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6 November 1991; accepted 9 March 1992

TECHNICAL COMMENTS

Neocortex Development and the Cell Cycle

S. K. McConnell and C. E. Kaznowski report (*1*) that environmental factors can determine the laminar fate of ferret neocortical neurons during the last mitotic division of their ventricular zone precursors. They suggest that the decision of a cortical ventricular zone precursor to generate a deep-layer neuron is made in late S-phase near the transition into G2 of the cell cycle. There is an alternative explanation for the data that is consistent with other findings that suggest a laminar fating of earlier ventricular zone precursors to the neocortex.

McConnell and Kaznowski find that, among migrating neurons, 90% of E29 cells labeled with [^{3}H]thymidine and transplanted 2 hours later into the neonatal host ventricular zone migrate to the superficial (2/3) neocortical layers. However, 90% of these cells transplanted 6 hours later (removed after 4 hours and transplanted 2 hours after that) migrate to the deep (5/6/subplate) layers. Thus, within a 4-hour period near the end of S-phase, 90% of cortical cells must change their laminar fate. If these cells have an S-phase of 8 hours and an unsynchronized cell cycle as stated in (*1*), then only the 25% of the cells that are in the first 2 hours of S-phase and transplanted 2 hours after labeling [rather than the observed 90% in (*1*), figure 2A] should have escaped the deep-layer decision phase in the last 4 hours of S-phase. If the deep-layer decision phase of the cell cycle happened later in S-phase or in G2, then fewer cells transplanted at 6 hours after labeling should have reached the decision phase before transplantation.

Only 20% or less of the transplanted E29 cells actually migrate out of the host ventricular zone into the host neocortical lamina [as detailed by McConnell (*2*)]. It is possible that one of the dissociation, culturing, or transplantation procedures used in (*1*) selected for different 20% subpopulations to migrate among the cells transplanted 2 hours, rather than 6 hours, after labeling. This explanation implies that there were heterogeneous ventricular zone populations among which to select. Heterogeneous ventricular zone populations have been revealed by combining retroviral lineage tracing and [^{3}H]thymidine autoradiography (*3*). In addition, data about cortical genotype ratios in mice produced from blastocyst chimeras have suggested that separate precursor populations may give rise to deep and superficial layer cortical neurons (*4*). We have recently found (*5*) through retroviral lineage tracing of the progeny of individual ventricular zone cells that many mammalian neocortical neuronal clones are restricted to deep, rather than superficial, layers. However, these studies (*3–5*) test for fating or specification of cells, but not if the cells are irreversibly committed to a phenotype; nor do they test whether the commitment can be overcome by environmental cues.

McConnell and Kaznowski also find that most cells lightly labeled with [^{3}H]thymidine (that have presumably divided more than once in the host tissue) migrate to superficial cortical lamina. Once again, these cells may represent a selected subpopulation that is already fated to produce superficial cortical neurons. One would expect continued division by these cells, although the postnatal host tissue environment may artificially limit the total number of divisions by these precursors.

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31 October 1991, accepted 4 February 1992

Response: Our experimental design specifically addressed the possibility that the ventricular zone might contain a heterogeneous mixture of precursor cells [reference 20 in (*1*); (*2*)]. Van der Kooy posits that different subpopulations of precommitted cells migrate selectively when transplanted at different times in the cycle. In other words, the postmitotic daughters of committed upper-layer precursors migrate if and only if they are transplanted in S-phase (at 0 hours), and the daughters of committed deep-layer precursors do not migrate when transplanted in S-phase. At later times (4 to 24 hours) the situation would have to reverse: the postmitotic daughters of committed upper-layer precursors never migrate when cells are removed at or after 4 hours after labeling, whereas the postmitotic daughters of committed deep-layer precursors only migrate when removed at or after 4 hours. Such a complex set of rules and behaviors seems a far less likely explanation than a simple change in fate of a single population of cells. However, the possibility of differential selection or survival of precommitted cells is an important problem to address in any transplantation study, so we performed an additional analysis of our data.

Our hypothesis—that S-phase environment determines cell fate—generates an easily examined prediction. If cells are sensitive to environmental determinants in

S-phase, then cells lightly labeled with [^{3}H]thymidine (which have therefore gone through an additional round of cell division after transplantion) should always change their fates and migrate to the upper layers, regardless of the time interval between thymidine labeling and transplantation. In contrast, if precommitted precursor cells are transplanted, lightly labeled cells should migrate to a mixture of upper and deep layers. We found that lightly labeled cells migrated to the upper layers, adopting the fate typical of their new environment, regardless of the time interval. This finding is incompatible with the selection hypothesis of van der Kooy.

Van der Kooy argues that the distributions of cells in the cell cycle do not perfectly match the timing of the commitment event, which he has assigned to mid–S-phase despite the fact that our data suggest it to be near the S to G2 transition (*1*). We have not identified the exact point in the cell cycle when laminar commitment occurs, but we did not make any strong claims to this effect. Furthermore, our data are consistent with the hypothesis that a cell cycle–dependent event controls determination, for three reasons.

First, cell cycle progression may be most accurately measured not linearly, in hours, but by actual progression through S-phase, as measured by cellular DNA contents. It is well known from studies of cultured cell lines (*3*) that rates of progression through S-phase are variable. Van der Kooy does not appear to take into account the possibility that progression through S-phase proceeds nonlinearly and that the important decision point is related not to time spent in S-phase but to progression through the cycle, as measured by DNA replication.

Second, as we explained in our paper (*1*), 0-hour cells were labeled with [^{3}H]-thymidine in vitro rather than in vivo, so there was less time (roughly an hour) between cell labeling and transplantation at this time point. The actual time that cells had to progress through the cell cycle when one compares the 0-hour and 4-hour time points was closer to 5 hours than the 4-hour period cited by van der Kooy.

Third, our estimate of the length of S-phase is rough and, without knowing the variability in the fractions of cells that migrate to the deep layers at different times, there may be no significant statistical differences between van der Kooy's projections and our data.

Van der Kooy argues that cell lineage experiments reveal "that many mammalian neocortical clones are restricted to deep, rather than superficial, layers." We await the publication of these data and warn that conclusions derived from studies of cortical cell lineages that use single retrovirus vectors have been called into question by recent work of Walsh and Cepko (*4*), who have shown that spatial clustering cannot be used to accurately determine clonal boundaries in the cortex. As van der Kooy points out, cell lineage studies reveal only the normal fates of cells and do not test their developmental potential. The latter was and remains the goal of our studies.

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2. Our experiments were designed to distinguish between two hypotheses, (i) that laminar fate was determined by cell cycle position and (ii) that the ventricular zone contains a mixture of precommitted cells. In previous work [S. K. McConnell, *J. Neurosci.* **8**, 945 (1988)], cells transplanted after a mixture of time intervals from 1 to 4 hours after labeling migrated to a mixture of the upper and deep layers. We reasoned that either the precursor cell population was heterogeneous, in which case the exact interval between labeling and transplantation should be irrelevant to the laminar fates of daughter cells, or the precursor cells were homogeneous but were receiving temporally mixed cues, in which case one should be able to find time points at which cells behave homogeneously. Indeed we found the latter to be the case.
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22 November 1991; accepted 4 February 1992