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Cell Proliferation in the Developing Mammalian Brain

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BOX 1. Nomenclature

Stem cells—Cells that can produce neurons, glia, progenitor cells and also more stem cells.

Progenitor cells—Cells that can produce one lineage (e.g., neurons or glia) and more progenitor cells. In some systems the distinction between progenitor and stem cells may be a matter of degree of "stemness" (Blau *et al*., 2001).

Neurogenesis—The production of the cells of the nervous system (both neurons and glia).

Neuronogenesis—The production of neurons.

Neuronogenetic interval (NI)—The period of time during which neurons arising in the PVE become permanently post-proliferative.

Pseudostratified ventricular epithelium (PVE)—A population of proliferating cells that lines the ventricles of the brain; the PVE is, for the most part, co-Extensive with the VZ.

Secondary proliferative population (SPP)—A population of proliferating cells that is adjacent to the ventricular zone; the SPP is, for the most part, coextensive with the SVZ.

Ventricular zone (VZ)—A cytoarchitectonically defined layer that is adjacent to the ventricles of the brain.

Subventricular zone (SVZ)—A cytoarchitectonically defined layer that is adjacent to the VZ and that appears after the VZ.

As the neural tube closes, the future brain consists of a single layer of cells that lines the lumen of the tube. The lumen is the developing ventricular system of the brain, and the layer of cells is the ventricular zone (VZ). The proliferating cells of the VZ will either directly or indirectly give rise to all of the cells of the developing central nervous system (CNS).

There are four major proliferative populations in the developing brain. The first of these to appear is the ventricular zone, which is the name agreed upon by the Boulder Committee (1970) as part of an effort to standardize and clarify a nomenclature that sometimes did not reflect accurately the known functions of the layers of the developing CNS. At the same time, the Boulder Committee recognized a second proliferative zone, the subventricular zone (SVZ) that develops later in much of the CNS. Two other proliferative populations arise in specific locations and give rise to specific populations of cells. These are the external granule cell layer of the cerebellum and the subhilar proliferative zone in the dentate gyrus. By the end of the developmental period, these four proliferative populations will give rise to all of the cells of the adult brain (with the exception of a small number that migrate into the brain from the periphery, and two of them, the SVZ and the subhilar zone in the dentate gyrus will continue to proliferate and produce neurons destined for limited areas of the nervous system into adulthood. The regulation of proliferation in these four proliferative zones is responsible for producing the right number of cells of the appropriate classes for all of the subdivisions of the CNS.

THE VENTRICULAR ZONE

The basic cellular organization of the VZ was recognized by both His (1889, 1897, 1904) and Ramon y Cajal (1894, 1909–1911). Both of these giants of the field recognized that the VZ was several cell diameters in thickness and that there were mitotic figures adjacent to the ventricular surface (Fig. 1). His thought that the mitotic figures were "germinal cells" ("*Keimzellen*") and that after mitotic division one daughter cell remained adjacent to the ventricular surface to divide again whereas the other became a postmitotic "neuroblast" that migrates away from the VZ and eventually develops into a neuron. His also thought that the remaining cells of the VZ were a syncytium of "spongioblasts" that give rise to the glial cells of the CNS. Cajal's views were similar to His' in that he also thought that the mitotic figures on the ventricular surface were a separate population of germinal cells that give rise to neuroblasts, but he did not think that the remaining cells of the epithelium,

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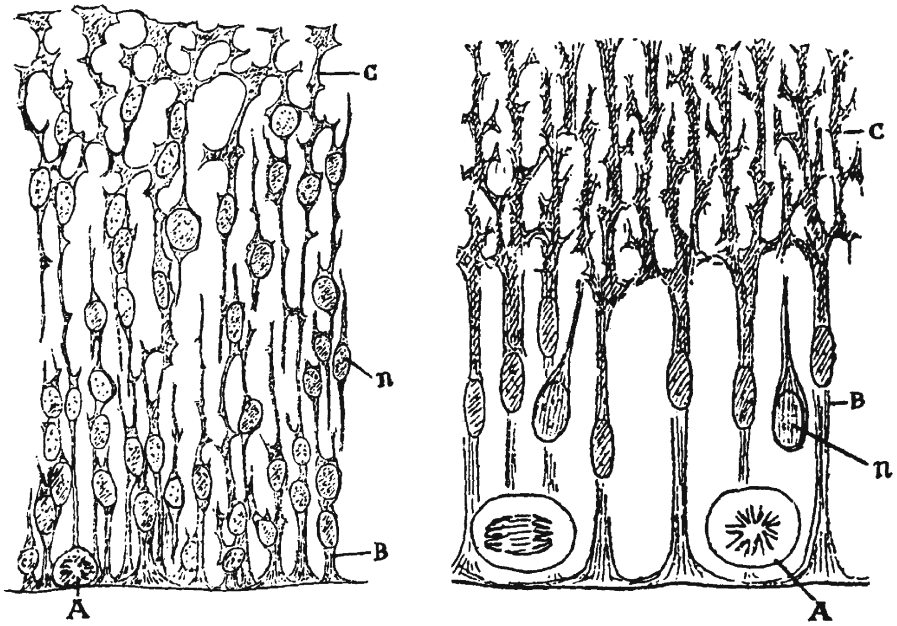

FIGURE 1. Early conceptions of the cellular organization of the early ventricular zone. On the left is a drawing from His (1889) and on the right is a drawing from Ramon y Cajal (1894). In both drawings the ventricular surface is at the bottom. His shows a germinal cell (A) in mitosis near the ventricular surface, the spongioblasts (B) forms a syncytium, and neuroblasts (n) migrating from the germinal zone at the ventricular surface to the marginal zone (C). Ramon y Cajal accepted the general conceptual framework put forward by His of the separate populations of germinal cells, neuroblasts, and spongioblasts, but he did not believe that the spongioblasts formed a syncytium (Jacobson, 1991). Thus, Ramon y Cajal's drawings of germinal cells and spongioblasts tend to look more like independent cellular entities, that is, more like our modern understanding of the structure of the ventricular zone.

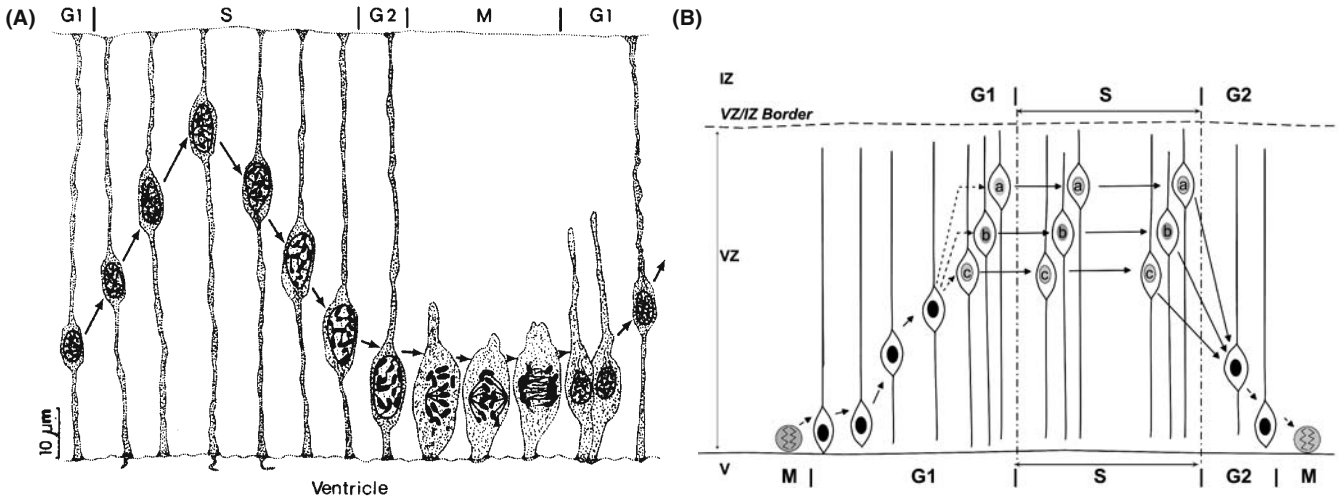

FIGURE 2. (A) A schematic diagram of Sauer's view of interkinetic nuclear migration (from Jacobson, 1991). During the cell cycle the cell nucleus moves to different levels of the ventricular zone. Mitosis occurs at the ventricular surface; the nucleus moves abventricularly during G1 and then again adventricularly before M. (B) A schematic diagram showing the correlation of interkinetic movements with the phases of the cell cycle (from Hayes and Nowakowski, 2000). During the cell cycle, both the direction and rate of movement of the nuclei are correlated with the phase of the cell cycle. Following mitosis, the nucleus of a PVE cells moves away from the ventricular surface. In the outer half of the VZ it enters the S-phase zone, where the nuclei labeled "a," "b," and "c" represent nuclei distributed throughout the thickness of the S-phase zone in the outer half of the VZ. During S, the nuclei do not seem to move, but as they finish S and enter G2, they move rapidly back to the ventricular surface.

that is, the spongioblasts, formed a syncytium but that they were independent cellular entities.

An alternative interpretation of the histological picture was presented by Vignal (1888), Schaper (1897a, b), and Koelliker (1896). These authors suggested that the so-called "germinal cells" adjacent to the ventricular surface were a transitional form of the "spongioblasts." Schaper (1897a, b) was most explicit about this; he suggested that the germinal cells of His "are not to be considered as a special type of cell in contrast to the main epithelial cells" but rather that they were part of the same population "in the process of continuous proliferation." In other words, Schaper suggested that the germinal cells and spongioblasts of His were really cells of the same type which move to different levels of the VZ during different phases of the cell cycle.

These issues were examined again in the late 1930s when F. Sauer (1935, 1936, 1937) undertook a careful cytological analysis of the VZ in the neural tube of chick and pig embryos. F. Sauer observed that the nuclei of the cells of the VZ were not identical in size or appearance and that a logical picture of the transitions of the cells through the cell cycle could be constructed from the distribution of these nuclei through the thickness of the VZ (Fig. 2(A)). With improved histological methods F. Sauer was also able to show convincingly that the VZ was not a syncytium, but that each cell had a distinct plasma membrane and that each cell was columnar in shape connected to both the ventricular and pial surfaces. He suggested, in essence, that after a mitotic division that the nuclei of the VZ cells move away from the ventricular surface during G1, that they remain in the outer half of the VZ during S, and that they return to the ventricular surface during G2 where they divide during M.

An important confirmation of these nuclear movements was made shortly after the introduction of the DNA precursor tritiated thymidine (^{3}H-thymidine) as a tracer. These key experiments showed that shortly after an exposure to ^{3}H-thymidine all of the labeled nuclei were in the outer half of the VZ but that a few hours later the labeled nuclei were adjacent to the ventricular surface (Sauer, 1959; Sauer and Chittenden, 1959; Sauer and Walker, 1959; Sidman *et al*., 1959) which unequivocally showed that at least some of the cells of the VZ comprise a single population which have nuclear movements that correlate with the cell cycle. These nuclear movements which are collectively referred to as interkinetic nuclear migration have since been confirmed to occur in all columnar and pseudostratified columnar epithelia. The interkinetic movements of the nuclei are the hallmark of the VZ in the developing CNS and none of the other proliferating zones exhibit such nuclear movements. In the VZ, more recent experiments using two DNA tracers (bromodeoxyuridine [BUdR] and ^{3}H-thymidine, Fig. 3) simultaneously and clearly show the separation of cells in G2 vs S (Hayes and Nowakowski, 2000). In addition, by changing the interval between the exposure to the two tracers it was possible to show that both the speed and the direction of movements of the nuclei is closely correlated with the phase of the cells cycle (Fig. 2(B)). These results show, as original suggested by F. Sauer, that during G1 the nucleus moves outwards away from the ventricular surface, that during S it is stationary, and that during G2, the nucleus moves rapidly toward the ventricular surface. The inward movement of the nucleus during G2 is quite rapid, occurring within 20–40 min. In contrast, the outward movement of the nucleus during G1 is about 4–10 times slower.

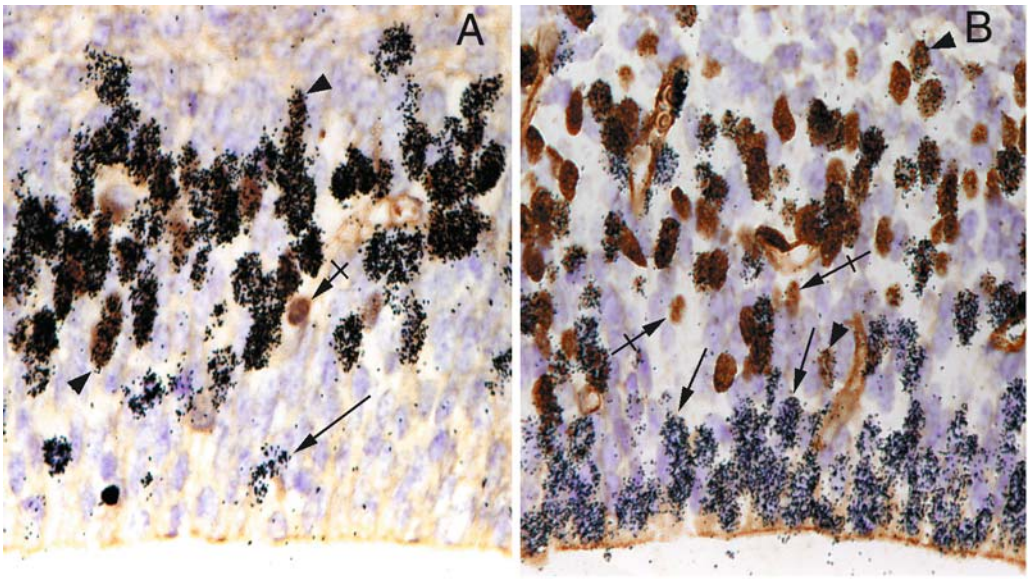

FIGURE 3. The labeling pattern in the ventricular zone (VZ) of an E14 mouse that received ^{3}H-TdR followed either 0.5 (A) or 2.0 (B) hr later by BUdR and was killed 0.5 hr after the BUdR injection. Some cells labeled only with ^{3}H-TdR are indicated by an arrow; cells labeled only with BUdR are indicated by a crossed-arrow. The cells labeled by the BUdR define the S-phase, and they are located in the S-phase zone in the outer half of the VZ. The ^{3}H-TdR-only labeled cells have left the S-phase during the period between the two injections. The 2 hr period (B) is long enough for many of the nuclei of the ^{3}H-TdR-only labeled cells to move towards the ventricular surface where they will divide.

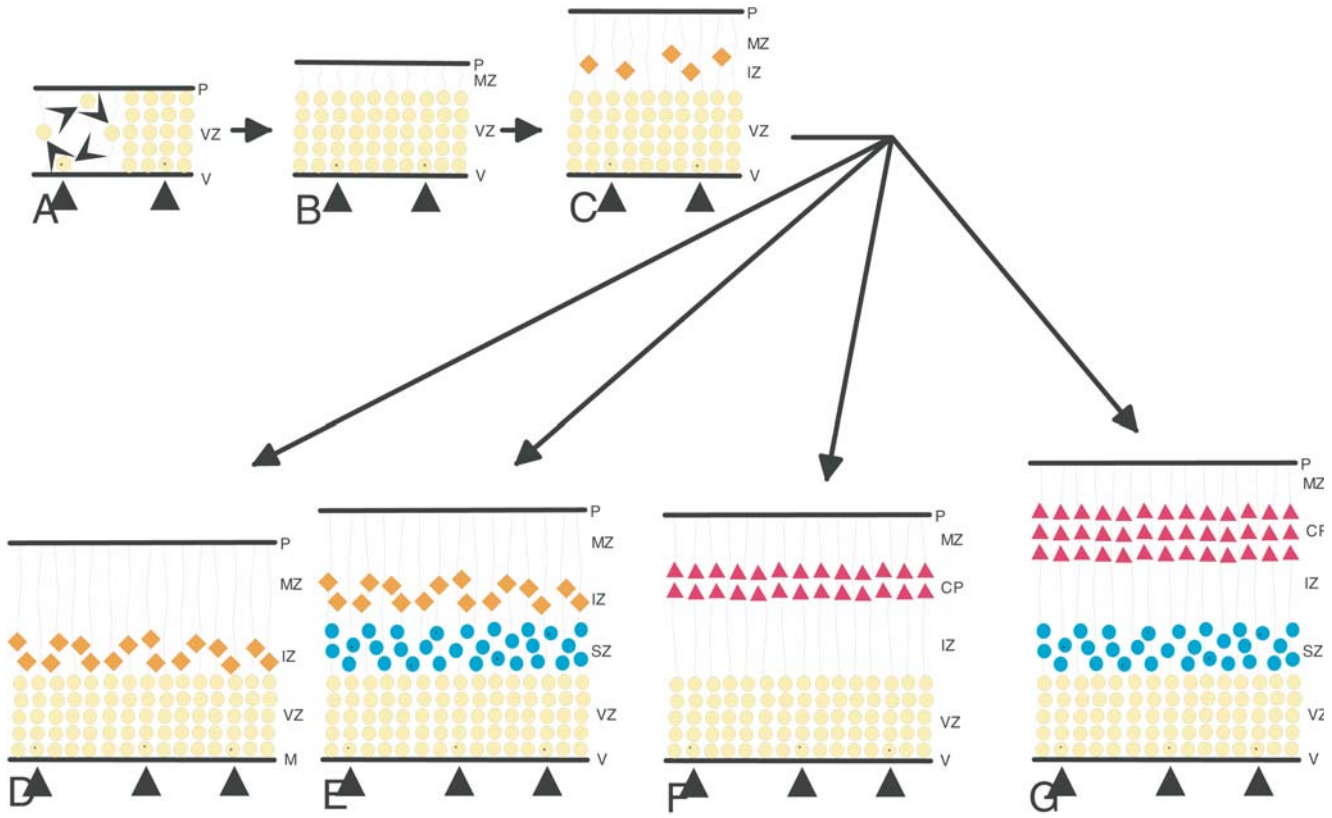

FIGURE 4. Radial differentiation of the neural tube. (A), (B), and (C) are schematic diagrams of the early stages of the radial differentiation of the neural tube through which every part of the CNS passes. (D), (E), (F), and (G) are schematic diagrams of various options for the later stages of the radial differentiation of the neural tube. Each of these options is characteristic of a different part of the neural tube. (A) At the time of closure of the neural tube its wall consists of a population of proliferating cells organized into a pseudostratified columnar epithelium, known as the ventricular zone (VZ). In this proliferative zone the nuclei of the cells are stratified, but each cell has processes that contact the ventricular (V) and pial (P) surfaces of the neural tube. As diagrammed on the right-hand side of the drawing, mitosis occurs at the pial surface (asterisks), and during the cell cycle the nucleus of each cell moves to a different level. DNA synthesis, for example, occurs in the outer half of the ventricular zone. This to-and-fro movement of the cell nuclei is known as interkinetic nuclear migration and means that all cells, even though they are apparently at different levels, are part of the proliferative population. (B) The next zone to appear during the radial differentiation of the neural tube is the marginal zone (MZ) which is an almost cell-free zone between the ventricular zone and the pial surface. (C) The intermediate zone (IZ), which contains the first postmitotic cells in the nervous system, is the next to form. This zone is located between the ventricular zone and the marginal zone. (D) In some parts of the neural tube, such as the spinal cord, the postmitotic cells derived from the ventricular zone aggregate and mature in a densely populated intermediate zone. (E) In some areas, such as the dorsal thalamus, a second proliferative zone, the subventricular zone (SVZ), is formed between the ventricular zone and the intermediate zone. In the subventricular zone, interkinetic nuclear migration does not occur; instead mitotic figures (asterisks) are found scattered throughout the thickness of the zone (DNA synthesis also occurs throughout the thickness of the subventricular zone). The postmitotic cells derived from both the ventricular and subventricular zones aggregate and mature in a densely populated intermediate zone. (Note, however, that any cells derived from the ventricular zone must cross the subventricular zone.) (F) In the hippocampus, the postmitotic cells derived from the ventricular zone migrate across a sparsely populated intermediate zone to form a cortical plate. (G) In the cerebral cortex, postmitotic cells derived from both the ventricular and subventricular zones migrate across a sparsely populated intermediate zone to form a cortical plate. Abbreviations: V: ventricular surface; VZ: ventricular zone; SZ: subventricular zone; IZ: intermediate zone; CP, cortical plate; MZ, marginal zone; P, pial surface.

The histological appearance of the VZ is remarkably constant, except for slight variations in thickness, with little variation regionally, as a function of time or in different species. However, the fact that different portions of the wall of the neural tube can develop into the highly different areas of the adult brain is *de facto* evidence that the output and capacity of the VZ must be remarkably variable. One early expression of this variability is the appearance of a second proliferative zone, in some regions of the CNS, but not in others. This second proliferative zone, which is known as the SVZ, appears adjacent to the VZ. This second zone, known as the SVZ, differs in several ways from the VZ (Fig. 4). It is attractive to speculate that the SVZ is a phylogenetically recently acquired specialization. For example, the hippocampus is classified as an archicortical (i.e., "old" cortex) structure and the neurons of its major subdivisions (areas CA1, CA2, and CA3) are all derived from the VZ (Nowakowski and Rakic, 1981). In contrast, in the neocortex (i.e., "new" cortex) the SVZ is substantial and, although it is unlikely to contribute large numbers of neurons to the neocortex (Takahashi *et al*., 1995a), it produces glial cells and also neurons in other parts of the

telencephalon (Goldman, 1995; Garcia-Verdugo, 1998). A similar contrast occurs in the developing diencephalon in which the hypothalamus lacks a SVZ whereas other diencephalic subdivisions have both ventricular and SVZ (Rakic, 1977). The SVZ appears early and becomes greatly enlarged in the ganglionic eminence, a population of proliferating cells that produces the striatum, parts of the basal forebrain, and a population of interneurons that migrate into the neocortex (Corbin *et al*., 2001; Wichterle *et al*., 2001; Anderson *et al*., 2002; Nery *et al*., 2002; Powell *et al*., 2003). The cells of the SVZ, in contrast, neither maintain an attachment to the ventricular or pial surfaces nor do their nuclei move as they move through the cell cycle (Sidman, 1970). The contributions of the SVZ to the adult brain are important and most of the glia for most of the brain are produced there (Goldman, 1995). In addition, in some areas of the brain a significant number of neurons are also produced in the SVZ (Garcia-Verdugo, 1998).

The VZs and the SVZs are cytoarchitectonic entities; that is, they are defined by their appearance in histological sections. The secondary proliferative population (SPP) arises from the primordial pseudostratified ventricular epithelium (PVE) (Smart, 1972; Altman and Bayer, 1990; Halliday and Cepko, 1992; Takahashi *et al*., 1993) but comes to have a more diffuse and widespread distribution through the cerebral wall overlying the VZ although it overlaps the PVE at the outer fringe of the VZ (Takahashi *et al*., 1993). The distribution of SPP to the architectonically defined SVZ, in the depths of the intermediate zone abutting the VZ, was originally emphasized by the Boulder Committee. The SPP is a principal spawning ground for neuroglial cells (Smart, 1961; Smart and Leblond, 1961; Privat, 1975; Mares and Bruckner, 1978; Smart and McSherry, 1982; LeVine and Goldman, 1988a, b; Levinson and Goldman, 1993). Neurons of the olfactory bulb (Hinds, 1968a, b; Luskin, 1993; Lois and Alvarez-Buylla,1994; Luskin and McDermott, 1994) and possibly, a small number of neurons destined for the neocortex (Reynolds and Weiss, 1992; Levinson and Goldman, 1993) may also undergo their terminal divisions in this proliferative population. The cells of the SPP, in contrast to those of the PVE, are not attached to each other as a pseudostratified epithelium (Rakic *et al*., 1974), and this population does not undergo interkinetic nuclear migration in the course of the cell cycle (Boulder Committee, 1970; Smart, 1972; Altman and Bayer, 1990).

The Cell Cycle in the Ventricular Zone

The use of the DNA precursors ^{3}H-thymidine and BUdR has also provided other insights into the behavior of the cells of the VZ. Notably, over the decades there have been a variety of methods used to measure the length of the cell cycle in various regions of the neural tube (for reviews see Sidman, 1970; Jacobson, 1991; Nowakowski *et al.*, 2002). In particular, the dynamics of the cell cycle for both the PVE and the SPP, that is, the proliferating cells of the SVZ, are now well known (Caviness *et al.*, 1995; Takahashi *et al.*, 1995a, b, 1996a, 1997). These studies have examined the entire period of time for mouse neocortical during the neuronogenetic interval (NI), defined as the period of time during which neurons arising in the PVE become permanently postproliferative. The results show that the amount of time required for a single cell to pass through one cell cycle, that is, from the beginning of one G1 to the beginning of the next G1, varies systematically during the development of the neocortex (Fig. 5). At the time of the production of the first neurons in the mouse neocortex, measurements of the cell cycle using cumulative labeling with BUdR show that the total length of the cell cycle (T_c) is about 8 hr, with an S-phase of about 3 hr, G2+M of about 2 hr, and G1 of about 3 hr (Takahashi *et al*., 1995). As development proceeds, the cell cycle lengthens until at the end of the period of neuron production T_c reaches ~18 hr; S and G2+M remain approximately constant at 3–4 hr and 2 hr,

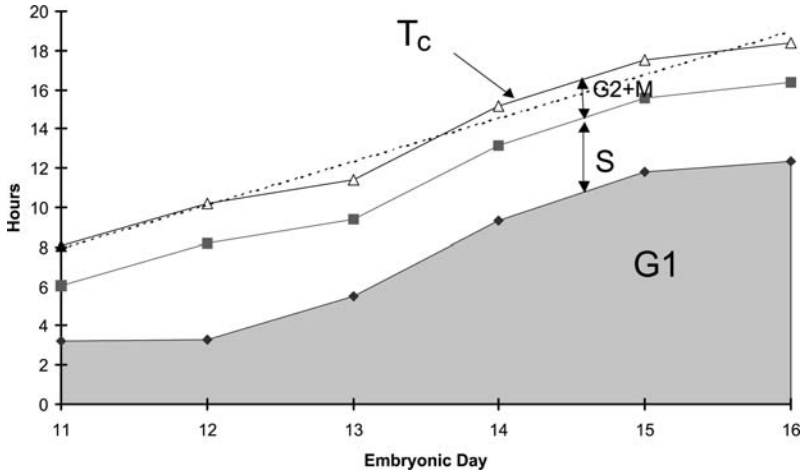

FIGURE 5. In the developing neocortex, the cell cycle lengthens systematically over the course of the six-day period during which neurons are produced. At the onset of E11, the cell cycle is ~8 hr, and by the end of the E16 it is over 18 hr. During this time the length of G2+M and S do not change systematically, and, hence, most of the lengthening is within G1.

respectively, and thus, virtually all of the change in T_c is due to an increase in the length of G1 (T_{G1}) (Takahashi *et al*., 1995b). The increase in T_{G1} is dramatic, from 3 hr early in development to almost 12 hr late in development. As a result of the lengthening of the cell cycle, there are different numbers of cell cycles on each of the six days of production of neurons for the neocortex. For example, on the first day of the NI (E11) the cell cycle starts at 8 hr and lengthens to over 10 hr; thus, there is time for approximately 2.5 cell cycles, where as on the sixth and last day of the NI (E16) the cell cycle is over 18 hr, and thus, there is sufficient time to complete only just over one cell cycle. By integration under the linear fit to the T_c (Fig. 5), it can be calculated that there is sufficient time for 11 cell cycles during the entire six-day period (Takahashi *et al*., 1995b).

The question of the range of cell cycle lengths present in the PVE during the developmental period has been addressed using different cell cycle measuring techniques. With cumulative labeling methods (Nowakowski *et al*., 1989), the percentage of labeled cells rises linearly if the population is proliferatively homogeneous, that is, the length of the cell cycle and the S-phase are similar for all cells. Thus, from these measurements we have estimated that the PVE of the mouse is 80–90% homogeneous (Takahashi *et al*., 1995a) with cell cycle parameters within 10% of the mean (Nowakowski *et al*., 1989). This means, of course, that as many as 10–20% of the cells might have cell cycle parameters outside of this range. The cumulative labeling method gives an estimate for a maximum value of T_c because it is derived from the detection of an inflection point in the slope of the rising labeling index. This inflection point corresponds to the time required to label the entire proliferative population and occurs when the last (or slowest cycling) proliferating cell that was not labeled by the first injection enters the S-phase and becomes labeled (see Nowakowski *et al*., 1989). In contrast, an alternative method for measuring cell cycle lengths, the percent labeled mitosis method, gives an estimate of the minimum value of T_c because it detects the time required for the first (or fastest cycling) proliferating cell to transit the entire cell cycle and enter M-phase for a second time (Kaufmann, 1968; Hoshino *et al*., 1973; Hamilton and Dobbin, 1983a, b). The difference between the maximum and minimum estimates of T_c is an estimate of the range in T_c for the slowest vs fastest cycling cells. When both methods are used to identify the range of the cell cycle lengths for the neocortical PVE, it was found that approximately 99% of the cells have a cell cycle within 5–7% of the mean (Hamilton and Dobbin, 1983a, b). This means that if there is present in the PVE a population of proliferating cells with either a longer or a shorter cell cycle, it comprises only about 1% of the total. Interestingly, this proportion corresponds to estimates of the proportion of true stem cells made by van der Kooy and colleagues (for review see Seaberg and van der Kooy, 2003).

Extensive data for changes in the cell cycle length are not available for other species, but there is some evidence that in the neocortex of primates, the cell cycle is longer (Kornack and Rakic, 1998), and that there are about 28 cell cycles required to make the monkey neocortex. In the human, the comparable period of time during which neurons are produced is much longer, about 120 days (Caviness *et al*., 1995). From this and other considerations, it has been estimated that about 34–35 cell cycles would be required to make all of the neurons of the human neocortex (Caviness *et al*., 1995).

Overall, this extensive analysis of cell proliferation in the neocortex indicates that the proliferating cells of the VZ form a coherent group of cells that have a similar cell cycle length that lengthens as development proceeds. In addition, most of the lengthening of the cell cycle is due to an elongation of the G1-phase. On the surface, this seems reasonable as the G1-phase of the cell cycle is generally considered to be regulatory. Another region of the brain for which cell cycle data is available that covers the entire period of neurogenesis is the retina (Alexiades and Cepko, 1996). The developing retina differs from the developing neocortex, however, in that both G1 and S lengthen. The lengthening of the cell cycle in the retina is detectable even when measured over a period of a few days (Rachel *et al*., 2002).

The Output from the Ventricular Zone

The output from the PVE is the population of neurons and other cell types that populate the mature brain and the cells that "seed" the SVZ. *A priori*, the mechanisms that control this output depend on four factors, the number of proliferating cells, the length of the cell cycle, the period of time that the proliferating population exists, and the proportion of daughter cells that exit vs remain in the proliferating population at each pass through the cell cycle. By definition the beginning of the NI coincides with the first cell cycle during which neurons are produced. Thus, for the first cell cycle of the NI and for each of the subsequent cell cycles, to a total of 11, some of the daughter cells of the proliferating population exit the cell cycle (Fig. 6). The daughter cells that exit from the cell cycle are called "Q" cells, signifying that they are proliferatively *q*uiescent (or that they *q*uit the cell cycle). The daughter cells that remain in the cell cycle are called "P" cells because they re-enter the S-phase and, hence, continue to proliferate. If, for the moment, the possibility of cell death within the proliferative population is ignored (considered in more detail below), it is clear that all of the daughter cells must select either a P or a Q fate and, hence, the proportions P and Q must add up to 1 (or $P + Q = 1$). Examination of the "old" tritiated thymidine birthday literature (e.g., Angevine and Sidman, 1961; Caviness and Sidman, 1973; Rakic, 1974; Stanfield and Cowan, 1979; Nowakowski and Rakic, 1981; Rakic and Nowakowski, 1981) shows that in various cortical structures different numbers of neurons are born on each of the various days of development. This means that P and Q must change dynamically as development proceeds (for review see Nowakowski *et al*., 2002).

From first principles, it seems clear that prior to the onset of neuron production, P must be 1 and Q must be 0. Similarly, at the end of the NI, in order to account for the disappearance and involution of the PVE at the end of the NI, P must be decreased to 0 and Q must be increased to 1. This means that during the NI, P decreases from 1 to 0, and Q increases from 0 to 1. In the neocortex, measurements of Q made on each of the days of the NI in both dorsomedial and rostrolateral cortex (Miyama *et al*., 1997)

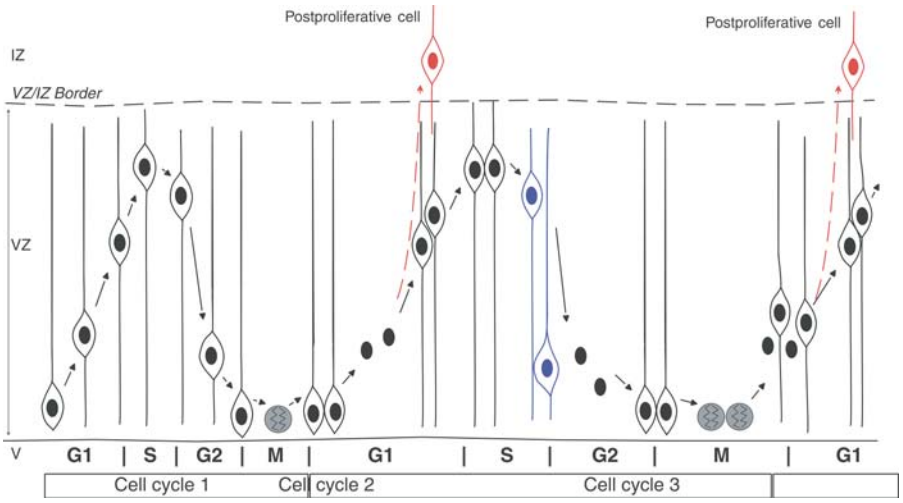

FIGURE 6. The cell cycle in the ventricular zone of the developing CNS: This schematic diagram illustrates the interkinetic movement of the nuclei of the cells comprising the proliferative ventricular epithelium of the ventricular zone (VZ). With each pass through the cell cycle the nucleus of a single cell moves from its starting position at the ventricular surface at the beginning of G1 to the border of the VZ where it enters S. During G2, the nucleus again moves down to the ventricular surface where it enters M and divides to form two cells. With each pass through the cell cycle some postmitotic neurons are produced. The postmitotic neurons migrate away from the VZ to produce the structures of the adult brain (in this case, the cerebral neocortex). During the production of the neocortex in the mouse, the cell cycle lengthens with each cell cycle and there are a total of 11 cell cycles.

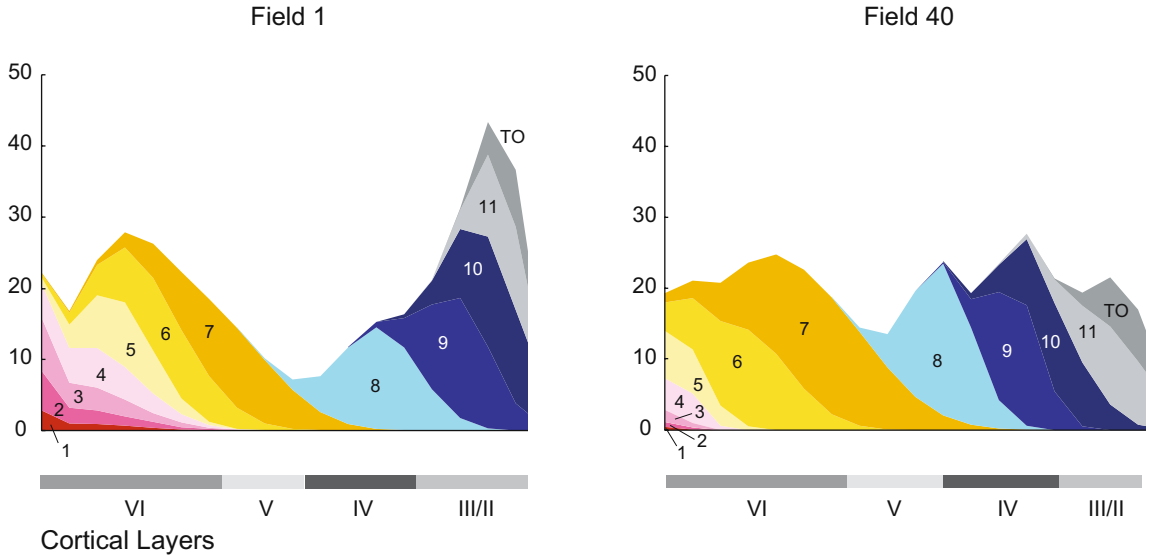

FIGURE 7. The laminar distribution of neurons produced during each of the 11 cell cycles of the Neuronogenetic Period for two nonadjacent cytoarchitectonic areas of the neocortex: Field 1, which is located dorsomedially, and Field 40, which is rostrolaterally. Each cell cycle produces neurons that are distributed in several layers, but there is a systematic change in the laminae of residence with each sequential cell cycle. Also, each layer receives neurons that are produced during more than one cell cycle. Note that the neurons of layers VI and V are produced during the first seven cell cycles, that is, during the period when the neopallium is still expanding (Fig. 8).

are shown in Fig. 7. In Fig. 7, the abscissa shows the 11 cell cycles of the NI, and the data for each of the embryonic days is plotted at its appropriate proportional position on this 11-cell-cycle scale. Thus, during this six-day NI of the neocortex, the nuclei of the PVE makes 11 round-trips through the cell cycle, and at each pass through the cell cycle the population produces an ever-increasing proportion of postmitotic neurons (Fig. 6) (Caviness *et al.*, 1995; Takahashi *et al.*, 1997). The path of $Q = 0 \rightarrow Q = 1$ increases monotonically; P is the complement of Q, and thus, the path of $P = 1 \rightarrow P = 0$ decreases monotonically (Fig. 8). The P and Q curves intersect at $P = Q = 0.5$, which is between cell cycle 7 and 8. This divides the NI into two qualitatively different periods. During the first period, when $Q < 0.5$ and $P > 0.5$, the proliferative population expands. Since the

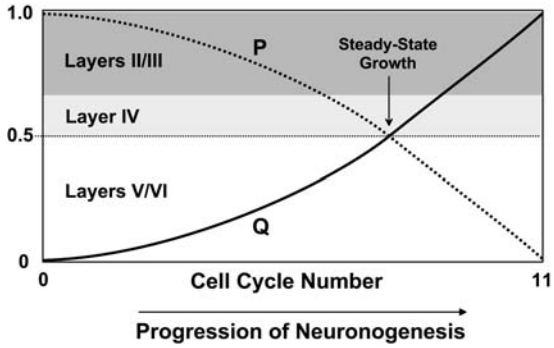

FIGURE 8. In the ventricular zone of the developing neocortex Q and P are complementary (i.e., $P = 1 - Q$), and as Q increases from 0 to 1, P decreases from 1 to 0. When P is > 0.5, more daughter cells re-enter the cell cycle than leave it, and the neopallium will expand. In the mouse, between cell cycle 7 and 8 a point is reached when this expansion stops, and both P and Q are equal to 0.5. This occurs approximately as the cells that will reside in the vicinity of the border between layers V and IV are produced.

VZ only increases slightly in thickness during this period, and the packing density of the cells remains constant (Takahashi *et al*., 1996a), the bulk of this expansion results in an increase in the surface area of the VZ and, hence, in the surface area of the entire developing neocortex. As Q increases to 0.5 and P decreases to 0.5, a point is reached where "steady-state growth" is achieved transiently. At this time, the number of P cells produced is exactly enough to replenish the proliferative population; an equivalent number of Q cells are produced that leave the PVE. As development proceeds, however, a second period, when $Q > 0.5$ and $P < 0.5$, is reached. During this second period, the proliferative population contracts. This is because fewer daughter cells re-enter the S-phase than are needed to maintain it. Since the ventricular surface does not contract during the developmental period and the packing density of the cells remains constant (Takahashi *et al*., 1996a, b), most of this must be reflected in a reduction of the thickness of the VZ. This, in fact, correlates with what is known about the development and involution of the VZ (Nowakowski *et al*., 2002). It has been suggested that other species follow the same pathway of changes for P and Q except that they take more or fewer cell cycles than the 11 cell cycles needed to make mouse neocortex (Caviness *et al*., 1995).

In the neocortex, the crossover point corresponding to the time when $P = Q = 0.5$, at which time the neocortical primordium ceases to expand, occurs as the NI passes through cell cycle 7. This point is important because it is when the expansion of the PVE stops. A cycle-by-cycle analysis of the laminar position of the neurons generated at each of the 11 cell cycles shows that this crossover point occurs as the last neurons of layer V are being produced (Fig. 7). This means that virtually all of the deep layers of the neocortex are produced during the first, expansion phase of the NI, and that virtually all of the superficial layers are produced during the second, extinction, phase. It is not known if the crossover point has similar significance in other regions of the developing CNS.

The pathway of changes in Q and P from $Q = 0 \rightarrow Q = 1$ and $P = 1 \rightarrow P = 0$ determines three properties of the proliferative population: (1) the life span of the PVE population, (2) the expansion of the PVE population and, hence, of the neocortical primordium, and (3) the output from the population, that is, the number of neurons produced both per cycle and also during the total NI. Each of these properties can be approached quantitatively (for review see Nowakowski *et al*., 2002). The life span of the NI is most closely regulated by changes in Q as it changes from $Q = 0 \rightarrow Q = 1$. Neuron production begins as soon as Q becomes greater than 0 and the PVE disappears when $Q = 1$ because at this point both daughter cells would have to leave the cell cycle. Thus, the mechanisms which determine the changes in Q, that is, the changes in the probability at each successive cycle that postmitotic daughter cells will exit the proliferative population, determine the number of cycles in the NI. At present, there is no clear molecular explanation for the changes in Q.

The expansion of the PVE is also specified by the changes in Q and P. In this case, the expansion at each cell cycle is dictated by P, that is, the probability that the daughter cells will re-enter the cell cycle. The amount of expansion is twice the value of P at each cell cycle. In addition, the expansion is multiplicative at each cell cycle. For example, for the first three cell cycles of the NI, P is 0.99, 0.96, and 0.92; thus, the total expansion for a "unit volume" of the PVE during these first three cell cycles is the product of these three numbers ($1.982 \times 1.93 \times 1.846$) or 7.061. In other words, during the first three cell cycles of the NI (which occur in just over a day), the PVE expands over seven-fold.

The output (or the number of neurons formed) from a "single unit" of the PVE at each cell cycle is specified by the changes in both P and Q. At each cell cycle, the output is equal to twice the number of cells present in the PVE at the beginning of the cycle times Q. A graph of this series (Fig. 9) shows that the predicted output rises gradually to a peak and then falls. This is, of course, expected because of the change in the size of the PVE,

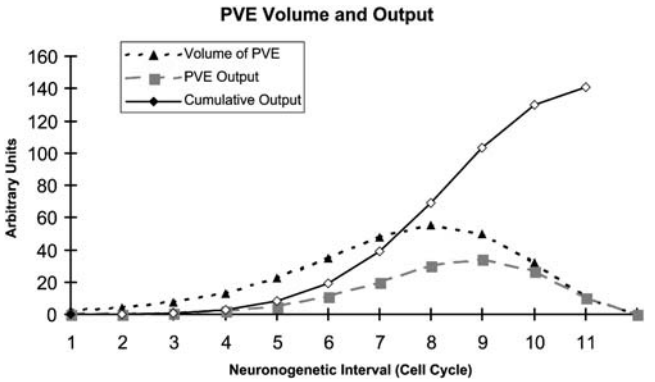

FIGURE 9. A graph of the changes in the PVE volume, PVE output per cell cycle and the cumulative PVE output as predicted by changes in P and Q (see Nowakowski et al., 2002). Note that the volume increases through cell cycle 8 when P first falls below 0.5.

which is controlled by *P*. It is also reassuring because it matches qualitatively the shape of the curves usually obtained from counts of the percentage of neurons born on sequential days during development (Angevine and Sidman, 1961; Caviness and Sidman, 1973; Rakic, 1974; Stanfield and Cowan, 1979; Nowakowski and Rakic, 1981; Rakic and Nowakowski, 1981). The cumulative total output at each cell cycle is simply the sum of the output for all of preceding cell cycles, and, thus, a graph of the cumulative output (Fig. 9) rises steadily to a total of over 140, indicating that in the mouse, on an average, a single PVE cell present at the beginning of the NI produces approximately 140 neurons.

The changes in the size of the PVE and developing cortical plate and the relationships of these changes can be seen more readily if results from a quantitative analysis (Nowakowski *et al*., 2002) are schematized (Fig. 10). At the beginning of the NI, the only cells present are the cells of the PVE; they are represented as a cube with a "unit volume" that is 1 unit high, 1 unit wide, and 1 unit deep. Since the PVE is about 6 cells high, such a unit volume would contain about 6^3 or 216 cells. During the first cell cycle, *P* is about 0.99 and *Q* is about 0.01. Thus, such a unit volume would produce only about two neurons on the first cell cycle and most of the daughter cells produced will remain in the proliferative population, and the PVE will expand. At the end of the third cell cycle the unit of the PVE has expanded to over seven times its original volume and produced about 1% of the neurons that will comprise the neocortex in the adult. Most of the cells of the neocortex are produced during the last few cell cycles (Fig. 10). This sequence of events corresponds at least qualitatively to histological observations. In principle, the neocortical VZ and, indeed, the VZ of the whole CNS contains many of these "units" arrayed across its surface. In the neocortex, it has been shown that the sequence of 11 cell cycles and the changes in *P* and *Q* in each are identical these "units," at least to the resolution that has been used so far (Miyama *et al*., 1997). The result of this arrangement is that different events in the sequence occur contemporaneously in different regions of the VZ (Fig. 11). The NI is initiated first in the rostrolateral cortex, and given the fact that there is more than a 24 hr difference between the rostrolateral cortex and the dorsomedial cortex (Miyama *et al*., 1997), when the NI is first initiated in the dorsomedial cortex, the rostrolateral cortex has already progressed into cell cycle 3 or even 4. This means that there is a gradient of maturation beginning in the rostrolateral cortex and spreading across the surface of the developing cortex. The gradient of maturation means that T_c, T_{G1}, *Q*, and *P* differ across the surface of the developing cortex (Fig. 11). Thus, at any given time the status of these proliferatively related parameters provides positional information. From the perspective of the cell cycle, this gradient divides the surface of the developing PVE into "cell cycle domains," that is, regions of the PVE in which all of the PVE cells are in the same cell cycle. As the developing cortex matures and each "unit" of the PVE progresses through the NI, these cell cycle domains "move" across the surface of the PVE defining a series of "waves" that radiate from the striatocortical fissure at the lateral edge of the neopallium (Fig. 11). In other regions of the CNS, the developmental progression of these "units" of the VZ presumably differ markedly with varied paths for $Q = 0 \rightarrow Q = 1$, and, hence, varied life spans of the VZ in different regions, and varied output and numbers of cells produced.

The Control of *P* and *Q* with Symmetric and Asymmetric Cell Divisions

The output of neurons by the PVE is controlled, in principle, by a variety of factors including the proportional representation of the three possible types of mitotic divisions: (1) symmetric nonterminal cell division (which produces two daughter cells that remain in the PVE and continue to proliferate), (2) symmetric terminal cell division (which produces two daughter cells that both migrate out of the PVE to become young neurons), and (3) asymmetric cell division (which produces one daughter cell that continues to proliferate and one that migrates out of the PVE). Changes in the proportions of these three types of mitotic divisions have been inferred from changes in the proportion of cells that enter vs leave the cell cycle (Takahashi *et al*., 1996a; Miyama *et al*., 1997), from time lapse cinematography (O'Rourke *et al*., 1992; Adams, 1996), from changes in the orientation of the mitotic apparatus (Smart, 1973; Chenn and McConell, 1995; Adams, 1996), and from immunohistochemistry (Chenn *et al.*, 1995). How are such changes effected within single lineages? How might they be distributed among lineages making various cortical cell types? For example, it has been suggested that there are specific populations "reserved" in the PVE to produce either specific cell types or cells that occupy specific laminae (Dehay *et al.*, 1993; Kennedy and Dehay, 1993; Luskin *et al.*, 1993). Since all of the cells in a given neighborhood of the PVE are proliferating (Takahashi *et al.*, 1995a, 1996a) with a similar cell cycle length (Cai *et al.*, 1997a), in the absence of cell death such a "reserved population" would have a specific pattern of repeated symmetric nonterminal mitoses and would expand for several cell cycles to produce relatively large lineages of a specific and characteristic size (8, 16, 32, 32, etc.) containing only proliferating cells. Similarly, lineages following other specific patterns of proliferation, for example, repeated asymmetric divisions, would produce lineages of other specific characteristic sizes, for example, a preponderance of even-sized or odd-sized lineages, etc. The alternative to such repeated patterns of mitosis-type is the absence of pattern in the sequence of cell divisions within a lineage, in which case no specific and characteristic lineage size distribution will be produced. In general, the size distribution of lineages obtained during defined periods of development will reflect the dynamic changes in the proportions of these three types of cell divisions and will, thus, reveal any extant repeated patterns of mitosis. Note that the presence of cell death to any significant extent (cf. Blaschke *et al.*, 1996; Thomaidou *et al.*, 1997) would modify the specific and characteristic lineage sizes obtained, but would do so in a predictable way.

To estimate the frequency of occurrence of each of these distinct behaviors individual retrovirally labeled lineages were studied; each lineage consisting of proliferating cells in the PVE in the developing neocortex at known numbers of cell cycles after infection with a retrovirus. In contrast to most previously

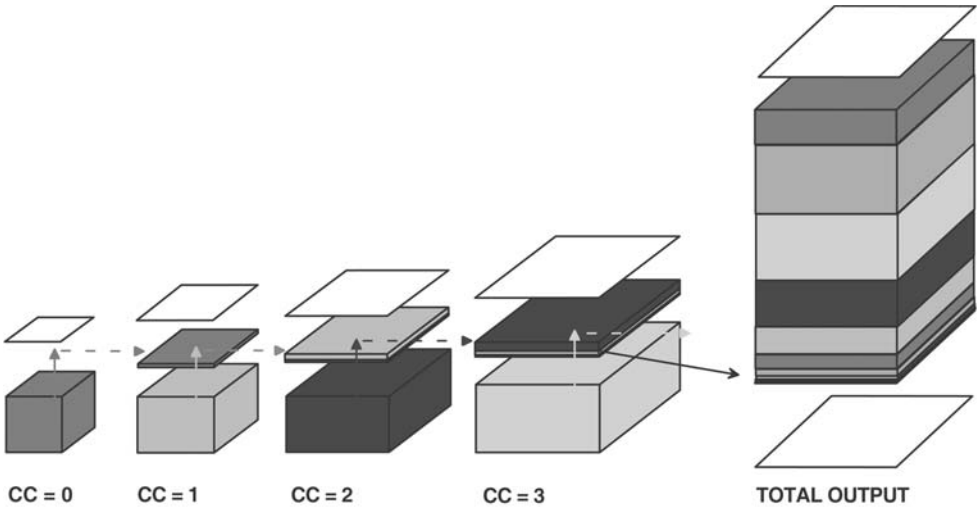

FIGURE 10. A visualization of the changes shown in the graphs of Fig. 12 and as given by the changes in *P* and *Q* per cell cycle (CC) (Nowakowski *et al*., 2002). At the onset of the neuronogenetic interval (NI) (CC = 0), a single unit of the PVE is shown. At the next cell cycle (CC = 1) the PVE has an increased volume; the output from the first cell cycle is shown in the position of the cortical plate. At CC = 2, the PVE has increased in volume again, and now the output from the first two cell cycles is shown in the position of the cortical plate. At CC = 3, the process is repeated. In the right-hand side of the figure, the diagram shows the final Total Output of all of the 11 cell cycles of the NI. Note that the output from the first three cell cycles corresponds to only a small part of the Total Output, whereas the output of the last three cell cycles comprises about 50% of the Total Output.

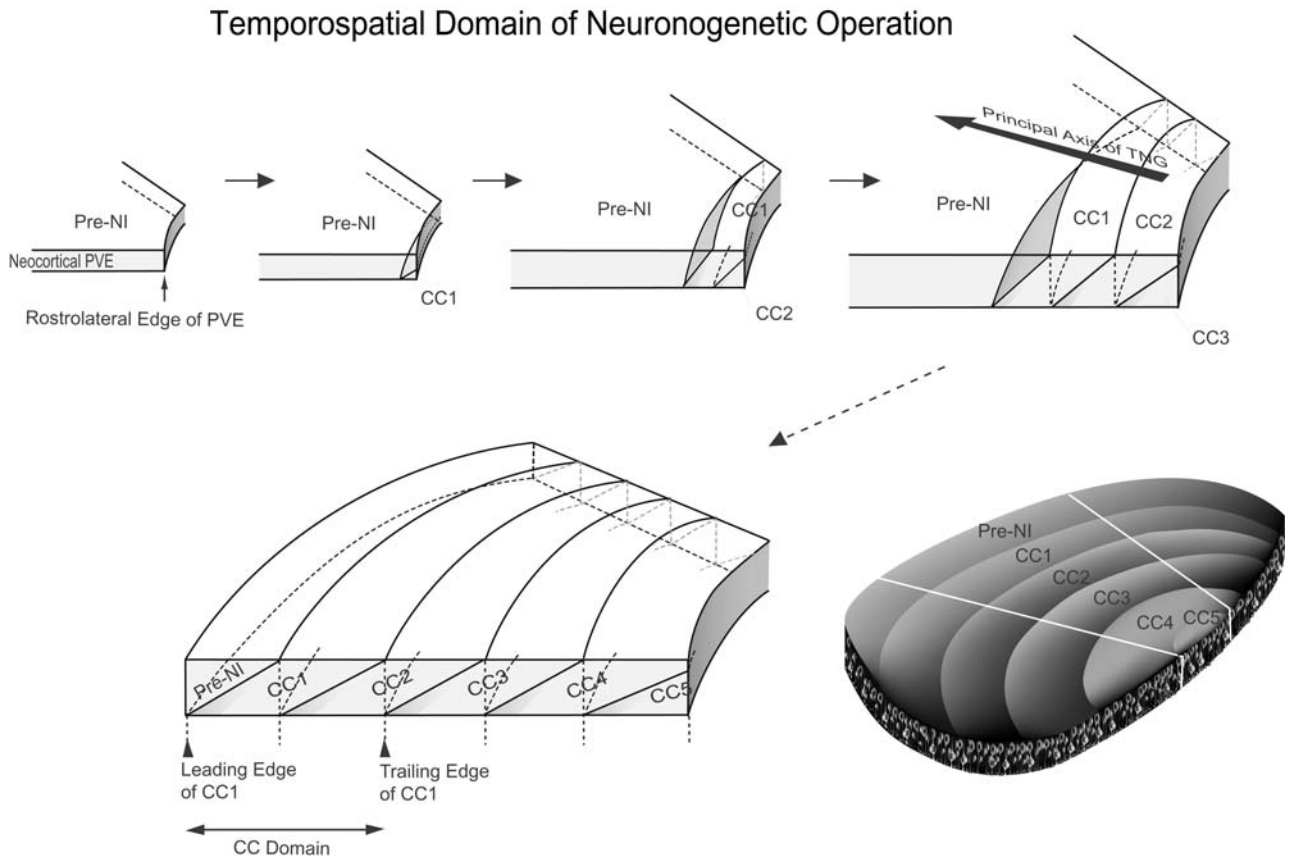

FIGURE 11. The sequence of dynamic changes in the length of the cell cycle (and in *P* and *Q*) is initiated in the rostrolateral-most portions of the neopallium and then spreads as a gradient of maturation across the neopallial surface. This wave-like progression of maturation means that at any given time there are "domains" of the PVE that are in different states. This is, in theory, sufficient to provide a basis for cell cycle length to serve as positional information that could be involved in the development of cytoarchitectonic subdivisions.

published experiments using this method (Price, 1987; Luskin *et al*., 1988; Walsh and Cepko, 1988, 1992; Williams *et al.*, 1991; Luskin, 1993; Mione *et al*., 1994, 1997; Lavdas *et al.*, 1996), the resulting labeled lineages were examined after short survivals, that is, during the period that cell proliferation continues to occur, and have focused on the size of the proliferating population, that is, the cells that remain in the PVE, rather than on the cells that migrate to the cortical plate. There are three influences (Fig. 12) that could act at each cell cycle to reduce the number of cells per lineage from the maximum number that would be produced in a pure population of symmetric nonterminally dividing cells. First, some cells of the lineage could leave the cell cycle to migrate and become young neurons (Q-cells, *Q* in Fig. 1). Second, some PVE cells could die (*D* in Fig. 12). Cell death could, in theory, occur at any time during development and has been well studied in the maturing neocortex during the postnatal period (Leuba *et al.*, 1977; Finlay and Slattery, 1983; Heumann and Leuba, 1983; Crandall and Caviness, 1984; Finlay and Pallas, 1989; Verney *et al.*, 2000). However, estimates of the magnitude of cell death occurring within the proliferative population and during the early period of cortical development vary greatly from $<$1.0% at any given time (Thomaidou *et al.*, 1997) to over 70% of the progenitor cells (Blaschke *et al.*, 1996). Thus, it remains unclear what role cell death in the proliferative population plays in the regulation of neuron number (Gilmore *et al*., 2000). Third, some PVE cells could move tangentially (*T* in Fig. 12), that is, away from their sisters and cousins (Fishell *et al.*, 1993; Tan and Breen, 1993; Walsh, 1993). Such tangential movements would not affect the actual numbers of cells in the proliferative population, but would affect the apparent number of cells identified in a lineage and would concomitantly increase the putative number of lineages identified.

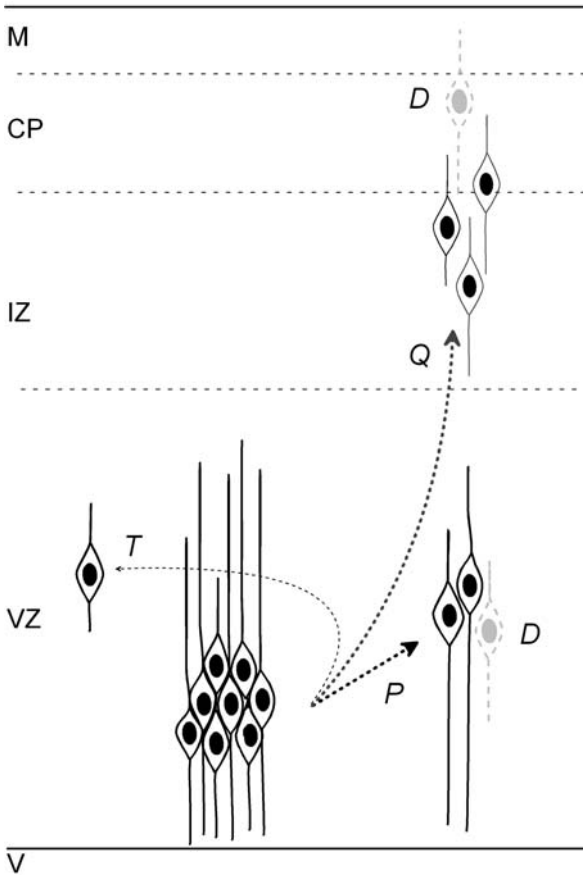

FIGURE 12. A schematic diagram depicting the influences in the ventricular zone that could affect lineage size in a single cell cycle. For a cluster of cells present at the beginning of G1 (in this example eight cells are shown) some cells could continue to proliferate (*P*), leave the proliferative population (*Q*), die (or lose the marker) in either the proliferative (*D*, VZ cell in gray with dashed lines) or postproliferative compartment (*D*, CP cell in gray with dashed lines), or move tangentially within the proliferative population (*T*). Abbreviations: M, marginal zone; CP, cortical plate; IZ, intermediate zone; VZ, ventricular zone; V, lateral ventricle.

The cells in each retrovirally labeled lineage in the developing VZ reside in clusters (or clades) of varying size (Cai *et al.*, 1997a). The size of these clusters is dependent on the proliferative behavior of the cells in the labeled lineage, and depends on the mixture of symmetric nonterminal, symmetric terminal, and asymmetric cell divisions. There are three hypothetical mixtures of these three types of cell divisions that could occur (Fig. 13). The three Models differ only with respect to their composition of types of cell division, that is, they each have different ratios of asymmetric, symmetric nonterminal, and symmetric terminal cell divisions; however, all three Models are based on the same *P*/*Q* values measured using double S-phase labeling methods (Takahashi, 1996b; Miyama *et al*., 1997). Importantly, the distribution of cluster sizes is best accounted for by the goodness-of-the-fit of the experimentally determined distribution with the distributions obtained from the model which assumes that all three types of cell divisions coexist during the entire NI, i.e., Model 1 of Fig. 13. Thus, these retroviral experiments: (1) provides evidence for the role of changes in *P*/*Q* in regulation of lineage size, (2) indicates that the amount of cell death and tangential movements in the PVE is low, and (3) indicate that the numbers of lineages that undergo a series of cell divisions with a repeated pattern is undetectable. In essence, these data suggest that the two daughter cells from a single cell division have their fate determined independently.

THE SUBVENTRICULAR ZONE

The SVZ was first recognized by Schaper and Cohen (1905) by the presence of mitotic figures in a location distant from the lateral ventricles. It was first shown definitively to have proliferating cells using ^{3}H-thymidine label *in vitro* using slabs of human brain (Rakic and Sidman, 1968). The proliferating cells of the SVZ differ in two major ways from those of the VZ (Fig. 4). First, the nuclei of proliferating cells of the SVZ do not move during the cell cycle, but reflecting the fact that cells of the SVZ, in contrast to those of the VZ, are not attached to each other as a pseudostratified epithelium, this population does not undergo interkinetic nuclear migration in the course of the cell cycle (Boulder Committee, 1970; Smart, 1972; Altman and Bayer, 1990). Second, the cell bodies of the SVZ cells do not have long

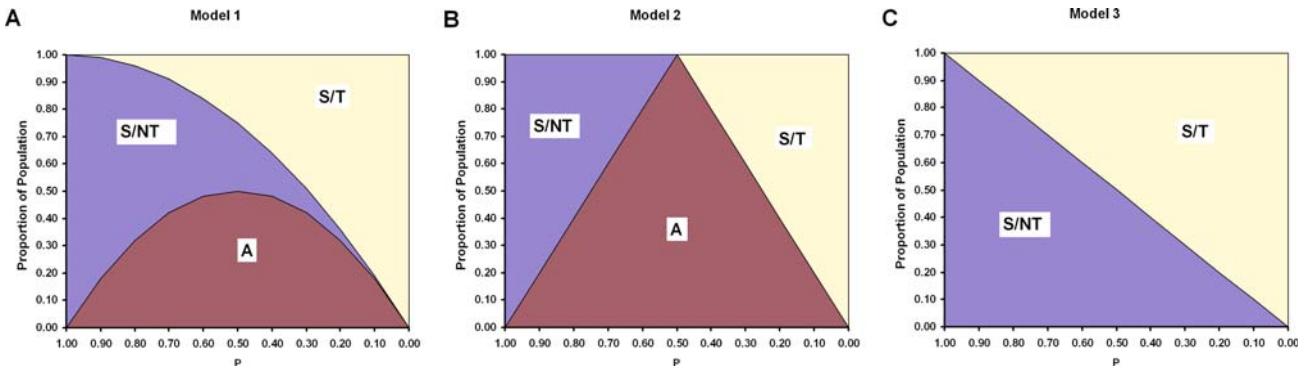

FIGURE 13. Schematic diagrams of the proportions of the changes in the proportions of symmetric non-terminal (S/NT), symmetric-terminal (S/T) and asymmetric cell divisions as a function of changes in P (abscissa) during the neuronogenetic interval. At any given time the sum of the proportions of the 3 types of cell divisions adds up to 1.0 (ordinate). The changes shown are the changes in the 3 types of cell divisions for the 3 different models developed for this study. For a detailed explanation of the assumptions of each model, see the text.

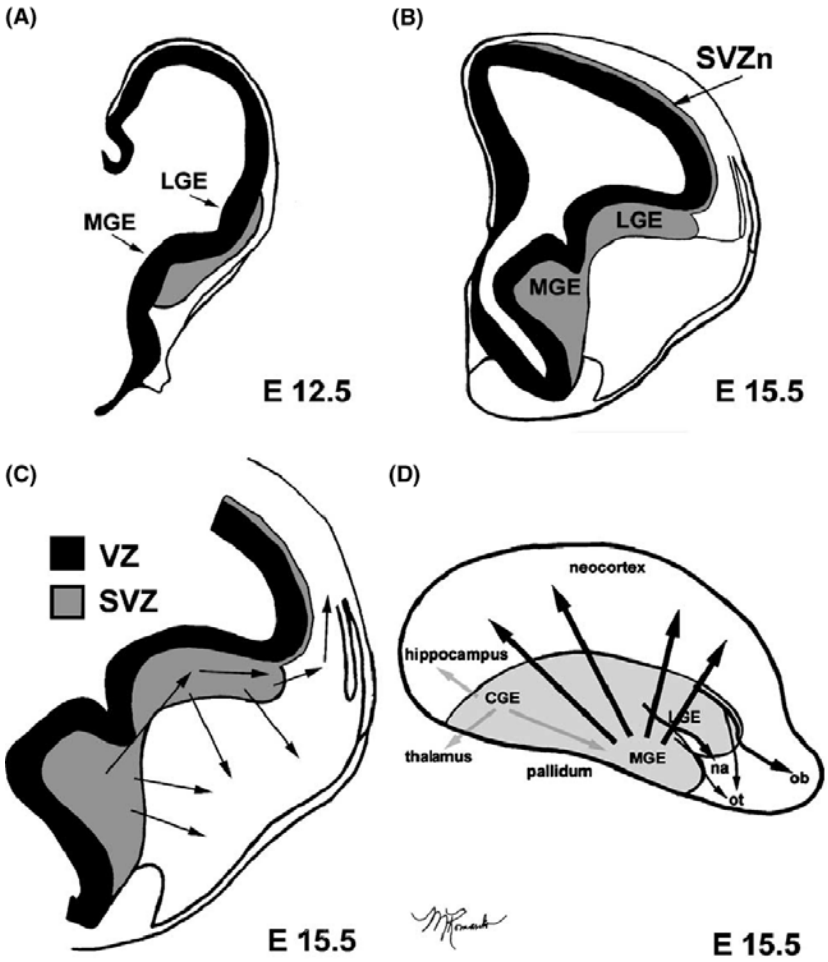

FIGURE 14. Contributions of the SVZ of the medial ganglionic eminence (MGE), lateral ganglionic eminence (LGE), and caudal ganglionic eminence (CGE) to early brain development. (A) A coronal view of the rodent forebrain germinal zones at E12.5. (B) The LGE and MGE are prominent structures in the E15.5 brain. By contrast, at this age the neocortical SVZ is unremarkable. (C) Directional movements of MGE and LGE cells as they migrate to the striatum, neocortex, and nucleus accumbens (na). Cells from the MGE also may migrate through the LGE en route to the neocortex. (D) Sagittal view of the rodent brain at E15.5 shows directional movements from the MGE, LGE, and CGE. Cells of the CGE migrate to the hippocampus, thalamus, pallidum, olfactory tract (ot), and olfactory bulb (ob). Panels (A), (B), (C) were adapted from Lavdas *et al*. (1999) and panel (D) was adapted from Wichterle *et al*. (2001). Figure modified from Brazel *et al*., 2003.

radially oriented processes, but rather they have shorter processes that remain confined to the SVZ (Rakic *et al*., 1974).

Regional Variation in the Subventricular Zone

Not all of the regions of the CNS have a SVZ. This and its distribution makes it attractive to speculate that the SVZ is a phylogenetically recently acquired specialization. For example, the hippocampus is classified as an archicortical (i.e., "old" cortex) structure and the neurons of its major subdivisions (areas CA1, CA2, and CA3) are all derived from the VZ (Nowakowski and Rakic, 1981). In contrast, in the neocortex (i.e., "new" cortex) the SVZ is substantial and may not contribute large numbers of neurons to the neocortex (Takahashi *et al*., 1995a). A similar contrast occurs in the developing diencephalon in which the hypothalamus lacks a SVZ whereas other diencephalic subdivisions have both ventricular and SVZs (Rakic, 1977). The spinal cord, much of the brain stem, and the retinal also lack a SVZ. In the neocortex, at least the time of appearance of the SVZ is approximately coincident with the time of the production of the first neurons (Nowakowski and Rakic, 1981).

The regional variation in the SVZ is far more complex than simply whether or not it is present (Brazel *et al*., 2003). Brazel and Levision (Brazel *et al*., 2003) have recognized a set of geographically defined subdivisions of the SVZ which all differ not only in location but also in the types of cells that they produced. These subdivisions are SVZa, anterior SVZ; SVZdl, dorsolateral SVZ; SVZge, postnatal equivalent of the ganglionic eminences; SVZn, neocortical SVZ; SVZspt, septal SVZ. By far the largest of these subdivisions are the lateral and medial ganglionic eminences which appear quite early in development in the position of the future basal forebrain (Fig. 14). These two proliferative areas persist as a fairly large proliferative zone through the first postnatal week in a rodent (Sturrock and Smart, 1980; Bhide, 1996). In all of its subdivisions during the early part of its existence, the SVZ is intermixed with the VZ along their borders (Boulder Committee, 1970; Altman and Bayer, 1990; Takahashi *et al*., 1993). Cells of the SVZ are also intermixed with nonproliferative cells including postmitotic neurons which arise from the VZ and intermingle with the proliferative cells of the SVZ in their ascent across the cerebral wall. In some regions of the brain the intermixed nonproliferating cells also include the somata of radial glial cells and probably other cells of glial lineage which have left the cell cycle during the epoch of neuronal migration but which may re-enter the cycle in the course of subsequent development of the cerebral wall (Schmechel and Rakic, 1979a; Schmechel and Rakic, 1979b). The SVZ in the lateral and medial ganglionic eminences is highly structured and the cells highly express members of the distal-less family in a pattern that is consistent with a maturation sequence (Fig. 15) (Panganiban and Rubenstein, 2002).

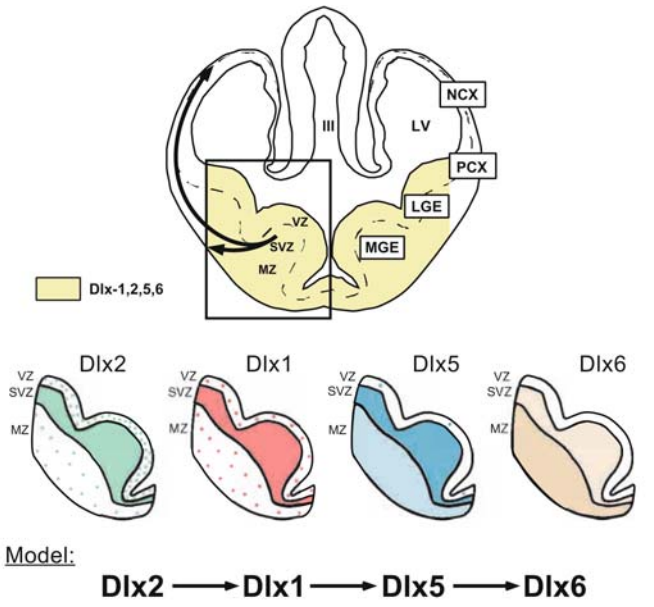

FIGURE 15. Expression domains of Dlx1, Dlx2, Dlx5, and Dlx6 during mouse brain development. (Top) Schema of a transverse section through the E12.5 mouse telencephalon showing the combined expression of Dlx transcripts. Most cells in the subpallial telencephalon express Dlx1, Dlx2, Dlx5, or Dlx6 at some stage of their differentiation. The arrows indicate the migration from the subpallium to the pallium (cortex) (Marin and Rubenstein, 2001). The boxed region on the left is used in the middle section to show the expression of Dlx2, Dlx1, Dlx5, and Dlx6. Dlx2 is primarily expressed in undifferentiated cells; it is expressed in scattered cells in the ventricular zone, in most cells in the subventricular zone and in scattered cells in the mantle zone. Dlx6 is primarily expressed in differentiated cells in the mantle zone. Dlx1 and Dlx5 are expressed in intermediate patterns. (Bottom) A hypothesized genetic and biochemical pathway that proposes the sequential role of Dlx2, Dlx1, Dlx5, and Dlx6 at different stages of differentiation. Telencephalic regions are as follows. Pallium: neocortex (NCX) and palliocortex (PCX). Subpallium: lateral ganglionic eminence (LGE). Medial ganglionic eminence (MGE). Stages of differentiation: ventricular zone (VZ); subventricular zone (SVZ); mantle zone (MZ). LV, lateral ventricle (ventricle of telencephalon); III, third ventricle (ventricle of the diencephalon). Figure modified from Panganiban and Rubenstein 2002.

Output of the Subventricular Zone

The SVZ produces both neurons and glia, but the types of cells produced differ both regionally and temporally. For example, although the neocortical SVZ coexists with the VZ for much of the time that neurons are produced for the neocortex (Takahashi *et al*., 1995b), and, thus, it is possible that the SVZ may produce a small number of neurons destined for the neocortex (Reynolds and Weiss, 1992; Levinson and Goldman, 1993), during this time virtually all of the daughter cells of the SVZ re-enter the cell cycle, and, thus, the proportion of neurons produced is estimated to comprise only at most 5–10% of the total (Takahashi *et al*., 1995b). The anterior part of the SVZ is, however, a major producer of the neurons of the olfactory bulb both during the prenatal and postnatal periods and also into adulthood (Hinds, 1968a, b; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Luskin and McDermott, 1994). Neurons are also produced by the lateral and medial ganglionic eminence. Many of these neurons take up residence locally and comprise the future striatum

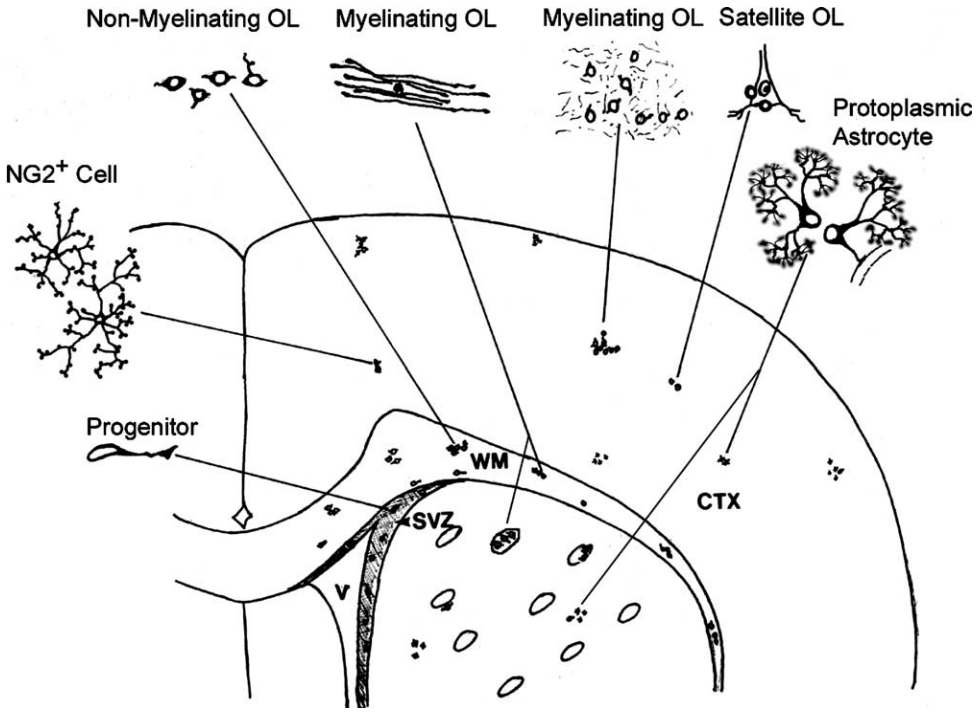

FIGURE 16. The descendants of the perinatal SVZdl. Depicted are the types of cells that are generated from postnatal day 2 SVZdl cells. Progenitors that leave the SVZdl and differentiate within the subcortical white matter become either myelinating or nonmyelinating oligodendrocytes. Few become astrocytes. Those progenitors that differentiate within the neocortex become myelinating oligodendrocytes as well as satellite oligodendrocytes and cells that label with the NG2 proteoglycan. Additionally, those progenitors that make contact with naked cerebral endothelial cells become protoplasmic astrocytes. Figure modified from Brazel *et al*., 2003.

(Bhide, 1996). Another group of them have an interesting fate in that they migrate laterally and populate the neocortex (Marin and Rubenstein, 2001; Letinic *et al*., 2002). This laterally migrating group produces many of the inhibitory interneurons (i.e., GABAergic) of the neocortex. An additional population of inhibitory interneurons (GABAergic) is produced in the ganglionic eminence of humans (but not in other primates or mice) that are destined for the dorsal thalamic nuclei in the thalamus (Rakic and Sidman, 1969; Letinic and Rakic, 2001). The migration of these telencephalic neurons into the dorsal thalamus forms a structure that is large enough to warrant a name, "corpus gangliothalamicum" (Rakic and Sidman, 1969). This special output population suggests that the SVZ proliferative population is available for recent evolutionary modification.

During perinatal life, the SVZ is a principal spawning ground for neuroglial cells (Smart, 1961; Smart and Leblond, 1961; Privat, 1975; Mares and Bruckner, 1978; Smart and McSherry, 1982; LeVine and Goldman, 1988b; Levinson and Goldman, 1993). The best studied of the gliogenic portions of the SVZ is the dorsal–lateral portion of the SVZ, that is, the SVZdl (Fig. 14). Mapping studies using retroviral markers (reviewed by Brazel and Levison, 2003) show that this zone produces a variety of glial cell types (Fig. 16). During postnatal life and into adulthood, parts of the SVZ persist as a population of stem/progenitor cells that seem to proliferate for the lifetime of the animal. These stem/progenitor cells produce both neurons and glia; the largest portion of these seems to be destined for the olfactory bulb, which they reach through the rostral migratory stream (Alvarez-Buylla and Garcia-Verdugo, 2002).

THE DENTATE GYRUS

The subhilar region of the dentate gyrus is a specialized proliferative population that produces the granule cells of the dentate gyrus. The presence of a proliferating population of stem and progenitor cells in the dentate gyrus of mammals was first described in the mouse (Angevine, 1964, 1965). This proliferating population initially arises from the VZ of the medial wall of the lateral ventricle, that is, near the anlage of the dentate gyrus, and migrates into the future position of the dentate hilus (Nowakowski and Rakic, 1981). It persists there during the developmental period and even throughout adulthood in all mammals studied including rodents (Kaplan and Hinds, 1977; Bayer, 1982; Bayer *et al.*, 1982; Stanfield and Trice, 1988), monkeys (Kornack and Rakic, 1999), and humans (Eriksson *et al*., 1998). Despite the persistence of this proliferative population into adulthood, the vast majority of the output of this proliferative population occurs between birth and P20, during which time approximately 80% of the neurons and glial cells of the murine dentate gyrus are born (Angevine, 1965; Bayer and Altman, 1975). However, there is also evidence that in the adult this proliferative population continues to give rise to neurons (and glia),

some portion of which survive, migrate into the granule cell layer, form connections, and become a permanent part of the dentate gyrus granule cell layer (Bayer, 1982; Bayer *et al.*, 1982; Crespo *et al.*, 1986; Stanfield and Trice, 1988) and exhibit important functional properties (van Praag *et al*., 2002). Importantly, it has been shown that during the adult period the number of granule cells increases (Bayer, 1982; Bayer *et al.*, 1982), the newly produced granule cells displace earlier generated granule cells (Crespo *et al*., 1986), and they grow an axon into the molecular layer of CA3 (Stanfield and Trice, 1988). In recent years, this proliferative population has been studied as an example of postnatal neurogenesis and stem cell proliferations. Proliferation in the subhilar region of the dentate gyrus has been shown to be affected by genetic differences (Kempermann *et al*., 1997; Hayes and Nowakowski, 2002), species differences (Kornack and Rakic, 1999), various treatments such as drugs (Eisch *et al*., 2000), stress (Tanapat *et al*., 1998; Gould and Tanapat, 1999), behavioral experiences (Kempermann *et al*., 1998a), hormones (Cameron *et al*., 1998; Tanapat *et al*., 1999), aging (Kempermann *et al*., 1998b), and exercise (van Praag *et al*., 1999).

Although proliferation in the dentate gyrus persists throughout the life span of the animal, there is a significant decline with age (Kuhn *et al.*, 1996; Kempermann *et al.*, 1998b); in mice at 18 months of age the reported number of BUdR labeled cells observed after 12 daily injections is only about 25% of the number observed after a similar labeling paradigm at 6 months of age (Kempermann *et al.*, 1998b). This decline could be due to a decrease in the number of proliferating cells, an increase in the amount of cell death (in either the proliferating population or the output population) during the 12-day period during which the BUdR injections were given, or both. (However, as yet untested is the possibility that the difference could be a result of changes in T_c and/or T_s with age, for example, by a lengthening of G1 or a shortening of S.) What is significant, however, is that the proliferation continues even in aged animals and that even though there is a large decline over a one-year period, the decline is relatively small when considered with respect to the length of a single cell cycle, which is about 12–14 hr in mice (Hayes and Nowakowski, 2002) and about 24 hr in rats (Cameron and McKay, 2001). Using the longer cell cycle, that is, ~24 hr, the changes due to age would indicate that the size of the proliferating population declines at a rate of <0.15% per cell cycle. (Note that the converse also would hold; that is, if the proliferating population is in fact a constant size, then an increase in the length of the cell cycle of ~0.15% per cell cycle could account for the age changes.)

THE RHOMBIC LIP AND THE EXTERNAL GRANULE CELL LAYER OF THE CEREBELLUM

The external granule cell layer of the cerebellum is unique among the proliferating populations of the CNS in that it is adjacent to the pial surface rather than the ventricular surface (Fig. 17). The external granule cell layer was first recognized as the source of the granule cells of the cerebellum near the end of the 19th century (Obersteiner, 1883; Schaper, 1897a, b; Ramon y Cajal, 1909–1911). The cells of the external granule cell layer originate from the rhombic lip and then migrate over the surface of the cerebellum. The rhombic lip also gives rise to neurons of the brain stem, chiefly of the inferior olivary nuclei but also of the cochlear and pontine nuclei (Harkmark, 1954; Taber-Pierce, 1973). In the human the cells migrating from the rhombic lip to the brain stem form a continuous band which was called the corpus pontobulbare by Essick (1907, 1909, 1912).

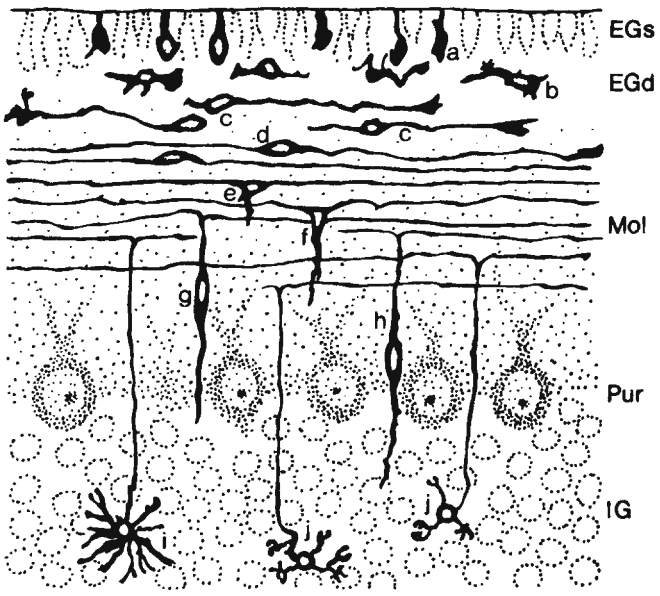

FIGURE 17. The external granule cell layer (EGL) lies beneath the pial surface of the developing cerebellum. These stem/progenitor cells divide in the EGL and migrate through the molecular layer (Mol), past the Purkinje cells into the internal granule cell layer (IG). Drawing from Jacobson (1991), modified from Ramon y Cajal (1909–1911).

The external granule cell layer is present in every vertebrate that has been examined. It is a single layer of cells that is about 6–8 cell diameters thick. Importantly, mitotic figures are scattered throughout the external part of the layer indicating that there is no interkinetic nuclear migration. In this regard, the external granule cell layer is similar to the SVZ. The internal part of the external granule cell layer is not a proliferative zone, but instead it consists of cells that are "waiting" to migrate. The major output of the external granule cell layer is the many cells that comprise the internal granule cell, which are arguably the most numerous neurons in the brain. The life span of the external granule cell is long in comparison with the VZ that produces the Purkinje cells of the cerebellum. For example, in the mouse, the Purkinje cells are produced in a three-day period from E10 through E13 but the internal granule cells are produced over a much more extended period from late in the postnatal period through the third week after birth (Miale and Sidman, 1961). The relatively long period of neuron production in the external granule cell layer is similar in other species including humans (Zecevic and Rakic, 1976).

It is interesting to note that the two major cell classes of the cerebellum, the Purkinje cells and granule cells, are produced in two distinct proliferative zones, the VZ of the fourth ventricle and the external granule cell layer, respectively, at quite different times during development. Thus, it is clear that the final product, that is, the normal cerebellar cortex with a proper number of both types of cells, requires an elaborate regulatory system that would need to include some sort of feedback system through which the early developing cell (the Purkinje cell) could influence the production of the later developing cell (the granule cell). This interaction is hinted at by the changes in the thickness of the external granule cell layer in the reeler mutant mouse where it achieves normal thickness only in places where the Purkinje cell dendrites are normally oriented toward the pial surface (Caviness and Rakic, 1978). Recent evidence indicates that this interaction is mediated by sonic hedgehog which is released from the Purkinje cells and which then binds to the Patched1 receptor on the proliferating cells of the external granule cell layer (Corcoran and Scott, 2001). Mutations in the Patched1 receptor may be involved in the development of medulloblastoma, one of the most common brain tumors of childhood (Corcoran and Scott, 2001; Pomeroy *et al*., 2002).

OVERVIEW

The four major proliferative populations of the developing brain each have a specific role during the development of the brain. They have two important tasks which are to (1) produce the right number of cells for the particular brain region—either too many or too few will result in abnormalities—and (2) to produce the right class of cells (neurons vs glia, and subtypes of each). The delineation of the regulation of these two tasks is a major goal of developmental neuroscience. Progress toward some aspects of this are detailed in other chapters of this book.

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