

1. Wyss-Coray, T. & Mucke, L. *Neuron* **35**, 419–432 (2002).
2. Lu, X. & Richardson, P.M. *J. Neurosci.* **11**, 972–978 (1991).
3. Yin, Y. *et al.* *J. Neurosci.* **23**, 2284–2293 (2003).
4. Yin, Y. *et al.* *Nat. Neurosci.* **9**, 843–852 (2006).
5. Neumann, S. & Woolf, C.J. *Neuron* **23**, 83–91 (1999).
6. Richardson, P.M. & Issa, V.M. *Nature* **309**, 791–793 (1984).
7. Leon, S., Yin, Y., Nguyen, J., Irwin, N. & Benowitz, L.I. *J. Neurosci.* **20**, 4615–4626 (2000).
8. Li, Y., Irwin, N., Yin, Y., Lanser, M. & Benowitz, L.I. *J. Neurosci.* **23**, 7830–7838 (2003).
9. Cao, Z. *et al.* *J. Neurosci.* (in the press).
10. Cai, D. *et al.* *Neuron* **35**, 711–719 (2002).
11. Meyer-Franke, A. *et al.* *Neuron* **21**, 681–693 (1998).
12. Neumann, S., Bradke, F., Tessier-Lavigne, M. & Basbaum, A.I. *Neuron* **34**, 885–893 (2002).
13. Qiu, J. *et al.* *Neuron* **34**, 895–903 (2002).
14. Rapalino, O. *et al.* *Nat. Med.* **4**, 814–821 (1998).

Chalk one up for ‘nature’ during neocortical neurogenesis

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During development, neurons destined for different neocortical layers are sequentially generated. Shen *et al.* report that this timing is programmed within individual progenitor cells and depends mainly on cell-intrinsic mechanisms.

What do neural stem cells ‘know’ and when do they know it? In the embryonic neocortex, this is a challenging question because different neuronal and glial cell types are generated in a specific temporal pattern from a common stem cell/progenitor pool. This process creates the mature neocortex, six layers of neurons with distinct morphological and functional identities. Cajal-Retzius cells are generated early and are essential for the migration of neurons generated later. Then deep layer neurons are generated, followed by neurons that migrate to increasingly superficial layers, in a so-called ‘inside-out’ pattern. Finally, glia are generated. The regulation of this process is poorly understood, but requires a cell-intrinsic program (‘nature’), gradual changes in cell-extrinsic cues (‘nurture’) or some combination. In this issue, Shen and colleagues¹ take advantage of a powerful *in vitro* system to place cell-intrinsic cues front and center.

The presence of multipotent progenitors in the neocortical germinal zones has been demonstrated by retroviral labeling *in vivo* and clonal analysis *in vitro*². However, cell transplantation indicates that, as development proceeds, the potential of neocortical progenitors gradually becomes restricted. For example, although early progenitors can contribute to upper layer (later) fates when transplanted to older embryos³, late progenitors cannot contribute to deep layer (earlier) fates when transplanted to early embryos⁴.

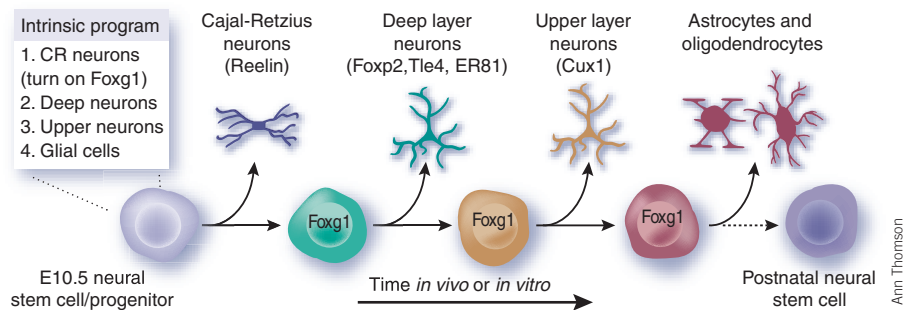


Figure 1 As early as E10.5, neocortical progenitors contain an intrinsic program that specifies the order in which different neuronal and glial cell types will be generated. As these cells mature and the program is executed, their developmental potential becomes restricted to the remaining steps in the program. The transcription factor Foxg1 contributes to this restriction by blocking the production of Cajal-Retzius (CR) neurons and permitting the generation of neocortical neurons (and eventually glia) characteristic of later fates.

Along with other studies^{5–7}, these findings suggest that both cell-intrinsic and cell-extrinsic cues are involved in determining cell fate.

In distinguishing the roles of these signals during neocortical development, the work of Temple and colleagues is among the most informative. They combine adherent progenitor culturing and time-lapse video microscopy to construct family trees of the cells generated from individual progenitors^{8,9}. For lineage analysis, this method is far superior to high-density adherent progenitor cultures and to the clonal ‘neurosphere’ assay in which single progenitors proliferate into balls of cells in suspension. The advantage of the Temple approach is that it permits complete documentation of all cell division, cell migration and cell death, and when combined with subsequent

marker staining, permits the assignment of specific cell fates. Because these cultures are extremely low density, the behavior of each clone is presumed to be encoded by information contained within the initially plated progenitor. With so few cells in a large relative volume, initial cell-cell contact is highly unlikely, nor can secreted molecules reach concentrations sufficient to exert effects.

This research group reported previously that some progenitors produce large clones containing neurons and glia (putative stem cells), and others produce smaller clones containing only neurons (apparent neuroblasts)¹⁰. Furthermore, among the stem cell clones, neurons are generated early during clonal expansion, whereas glia are generated later, mimicking the normal order of cell production *in vivo*. These

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data suggest that individual cells within the progenitor pool have intrinsic information regulating whether they will generate both neurons and glia, and in which order.

This finding raised an exciting question: is a similar intrinsic mechanism operating during the sequential generation of neurons destined for different neocortical layers? The new study clearly suggests that the answer is yes. Using both high-density and clonal progenitor cultures from as early as embryonic day (E) 10.5, Shen and colleagues show that the order and timing of neuronal subtype generation *in vitro* mimics the order and timing observed *in vivo*. First Cajal-Retzius neurons (which are Reelin positive) are generated, followed by cells expressing deep layer markers (Foxp2, Tle4 and ER81), and finally cells expressing an upper layer marker (Cux1). This remarkable finding suggests that, before E10.5, individual neocortical progenitors are intrinsically programmed for a complex pattern of neurogenesis (Fig. 1).

The new analysis also uncovered other interesting findings. First, the stereotypical neuronal birth order was observed whether the original plated cell was multipotent (generating neurons and glia) or was a neuroblast (generating only neurons). This result suggests that neuroblasts are not restricted to generating a single neuronal subtype, but retain a level of multipotency, albeit within the neuronal lineage. Second, progenitors often showed asymmetric division patterns while generating neurons of different laminar character in sequence. This finding suggests that the intrinsic program readout in progenitors is operating in conjunction with mechanisms regulating symmetric versus asymmetric cell division (such as Numb signaling¹¹). In addition, Cajal-Retzius and other neocortical neurons could be generated from a common progenitor. Thus, even if many Cajal-Retzius neurons migrate into the neocortex from other sources, as other studies suggest^{12,13}, at least some are likely to be born in the neocortical germinal zone. Finally, the authors showed that as neuroblasts, and more surprisingly stem cells (narrowly defined by their ability to generate both neurons and glia), carry out their intrinsic program, they lose the capacity to generate cell types from earlier in the program. Such a loss in potency has been suggested by transplantation studies⁴, but they could not draw conclusions about stem cells directly because only a small fraction of the transplanted cells were likely to be stem cells.

The finding that individual neocortical progenitors possess intrinsic information regulating birth order raises interesting questions regarding the molecular control of this process. For example, can progenitors 'count' the number

of divisions they have undergone and use that information to determine what type of neuron to generate next? Such a process might involve gradual chromatin remodeling, and/or a cascade of gene regulation events, with each step somehow triggered by successive cell divisions. In fly neurogenesis, the temporal regulation of a series of transcription factors generates diversity from individual neuroblasts¹⁴. Minimal evidence for such a mechanism exists during neocortical neurogenesis, but this possibility warrants increasing consideration. In addition, it will be of great interest to determine which signals, before E10.5, establish the intrinsic program in neocortical progenitors in the first place.

Although the new paper provides clear evidence in favor of an instructive cell-intrinsic program, the authors do not exclude cell-extrinsic cues. Indeed, they have shown that exposure to the extrinsic cue FGF2 can alter the cellular output of neocortical progenitors *in vitro*⁷. In addition, donor cells can be 'reprogrammed' after *in vivo* cell transplantation, supporting the existence of endogenous cell-extrinsic cues^{3,5,6}. Shen and colleagues looked for such cues either by exposing developing clones to media conditioned by high-density neocortical cultures from various ages (likely to contain secreted molecules that could act as extrinsic cues) or by permitting cell-cell contact between expanding clones and neocortical progenitors from various ages (thus testing for the existence of short-range and/or membrane-bound extrinsic cues). They did not detect any significant changes in clonal composition, suggesting that, at least in the context of these assays, putative extrinsic cues could not override the intrinsic program being carried out by individual progenitors. It remains possible that early-born neurons interact with one or more progenitors in their clone and instruct them to generate later-born neuronal subtypes. Documenting the proximity of individual cells as clones expand might suggest correlations between particular lineage patterns and apparent cell-cell interactions. As a first step in this direction, the authors note that Cajal-Retzius neurons, which are typically born first, migrate away from the rest of the clone, making them less likely to influence subsequent fate choices.

To address the molecular regulation of neuronal birth order, Shen and colleagues turned to the gene *Foxg1*, which encodes a winged-helix/forkhead transcription factor essential for normal forebrain development. *In vivo*, *Foxg1* represses Cajal-Retzius cell fate, as the loss of *Foxg1*, either before neurogenesis or during mid-neurogenesis, leads to excess Cajal-Retzius cell production¹⁵. Although that work found that a cell-intrinsic molecule could

control the order of neocortical neurogenesis, it did not rule out the possibility that *Foxg1* did so by regulating cell-extrinsic signals.

To test the role of *Foxg1* in their *in vitro* clonal system, Shen and colleagues used RNA interference. Knockdown of *Foxg1* expression by shRNA to ~50% of normal levels resulted in smaller clones with a higher fraction of neurons. Consistent with their *in vivo* work, they also found that many more Cajal-Retzius neurons were generated and that *Foxg1* knockdown led to the generation of Cajal-Retzius neurons from E12.5 progenitors, which would otherwise not produce them. However, unlike the *in vivo* work, the low-density clonal analysis clearly suggests that *Foxg1* acts cell intrinsically to regulate neuronal subtype generation. One obvious question is why no phenotype has been reported for animals heterozygous for a *Foxg1* mutation, when the authors saw such dramatic changes *in vitro* after a 50% reduction in *Foxg1* expression. It remains possible that a heterozygous phenotype has yet to be reported or that compensatory mechanisms exist *in vivo*, such as upregulation of the functional allele.

Overall the new work is an important advance toward understanding neocortical neurogenesis. Although we knew that cell-intrinsic mechanisms regulated the switch from neuronal to glial production, at least in part, there was no way to predict whether a similar mechanism regulates the higher-resolution process that generates different types of neocortical neurons. By showing that a cell-intrinsic program contributes to this process, this work provides a much needed foundation for studying the timing and sequence of neocortical neurogenesis. Future work should focus on how the generation of so many different neuronal subtypes can be encoded within an individual progenitor.

1. Shen, Q. *et al.* *Nat. Neurosci.* **9**, 743–751 (2006).
2. Temple, S. *Nature* **414**, 112–117 (2001).
3. McConnell, S.K. *J. Neurosci.* **8**, 945–974 (1988).
4. Frantz, G.D. & McConnell, S.K. *Neuron* **17**, 55–61 (1996).
5. McConnell, S.K. & Kaznowski, C.E. *Science* **254**, 282–285 (1991).
6. Fishell, G. *Development* **121**, 803–812 (1995).
7. Qian, X., Davis, A.A., Goderie, S.K. & Temple, S. *Neuron* **18**, 81–93 (1997).
8. Qian, X. *et al.* *Neuron* **28**, 69–80 (2000).
9. Davis, A.A. & Temple, S. *Nature* **372**, 263–266 (1994).
10. Qian, X., Goderie, S.K., Shen, Q., Stern, J.H. & Temple, S. *Development* **125**, 3143–3152 (1998).
11. Shen, Q., Zhong, W., Jan, Y.N. & Temple, S. *Development* **129**, 4843–4853 (2002).
12. Bielle, F. *et al.* *Nat. Neurosci.* **8**, 1002–1012 (2005).
13. Takiguchi-Hayashi, K. *et al.* *J. Neurosci.* **24**, 2286–2295 (2004).
14. Grosskortenhaus, R., Pearson, B.J., Marusich, A. & Doe, C.Q. *Dev. Cell* **8**, 193–202 (2005).
15. Hanashima, C., Li, S.C., Shen, L., Lai, E. & Fishell, G. *Science* **303**, 56–59 (2004).