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# Population Dynamics During Cell Proliferation and Neuronogenesis in the Developing Murine Neocortex

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**Summary.** During the development of the neocortex, cell proliferation occurs in two specialized zones adjacent to the lateral ventricle. One of these zones, the ventricular zone, produces most of the neurons of the neocortex. The proliferating population that resides in the ventricular zone is a pseudostratified ventricular epithelium (PVE) that looks uniform in routine histological preparations, but is, in fact, an active and dynamically changing population. In the mouse, over the course of a 6-day period, the PVE produces approximately 95% of the neurons of the adult neocortex. During this time, the cell cycle of the PVE population lengthens from about 8 h to over 18 h and the progenitor population passes through a total of 11 cell cycles. This 6-day, 11-cell cycle period comprises the “neuronogenetic interval” (NI). At each passage through the cell cycle, the proportion of daughter cells that exit the cell cycle (Q cells) increases from 0 at the onset of the NI to 1 at the end of the NI. The proportion of daughter cells that re-enter the cell cycle (P cells) changes in a complementary fashion from 1 at the onset of the NI to 0 at the end of the NI. This set of systematic changes in the cell cycle and the output from the proliferative population of the PVE allows a quantitative and mathematical treatment of the expansion of the PVE and the growth of the cortical plate that nicely accounts for the observed expansion and growth of the developing neocortex. In addition, we show that the cells produced during a 2-h window of development during specific cell cycles reside in a specific set of laminae in the adult cortex, but that the distributions of the output from consecutive cell cycles overlap. These dynamic events occur in all areas of the PVE underlying the neocortex, but there is a gradient of maturation that begins in the rostrolateral neocortex near the striatotelencephalic junction and which spreads across the surface of the neocortex over a period of 24–36 h. The presence of the gradient across the hemisphere is a possible source of positional information that could be exploited during development to establish the areal borders that characterize the adult neocortex.

The neocortex of mouse, and indeed of all mammals, has a six-layered organization. Moreover, there are strikingly similar numbers of neurons per unit area in different species (Rockel et al. 1974, 1980) albeit with some variation in different cytoarchitectonic areas (Beaulieu and Colonnier 1989; Beaulieu 1993). In contrast, however, the surface area of the neocortex is remarkably variable and accounts for most of the differences in neuron number in different species; for example, the mouse neocortex has a surface area that is about 1/400 of the surface area of the monkey neocortex (Haug 1987). These basic

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facts illustrate that both neuron number and the surface area of the neocortex are critical determinants of the adult structure; it follows that the regulation of neuron number and surface area must be an important feature of development. The regulation of neuron number per se must be under the control of only two developmental processes: i.e., cell proliferation and neuron death. In this chapter we will describe the dynamic changes that occur in the proliferating, migrating, and differentiating populations during neocortical development and illustrate how both the neuron number in the developing neocortex, as well as its surface area, are regulated by basic cell biological processes.

During development, most neurons destined for the neocortex arise from a pseudostratified epithelium that lines the lateral ventricles (i.e., a pseudostratified ventricular epithelium, PVE, see Table 1). For the most part, the cells of the PVE are located in the ventricular zone (VZ; Boulder Committee 1970). During late development a secondary proliferative population (SPP) appears in the location of the subventricular zone (SVZ). The PVE and SPP overlap somewhat at the border between the VZ and the SVZ. The PVE itself is relatively uniform in appearance both across the surface of the ventricles and as development proceeds; it approximately doubles in width early in the neuronogenetic interval but increases dramatically in surface area. Despite its uniform appearance, however, the behavior of this apparently homogeneous population of proliferating cells is not only greatly complex but changes systematically as development proceeds. At various stages during development, the output from the PVE comprises the cells of the secondary proliferative population and neurons and glia of the neocortex, and, thus, the proliferating cells of the PVE meet the definitions of both stem and progenitor cells (see Table 1 for definitions). It is not clear, however, whether the PVE cells are all stem cells, all progenitor cells, or some mixture of cells with multiple potentials. For this

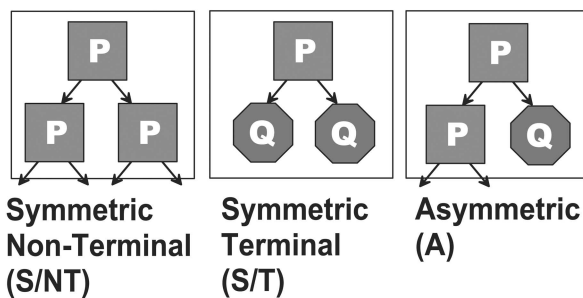
**Table 1.** Nomenclature used

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 postproliferative.
Pseudostratified ventricular epithelium (PVE)	Population of proliferating cells that lines the ventricles of the brain; the PVE is, for the most part, co-extensive with the VZ.
Ventricular zone (VZ)	Cytoarchitectonically defined layer that is adjacent to the ventricles of the brain.

reason, we shall refer to them as stem/progenitor cells, as PVE cells or as proliferating cells, whichever is appropriate for the context. However, as we shall see, the major output of the neocortical PVE is the neurons of the neocortex; thus, it seems likely that the majority of the PVE cells are neuronal progenitor cells and that their major function is neuronogenesis.

The principal behavior of a proliferating population is cell division. This involves passage through the cell cycle beginning at the end of one mitotic division and concluding with another mitotic cell division. With respect to the proliferative (as opposed to the histogenetic) fate of the daughter cells, there are a priori only three types of cell divisions (Fig. 1): symmetric, non-terminal cell division; symmetric, terminal; and asymmetric. Between each mitosis, the cells of the proliferating population pass through four distinct phases: G1, S, G2, and M. The S-phase of the cell cycle is the DNA synthetic phase during which the DNA content of the nucleus is replicated. This allows the markers of DNA synthesis to be used to define the S-phase, to study proliferating cells and to determine the kinetics of the movement of the proliferating cells through the cell cycle. The most widely used S-phase markers are thymidine analogs, in particular tritiated thymidine ( $^3\text{H-TdR}$ ) and halogenated uridine, usually bromodeoxyuridine (BUdR), although iododeoxyuridine (IUdR) and chlorodeoxyuridine (CUdR) have also been used.  $^3\text{H-TdR}$  has been available since 1959 (for a review, see Sidman 1970); generally, the tritium is in the methyl group so that the demethylated form (i.e., uridine) does not lead to incorporation into RNA. The incorporation of  $^3\text{H-TdR}$  into cells is detected autoradiographically. The halogenated uridines are detected by immunohistochemistry using monoclonal antibodies to single-stranded DNA (Gratzner

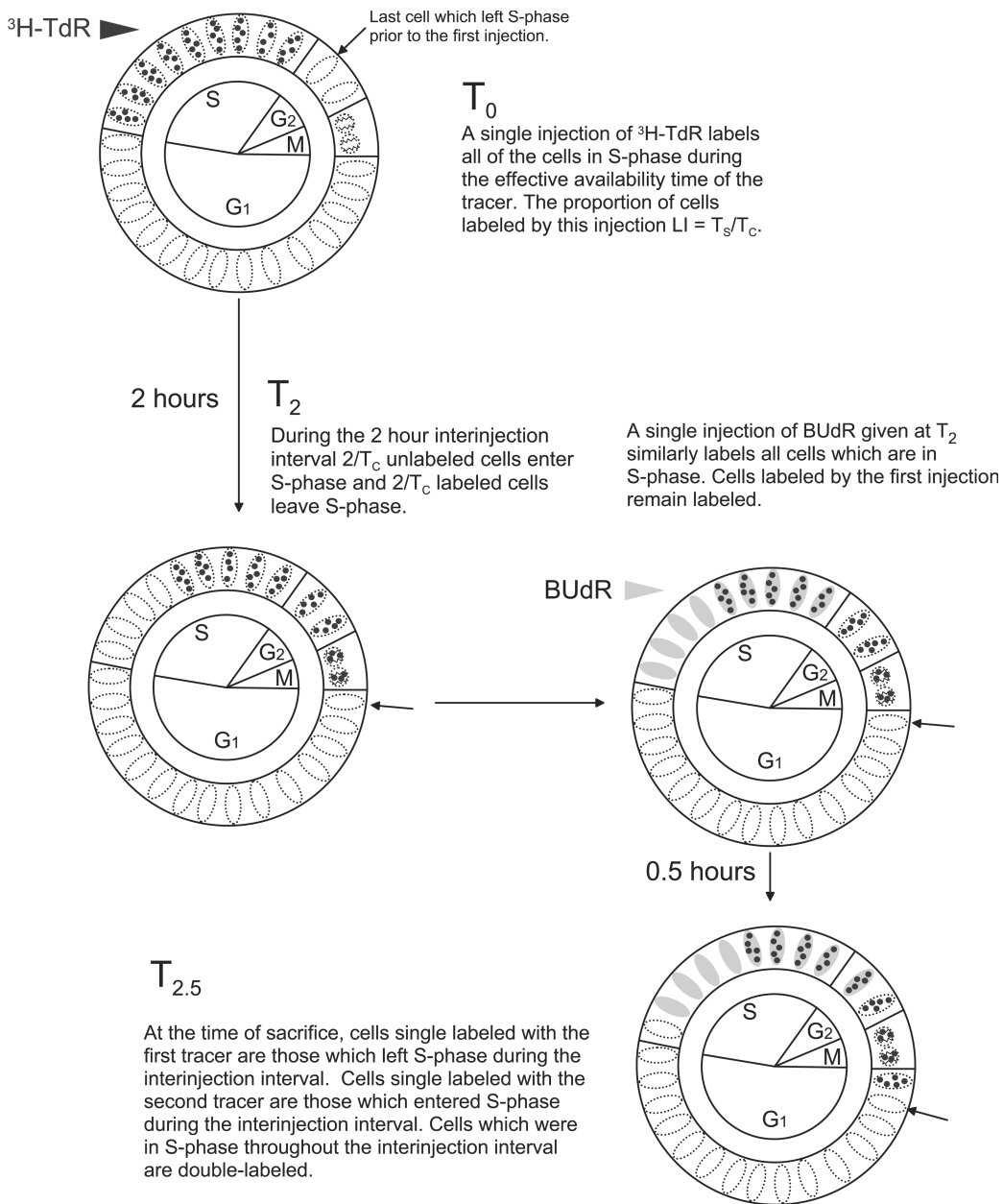
### 3 Types of Cell Divisions



**Fig. 1.** With respect to the decision to re-enter the cell cycle, there are only three types of cell divisions. (1) A proliferating cell can divide to make two daughter cells, both of which re-enter the cell cycle and continue to proliferate (P cells); this is a symmetrical, non-terminal cell division. (2) A proliferating cell can divide to make two daughter cells, neither of which re-enters the cell cycle (Q cells); this is a symmetrical, terminal cell division. (3) A proliferating cell can divide to make two daughter cells of which only one re-enters the cell cycle (P cell) and the other does not (Q cell); this is an asymmetrical cell division

1982). In the developing nervous system, the incorporation of either  $^3\text{H-TdR}$  or  $\text{BUdR}$  does not seem to affect adversely cell proliferation, migration or differentiation of neurons (Miller and Nowakowski 1988; Nowakowski et al. 1989), and, when detection methods that have similar sensitivity are used, the effective loading and clearance times are similar (Hayes and Nowakowski 2000). The loading time, i.e., the time needed to reach detectability, for both  $^3\text{H-TdR}$  and  $\text{BUdR}$  is short, about 10 min; thus, only a short survival (e.g., 0.5 h) is needed to label S-phase cells (Takahashi et al. 1992; Hayes and Nowakowski 2000). Both markers remain at a sufficiently high concentration that rapid labeling of DNA continues for about 30–40 min (Hayes and Nowakowski 2000), which is consistent with the measured declines in the plasma levels (Nowakowski and Rakic 1974), but continued incorporation at low levels persists for 4–8 h after a single injection of either marker (Hayes and Nowakowski 2000). After this time, the levels of the markers apparently fall to a point where even continuous exposure of the proliferating cells during the entire S-phase is insufficient to produce enough incorporation to reach detection (Hayes and Nowakowski 2000).

The availability of S-phase markers provides a convenient point-of-entry for the study of the dynamics of cell proliferation. As described above, a single injection of an S-phase marker will label all the cells in the S-phase; the proliferating cells in the other phases of the cell cycle remain unlabeled (Fig. 2). Thus, if the tissue is collected after a short period of time (e.g., 0.5 h), a snapshot view of the location and number of cells in the S-phase is obtained. Assuming that the cells are randomly distributed in the cell cycle, the proportion of the proliferating population that will be detected is proportional to the length of the S-phase ( $T_s$ ) divided by the length of the complete cell cycle ( $T_c$ ) or  $T_s/T_c$ . However, if sufficient time is allowed to elapse, some of the labeled cells will exit S and enter G<sub>2</sub>, and other cells will enter S from G<sub>1</sub> (for details, see Hayes and Nowakowski 2000). In the developing neocortex, a 2-h period approximates the length of the G<sub>2</sub> + M phases (Takahashi et al. 1995; Hayes and Nowakowski 2000), so, for approximately 2.5 h after exposure to the label, all of the labeled nuclei will be distributed in S, G<sub>2</sub> or M. If at this time (i.e., 2 h after injection of the first tracer) a second S-phase marker is given, a fraction of the proliferating cells proportional to the elapsed time (i.e.,  $2/T_c$ ) will exit the S-phase and enter G<sub>2</sub> (Fig. 2). At the same time, some cells will enter S from G<sub>1</sub>; these cells will not be labeled by the first marker but will be labeled by the second. The cells that remained in S during the 2-h interinjection interval will become double-labeled with the second marker (C and D in diagram on the right). This paradigm will effectively divide the proliferating population into four different types of cells, based on labeling characteristics. (1) The cells that exited S during the interinjection interval will be labeled only with the first S-phase marker. (2) The cells that entered S during the time between the injections and which did not get labeled by the first marker will be labeled only with the second S-phase marker. (3) Cells that were in S at the time of both injections will be labeled by *both* markers. (4) Proliferating cells that were



**Fig. 2.** Labeling paradigm used to identify cohorts of cells in specific phases of the cell cycle or that are born during a narrow window of time. The paradigm depends on the specificity of the S-phase labels, tritiated thymidine ( $^3\text{H-TdR}$ ) or bromodeoxyuridine (BUdR). Either S-phase label is incorporated into cells synthesizing DNA at the time of the exposure, but cells in other phases of the cell cycle are unlabeled. By injecting the two tracers at different times, in this case with a separation of 2 h, cells that are labeled with only one tracer, with both tracers, or with neither tracer are produced



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