, cerebral cortex, neurogenesis, retroviral vectors.

Introduction
The studies presented in this paper are concerned with two processes that occur during the development of the cerebral cortex. The first of these is the process whereby the progenitor cells, primarily of the ventricular zone, give rise to all the differentiated cell types that make up the cortex. The other is the process of histogenesis, by which these cells migrate to their correct position in the cortical structure. Both of these phenomena have been widely studied (see McConnell, 1988, for a recent review), but analysis of the formative events involved has been hampered by a lack of information about the cell lineage relationships of the cerebral cell types. We need to know, for instance, if one ventricular zone cell can give rise to the whole range of neural cell types, or if there are committed progenitor cells, each of which gives rise to one, or a restricted subset, of the cell types. This is important because, until the nature and timing of the developmental decisions are understood, it is difficult to determine what factors influence, or help implement, the determination of cell fate.

These questions have been addressed in the monkey cerebrum by Levitt et al. (1981) who showed that there were dividing ventricular zone cells that contained glial fibrillary acidic protein (GFAP), an astrocyte-specific marker. This was taken as evidence for the existence of progenitor cells, committed to the generation of astrocytes. However, as has been pointed out elsewhere, intermediate filament proteins often appear transiently in development, in cells that will not express them in their adult form (Choi & Kim, 1984; Ogawa et al. 1985; Bennett & DiLullo, 1985).

To study cell lineage effectively, a marker is required that can be introduced into an individual ventricular zone cell, such that the cell, and all its progeny, are indelibly labelled. We and others (Sanes et al. 1986; Price et al. 1987) have recently shown that retroviral vectors can be used as lineage markers in this way (see Price, 1987 for a review). This has
allowed us to address questions of cell lineage, and also to consider certain aspects of histogenesis.

During histogenesis of the cerebral cortex, the two-dimensional, pseudostratified, sheet of ventricular zone cells expands in the third dimension to give the mature structure. Various hypotheses have suggested that neurones migrate strictly radially, so ensuring that a clone of related cells comes to lie in a column in the adult cortex (Meller & Tetzlaff, 1975; Rakic, 1978, 1988; Smart & McSherry, 1982). The clonal derivation of cortical columns might, therefore, underlie the functional organization of the cortex, which is thought also to be columnar (Mountcastle, 1978). It has been proposed that the radial glial cells could play a central role in maintaining the strictly radial migration (Rakic, 1978, 1988). These models predict, therefore, that there would be a restricted horizontal spread of clonally related neurones.

Despite the attraction of such hypotheses, they have been difficult to test because there has been no way of seeing precisely how clones expand during migration. However, the retroviral marking technique can also provide this information, by allowing us to label individual progenitor cells and then, subsequently, to observe how the clones to which they give rise are distributed in the adult cerebrum.

In this paper, we describe experiments in which we label individual ventricular zone cells in utero on embryonic day 16 (E16) and perform clonal analysis on these animals 14 days after birth. We present data that indicate that there are at least two types of progenitor cells in the ventricular zone at the time of injection. We also show that both neuronal and glial cells are capable of considerable lateral migration during the process of cerebral histogenesis.

Materials and methods

Production of retrovirus

The virus used in these studies was the BAG vector, which has been previously described (Price et al. 1987). Viral producer cells were maintained in Dulbecco's modified Eagle's medium plus 10% calf serum (CS: Serolab). Concentrated viral stocks were generated by removing the medium for producer cells, filtering it through a 0.45 μm filter, and centrifuging it at 14000 revs min⁻¹ in a Beckman SW28 rotor overnight. Concentrated virus was resuspended in CS and frozen at -20°C until required.

Viral titre was defined as the number of X-gal stained infected cells found in these experiments was: what constitutes a clone? In a previous study using this approach in the retina (Turner & Cepko, 1987), clones were found to be discrete radial arrays of cells

Viral injections into embryos

Pregnant rats on day 16 of pregnancy (plug day being day zero) were anaesthetized with a mixture of 10 mg of Ketamine ('Vetelar', Parke-Davis, Gwent, UK) and 4 mg of xylazine ('Rompun'), Bayer, Bury St. Edmunds, UK) injected intraperitoneally. A single incision along the midline of the abdomen exposed the uterus, and approximately 1–2 μl of an appropriate concentration of the BAG virus, was introduced into the left cerebral vesicle of each embryo by injection through the uterine wall with a 30G needle and a 50 μl Hamilton syringe. The incision was closed by suture and the female rat was given 60 μg buprenorphine (‘Temgesic’, Reckitt & Colman, Hull, UK) and 2000 i.u. penicillin and streptomycin (Gibco, Ohio, USA) by intraperitoneal injection. The pups were born normally, and they were left undisturbed for 14 days, at which point they were killed by perfusion with 2% paraformaldehyde in 0.1 M-Pipes buffer containing 1-25 mM-EGTA and 2 mM-magnesium chloride. Some uninjected animals were taken as controls and treated similarly. The brains of the pups were fixed by immersion in the same fixative for several hours, followed by an overnight immersion in 30% sucrose solution. At this point, the brains were screened for any visible defects that might have been the result of the injection. A small number of brains were scarred at the site of injection and were discarded. The brains were then frozen on dry ice and serially sectioned at 20 μm on a cryostat.

β-galactosidase histochemistry

Sections were treated for 10 min with 0.01% w/v sodium deoxycholate, 0.02% v/v Nonidet NP40 and 2 mM-magnesium chloride in phosphate-buffered saline pH 7.4 (PBS). They were then reacted with 1 mg ml⁻¹ X-gal (5-bromo-4-chloro-3-indolyβ-D-galactopyranoside) in PBS+0.01% sodium deoxycholate+0.02% Nonidet NP40+2 mM-magnesium chloride+20 mM of both potassium ferricyanide and potassium ferrocyanide. After further washes in PBS, the sections were counterstained briefly in Orange G (1% solution in 2% phosphotungstic acid), dehydrated through alcohols and mounted.

Computer-aided 3-D reconstruction

Camera-lucida drawings of serial sections were reconstructed using a PDP11 computer and the SSRCON software (Shepherd et al. 1984). The reconstructions presented here are redrawn from computer printouts.

Results

Definition of clonality

A primary issue in the interpretation of the pattern of infected cells found in these experiments was: what constitutes a clone? In a previous study using this approach in the retina (Turner & Cepko, 1987), clones were found to be discrete radial arrays of cells
Fig. 1. Clones of grey matter cortical astrocytes. (A) A low-power photomicrograph of part of an astrocyte clone, which is spread over a single cortical section from superficial to deep laminae. The pial surface is just visible in the top left-hand corner. Scale bar, 100 μm. (B, C) Higher power photomicrographs of parts of astrocyte clones, similar to that shown in A. Note the filled staining pattern that appears to cover the cell body and all the cell process, including the characteristic blood-vessel end feet (arrowed). These are particularly clear in the case of the two, closely adjacent cells in C, both of which have blood-vessel end feet. Scale bars, 10 μm.
Fig. 2. Pyramidal and nonpyramidal neurones and horizontal cells. (A) A single lamina II pyramidal neurone, characterized by its triangular soma and prominent apical dendrite. In this, and all the other micrographs in this figure, the pial surface is towards the top of the page. Note that, unlike the astrocyte clones, this is the only stained cell in this section, and that the staining seems to be limited to the nuclear region, and to a small spot of staining in the apical dendrite, indicated by an arrow. Scale bar, 10 μm. (B,C) Nonpyramidal neurones. Again the staining is predominantly nuclear, plus the one or two points of staining in the most-prominent dendrite (arrows). Scale bars, 10 μm. (D) Nonpyramidal bipolar neurone of lamina V. In this cell, the staining fills the cell body and the single apical process. We know the cell is bipolar because we could trace its basal process into the adjacent section. Scale bar, 10 μm. (E) Two horizontal cells in the nascent white matter. Note how the cell bodies are orientated horizontally along the fibre tract. These cells had processes running horizontally in adjacent sections. The dark yellow area just visible at the bottom of the photomicrograph is the ventricular zone. Scale bar, 20 μm.
and their identification presented little difficulty. In these experiments in the cerebrum, clusters of cells were not so discrete. Nonetheless, small groups of stained cells were observed, that were a considerable distance (i.e. at least 0.5 mm) from other such groups. Although the inoculum was into the left cerebral vesicle, these clusters were found throughout the brain, even as far caudally as the hindbrain. Thus, most of the clones were not near the site of injection and were unlikely to have been disturbed by the injection process. It was found through trial and error that the injection of virus at a concentration such that 1 μl represented 100–1000 infective particles (as titred on NIH-3T3 cells) gave us up to 50 clusters throughout the forebrain and midbrain regions. More caudal areas were mostly ignored in these studies. As only a proportion of these were in the cerebrum (see below), this gave what we considered to be about the correct density of infective events so that clusters could safely be considered to be clones. This analysis suggests that the in vivo titre is about 10% of that found in vitro. This is similar to what Turner & Cepko (1987) found in the retina, and seems reasonable given that almost certainly some of the inoculum does not reach its target and that ecotropic virus probably has a slightly lower titre on rat cells than on mouse cells like NIH-3T3. We have analysed ten animals from four litters that had the appropriate number of viable nuclei. Clones, and those animals provide the body of data presented here.

Types of clone

Essentially all the clones we found in the cerebral cortex could be classified into three types. The largest type, in terms of cell number per clone, contained polymorphic cells that had small round perikarya and extensive stained processes (Fig. 1). These cells were predominantly, if not exclusively, astrocytes. This conclusion was based on a number of criteria. The cells had the morphology of astrocytes. Many of them could be seen to have end feet on blood vessels or, less frequently, on the pial surface (Fig. 1B,C). They had small cell bodies and these clones never included cells that had a clear neuronal morphology. For instance, these clones never included pyramidal cells, or any of the more prominent nonpyramidal morphological types such as bipolar cells.

The majority of astrocyte clones had between 20 and 30 cells. The largest we found was 35 cells and the smallest was 12. They were found throughout the cortical laminae (Fig. 1A); one clone could be spread from lamina VI through to the pial surface, but they did not extend into the white matter. Also, they tended to be found in small clusters of two or three cells (Fig. 1A), giving the impression that, after migrating into the cortical plate, a cell had divided once or twice more in situ. The question of the horizontal spread of such clones will be dealt with below.

The second type of clone we found was distinctly different, in all of its major features, from the clones of putative astrocytes (Fig. 2). First, the cell morphology and pattern of X-gal staining was very different. Some of these cells had the morphology of pyramidal cells. They could be identified by the shape of their cell bodies and their major apical dendrite (Fig. 2A). Other cells with this pattern of staining had the morphology of nonpyramidal neurones (Fig. 2B–D). Their cell bodies were more variable in shape than the pyramidal neurones, but they also tended to have one or two major processes which most commonly were orientated vertically, i.e. either directly towards or away from the pial surface. This was most unlike the morphology of the astrocytes whose cell bodies were smaller and whose processes were greater in number and much more variable in orientation. (Compare Fig. 1 with Fig. 2.)

Unlike the astrocytes, the staining of neurones was usually limited to the nuclear region and to occasional small points of staining in the cell processes (Fig. 2). In many cases, unstained areas of cytoplasm were clearly visible in the cell body and major processes. The small points of staining were usually restricted to the cell’s principal dendrite in both pyramidal and nonpyramidal cells. Very occasionally, we saw cells that by morphological criteria were neurones and were part of neuronal clones of the type described here, but nonetheless had cytoplasmic rather than nuclear staining. Such a cell is shown in Fig. 2D. It is a bipolar neurone in lamina V, similar to those described by Feldman & Peters (1978) and it has a morphology quite unlike the astrocytes described above although it is completely stained.

Fig. 3 is a histogram illustrating the number of neurones per clone, based on the data from one animal. The majority of neuronal clones contained
just one neurone, with the average number being 1-5. In fact, the range over all the animals we have looked at so far has been between 1 and 6 neurones per clone. The single clone in Fig. 3 containing 8 neurones was atypical in a number of respects and will be dealt with separately below.

With this series of injections, all of which were administered on E16, most of the neurones that we observed were in laminae II–IV. All of the neurones shown in Fig. 2A–C were from this supragranular region. This is in accord with what one would expect from the thymidine-labelling studies of other workers (Berry & Rogers, 1965; Miller, 1986), and is another distinctive difference between the neuronal and astrocyte clones. Neuronal clones were not limited to one lamina; we have seen examples crossing the boundaries between laminae II and III, and laminae III and IV (Figs 4, 5). We have seen clones with cells in all of these three supragranular laminae.

We have in addition observed four cases of large stained neurones from laminae V or VI (data not shown). Interestingly, all of these clones were single cell clones, so we have yet to observe a clone that contains a large infragranular neurone and supragranular neurones. Clearly, this does not represent sufficient cases to draw any conclusions, but it leaves the possibility open that a different progenitor cell generates the large infragranular neurones from the one that generates the supragranular ones.

There is a third type of clone that we see. Its principle characteristic is that the cells are confined to the ventricular, subventricular, or nascent, white matter regions (Fig. 2E). The cells are small and are generally orientated horizontally as if they were lined up along the fibre tracts. They are variable in morphology, some being small oval cells with no apparent processes, others being monopolar or bipolar. Some are multipolar with processes that can be traced for several tens of micrometres running parallel to the fibre tract. They all seem to have the filled nuclear staining pattern of astrocytes rather than the neuronal nuclear staining, although they never have blood vessel end feet. We call this morphologically heterogeneous group of cells 'horizontal cells', but as yet we do not know their real identity.

None of the above was ever seen in uninjected control animals. In addition, one or two animals in injected litters were often left uninjected, mainly because they were orientated inconveniently in the uterus. In such litters, there was invariably the appropriate number of negative animals, although these could not be identified a priori as the uninjected animals. Some structures in control animals did tend to stain with X-gal. These included the choroid plexus, blood vessel endothelial cells and some neuronal subpopulations, particularly in the cerebellum and brain stem (although not the cerebrum). The conditions we have used for staining have been optimized to minimize this endogenous β-galactosidase and it was not a problem in these experiments.

**Codistribution of clones**

The above data suggested the existence of three different types of clones. However, when we studied the distribution of the different clones, we found that the horizontal cell clones were frequently colocalized with the neuronal clones. By colocalization, we mean that the horizontal cells were found in the same section immediately deep to the stained supragranular neurones or, in 3-D reconstructions of clones, the cluster of horizontal cells were found to be overlapping the stained neuronal cells (see below). This colocalization was never found to be the case for neurones and astrocytes (with one exception described below), or horizontal cells and astrocytes, even though astrocyte clones covered a greater cortical space and so would be more likely to coincide with other clones just by chance.

The number and colocalization of clones is shown in Table 1. About half of the clones were neuronal, about 15% were astrocytic and 14–27% were horizontal or horizontal plus neuronal. However, the variation here is large and probably reflects the arbitrary sample generated by the random retroviral infections. Clones could, with very few exceptions, be easily classified as to which types they belonged; we found no intermediate type clones.

As mentioned above, we found just one clone that contained neurones, astrocytes and horizontal cells. This is the clone in Fig. 3 that contained eight neurones. It is possible that this represents the superimposition of an astrocyte and a neuronal/horizontal cell clone. An alternative explanation, however, is that this is a single clone and that the infected cell was a pluripotential precursor, presumably the

<table>
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<th>Type of clone</th>
<th>AC08-5</th>
<th>AC08-6</th>
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<tbody>
<tr>
<td>Neuronal</td>
<td>2 (14%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>pyramidal alone</td>
<td>0</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>nonpyramidal alone</td>
<td>7 (50%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>pyramidal and nonpyramidal</td>
<td>0</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Horizontal</td>
<td>1 (7%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Horizontal and neuronal</td>
<td>1 (7%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Not identifiable</td>
<td>0</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Noncortical</td>
<td>3 (21%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>14</td>
<td>30</td>
</tr>
</tbody>
</table>

*Table 1. Types of clones found in two infected animals*
precursor of both the astrocyte and neuronal/horizon-
tal cell progenitors. This latter explanation seems
the more likely because of the large number of
neurones in the cluster. Eight is well above the
average number of neurones per clone, so if this
cluster is the result of superimposed clones, it has to
be proposed that at least three infective events were
involved – two neuronal/horizontal cell types and one
astrocyte type. This seems unlikely, but to resolve the
question we need to label earlier in development
when such pluripotential precursor cells will presum-
ably be more prevalent.

The distribution of neurones in clones
In addition to the question of cell lineage, we were
interested in how clonally related neurones were
distributed. Were related neurones arranged in
columns with the more recently generated neurones
found in more superficial locations or was some other
arrangement predominant? We found no evidence
for a columnar arrangement of clones. Related
neurones were sometimes in the same lamina and
sometimes in adjacent laminae. The two neurones
shown in Fig. 4A are fairly typical; one is in lamina II,
the other in lamina III. The horizontal distance
between them, medial to lateral (as indicated by the
dashed line) was 80 \( \mu \)m. Related neurones were often
as much as 200 \( \mu \)m apart in this dimension. Fig. 4B
shows two lamina III small pyramidal cells, that are
separated by 180 \( \mu \)m.

Both Fig. 4A and B show photomicrographs of
coronal sections, so the separation between the
related cells is in the mediolateral dimension. From a
direct observation of such data, we could not ascer-
tain whether clones could also be dispersed ortho-
gonal to the plane of section, in the rostrocaudal
dimension. To address this question, we performed
computer-aided 3-dimensional reconstruction of
serial sections (Shepherd et al. 1984). Fig. 5 shows a
reconstruction of a clone which contains both
neurones and horizontal cells. Fig. 5A shows a single
section containing three stained cells. The two cells
towards the ventricular surface were horizontal cells
and the third cell in the superficial cortical plate was a
lamina II nonpyramidal neurone. These were not the
only cells in this cluster, however, as can be seen in
Fig. 5B in which the whole series of sections from this
region has been superimposed. There were in fact
three neurones in the cortical plate region and nine
horizontal cells. Fig. 5C shows these same sections
rotated through 90° so that they are viewed in the
mediolateral direction. The three neurones are rep-
resented by the three upper most triangles, and the
cluster of triangles towards the left-hand edge of the

Fig. 4. The mediolateral spread of related neurones.
Both A and B show two related neurones that belong to
the same clone. In each case, the dashed line indicates
the distance between the labelled cells in the horizontal
dimension. In A that distance is 80 \( \mu \)m; in B it is 180 \( \mu \)m.
J. Price and L. Thurlow

Figure represent the horizontal cells. The section on the extreme right of the series—the most caudal—contained one neurone, the deepest neurone of the series, a lamina IV cell. Moving to the left through the series, i.e. rostrally, the two more superficial neurones are encountered, both in lamina II, and the series of horizontal cells which continue through a number of sections in this rostral direction. The two superficial neurones are in section numbers 3 and 8 and so are about 100 \( \mu m \) apart. The lamina IV neurone appears to be even further removed from these two cells, but this conclusion depends on the sections being precisely coronal, and it is hard to be certain that this is the case.

Discussion

We have introduced a retroviral lineage marker into rat cerebral vesicles on E16 and analysed the resultant clones on P14. Our findings allow us to make the following two principal conclusions:

1. We can identify two types of progenitor cell. One gives rise to astrocytes of grey matter; the other gives rise to both pyramidal and nonpyramidal neurones, and to cells of the nascent white matter, which we have called horizontal cells.

2. Clones of neurones do not take up a radial conformation. Related neurones can have a range of relationships one to another. They can be in the same lamina or in different laminae and they can be separated from each other horizontally by as much as 200 \( \mu m \). Walsh & Cepko (in press) have performed similar experiments and have also come to this conclusion. Luskin et al. (1987) in a preliminary report described clones as forming ‘interrupted columns that spanned several laminae’; this may or may not be consistent with our findings.

We will discuss these two conclusions separately.

Cell lineage in the cerebral cortex

The first of the above conclusions is based on the observation that we found two types of clone. One contained grey matter astrocytes, as shown by their blood vessel and pial end feet, and their small polymorphic cell bodies; the other contained neurones, either pyramidal or nonpyramidal, as indicated by their somatic morphology. In addition to neurones, about 20\% of this second type of clone

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**Fig. 5.** Computer-aided, 3-D reconstruction of a neuronal/horizontal cell clone. The solid lines represent the pial and ventricular surfaces of the cortex; the dashed lines in A represent the outline of the hippocampal formation. The triangles represent cells, the three towards the pial surface being neurones, those towards the ventricular surface being horizontal cells. (A) A single coronal section; the midline is at the left edge of the figure. (B) The series of superimposed coronal sections, from which that shown in A was taken. Note that from this perspective, all the cells in the clone occupy the same radial segment. (C) The same data as shown in B, rotated through 90° such that it is now viewed in the mediolateral direction. Some sections that had no stained cells have been omitted for clarity. The three triangles towards the top of the figure represent the neurones; those towards the bottom left represent the horizontal cells.
contained 'horizontal' cells. These were cells of varied morphology that were limited to the white matter, in which they were orientated horizontally, along the fibre tract. Their location suggests them to be glia; there are occasional neurones in this region (see, for example, Hendry et al. 1984), but they have already been generated at E16, the time of virus injection (Raedler & Raedler, 1978; Rickmann et al. 1977; Luskin & Schatz, 1985). Many of the horizontal cells were process-bearing cells that could have been fibrous astrocytes or oligodendrocytes, and some of them were bipolar cells, similar to the O-2A cells found in cultures of optic nerve (Raff et al. 1983). We think that the horizontal cells may be the cerebral equivalent of the 0-2A lineage.

We observed that the horizontal cells are sometimes found alone in clones, and sometimes with neurones, but that the majority of neuronal clones only contained neurones. This could be interpreted to mean that there are three progenitor cell types: one that gives neurones, one that gives horizontal cells and one that gives both. A simpler explanation is that there is one type of progenitor cell that gives neurones and horizontal cells. At E16, some of these progenitors are generating neurones, and will continue to do so for a few more cell cycles. This would be the source of the neurone-only clones. Some progenitors, however, will carry on dividing but will switch to the production of horizontal cells, generating mixed clones. The clones that contain only horizontal cells would be the result of infecting a progenitor that had already made that switch.

This explanation is supported by the type of clone that we have reconstructed in Fig. 5. It is likely that the lamina IV cell was the first cell to be generated, followed by the two more superficial neurones, then the horizontal cells. Thus, the cells are spread in the rostrocaudal axis as a result of the sequence in which they were generated (Fig. 5C). However, although we can be sure a common neuronal/horizontal cell progenitor exists, we cannot be sure of the exact sequence of neuronal and horizontal cell generation in these mixed clones.

Clearly marked clones of two or three cells often contained both pyramidal and nonpyramidal neurones. So this progenitor can generate more than one cell type, right up to its final few divisions. This is very similar to the situation in retina as described by Turner & Cepko (1987). In their case, a progenitor cell could give either rod photoreceptors, bipolar cells, amacrine cells or Müller glia, and a small clone could have a variety of combinations of these cell types. This suggests that these multipotential progenitors generate naïve postmitotic cells, which subsequently learn, by some interaction with their specific environment, what their fate is to be. There is evidence from studies of mutants in the mouse cerebral cortex (Caviness, 1982), and X-ray perturbation experiments (Jensen & Killackey, 1984), that neurones can achieve their appropriate cell fate despite failing to migrate to the correct site. This implies that the neurones knew their fate prior to migration although the possibility exists that the determination is postmigratory, and yet occurred in the aberrant location in which the ectopic neurones found themselves. In conjunction with our findings, these data suggest that neurones must acquire their identity during the period between their final mitosis and the early stages of histogenesis. This would most likely be the result of cell–cell interactions between the mitotic cells, immediately postmitotic cells, those cells that are already undergoing differentiation, and possibly incoming afferents. Examples of such mechanisms exist, probably the best defined being the Drosophila retina (Ready et al. 1986).

Throughout the above discussion, we have used the term 'progenitor cell'. We have preferred this to others such as 'stem cell', which may have been taken to imply a particular mode of division. We cannot tell, from these studies, whether the generation of neural cell types takes place by a symmetrical or an asymmetrical mode of division, or by some combination of both.

It is noteworthy that, in many neurones, the β-galactosidase was apparently localized almost exclusively in the nuclear region, presumably in the nucleoplasm, although this point could not be determined by light microscopy. In some neurones, and all glial cells, the staining was found throughout the cell. Most astrocytes were too small to say if the staining was nuclear as well as cytoplasmic, but in some cases the nucleus appeared unstained. Why neurones differ in their degree of cytoplasmic staining is unclear, but there was no correlation with morphological type – pyramidal cells, for instance, fell into both groups. Kalderon et al. (1984) in their study of nuclear localization signals observed that β-galactosidase was transported to the nucleus without any additional localization signal, so β-galactosidase probably contains a cryptic nuclear localization sequence. We do not know why this property should vary from cell to cell. Presumably the nuclear localization signal is a weak one, and as the cell produces more β-galactosidase, the protein tends to spill over into all the cytoplasmic processes.

Regarding our conclusions on cell lineage, two further points should be made. First, these conclusions require that we have correctly identified clusters of stained cells as clones. This point has been previously discussed (Price, 1987), but regarding this particular case, it should be noted that, at the infection density used, neuronal and astrocyte clones
never overlapped; only neuronal and horizontal cell clones colocalized. Thus, if this colocalization is the result of multiple infections, there has to be an explanation of why neurone and astrocyte clones are never colocalized.

The second point is that all the cell identification in this paper uses morphological criteria. This clearly has its limitations and we need to consider if the cells could have been misidentified. For some of the cell types, morphological criteria alone are inadequate. This is the case with the cells that here we have termed 'horizontal'. For the neurones and astrocytes, however, we are confident in our identification; these cell types have a distinctive morphology. In addition, there is circumstantial evidence supporting our major conclusion. For instance, the neuronal clones are largely limited to the supragranular layers, as would be predicted from thymidine birthdating studies (Berry & Rogers, 1965; Miller, 1986). If neuronal clones contained astrocytes, why are they also limited to these laminae, especially as they are not so limited in the putative astrocyte clones? Similarly, many of the cells we consider to be astrocytes, are unlikely to be neurones because they are in laminae, the neurones of which were already generated at the time of viral injection. Nonetheless, these studies would be enhanced if we had a means of immunohistochemically labelling the X-gal stained cells with cell-type-specific antibodies. Such experiments have technical problems, which we are trying to overcome.

Cortical histogenesis

In the 3-D reconstruction described above, it was clear that the related neurones had spread in the horizontal dimension by at least 100 µm. In the lateral dimension, the spread reached 200 µm (Fig. 4). In considering the implications of this result, two points should be made clear. First, it is difficult to be sure that we have found the upper limit of clonal spread. We have reconstructed relatively few clones, so 100 µm may not be the maximum possible rostrocaudal spread. In the mediolateral dimension, we operationally defined clusters of cells separated by more than 300 µm as separate clones, and the overwhelming majority of clones fell unequivocally within this limit. But in a very small number of cases, the possibility arose that neuronal clones had spread over more than 200 µm. Certainly, astrocyte clones spread more widely than this. We have not resolved the limit of astrocyte migration, but some astrocyte clones were spread over distances approaching a millimetre.

The second point is that, although clones spread both rostrocaudally and mediolaterally, there could still be barriers to horizontal movement. However, if barriers exist within the cerebrum, across which cell mixing does not occur, they would have to be at least 200 µm apart, or wider if all cell mixing, including glial cells, were to be included.

How do our findings fit with current theories of cerebral development? Anatomical and physiological studies from a variety of cortical regions and species have shown that the cerebrum is organized in a columnar fashion (see Mountcastle, 1978). Estimates of the column size have varied, but there is good evidence for columns of around 300–500 µm. Such columns have been defined physiologically in monkey, cat and rat visual cortex (Hubel & Wiesel, 1977; Dräger, 1975; Mangini & Pearlman, 1980), and somatosensory cortex of the rat and cat (Mountcastle, 1978; Carreras & Andersson, 1963; Armstrong-James, 1975; Welker, 1971). Anatomical studies have also suggested that columns of approximately this size are the 'modular units' of cortical organization (Woolsey & Van der Loos, 1970; Szentágothai, 1975; Mountcastle, 1978). Could columns of such dimensions also be developmental units, i.e. polyclonal compartments, the cells of which would not mix with those of other compartments? Our data would fit such a concept only if glial cells were excluded from consideration, an arbitrary limitation that seems unlikely to us.

In the visual cortex, orientation columns have been described (Hubel & Wiesel, 1977) that are 30–50 µm wide. Our data would preclude a clone being limited to such dimensions, but it is not clear in any case what the anatomical basis of this repeat unit is (Szentágothai, 1975), nor is it clear how widespread such units are. They are not thought to occur in the rodent visual cortex (Dräger, 1975; Mangini & Pearlman, 1980).

Several models of cerebral development have predicted strictly radial modes of neuronal migration (Rakic, 1978, 1988; Smart & McSherry, 1982), and Rakic has proposed that the radial glial scaffold maintains an exact radial movement (Rakic, 1978). What Rakic observed, and we have confirmed (data not shown), is that young neurones migrate in columns through the intermediate zone. It was tempting to suppose that such a developmental column was a clone (or a polyclone, Rakic, 1988), and that by retaining its radial orientation, it became a functional column in the adult animal. Our results do not preclude developmental columns forming the basis of physiological columns. However, our data make it unlikely that the cells of a developmental column are clonally related.

There is a body of data, both anatomical and physiological, that suggests that the columnar organization of the cerebrum is plastic and dependant on its afferent input (for example, see Hubel & Wiesel, 1977). This is reflected in the correlation between the degree of columnar organization of different regions
of cortex, and the nature and extent of their input (Rockel et al. 1980). Columns can even be generated in the frog optic tectum, in which they are not normally found, by the experimental manipulation of its afferent input (Constantine-Paton & Law, 1978; Fawcett & Willshaw, 1982). Thus, how clones of neurones distribute within the cortical plate initially may have no bearing on their organization into columns. If afferent input does organize cortex into columns, what organizes the distribution of afferent fibres? Cooper & Steindler (1986) have suggested that glial cells may delineate the cortical somatosensory columns relatively early in corticogenesis. Perhaps in this way, glial cells provide the scaffold into which the other cortical components are fitted.

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References


or an oligodendrocyte depending on the culture medium. Nature, Lond. 303, 390–396.


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