A Gradient in the Duration of the G1 Phase in the Murine Neocortical Proliferative Epithelium

Neuronogenesis in the neocortical pseudostratified ventricular epithelium (PVE) is initiated rostrolaterally and progresses caudomedially as development progresses. Here we have measured the cytokinetic parameters and the fractional neuronal output parameter, Q, of laterally located early-maturing regions over the principal embryonic days (E12–E15) of neocortical neuronogenesis in the mouse. These measures are compared with ones previously made of a medial, late-maturing portion of the PVE. Laterally, as medially, the duration of the neuronogenetic interval is 6 days and comprises 11 integer cell cycles. Also, in both lateral and medial areas the length of G1 phase (T\textsubscript{G1}) increases nearly 4-fold and is the only cell cycle parameter to change. Q progresses essentially identically laterally and medially with respect to the success of integer cell cycles. Most importantly, from E12 to E13 there is a steeply declining lateral to medial gradient in T\textsubscript{G1}. The gradient is due both to the lateral to medial graded stage of neuronogenesis and to the stepwise increase in T\textsubscript{G1} with each integer cycle during the neuronogenetic interval. To our knowledge this gradient in T\textsubscript{G1} of the cerebral PVE is the first cell biological gradient to be demonstrated experimentally in such an extensive proliferative epithelial sheet. We suggest that this gradient in T\textsubscript{G1} is the cellular mechanism for positionally encoding a protomap of the neocortex within the PVE.

Introduction

The neocortex arises from a pseudostratified ventricular epithelium (PVE) which borders the ventricular cavity deep within the embryonic cerebral vesicles (for references see Takahashi et al., 1993, 1995a). The appearance of this epithelium is homogeneous throughout, revealing nothing of the neocortical architectonic diversity to which it will give rise. Several lines of evidence suggest that, despite the homogeneity of its appearance, substantial regional specification, and even a detailed neocortical ‘protomap’ (Rakic, 1988), are already present in the PVE even at a time before most neurons have been produced. For example, at a coarse-grained level of resolution, there are delimited patterns of early gene expression within the PVE which appear to recognize major subdivisions of the forebrain (Bulfone et al., 1993; Puelles and Rubenstein, 1993; Shimamura et al., 1995). Further, there appear to be barriers to the migration paths that neurons arising from the PVE follow at interfaces of neocortical and striatal PVE (Fishell et al., 1993). Within the neocortical anlagen itself, differences in the density of the proliferative population and differences also in cell cycle kinetics in adjacent regions of the PVE appear to anticipate the final differences in neuronal density which will be characteristic of the adjacent areas 17 and 18 (Dehay et al., 1993).

If neocortical regional specification is encoded within the proliferative population of the PVE, it is reasonable to expect that the mechanisms of regional encoding will be regulated coordinately with the mechanisms regulating the proliferative process. Some insight into the possible cellular basis for such encoding has been provided by previous work showing that neuronogenesis is initiated non-synchronously across the neocortical PVE. With respect to the neocortical epithelium, the first neurons to be formed arise far rostrolaterally near the striatum. In time the process sweeps rostrocaudally and lateromedially (Hicks and D’Amato, 1968; Caviness and Sidman, 1973; Sidman and Rakic, 1973; Fernandez and Bravo, 1974; Bisconte and Marty, 1975a,b; Smart and McSherry, 1982; Smart and Smart, 1982; McSherry, 1984; Luskin and Shatz, 1985; McSherry and Smart, 1986; Bayer and Altman, 1991).

This long-recognized feature of neocortical neuronogenesis tantalizingly suggests that some graded process might be operating in the PVE which would be suitable to a positional encoding morphogenetic role. We report here an analysis in the PVE of mouse which is concerned with a search for spatially graded parameters of cell proliferation that might serve such a morphogenetic role. The parameters to be considered are the duration of the cell cycle (T\textsubscript{C}) and its phases, the growth fraction (GF), and P and Q (Takahashi et al., 1993, 1994, 1995a, 1996b). P corresponds to the fraction of postmitotic cells which ‘elect’ to remain in the PVE and to sustain the proliferative pool. Q, the complement of P, corresponds to the fraction which quits the PVE as permanently postmitotic neurons. The full set of proliferative parameters has already been measured for the PVE in the dorsomedial murine neocortical cerebral wall, a region which lies near the late-maturing caudomedial pole (Takahashi et al., 1993, 1994, 1995a, 1996b). In the present analysis, these parameters will be determined for comparison at more laterally positioned regions of PVE, i.e. at regions which are early maturing. The experimental goal of the analysis is to characterize the lateral to medial differences in the proliferative process with the expectation that the characterization will reveal a gradient in neuronogenetic process. A longer-term objective is to formulate testable hypotheses relating to the larger developmental significance of the spatially graded proliferative process in the neocortical PVE, specifically as the graded process may serve a positional encoding, i.e. a neocortical mapping function.

Materials and Methods

Animals and Histology

CD1 mice were maintained on a 12 h (7:00 a.m.–7:00 p.m.) light–dark schedule. Conception was ascertained by the presence of a vaginal plug, with the day of conception considered to be embryonic day 0 (E0). Plug-checks were conducted at 9:00 a.m. For histological processing, embryos were removed by hysterotomy from deeply anesthetized dams, the brains sectioned coronally at 4 µm, and the sections processed for bromodeoxyuridine (BrdU) immunocytochemistry and autoradiography as previously described (Takahashi et al., 1992, 1993, 1994).

Experimental Design

There are two general experimental designs. Experimental design 1 is concerned with the cell cycle parameters, i.e. T\textsubscript{C} and the duration of cell...
cycle phases, G1 ($T_{G1}$), S ($T_s$), G2 and M and measures of GF. Experimental design 2 is concerned with estimation of $P$ and $Q$. Experimental designs 1 and 2 are identical to those previously used in analyses of proliferative behavior of the dorsomedial PVE and secondary proliferative population (SPP) (Takahashi et al., 1993, 1994, 1995b, 1996b) and will be reviewed only in outline here.

The full analysis in lateral (LCZ, Fig. 1) and a more limited analysis of $T_{G1}$ in intermediate (ICZ) cerebral zones were undertaken at approximately midhemisphere along the rostrocaudal axis of the hemisphere (Figs 1–3). These zones were located at the same coronal levels as the dorsomedial cerebral zone (DCZ, Fig. 1) studied in prior analyses (Takahashi et al., 1992, 1993). Quantitative analyses in LCZ and ICZ were undertaken in sectors of the ventricular zone (VZ) which were 100 µm in the mediolateral dimension and 4 µm (corresponding to section thickness) in the rostral-caudal dimension. The sector was divided in its radial dimension into bins 10 µm in height, and the bins numbered 1, 2, 3, and so on from the ventricular margin (Takahashi et al., 1992, 1993). Calculations were performed with Microsoft Excel with the exception of integer cell cycle calculations which were performed by numerical integration using Mathcad.

**Cell Cycle Parameters (Experimental Design 1)**

Pregnant mice were injected i.p. with BUdR (Sigma; 50 µCi/g body wt) at 9:00 am on each of E11–E15 (Takahashi et al., 1995a). The injections given at 9:00 am were repeated at 3 h intervals until the proportion of labeled nuclei (labeling index, LI) in the VZ reached a maximum (Takahashi et al., 1992, 1993). The $T_{G1}$ and $T_s$ are estimated from the slope of the progression of LI with time and its intercept. The combined length of G2 and M phases ($T_{G2+M}$) was determined as the time required for all mitotic figures at the ventricular margin to become labeled. $T_{G1}$ was estimated as $T_{G1} = (T_s + T_{G2+M})$ (Takahashi et al., 1993). The GF corresponds to the fraction of cells labeled where LI is at its maximum value. Measurements of cell cycle kinetics and GF were based upon analysis of brains of four embryos for each time point at each age.

**Q and P Fractions (Experimental Design 2)**

The determination of $Q$ and $P$ for the PVE, as previously described (Takahashi et al., 1994, 1996b), requires an initial set of analysis through which the total proliferative pool of the cerebral wall is partitioned into its PVE and SPP subpopulations. A second set of analyses is required to measure the fraction of $Q$ or $P$ for the respective PVE and SPP subpopulations.

**PVE–SPP Partition**

The majority of cells of the SPP, variably >80% depending upon the embryonic day (Takahashi et al., 1994, 1995b, 1996b), are distributed through the intermediate (IZ) and subventricular (SVZ) zones of the cerebral wall overlying the VZ. This larger component of the SPP population is spatially distinct and readily distinguished from the PVE. A small component of the SPP, variably 10–20%, lies within the outer 1–2 bins of the VZ and must be distinguished experimentally from cells of the PVE for determination of $Q$ and $P$ for PVE. Partition of PVE and SPP is enabled by the fact that cells of the PVE, but not those of the SPP, undergo interkinetic nuclear migration in the course of the cell cycle (Sauer, 1935; Takahashi et al., 1995a).

The cells in S phase of both PVE and SPP are labeled initially by i.p. injection into pregnant dams of tritiated thymidine ($[^3]H$)TdR, 5 µCi/g body wt) at 7:00 a.m. on each of the embryonic days E11–E15. An hour after the $[^3]H$TdR injection, i.e. at 8:00 a.m., cells in S phase are exposed to BUdR (50 µg/g body wt), with the result that only those nuclei that were still in S phase in the 7:00–8:00 a.m. interval were labeled only with $[^3]H$TdR ($1 h$ cohort). Because of interkinetic migration, the nuclei of cells of the PVE labeled in S phase with $[^3]H$TdR will shift inward toward the ventricular margin as they advance through G2 into prophase (for references see Takahashi et al., 1996a). Nuclei of cells of the SPP, in contrast, will not shift inward toward the ventricle. To some extent, though not completely, the displacement of the nuclei of the PVE portion of the 1 h cohort toward the ventricle enables classification of cells of the cohort within the VZ as belonging to either the PVE or SPP. A separation of populations (i.e. PVE and SPP) was done by using a least-squares fit of two (or more) normal distributions to a bimodal (or multimodal) histogram. This method provided an objective and unbiased estimate of the size and mean position of separate populations whether or not there was overlap in their distributions. The steps required for separating the two populations are explained fully in the Results section, as they are used.

**Measurement of Q and P**

For measurement of $Q$ and $P$ a different set of embryos was used from those used for separating PVE cells from the component of SPP cells. In this experiment, proliferating cells of the embryonic cerebral wall were also exposed sequentially to the S-phase markers $[^3]H$TdR and BUdR, but the interinjection interval was 2 h rather than 1 h. Two separate schedules of $[^3]H$TdR injection followed in 2 h by either single BUdR or sequential BUdR injections make possible the experimental determination of separate values for the numbers of $Q$ and $P$ cells ($N_{Q,P}$) and the numbers of $Q$ cells ($N_Q$) in a primary 2 h cohort of proliferative cells (Takahashi et al., 1996b).

Cells labeled only with $[^3]H$TdR were scored after $T_s - T_i$ with respect to their bin location in the cerebral wall. Data was collected from the standard coronal sector from the brains of 16 embryos at each age: eight brains (four brains from each of two separate litters) were used to obtain $N_{Q,P}$ and eight brains (four brains from each of two separate litters) were used to obtain $N_Q$. The number of $[^3]H$TdR-only labeled cells was counted on six non-adjacent sections for each brain; then the average and SEM values for each set of eight embryos were calculated to obtain $N_{Q,P}$ and $N_Q$ for each day of the neurogenetic interval (Takahashi et al., 1994). The number of $[^3]H$TdR-only labeled cells per bin was computed by first
averaging the values obtained from six non-adjacent sections from single brains and then averaging the values obtained from eight brains.

Results

Cerebral Stratification

Neuronogenesis is initiated rostrolaterally in the PVE and progresses caudomedially in time. Histogenetic consequences are demonstrable by virtue of the relative maturity of the cerebral wall in the LCZ and the DCZ during the developmental period. In both the LCZ and the DCZ the individual strata increase in width over the course of the neuronogenetic interval, but there is a clear and orderly lateral to medial progression in the state of maturity. The LCZ of the cerebral wall of mouse at E11 has two architectonic strata, the relatively wide VZ and a narrow overlying primitive plexiform zone (Figs 2 and 3). Subsequently, in the interval between E13 and early E14, the full late embryonic stratification pattern emerges including from the ventricular surface outward: VZ, SVZ, IZ and the cortical strata. The cortical strata, from deep to superficial, are subplate, cortical plate and molecular layer. The time of this architectonic transition in the LCZ (late E13) is ∼24 h in advance of its occurrence in the DCZ (late E14, Takahashi et al., 1996b). It should be noted that during the entire interval from E11 to E16, the PVE is approximately coextensive with the VZ throughout the neocortical neuronogenetic interval (Takahashi et al., 1993, 1994). However, in contrast to other strata of the cerebral wall, there is no apparent lateral to medial gradient in the cytoarchitectonic appearance of the PVE (Fig. 4).

Cell Cycle Parameters

The results of the BuDR cumulative labeling (experimental design 1) during the E11–E15 interval are shown in Figure 5. At each age the LI maximizes at or near 1.0, i.e. the apparent GF of the PVE is constant at or near 1.0 throughout the neuronogenetic interval. $T_c$ in the LCZ increases from 9.5 h at E11 to 21.0 h at E15 (Table 1, Fig. 6). $T_S$ is ∼4 h during this interval with only small and unsystematic variations through the interval. In contrast, $T_G_1$ increases >3-fold from 3.4 h at E11 to 12.5 h at E15 (Table 1, Figs 6, 10A, C). Between E11 and E14 all mitotic figures are labeled within 2.0 h and on E15 within 2.5 h, indicating that the combined length of G2 and M phases is constant at 2.0 h through E14 but somewhat longer on E15 (Takahashi et al., 1994, 1995a). Thus, prolongation of the cell cycle over the neuronogenetic interval essentially reflects prolongation of G1 phase (Fig. 6). $T_G_1$ was determined in ICZ on E12 and E13, i.e. the embryonic dates on which the values of $T_G_1$ in DCZ and LCZ were substantially different. On both E12 and E13, $T_G_1$ in the ICZ was intermediate between $T_G_1$ in the DCZ and LCZ (Table 1). The earlier determinations of cell cycle parameters of the PVE for the DCZ (Takahashi et al., 1995a) are included in Table 1 for direct comparison with parameters in the LCZ and ICZ.
Figure 4. The murine cerebral wall during the neuronogenetic period. At E11 (A,B) the cerebral wall is stratified only as ventricular (VZ) and primordial plexiform (PPZ) zones. By E13 (C,D), the molecular layer (ML), cortical plate (CP) and intermediate zone (IZ) have formed within the PPZ of the lateral cerebral zone (LCZ) but not in the dorsomedial cerebral zone (DCZ). At E15 (E,F) the stratification of DCZ and LCZ includes ML, CP, subplate (SP), IZ, subventricular zone (SVZ) and VZ. The growth pattern of strata in the DCZ (A,C,E) (Takahashi et al., 1995a) is similar to that of the LCZ (B,D,F) but lags behind that of the LCZ by ∼24 h. The width but not the architectonic appearance of the VZ in DCZ and LCZ change through the interval E11–E15. Coronal plane, scale bar = 100 µm.

Figure 5. The progression of the LI of the PVE with cumulative labeling with BUdR. Cumulative exposure to BUdR was initiated at 9:00 a.m. on each of the days E11–E15. With each plot, the maximum LI provides an estimate of the growth fraction. The time at which the maximum LI is reached is equal to the length of the cell cycle minus the length of S phase \( T_C - T_S \), and the y-intercept is equal to \( T_S/T_C \). Although the slope of LI progression was obtained by a least-squares fit considering all of the data for each of the time points (Takahashi et al., 1993), only the mean values for each time point are shown here. SEM are <5% of the mean value for each point in the full series of experiments.

Figure 6. The progression in the lengths of the cell cycle phases of the PVE. The length of the overall cell cycle \( T_C \) is approximately doubled over the interval E11–E15, with this increase due essentially to increase in the length of G1 phase \( T_{G1} \). There is no systematic change in either the length of S phase \( T_S \) or of the combined G2 and M phases \( T_{G2+M} \) over the same interval.
Q and P of the PVE of the Lateral Cerebral Zone

Partition of the Entire Proliferative Population into PVE and SPP

The distribution (per 10 µm bin, see Materials and Methods) of the combined PVE and SPP populations in G2-prophase (\([3H]\)TdR-only labeled cells of the 1 h cohort) is plotted for E12–E15 in Figure 7. At each age such cells are located in all bins of VZ, SVZ, and IZ. Within the overall distribution, there are three subdistributions signaled by nadirs in bin 5 and variably in bins 7–11. An inner, or juxtaventricular, subdistribution extends through bins 1–5 (bin 5 marks, approximately, the median bin of the PVE as determined in the cumulative labeling experiments). This is the largest of the three subdistributions. It corresponds to a ‘pure culture’ of PVE cells swept toward the ventricle as a result of the interkinetic nuclear movement in G2 phase (Takahashi et al., 1995b, 1996a).

An intermediately positioned subdistribution extends through the outer VZ to overlap the SVZ. This subdistribution, substantially smaller than the other two, is a mix of PVE cells which are in early G2 phase before nuclear displacement (and hence still located within the outer half of the PVE) and SPP cells in G2-prophase. An outer subdistribution is located entirely superficial to the VZ–SVZ boundary and confined to the SVZ–IZ. It represents cells in G2-prophase of the SPP (Takahashi et al., 1995b).

Best-fit curves to the inner and outer subdistributions, assuming normal distributions, provide estimates of the relative contributions of PVE and SPP to the intermediate subdistribution in the outer VZ (see text).

Q and P Fractions of the PVE

The total number of PVE cells in the 2 h cohort, including both the cells of the P and Q fractions \(N_{P+Q}\) for PVE, Table 2(4), is estimated from its fractional contribution \(N_{P} / N_{VZ}\) to the total 2 h cohort \(N_{VZ}\) (Table 2(2)). The number of P fraction cells for the PVE \(N_{P}\) for PVE) is estimated primarily as a range \(N_{P} = \text{maximum number assumes that all P fraction cells within the VZ belong to the PVE. The minimum value assumes that all cells in the outer bin of the VZ belong to the SPP. We have taken as our estimate of NP for PVE, the median of the maximum and minimum estimates [Table 2(5)], realizing that this must involve an error of a few percentage points at most. P

Table 1
PVE cell cycle parameters

<table>
<thead>
<tr>
<th>Age</th>
<th>TC (h)</th>
<th>IC (h)</th>
<th>LC (h)</th>
<th>TC (h)</th>
<th>IC (h)</th>
<th>LC (h)</th>
<th>TC (h)</th>
<th>IC (h)</th>
<th>LC (h)</th>
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<tr>
<td>E11</td>
<td>8.1</td>
<td>–</td>
<td>9.5</td>
<td>2.8</td>
<td>–</td>
<td>4.2</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>E12</td>
<td>10.2</td>
<td>10.7</td>
<td>11.3</td>
<td>4.9</td>
<td>4.5</td>
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<td>2</td>
</tr>
<tr>
<td>E13</td>
<td>11.4</td>
<td>12.1</td>
<td>14.1</td>
<td>3.9</td>
<td>3.9</td>
<td>3.4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E14</td>
<td>15.1</td>
<td>–</td>
<td>18.1</td>
<td>3.8</td>
<td>–</td>
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<td>E15</td>
<td>17.5</td>
<td>–</td>
<td>21.0</td>
<td>3.7</td>
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<td>6.0</td>
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<td>18.4</td>
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<td>–</td>
<td>–</td>
<td>2</td>
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Figure 7. The distribution of the combined PVE and SPP populations in G2-prophase \(\([3H]\)TdR-only labeled cells of the 1 h cohort) for E12–E15. The analysis is undertaken in a coronal sector of the lateral cerebral zone (LCZ) which is 100 µm in its medial to lateral dimension and 4 µm (corresponding to section thickness) in its rostral to caudal dimension. The sector is divided in its radial dimension into bins 10 µm in height, and the bins are numbered 1, 2, 3, and so on from the ventricular margin outward (Takahashi et al., 1992, 1993). There are inner (PVE), intermediate (mixed PVE and SPP) and outer (SPP) subdistributions spanning VZ and overlying cerebral wall and signaled by nadirs in bin 5 and variably in bins 7–11. Best-fit curves to the inner and outer subdistributions, assuming normal distributions, provide estimates of the relative contributions of PVE and SPP to the intermediate subdistribution which corresponds to the outer VZ [see text].

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progression of $T_c$ and $T_{G1}$ is extrapolated to these times of onset and termination, we derive a range for the two of 8.0–19.3 h and 3.0–12.5 h respectively, and determine, further, that the neurongenic interval in the LCZ is divisible (using numerical integration) into approximately 11 integer cell cycles (precisely 10.9 cycles; see also Takahashi et al., 1995a). That is, for both LCZ and DCZ the neurongenic interval is divisible into 11 integer cell cycles.

**Comparative Proliferative Behavior in LCZ and DCZ**

Although the overall neurongenic interval is ~6 embryonic days and divisible into 11 integer cell cycles in both the LCZ and DCZ of the PVE, the process in the LCZ at any time during the neurongenic interval is in advance of that in the DCZ. If progression through the neurongenic interval in LCZ and in DCZ are transformed with respect to integer cell cycle, graphs of the progression of both $T_{G1}$ and $Q$ are observed to be closely similar for the LCZ and DCZ (Fig. 10C,D). Even though the progression in both $T_{G1}$ and $Q$ per integer cell cycle are approximately identical in the LCZ and DCZ, the embryonic date of occurrence of a given integer cycle is advanced ~24 h in the LCZ relative to the DCZ. Thus, at any moment of the neurongenic interval, $T_{G1}$ and $Q$ are distributed as gradients across the lateral to medial axis of the PVE. The lateral to medial gradient in $T_{G1}$ is relatively flat at E11–E12, early in the neurongenic interval, and increases sharply between E12 and E13 (arrow in Fig. 10A). It flattens again after E14 as $T_{G1}$ approaches asymptote in both the LCZ and DCZ. In contrast, the steepness of the lateral to medial gradient in $Q$ is relatively invariant throughout the entire neurongenic interval (Fig. 10B).

**Discussion**

**Uniformity of Neurongenesis throughout the PVE**

For almost 30 years it has been known that neocortical neurongenesis is initiated rostrilaterally but then spreads caudomedially across the surface of the cortex, and that during the ensuing days neurons produced at the same time but in different medial to lateral positions have different fates (Hicks and D’Amato, 1968; Caviness and Sidman, 1973; Sidman and Rakic, 1973; Fernandez and Bravo, 1974; Bisconet and Marty, 1974b; Smart and McSherry, 1982; Smart and Smart, 1982; McSherry, 1984; Luskin and Shatz, 1985; McSherry and Smart, 1986; Bayer and Altman, 1991). However, at the time that these differences in neurongenesis exist the cytoarchitectonic appearance of the proliferating cells in the PVE is essentially identical regardless of where in the hemisphere or when during

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**Table 2**

<table>
<thead>
<tr>
<th>Age</th>
<th>$N_{1+Q}$</th>
<th>%PVE</th>
<th>$N_{1+Q}$ for PVE</th>
<th>$N_{1+Q}$ for PVE</th>
<th>$N_{1+Q}$ for PVE</th>
<th>$N_{1+Q}$ for PVE</th>
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<tbody>
<tr>
<td>E12</td>
<td>11.06</td>
<td>92</td>
<td>10</td>
<td>6.96–7.98</td>
<td>7.5</td>
<td>0.74</td>
</tr>
<tr>
<td>E13</td>
<td>14.17</td>
<td>86</td>
<td>12</td>
<td>5.73–6.79</td>
<td>6.3</td>
<td>0.52</td>
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<tr>
<td>E14</td>
<td>13.00</td>
<td>83</td>
<td>11</td>
<td>2.94–3.21</td>
<td>3.1</td>
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<td></td>
<td></td>
<td></td>
<td>2.29–2.88</td>
<td>2.6</td>
<td>0.23</td>
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<table>
<thead>
<tr>
<th>Range</th>
<th>Median</th>
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</table>

Experiments were initiated at 9:00 a.m. on each of gestational days E12–E15 (column 1). The PVE as percentage of the total proliferative population of the cerebral wall (column 3) is estimated by a curve-fitting strategy (Fig. 8 and text) from the 1 h cohort experiment. The total number of PVE cells in the 2 h cohort, including those in the P and Q fractions ($N_{1+Q}$, column 4), is derived as the product of column 3 and the total value for $N_{1+Q}$ for the entire 2 h cohort including cells of both PVE and SPP (column 2). The number of P fraction of the PVE of the 2 h cohort ($N_P$) is estimated as a range and as the median of this range. The maximum value of the range is the total number of P fraction included in all bins estimated to contain PVE cells, while the minimum value of the range is the total number of P fraction cells included in 1 less bin. The median is the average of the maximum and minimum values. $P$ (column 6) is the complement of $P$ fraction ($1 - P$).
the neuronogenetic interval it is examined. Here we have shown that the homogeneous appearance of the PVE masks underlying ‘behavioral’ differences in the proliferating cells that comprise it and that the ‘behavior’ of the PVE cells closely corresponds to, but presages, the differences in maturation that will appear in the overlying cortical plate as development proceeds. The basic finding of our analysis is that within the PVE the proliferative processes associated with neuronogenesis are initiated sequentially, i.e. from lateral areas (LCZ) to medial areas (MCZ), across its tangential dimension, but once initiated, the processes proceed essentially identically across the lateral to medial axis of the PVE. Thus, independently of cortical position, i.e. both in DCZ or LCZ, neuronogenesis occurs over the course of 11 integer cycles during a period of 6 days. The 6 day period and the first of the 11 cell cycles are initiated first in the LCZ. Then there is an ~24 h delay and almost three cell cycle lag between the time of initiation of neuronogenesis in the LCZ and the onset of neuronogenesis in the DCZ. Intermediate cortical areas have neuronogenesis initiated at an intermediate time. Also independent of cortical position, i.e. whether in DCZ or LCZ, $T_C$ and $Q$ advance essentially indistinguishably with each succeeding integer cycle. Moreover, the pattern of advance of $T_C$ in both DCZ and LCZ is due almost completely to the increase in $T_{G1}$, and the estimated value of the GF is close to 1.0. Earlier analyses by Waechter and Jaensch (1972) in rat and of Schmahl (1983) in mouse were the first to establish that the lengthening of $T_C$ reflected a lengthening of $T_{G1}$. Schmahl (1983) had also detected medial to lateral differentials in $T_{G1}$ through the interval E11–E16 (conception = E0) but the values laterally were greater than those medially at E15 and E16 but less than those medially at E11 and E12.

**PVE Gradients in $T_{G1}$ and $Q$**

The combination of the staggered onset of progression through the neuronogenetic interval in LCZ and in DCZ and the similarity of progression in cytokinetic parameters once neuronogenesis is initiated results in a development of gradients across the surface of the PVE. To a considerable extent, the development of the gradients and changes in the steepness of the gradients are a result of the precision of the progression of the cytokinetic parameters. The precision of the similarity of the cytokinetic parameters in the LCZ versus the DCZ can be appreciated if they are plotted with respect to integer cell cycle. On such a plot (Fig. 10C,D), the progression of $T_{G1}$ and of $Q$ in LCZ are observed to be essentially identical to the progression of $T_{G1}$ and of $Q$ in DCZ. This demonstrates that $T_{G1}$ and $Q$ are the developmentally regulated parameters of the proliferative process and that the regulation of these parameters is independent of time but dependent on the state of maturation of the PVE. When the same data are plotted as a function of time (i.e. not transformed with respect to elapsed integer cell cycle number) the developmental gradients within the PVE are revealed. Thus, on E12–E13 the

**Figure 9.** The distributions of cells of the $P$, $Q$ and $P + Q$ fractions for the total PVE and SPP across the cerebral wall in the LCZ on each of the days between E12 and E15. The analysis is undertaken in a coronal sector of the cerebral wall which is 100 $\mu$m in its medial to lateral dimension and 4 $\mu$m (corresponding to section thickness) in its rostral to caudal dimension. The sector is divided in its radial dimension into bins 10 $\mu$m in height, and the bins are numbered 1, 2, 3 and so on from the ventricular margin (Takahashi et al., 1992, 1993). The double-headed horizontal arrow in each plot indicates the height of the ventricular zone (VZ) at each age. The numbers of cells in the $P$ ($N_P$) and in the $P + Q$ fractions ($N_{P+Q}$) for each bin are determined as described in Materials and Methods. The values for $Q$ fraction cells ($N_Q$) in each bin are estimated as $N_{P+Q} - N_P$. The bins, 10 $\mu$m in height, are the $x$-axis. At E12, SEM is <20% of the value when the value is >1.3; >1.0 at E13; >0.8 at E14; and >1.5 at E15.
The existence of a PVE gradient in $T_{G1}$ is a reflection only in part of the rostromedial to caudomedial temperospatial delay in the onset of neuronogenesis. Critical to the existence of this gradient is also the fact that $T_{G1}$ advances with each successive integer cycle. Thus, were $T_{G1}$ invariant throughout the neuronogenetic interval there would be no gradient in $T_{G1}$ despite the rostromedial to caudomedial temperospatial delay in the onset of neuronogenesis. It is further of note that the gradient in $T_{G1}$ might not exist or it might have a radically different character if $T_{G1}$ varied unsystematically rather than systematically with precise progression with each integer cell cycles. The forceful implication here is that a systematic pattern of $T_{G1}$ advance with successive integer cycles is critical to the existence of the gradient in $T_{G1}$ and the pattern of $T_{G1}$ advance with progression of integer cycle is a critical determinant of the character of the PVE gradient in $T_{G1}$.

The $T_{G1}$ Gradient and the Proliferative Process

The slope of the $T_{G1}$ gradient is gentle both early and late but steep midway through the histogenetic process (Fig. 11). The actual mechanisms through which it is regulated, those which initiate and impose the rostromedial to caudomedial time delay in initiation of neuronogenesis across the PVE and those which determine the advance of $T_{G1}$ with integer cycle, are currently unknown. However, the apparent precision and coherency of this gradient suggests that it is regulated by cell-cell communication and is not a manifestation of independent events regulated cell autonomously in each cell of the PVE. Whatever the regulatory mechanisms, the gradient in $T_{G1}$ may itself serve a central regulatory histogenetic role. We hypothesize that the gradient in $T_{G1}$ is a morphogenetic gradient in the sense that it would be suited to serve those histogenetic processes which require the cell to ‘know its position’ within the proliferative developmental field (Goodwin and Cohen, 1969; Wolpert, 1969, 1981; Meinhardt, 1982; Nusslein-Volhard, 1996). Specifically, we suggest that the gradient in $T_{G1}$ may play a unique, positionally encoding histogenetic role for specification of the topology of the neocortical architectonically encoded neural systems map.

Position Encoding: An Oscillatory Frequency Shift Model

The existence of a gradient in $T_{G1}$ across the neocortical PVE means that this proliferative epithelium has the properties of a classic developmental field (Goodwin and Cohen, 1969; Wolpert, 1969, 1981; Meinhardt, 1982; Nusslein-Volhard, 1996). In this context the PVE is a boundary-delimited epithelial sheet with two tangential dimensions, one rostral-to-caudal and the other medial-to-lateral, with $T_{G1}$ varying along each dimension. Importantly, at any moment within the boundaries of this
development of the mammalian central nervous system within the framework of classical developmental field theory. In this instance the field gradient corresponds to a shift in the frequency of the cell cycle oscillation (i.e. $T_e$ or $T_{G1}$) and thus is akin to the cell cycle oscillatory phase shift model originally proposed as a positional encoding mechanism in invertebrates by Goodwin and Cohen (1969).

The significance of field theory is that it introduces a cell biological mechanism by which each cell arising within the field, in this instance the neocortical PVE, would be instructed by its unique positional value to elect a specific path of differentiation among a larger range of paths which it would be competent to follow (Wolpert, 1969, 1981). Neighboring cells would have similar positional values and, hence, would adopt similar cell and molecular biological paths of differentiation, while cells in other regions of the field would be instructed by their different positional values to adopt different positionally encoded cell and molecular biological paths of differentiation. In this way, patterns of regional differentiation would be instructed to emerge within an epithelium which would have been homogeneous from before the outset of neuronogenesis (Goodwin and Cohen, 1969; Wolpert, 1969, 1981; Meinhardt, 1982). The specific qualities to differentiate within and to characterize the various regions of the field would be inherent in the different paths of differentiation assigned by positional values to the cells of the field. We suggest that cell biological mechanisms that regulate $T_{G1}$ are coordinate with or the same as those which determine a combinatorially interactive profile of activation of transcription, which then directs instantiation of the topology of the neocortical map. That is, we suggest that the temperospatial information inherent in the $T_{G1}$ gradient acts 'upstream' in an instructive positional sense from the transcriptional array that confers the specific histotypic character of each neocortical map subdivision. With respect to cell cycle regulation, the direction of information flow suggested here is opposite to that suggested for mapping of body topology in Drosophila (Foe, 1989; Foe et al., 1993). In Drosophila, mechanisms regulatory to G2, specifically those governing zygotic transcription of the cdc2 phosphatase, cdc25string, have been suggested to be interpreters of a body topology already instantiated within a patterned distribution of gene expression already established in the course of blastoderm development (Edgar and O’Farrell, 1989; Follette and O’Farrell, 1997).

We suggest that the encoding mechanism should operate across the expanse of the PVE to set mapping fates at a specific stage of the neocortical development. Encoding would occur with respect to the proliferative population. The Q cells arising ‘postencoding’ would be the ‘interpreters’ of the map and would translate it to the neocortex. Orderly topological translation would require that neighborhood relationships of postmigratory cells approximate those of the progenitors of the Q fraction cells. We suggest that there are two stages during neocortical development when the mapping fates might be ‘set’. First, this might occur in the course of integer cell cycle 8 because this is specifically when $Q = P = 0.5$ both for MCZ (Takahashi et al., 1996b) and LCZ (present study). This is the ‘moment’ (which would sweep as a wave rostrolaterally to caudomedially) when the tangential dimension of the PVE has reached its maximum (Takahashi et al., 1996b), and thus when the PVE might be considered first to become homeomorphic (i.e. one-to-one mappable) with the neocortex. The second or alternative formulation is that the positionally encoding moment for each region might occur somewhat earlier in the E12–E13 window.

![Figure 11](image-url)
when the difference in $T_{G1}$ between the DCZ and LCZ is maximal and the $T_{G1}$ gradient is steepest across the PVE. During this earlier time period, the potential spatial resolution of the encoding mechanism would be maximized. The neocortical architectonic map is, in either case, suggested to be an instantiation of a topological pattern or ‘map’ primarily encoded in the PVE prior to the time that most of the neocortical neurons have been generated. This formulation provides a specific cellular and proliferative basis for the generation of the protomap organization of the PVE that has been proposed by Rakic (1988).

The Evidence from Neocortical Histogenesis

Circumstantial evidence is in accord with the hypothesis formulated in the foregoing section. We have referred earlier to regional differences in proliferative cell density and cell cycle kinetics in the PVE of origin of fields 17 and 18 in primate (Dehay et al., 1993) and to the delimitations of forebrain regions of the PVE by early patterns of gene expression (Bulfone et al., 1993; Puelles and Rubenstein, 1993; Shimamura et al., 1995). There is the additional circumstantial evidence that the specific topology of the instantiated neocortical map in rodents arises independently of its principal neocortical afferent systems and, in particular, independently of the thalamocortical projection (Caviness, 1988; O’Leary et al., 1994). It is equally certain that later the differentiated features of each neocortical mapping unit substantially come to reflect information relayed via the thalamocortical projection (O’Leary et al., 1994). Thus, reciprocal thalamocortical and cortico-thalamic afferent systems both arise and distribute to target structures at virtually the same time, with neither having a temporal priority (Almendral et al., 1987; De Carlos and O’Leary, 1992; Erzurumlu and Jhaveri, 1992).

Ascending and descending systems encounter each other first in the internal capsule and follow independent, rather than mutually fasciculated, trajectories to their target structures (Woodward et al., 1990; De Carlos and O’Leary, 1992; Bicknese et al., 1994).

We draw attention to the present observation that the neuronal population being produced in the PVE at the time when the $T_{G1}$ gradient is steepest is that of the infragranular layers (Caviness, 1982; Takahashi et al., 1996a,b). It is pertinent that the thalamocortical afferents do not map topologically to the murine cortex until E16–E17 (Caviness, 1988) which is much later (2–3 days) than when the $T_{G1}$ gradient in the PVE is maximum. Importantly, the neurons of layers VI and V, formed on E13–E14, arrive in and become established within the cortex following migration on E16–E17 (Caviness, 1982, 1988; Takahashi et al., 1996a), i.e. at about the same time that the thalamocortical afferents arrive. Early patterns of gene expression within this early formed neuronal population, established virtually from the time that their migrations are completed (Barbe and Levitt, 1991; Cohen-Tannoudji et al., 1994; Frantz et al., 1994a,b), further substantiate the view that the fine grain of neocortical mapping is initially instantiated with respect to the infragranular neuronal population. Thus, the events in the PVE not only precede but produce the cells at the right time to coordinate with the later arrival of the major afferent systems associated with the neocortical map.

Further consistent with the hypothesis that regional specification is primarily conferred with respect to cells of layers VI and V, i.e. cells formed when the positional encoding potential of the gradient in $T_{G1}$ would be maximal, is the observation that a substantial proportion of neurons of clonal origin within the PVE destined for both supragranular and infragranular layers of the cortex retain after migration to the cortex the same neighborhood relationships to each other which existed in the PVE before their migrations (Walsh and Cepko, 1992; Walsh and Cepko, 1993; Kornack and Rakic 1995; Reid et al., 1995). Thus, mapping relationships that might be gradient encoded into this population while still in the PVE would be retained after their translation into the cortex. Where all neurons of a clone are recovered and mapped, however, it appears that those neurons of a clone which retain their neighborhood relationships after migration are only a portion of the neurons which actually arose within that clone. Some become dispersed, often widely so, across a vast expanse of neocortex (Walsh and Cepko, 1993; see also Nakatsuji et al., 1991; Tan and Breen, 1993; Tan et al., 1995). Wide dispersion after migration appears to be especially characteristic of the later generated neurons of the supragranular layers (Walsh and Cepko, 1992, 1993). It is less characteristic of the infragranular neurons which the present hypothesis holds to be the population within which the neocortical map is initially instantiated.

Prospectus

In summary, our data demonstrate that there is a gradient in $T_{G1}$ across the PVE during the early development of the cerebral cortex. This gradient arises both because the initiation of neuronogenesis is staggered, starting rostrolaterally and propagating caudomedially, and because $T_{G1}$ increases with each of the 11 integer cell cycles that comprise the neuronogenetic interval. The role of the $T_{G1}$ gradient in specification of the topology of the neocortical map is for the present principally hypothetical, but the existence of such a gradient could plausibly account for the formation of a neocortical protomap (Rakic, 1988) within the PVE in the course of early cortical development. Moreover, the existence of the $T_{G1}$ gradient implies that the proliferative behavior of all cells of the neocortical PVE, like that of other morphogenetic systems (e.g. Goodman and Doe, 1993), is coordinated by mechanisms of cell–cell communication (LoTurco et al., 1995; Kriegstein et al., 1996). That the oscillatory behavior of the overall population could be regulated through modulation of $T_{G1}$ is to be expected from the general experience that it is only during the G1 phase of the cell cycle that the oscillatory behavior of the proliferative cell may be modulated by cell-external influences (Pardoe, 1989; Murray and Hunt, 1993; Massague and Poljak, 1995; Coats et al., 1996). The cellular and molecular mechanisms which establish and regulate the $T_{G1}$ gradient remain to be explored experimentally.

Notes

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