Molecular Neurobiology

ISSN0893-7648/06/33(1): 033–050/$30.00

From Radial Glia to Pyramidal-Projection Neuron

Transcription Factor Cascades in Cerebral Cortex Development

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Abstract

Pyramidal-projection neurons are glutamatergic neurons that develop from progenitors in the ventricular and subventricular zones of the embryonic cortex. Recently, much has been learned about the cortical progenitor cells and the cellular and molecular mechanisms by which they produce projection neurons. We now know that radial glia are the progenitors of most or all projection neurons and that they generate neurons by two distinct mitotic sequences: direct neurogenesis to produce a single daughter neuron or indirect neurogenesis to produce two to four neurons via intermediate progenitor cells. The underlying genetic programs for proliferation and differentiation are controlled and implemented by specific transcription factors, whose interactions largely determine the cortical surface area, thickness, and neuronal subtype composition. In turn, transcription factor expression is modulated by extrinsic signals from patterning centers and adjacent cells and by intrinsic signals distributed asymmetrically within progenitors and daughter cells. Together, the new findings provide a coherent framework for understanding cortical neurogenesis.

Index Entries: Basal progenitor; intermediate progenitor cell; Pax6; subventricular zone; Tbr1; Tbr2; ventricular zone.

Introduction

Pyramidal-projection neurons are the predominant type of cortical neuron, accounting for 75 to 85% of the total neuronal population in diverse mammalian species *(1–3)*. They use glutamate as neurotransmitter, make long excitatory connections to other cortical areas and subcortical nuclei, and typically—but not always—display a pyramidal-shaped cell body. The remaining 15 to 25% of cortical neurons are interneurons, which use γ-aminobutyric acid as neurotransmitter, make short inhibitory "local circuit" connections, and display diverse

Received April 13, 2005; Accepted June 9, 2005.
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nonpyramidal morphologies *(1–3).* The distinctions between mature projection neurons and interneurons reflect their origins from different regions of the developing forebrain. Projection neurons are derived mainly from the neuroepithelium of the embryonic cortex (dorsal telencephalon) and migrate radially to form cortical columns, but most interneurons are produced in the basal telencephalon and migrate tangentially into the cortex *(4–6).* Accordingly, progenitor cells for projection neurons and interneurons have distinct molecular, migrational, and morphological properties related to telencephalic regionalization *(7).* This article covers only projection cell neurogenesis, with an emphasis on transcription factors (TFs) and differentiation sequences, from uncommitted progenitor cell to postmitotic pyramidal neuron.

Cortical neurogenesis is a complex process in which proliferation and differentiation are coordinated simultaneously with other fundamental mechanisms of cortical development, such as forebrain regionalization, areal patterning, and laminar fate specification. Unsurprisingly, many of the TFs that regulate cortical neurogenesis also control aspects of these other mechanisms *(8).* This complexity presents difficult challenges to the analysis of TFs by gain- and loss-of-function methods, because manipulations of TF gene expression rarely perturb neuronal output exclusively. Therefore, neurogenesis cannot be considered in isolation but should be approached with regard to the context of other mechanisms.

Historical Perspectives of Cortical Progenitor Cells

In the late 19th century, histologists such as Golgi, Magini, and His observed that mitotic activity in the developing cortex (and throughout the nervous system) occurred mainly at the ventricular (apical) surface of the neuroepithelium. Thus, the ventricular zone (VZ) was recognized as the main histological compartment containing neuronal and glial progenitors. Since then, the morphological features of neuronal and glial progenitors in the VZ have been debated, and different views prevailed alternately throughout the 19th and 20th centuries. The following discussion briefly highlights previous leading theories of cortical progenitor cells. For interested readers, more extensive historical articles are available *(9–11).*

In the late 19th century, His and Cajal *(12)* promulgated the theory that the VZ contains distinct neuronal and glial progenitors, characterized as rounded "germinal cells" and radially aligned "spongioblasts," respectively (Fig. 1A). This theory was later contradicted by evidence that spongioblasts and germinal cells were actually different morphological forms of the same cell type observed at different phases of the cell cycle. Therefore, in 1970, the Boulder Committee *(13)* expressed its consensus view that the VZ contains a single class of multipotent progenitor cells, which they called "ventricular cells." In the early 1980s, Rakic and colleagues *(14,15)* revived the theory of His and Cajal in modified form. They reported that immunoreactivity for glial fibrillary acidic protein (GFAP) distinguished between $GFAP^+$ glial progenitors and $GFAP^-$ neuronal progenitors in developing primate cortex. The $GFAP^+$ progenitors were proposed to generate mature astrocytes as well as radial glia, which were viewed as mainly structural elements for guiding the migrations of newborn cortical neurons *(16).* The $GFAP^-$ progenitors were proposed to generate cortical neurons and were viewed as a distinct, albeit poorly understood, cell type. Until recently, this view of cortical neurogenesis prevailed.

In addition to mitotic activity at the ventricular surface, there were also repeated observations of cell division away from the ventricular surface—especially just outside the VZ. The first reports of mitotic activity outside the VZ appeared in the late 19th century in work by histologists such as Rauber and Merk; others confirmed their findings throughout the 20th century (reviewed in refs. *13* and *17*). In 1970, the Boulder Committee *(13)* introduced a revised

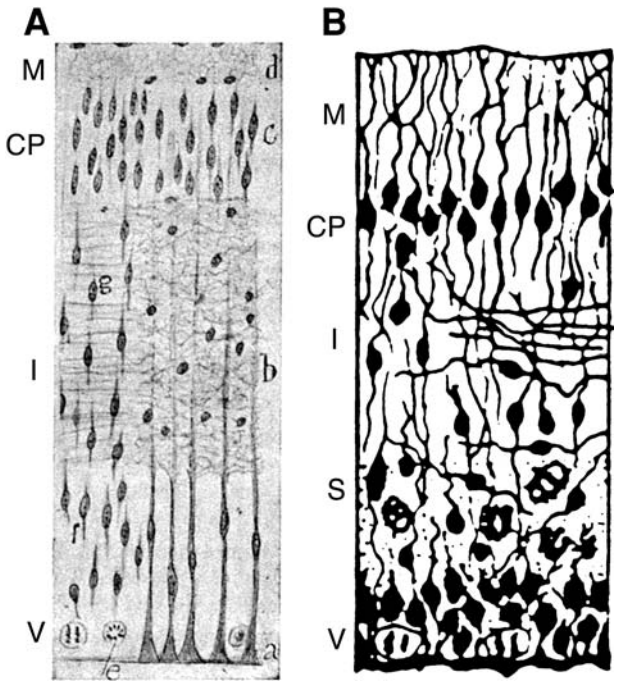

Fig. 1. Previous concepts of cortical progenitor cells. **(A)** At the end of the 19th century, the theory espoused by His and Cajal held that neuronal progenitors or "germinal cells" divided mainly at the ventricular surface and were distinct from radially oriented glial progenitors ("spongioblasts"). This illustration by Cajal *(12)* was adapted from a drawing by His. It shows the fetal human cortex at the beginning of the third gestational month. One mitotic figure at the ventricular surface is labeled "e," and another mitotic figure is shown to its left. (Reproduced with permission from ref. *12*.) **(B)** In 1970, the consensus view of the Boulder Committee *(13)* was that mitotic neuronal progenitors and spongioblasts were different forms of the same "ventricular cells," essentially equivalent to radial glia. Ventricular cells were believed to be the ultimate progenitors of all neurons and glia, similarly to the current theory. The SVZ was distinguished as a separate layer, believed to contain neuronal and glial progenitors. This illustration from the Boulder Committee report *(13),* drawn by Rakic, shows a generalized vertebrate cortex during the middle of neurogenesis. Mitotic figures at the ventricular surface and in the SVZ are drawn with hatched nuclei, in contrast to other cells, which have solid black nuclei. (Reprinted with permission from ref. *13*) CP, cortical plate; I, intermediate zone; M, marginal zone; S, subventricular zone; V, ventricular zone.

nomenclature of the developing cortex, in which the subventricular zone (SVZ) was named and recognized as a progenitor-containing compartment distinct from the VZ (Fig. 1B). In 1973, Smart *(17)* published a quantitative study of surface and nonsurface mitotic activity in the developing mouse cortex. Smart's work documented that nonsurface mitoses appeared in the cortex as early as embryonic day (E) 11 and increased markedly thereafter, accounting for up to 20% of mitotic figures in lateral segments of the cerebral wall on E12. Notably, Smart's careful analysis indicated that nonsurface mitotic activity was substantial even before the SVZ formed a histologically distinct layer (on approx E13–E14 in mice).

The question of whether progenitors dividing in the SVZ and other nonsurface positions generated neurons, glia, or both lineages has engendered controversy. The opinion of the Boulder Committee was that the SVZ "gives rise to special classes of neurons and to all macroglia" *(13).* On the basis of other studies cited in his article, Smart *(17)* suggested that the majority of daughter cells from nonsurface mitoses differentiated into neurons. Smart further asserted that rather than forming a separate progenitor population, "at least some" nonsurface progenitors were daughter cells derived directly from ventricular surface mitoses. More recently, Takahashi and colleagues *(18)* took the opposite position, postulating that progenitors in the SVZ and basal VZ formed a distinct "secondary proliferative population," characterized as "principally the progenitor population to the neuroglial population of the mature neocortex," and "possibly, also a small number of neurons destined for the neocortex."

Such interpretations regarding the identities of cortical progenitors and their progeny (neurons or glia) were limited by the inability to observe and track dividing cells and daughter cells over time and by the lack of molecular markers to identify cell types. Histological methods revealed only a "snapshot" of the developing cortex. Even cell birthdating with [^{3}H]–thymidine (or later, bromodeoxyuridine) could only track cohorts of cells.

These technical barriers were finally overcome at the start of the 21st century, when new methods enabled direct lineage tracing of cortical progenitor cells and their progeny, and new markers facilitated molecular characterization of cell types.

Projection Neurons Are Produced From Radial Glia and Intermediate Progenitor Cells

By the late 1990s, the stage was set for reconsidering the possibility that radial glia might produce neurons as well as astrocytes, and several groups pursued this idea using diverse approaches. Malatesta and colleagues *(19)* purified radial glia by fluorescence-activated cell sorting and characterized their clonal progeny (expanded in vitro) using molecular markers. In a 2000 article, they reported that a large proportion of radial glia (on the order of two-thirds in E14 and E16 mouse cortex) were pure neuronal progenitors *(19).* Two groups soon confirmed their observations in vivo , and both used time-lapse video imaging to directly observe neurogenesis from radial glia. Kriegstein and colleagues *(20)* used retroviral vectors to genetically label individual radial glia with a transgene for enhanced green fluorescent protein (GFP). They characterized the GFP-labeled progeny at different time intervals after retroviral transfection and found that many of the progeny were neurons. Time-lapse imaging of the GFP-transfected cortex in slice cultures demonstrated that single neurons were produced directly from radial glia by asymmetrical division at the ventricular surface. Each asymmetrical division also produced a radial glia, leading to self-renewal of these progenitors. In 2001, Miyata and colleagues *(21)* reported similar findings from time-lapse imaging of cortical slice cultures labeled by focal application of a fluorescent tracer known as DiI. These papers established that at least some projection neurons were produced directly from radial glia dividing at the ventricular surface (Fig. 2A). Subsequent studies based on genetic lineage tracing in mice showed that radial glia are the ultimate progenitors of the vast majority of telencephalic neurons, including virtually all cortical projection neurons *(22,23).* Additionally, some early born cortical neurons may be derived from neuro-epithelial cells, which are morphologically similar to radial glia but lack molecular and ultrastructural characteristics of glial cells *(24).* Because neuro-epithelial cells behave similarly to radial glia regarding neurogenesis and radial glia acquire their characteristic features progressively (leading to different definitions in some studies), neuro-epithelial cells are grouped with radial glia for purposes of this article.

More recent studies using time-lapse imaging and lineage analysis have shed light on the fates of cells produced in the SVZ and other nonsurface positions. Three papers published in 2004 showed that projection neurons arise not only directly from radial glia but also indirectly via intermediate progenitor cells (IPCs) that divide at nonsurface positions in the basal VZ, SVZ, and intermediate zone (IZ; refs. *25–27*). All three groups reported virtually identical observations pertaining to nonsurface-dividing cells (Fig. 2B). First, a radial glia divides asymmetrically at the ventricular surface to self-renew and produce a rounded daughter cell—the IPC. Second, the IPC migrates away from the ventricular surface, usually into the SVZ, and extends multiple short processes. Third, the multipolar IPC divides symmetrically at a nonsurface position to produce two immature, multipolar neurons. Fourth, the immature neurons migrate into the cortical plate and differentiate as projection neurons. Infrequently, the multipolar IPC divides symmetrically to produce two IPCs, which re-enter mitosis, to finally produce four neurons from the original radial glia division *(25).* Because the nonsurface-dividing progenitors arise from radial glia and produce neurons after just one additional division (or less frequently, two divisions), Kriegstein's group *(25)* called them "intermediate progenitor cells."

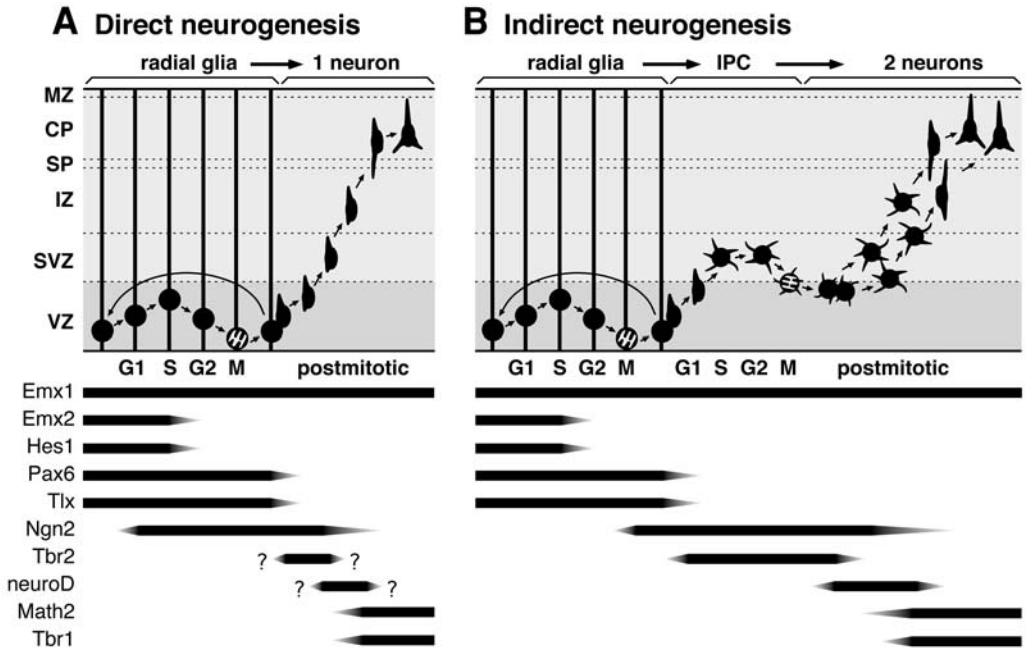

Fig. 2. Direct and indirect pathways of cortical neurogenesis correlated with TF expression. **(A)** Direct neurogenesis. A radial glia divides at the ventricular surface to self-renew and produce one neuron, which migrates radially into the cortical plate. **(B)** Indirect neurogenesis. A radial glia divides at the ventricular surface to self-renew and produce one IPC. The IPC migrates into the SVZ or basal VZ and divides away from the ventricular surface to produce two (or, less often, four) neurons, which migrate radially into the cortical plate. TF expression is indicated below each diagram. Emx1 is expressed in progenitor cells and throughout the differentiation sequence to postmitotic neurons *(98).* Emx2 is expressed in the VZ (where it promotes symmetric progenitor proliferation *[53]*) and in Cajal–Retzius neurons *(99,100). Hes1* is expressed only in the VZ, promotes progenitor cell identity, and suppresses neuronal differentiation *(90,91,101).* Pax6 is expressed in radial glia and is rapidly downregulated in newborn neurons and IPCs *(60,74). Tlx* is expressed in the VZ, where it prevents precocious neuronal differentiation and promotes SVZ histogenesis *(37,42).* Ngn2 protein is expressed in a subset of progenitors (presumably neurogenic) as well as some immature neurons *(27,102).* Tbr2 is expressed in the SVZ and basal VZ (in cells identified as IPCs) and in preplate neurons *(60).* It is unknown if Tbr2 is expressed transiently in the direct pathway. neuroD is expressed mainly in SVZ cells (including some IPCs) and in the IZ as well as preplate neurons during early corticogenesis (refs. *75–77* and *103;* unpublished results, 2005). Math2 and Tbr1 are expressed by postmitotic neurons, mainly in the IZ and cortical plate *(43,84,103).* Zones of the developing cortex are indicated to the left of diagram **(A).** CP, cortical plate; MZ, marginal zone; SP, subplate.

Other groups referred to them as "basal progenitors" or "nonsurface–dividing cells," referring to their location at the time of mitosis *(26,27).* Because some basal/nonsurface mitoses may be gliogenic *(28),* the IPC terminology is used in this article to refer specifically to neurogenic nonsurface-dividing progenitors.

As implied earlier, time-lapse imaging revealed that IPCs display multipolar morphology and migrate slowly in radial and tangential directions by dynamic extension and retraction of short processes *(25).* This "multipolar migration," in which cells appear to move independently of radial glia guidance, is typical of many cells in the SVZ and IZ, including not only IPCs, but also many newborn projection neurons *(29).* Newborn projection neurons also use other modes of migration, dubbed "locomotion" and "translocation," to migrate rapidly along the radial axis *(30).*

Locomotion is believed to be guided by radial glia *(31).* These different modes of migration are used dynamically by subsets of newborn projection neurons at different stages of development and during different phases of migration through the VZ, SVZ, IZ, and cortical plate *(25,29,31).* Additionally, during late stages of neurogenesis, some newborn projection neurons also migrate retrogradely (towards the VZ) and contact the ventricular surface before migrating into the cortical plate *(25).*

Estimates of IPC contributions to cortical neurogenesis suggest that a substantial fraction—possibly a majority—of neurons in upper and lower cortical layers may be derived from IPCs. The methodology employed by Huttner's group was especially amenable to quantitative analysis *(26).* Their imaging technique relied on GFP reporter expression from the endogenous *Tis21* promoter (i.e., *Tis21*–GFP knock-in mice). The *Tis21* gene encodes a cytoplasmic antiproliferative protein that is expressed during G1 phase of mitosis in progenitors fated to undergo neurogenic division *(32).* In the study by Huttner et al. *(26),* cell counting of GFP-labeled mitoses at surface (apical) and nonsurface (basal) positions revealed that most neurons born very early in cortical development (on E10.5 and E11.5) were produced directly from radial glia. Indirect neurogenesis was detected at these very early ages but accounted for only a minority of neuron production. Subsequently, on E12.5 and E13.5, the majority of neurons were produced from IPCs *(26).* Miyata and colleagues *(27)* provided similarly high estimates of IPC contributions to neurogenesis during the middle of corticogenesis. Surprisingly, Kriegstein et al. *(25)* found that during later stages of cortical neurogenesis, when the SVZ is most prominent, radial glia actually produced more neurons than did IPCs. Additional studies are needed to determine precisely what proportions of projection neurons are derived from radial glia and IPCs at each stage of cortical development.

In summary, it is evident that two main pathways exist for neurogenesis in the developing cortex: (a) direct neurogenesis of one neuron from an asymmetrically dividing radial glia (Fig. 2A) and (b) indirect neurogenesis of two (or, sometimes, four) neurons from one radial glia via an IPC (Fig. 2B). A proposed third pathway is "symmetric terminal" division, in which a radial glia divides symmetrically to produce two neurons, without self-renewal *(33).* This type of neurogenic division is suggested to occur at the end of neurogenesis, when progenitor cells are no longer necessary and can be depleted. The existence of symmetrical terminal divisions has been supported by quantitative studies based on parameters of lineage size distribution and cell cycle dynamics *(33).* On the other hand, symmetrical terminal divisions have not been observed directly in imaging studies—not even during late corticogenesis *(25).* Rather, Kriegstein and colleagues *(25)* reported that the end of neurogenesis was marked by "final radial glia" divisions, in which a radial glia divided asymmetrically to produce an astrocyte and a neuron or IPC. Therefore, the putative "third pathway" of projection cell neurogenesis is unconfirmed. Significantly, the observations of Kriegstein and colleagues *(25)* also imply that some radial glia produce both neurons and glia—that is, they are multipotent. Other studies have suggested that most radial glia are committed to either neuronal or glial lineages from early corticogenesis *(10,11,14,15, 19,34).* These issues of radial glia fate potential and heterogeneity continue to be a focus of intense research.

The significance of distinct radial glia and IPC pathways for cortical neurogenesis is presently unknown, but at least two possible implications merit consideration. First, the indirect pathway may provide a mechanism for increasing the number of neurons in a radial column (i.e., cortical thickness) during the constrained time limitations of cortical neurogenesis. IPCs amplify the neuronal output by producing two (or, sometimes, four) neurons from each asymmetrical radial glia division. Because the number of radial glia is an important control point for regulating cortical surface area *(16,35),* the balance of IPC (indirect) and

radial glia (direct) neurogenesis may control the relationship between cortical surface area and thickness. Second, IPCs and radial glia might produce different subtypes of projection neurons (e.g., destined to migrate into different layers or to make different axon projections). Therefore, the SVZ, where IPCs are primarily located, has been proposed as the proliferation zone for upper-layer cortical neurons *(36–41).* However, IPCs also appear to generate the majority of neurons during early neurogenesis, when deep layers are produced *(26).* The significance of dual neurogenic pathways remains an important avenue for further investigation.

Transcription Factors Regulate Fundamental Processes in Corticogenesis

Important progress in understanding cortical neurogenesis has also come from analysis of molecular expression in progenitor cells as well as from gene targeting to alter molecular expression. Such studies have revealed a key role for TFs as markers of distinct progenitor and neuron types and as regulators of progenitor proliferation and cell fate. TFs bind to DNA and activate or repress suites of downstream genes and, therefore, are well-positioned to regulate broad aspects of mitotic activity, fate choice, and differentiation. Other types of molecules also play important roles, but TFs are a large group of principal actors, and their functions and interactions can be explained most effectively in a focused discussion.

Before discussing specific TFs and their functions, two general principles are worth noting. First, the TFs that regulate cortical development belong to large superfamilies of TFs, whose members control the development of tissues and organs throughout the embryo. Transcriptional regulators of cortical development represent virtually all the important TF superfamilies, including homeodomain, paired-domain, basic helix–loop–helix (bHLH), winged helix, nuclear orphan receptor, Ets, zinc finger, and T-domain families. Second, TFs in the developing cortex are often expressed in specific regions, areas, gradients, zones, or layers, and these patterns frequently contain clues to TF functions. For example, Tlx (an orphan nuclear receptor homologous to *tailless* TF in *Drosophila*) is expressed mainly in the cortical VZ (a progenitor compartment), and it regulates proliferation and forebrain regionalization *(37,42).* Conversely, Tbr1 (a T-domain TF) is expressed in the IZ and cortical plate (where postmitotic neurons are located), and it regulates aspects of neuronal differentiation and layer-related subtype specification *(43).* On the basis of their expression patterns, many TFs can reliably be correlated with specific steps in proliferation and differentiation via direct and indirect pathways of neurogenesis (Fig. 2).

Before the onset of neurogenesis, TFs in the cortical neuro-epithelium function mainly to promote forebrain regionalization, areal patterning, and progenitor proliferation and suppress neuronal differentiation. Forebrain regionalization refers to the establishment of distinctions and boundaries between embryonic cortex (dorsal telencephalon) and subcortical structures (ventral telencephalon). Emx1 and Emx2 (homeodomain TFs), Pax6 (a paired and homeodomain TF), Tlx, and neurogenin (Ngn)-1 and Ngn2 (bHLH TFs) are among the TFs contributing to regionalization *(7,44,45).* Importantly, each of these TFs also plays essential roles in other aspects of cortical development, such as areal patterning and neurogenesis. Areal patterning refers to parcellation of the cortex into specialized subdivisions with unique functions, such as motor, somatosensory, auditory, and visual. This process depends on setting up rostrocaudal and mediolateral gradients of TF expression, which are modulated by diffusible signals from patterning centers at the anterior, medial, and lateral boundaries of the cortex *(8,46–48).* Emx2 and Pax6 expression gradients are particularly important in areal patterning, as demonstrated by experimental perturbations of their gradients *(49,50).*

As neurogenesis begins, cortical neuroepithelial cells assume the morphological and

molecular properties of radial glia and shift away from pure proliferation to a combination of proliferation and neuronal differentiation *(34).* Proliferation and neuronal differentiation can be seen as opposing forces in cortical development, because proliferation expands or maintains the progenitor pool, whereas differentiation depletes it. At the end of corticogenesis, progenitors choose exclusively differentiation (neuronal and glial) at the expense of expansion and self-renewal. Because of the importance of maintaining the proper balance between proliferation and differentiation at each stage of cortical neurogenesis, a large number of TFs are involved in regulating this choice. The bHLH molecules are foremost among the transcriptional regulators of proliferation and neurogenesis *(51).* This large superfamily includes members that maintain proliferative activity and suppress differentiation (Id and Hes families) as well as "proneural" TFs that promote neuronal fate commitment, suppress glial differentiation *(52),* and induce exit from the mitotic cycle (e.g., Ngn1 and Ngn2). Proliferation, self-renewal, and neurogenesis are also regulated by other, non–bHLH types of TFs. Emx2 and Tlx favor progenitor proliferation *(37,53),* whereas Pax6 promotes neurogenesis *(45,54).* Interestingly, Lef1/TCF (a TF activated by the canonical Wnt signaling pathway) apparently favors proliferation in early corticogenesis but favors neuronal differentiation in late corticogenesis *(35,55).*

Once the commitment to neurogenesis is made, additional TFs consolidate differentiation of the projection neuron phenotype and begin to specify projection neuron subtypes related to laminar fate. These processes begin even as progenitors undergo their final mitotic cycle *(56–59),* and they continue as postmitotic neurons migrate to their destinations within the cortical plate. Neuronal differentiation factors include bHLH TFs such as neuroD, neuroD2, and Math2, which are all expressed in newborn cortical projection neurons (at least transiently) *(51).* Two T-domain TFs, Tbr2 and Tbr1, are expressed sequentially in many (possibly all) projection neurons during differentiation, but their role in determining general properties of projection neurons is not fully understood *(43,60).*

Laminar fate, an important aspect of projection neuron subtype *(61),* is tightly linked to cell birthday *(62–64)* and is determined in progenitor cells during the last mitotic cycle that will produce the neuron *(56).* Interestingly, the sequential production of neuron types in the cortex has many similarities to neurogenesis in *Drosophila,* in which genetic screens have identified a group of TFs that control "temporal identity" and whose sequential expression directs progenitors to produce a series of neuron types *(65).* It has been hypothesized that laminar fate in the cortex could be directed by a similar sequence of temporal regulator TFs *(65, 66).* However, TFs with temporal regulator properties have not been identified in the mammalian cortex, and cortical neurons need not be generated in a fixed sequential order *(67).*

Several TFs have been linked to laminar fate in the cortex. Er81 (an Ets domain transcription factor) is expressed in some progenitor cells and, subsequently, in a subset of layer 5 projection neurons, suggesting that Er81 may specify layer 5 fate *(58).* Similarly, Cux2 (a homeodomain TF) is expressed in mitotically active SVZ cells and in upper-layer neurons, suggesting that it may specify upper-layer fate *(38,39).* Foxg1 (a winged-helix TF) has been proposed as a relatively specific suppressor of Cajal–Retzius cell fate *(59),* but the increased production of Cajal–Retzius cells in *Foxg1*$^{-/-}$ mutant mice has also been attributed to altered areal patterning with expansion of the cortical hem *(68),* which is an apparent source of Cajal–Retzius cells *(69).* In postmitotic projection neurons, several TFs are expressed in layer-related patterns, including Tbr1 *(43),* Foxp2, and Foxp1 (winged-helix TFs; ref. *70*); Nurr1 (a nuclear orphan factor; ref. *71*); Fez1 (a zinc-finger TF; ref. *72*); and others *(61).* Large-scale analysis of gene expression suggests that different subtypes of projection neurons are defined by the combinatorial expression of many TFs and other molecules *(73).*

The recent characterization of IPCs as neuronal progenitors has brought additional correlations with TF expression. Actually, the identification of IPCs as distinct progenitors was presaged by studies of Pax6 expression. Götz and colleagues *(74)* discovered that although radial glia are $Pax6^+$, a separate subset of cortical progenitor cells located mainly in the SVZ and basal VZ are $Pax6^-$. Subsequently, Englund et al. *(60)* found that the $Pax6^-$ progenitors are IPCs and that the IPCs specifically express Tbr2. Low levels of Pax6 were detected in a minority of $Tbr2^+$ cells (mainly in the VZ), which were presumably newly generated IPCs that inherited Pax6 passively from radial glia. Another subset of $Tbr2^+$ cells, including some in M phase, expressed low levels of NeuN, which is a neuronal marker; these were interpreted as IPCs making the transition into newborn neurons. As these newborn neurons migrated out of the VZ and SVZ and into the IZ and cortical plate, they upregulated expression of Tbr1 and downregulated Tbr2. These observations led to delineation of a TF sequence, Pax6 → Tbr2 → Tbr1, in the differentiation of radial glia → IPCs → postmitotic neurons (Fig. 2B; ref. *60*). It is unknown whether Tbr2 is expressed in the direct pathway from radial glia → postmitotic neurons. If so, then Tbr2 must be expressed very briefly, because radial glia do not express Tbr2, nor do newborn neurons in the upper IZ and cortical plate. Nevertheless, it is possible that neurons produced directly from radial glia express Tbr2 transiently as they migrate from the ventricular surface to the lower IZ.

The finding that IPCs express Tbr2 suggests that they are committed to a glutamatergic neuronal fate, although they have not yet completed mitotic activity. This conclusion is implied because both Tbr2 and Tbr1 have only been detected in glutamatergic neurons and their precursors and have never been detected in γ-aminobutyric acid-ergic interneurons or glia (ref. *43* and unpublished observations). This generalization is true not only in the cortex but also among cells that express Tbr2 and Tbr1 in the olfactory bulb, basal forebrain, and cerebellum (manuscript in preparation, 2005). However, further studies (e. g., transplantation) are needed to conclusively determine if IPCs retain the potential to differentiate into cell types other than glutamatergic neurons.

The emerging view of cortical neurogenesis suggests that differentiation from radial glia to pyramidal neuron proceeds through a sequence of stages that are associated with the expression of specific TFs in the direct and indirect pathways. Consistent with this view, my lab has obtained new preliminary evidence that neuroD may mark a distinct stage of projection neuron differentiation. *neuroD* messenger RNA is detectable mainly in the upper SVZ and lower IZ of the developing neocortex *(75–77).* We have found that neuroD protein is co–expressed with Tbr2 in a subset of cells in the SVZ and with Tbr1 in a subset of cells in the lower IZ (unpublished observations, 2005). These observations suggest that neuroD may mark the transition from late IPC to early postmitotic migrating neuron.

TF Cascades and the Regulation of Cortical Neurogenesis

Genetic studies of TF functions have started to uncover interactions and mechanisms that regulate projection cell fate specification. As noted earlier, the molecular mechanisms of neuronal fate specification have their beginnings in proliferating progenitor cells. Radial glia and IPCs make the commitment to neurogenesis (as indicated by *Tis21* expression) during G1 phase *(26),* and laminar fates are selected around the S/G2 transition *(56).* As neurogenic progenitors proceed through M phase, they are then faced with a decision of which daughter cells will adopt neuronal and progenitor (self-renewal) fates.

The series of fate choices inherent in neurogenesis are coordinated by multiple cell-intrinsic and -extrinsic molecular systems acting throughout the cell cycle. Intrinsic factors (mainly TFs) not only regulate proliferation and neurogenesis but also limit the range of

available fate choices. Most radial glia appear to be restricted to either neuronal or glial lineages *(10,11,14,15,19,34),* and laminar fate potential is progressively restricted as corticogenesis proceeds *(78).* The intrinsic factors are modulated by extrinsic signals from neighboring cells. For example, signaling through the Notch transmembrane receptor promotes radial glia fate *(79),* and the laminar fates of neurons can be affected by short-range cues within the cortex *(67,80).* Finally, proliferation and cell fate are also influenced by asymmetrical cellular inheritance of factors such as Numb (a negative regulator of Notch), Numblike, epithelial growth factor receptor, and apical plasma membrane components *(81–83).*

In radial glia that are neuronal progenitors, the available fate choices include radial glia (self-renewal), neuron, or IPC. Several TFs have been implicated in promoting either self-renewal or neuronal fates. Recent studies of *Pax6, Tlx, Ngn1,* and *Ngn2* suggest that these TF genes may be particularly important at different stages (i. e., during early or late corticogenesis) *(84).* Additionally, TFs may potentially select direct or indirect pathways of neurogenesis. Most previous studies have not distinguished between direct and indirect pathways of neurogenesis, because until recently, the indirect pathway was not characterized. Nevertheless, a re-examination of previous work suggests that some TFs may regulate IPC production (i.e., the indirect pathway) selectively.

The TF most convincingly linked to IPC production is Ngn2. Retrovirally mediated overexpression of Ngn2 in vivo causes the cortex to shift from mainly surface mitotic divisions to mainly nonsurface divisions *(27),* suggesting that high-level Ngn2 expression favors IPC production. In turn, the *Ngn2* gene is directly regulated by Pax6 at high levels *(45),* and retrovirally mediated *Pax6* overexpression instructs neurogenesis *(54).* Mice deficient in *Pax6* have fewer cortical neurons, and sorted radial glia cells have reduced neurogenic potential *(54). Pax6* mutants show decreased expression of *Ngn2* and *Tbr2* (an IPC marker) in cortical regions *(44,54,85,86).* Furthermore, *Pax6* mutants have a defective SVZ (where IPCs are mainly located), as indicated by decreased expression of SVZ markers *Svet1* and *Cux2 (36,38,39).* Together, these data suggest that Pax6 may promote indirect neurogenesis by activating *Ngn2* expression. However, it remains uncertain whether Pax6 regulates indirect or direct neurogenesis selectively. Against this idea, transduction of Pax6 retrovirus into dissociated cells from E14 mouse cortex resulted in increased production of single-cell clones *(54),* which could be interpreted as direct neurogenesis in vitro (with the caveat that cell culture conditions may alter proliferation). Moreover, *Pax6* loss-of-function mutants actually have increased nonsurface mitotic activity in the cortex *(87,88).*

The abundant nonsurface mitotic figures can be interpreted as IPCs, suggesting that Pax6 suppresses indirect neurogenesis, or as ectopic surface mitoses (radial glia), which divide in abnormal nonsurface locations because of a disturbance of progenitor and neuron migration *(87).* Further complicating the issue, some defects in the *Pax6* mutant cortex arise from abnormal dorsal–ventral identity and ectopic expression of molecules associated with striatal progenitors *(85,86).* The complex functions of Pax6 in forebrain development requires additional study to resolve its specific role in regulating indirect versus direct neurogenesis in the cortex.

Tlx is another TF that may promote the IPC pathway selectively. Similarly to *Pax6* mutant mice, *Tlx* mutants have specific defects of the SVZ and late-born cortical neurons *(37,84).* Interestingly, during early telencephalic development, *Tlx* and *Pax6* cooperate genetically to establish the pallio–subpallial boundary *(42).* Their similar roles in SVZ formation and upper-layer neurogenesis suggest that they may further cooperate during late cortical development *(84).*

A counterbalance to Ngn2, Pax6, and Tlx may be provided by inhibitors of neurogenesis that block IPC production. Similarly to *Ngn2,* the most important inhibitors of neurogenesis, *Hes* and *Id* genes, belong to the bHLH superfamily. Whereas Hes TFs bind DNA directly

and repress transcription, Ids are "dominant-negative" HLH molecules, which lack the basic DNA binding domain. They inhibit differentiation by binding E proteins and sequestering them from proneural and neuronal differentiation bHLH factors such as Ngn2 and neuroD *(51).*

Mice with mutations of *Hes* and *Id* genes generally show precocious neurogenesis in the cortex and other brain areas. Variations of this theme have been observed in *Hes1 (89,90), Hes5 (89), Hes1/Hes5* double-mutant *(91), Id4 (77),* and *Id1/Id3* double-mutant *(92)* mice.

Hes1 and Id4 may inhibit IPC production with some selectivity, relative to direct neurogenesis. Despite precocious neurogenesis, *Hes1*$^{-/-}$ embryos demonstrate no apparent depletion of cortical progenitor cells *(90).* Significantly, in contrast to wild-type progenitor cells, which typically divide to produce one neuron and one progenitor cell, *Hes1*$^{-/-}$ progenitor cells (from E13.5 forebrain) typically divide to produce two neurons, like IPCs *(90).* These data suggest that the *Hes1*$^{-/-}$ cortex may contain an increased proportion of IPCs. Alternatively, the *Hes1*$^{-/-}$ phenotype may reflect failure of self-renewal, although if this were the case, one would expect to see progenitor cell depletion.

Id4 mutant mice show even stronger evidence of increased IPC production. *Id4*-null mice display a unique phenotype characterized by precocious neurogenesis, decreased cortical surface area, and increased cortical thickness *(77).* The VZ and SVZ are thickened, nonsurface mitoses are dramatically increased, and *Tbr2, Ngn2,* and *NeuroD* expression are upregulated in at least some cortical areas *(77).* These features can be interpreted to suggest that IPC production is selectively increased at the expense of direct neurogenesis from radial glia (resulting in a thicker cortex) and at the expense of radial glia self-renewal (resulting in a reduced cortical surface area).

The hypothesis that IPC neurogenesis is selectively promoted and inhibited by specific TFs (Fig. 3) would be strengthened if differential effects on direct (radial glia) and indirect (IPC) neurogenesis could be verified quantitatively.

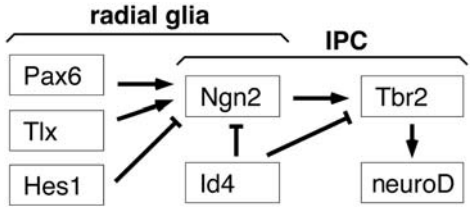

Fig. 3. Transcription factors implicated in regulating IPC production from radial glia. Tbr2 is expressed in IPCs but not in radial glia *(60).* NeuroD is also expressed in some IPCs, mainly in the SVZ (refs. *75–77* and *103;* unpublished data, 2005). Ngn2 overexpression induces cortical progenitor cells to divide at nonsurface positions *(27),* suggesting that Ngn2 promotes IPC production. Pax6 at high levels is a transcriptional activator of the *Ngn2* gene *(45),* and Pax6 is required for histogenesis of the SVZ *(36, 38,39),* where IPCs are predominantly located. Similarly, Tlx is necessary for SVZ histogenesis *(37).* Hes1 and Id4 appear to inhibit IPC production. Whereas *Hes1* is expressed only in the VZ (*101*), *Id4* is expressed in the VZ, SVZ, IZ, and cortical plate *(104).* E13.5 *Hes1*$^{-/-}$ progenitors demonstrate an increased propensity to divide symmetrically to produce two neurons, typical of IPCs *(90).* In *Id4*$^{-/-}$ embryos, the IPC marker Tbr2 is upregulated, as are neuroD and Ngn2 *(77).* Therefore, indirect neurogenesis may be controlled by a balance of TFs that promote (Ngn2, Pax6, and Tlx) or inhibit (Hes1, Id4) IPC production from radial glia.

Tis21–GFP mice could be valuable for this purpose, because GFP is expressed only in neurogenic progenitors, and double-labeling with phospho–histone H3 reveals neurogenic mitoses in surface and nonsurface positions *(26).* By breeding this reporter into other genetic models, it should be possible to measure changes in the amount of direct and indirect neurogenesis caused by genetic perturbations.

Projection Cell Neurogenesis, Cortical Surface Area, and Cortical Thickness

The developing cortex can be conceptualized as an array of radially organized proliferative

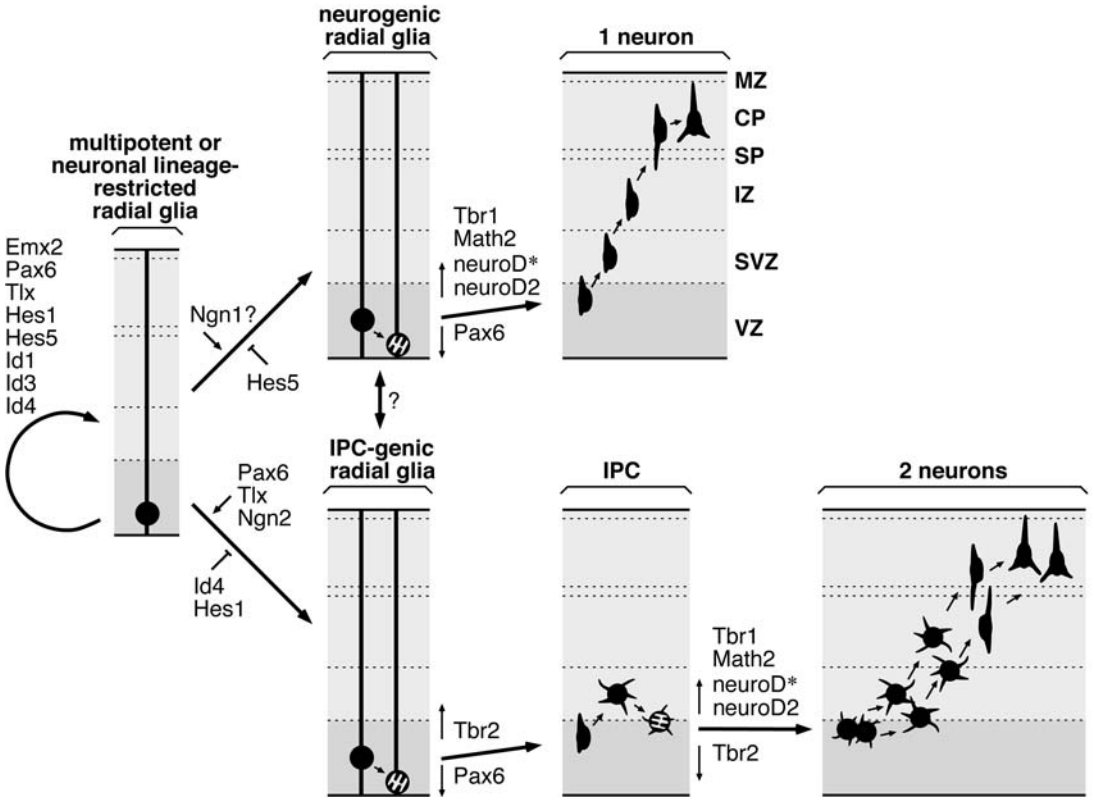

Fig. 4. Model for TF regulation of direct and indirect pathways of cortical neurogenesis. Projection neurons are ultimately produced from multipotent or neuronal lineage-restricted radial glia. Subsets of these radial glia express TFs that pattern cortical identity (such as Emx2, Tlx, and Pax6), promote symmetrical progenitor divisions (Emx2), and maintain the proliferative state (including Emx2, Hes1, Hes5, Id1, Id3, and Id4). Individual radial glia produce neurons (upper, direct neurogenesis pathway) or IPCs (lower, indirect neurogenesis pathway) by asymmetric division. The neurogenic and IPC-genic forms of radial glia may be interconvertible. In the direct pathway, Ngn1 and Hes5 are indicated as likely regulators of direct neurogenesis (although both probably also regulate indirect neurogenesis—that is, they are not selective for either pathway). The direct transition from radial glia to newborn neuron is correlated with downregulation of radial glia marker Pax6 and upregulation of postmitotic neuronal markers Tbr1, Math2, and neuroD2 *(60, 84, 105).* Indirect neurogenesis is promoted by Ngn2 *(27)* and is inhibited by Id4 *(77)* and Hes1 *(90),* although it is unclear whether any of these TFs are truly selective for the indirect pathway. Pax6 and Tlx appear to cooperatively promote indirect neurogenesis, especially in later stages of corticogenesis when the SVZ is prominent *(36–39,84).* The transition from radial glia to IPC involves upregulation of Tbr2 and downregulation of Pax6 *(60).* The subsequent transition from IPC to neuron is correlated with downregulation of Tbr2 and upregulation of Tbr1, Math2, and neuroD2 *(60, 84, 105).* NeuroD is expressed transiently (denoted by *) by late IPCs in the SVZ and by early postmitotic neurons in the lower IZ (refs. *75–77;* and unpublished observations, 2005).

units that produce ontogenetic columns (the "radial unit model" of Rakic *[16]*). According to this model, cortical surface area is directly related to the number of radial units, and cortical thickness is directly related to the neuronal output from each radial unit. Clearly, proliferation and neurogenesis are not the only factors affecting cortical dimensions, but they are major early determinants. Other significant factors may include programmed cell death, interneu-

ron migration from the basal telencephalon, cell size, and glial cell proliferation. The current understanding of progenitors, neurogenesis, and TFs (Fig. 4) suggests possible cellular and molecular mechanisms by which the number of radial units and the total neuronal output of each proliferative unit may be modulated.

The cellular counterparts of radial units are believed to be the pool of self-renewing neuronal progenitors, which includes neurogenic and "IPC-genic" radial glia (Fig. 4). Predictably, cortical surface area should be reduced in mutants where expansion of the radial glia neuronal progenitor pool is decreased. Emx2 has been implicated in promoting symmetric progenitor proliferation *(53),* and *Emx2* mutant mice have an approx 30% reduction of cortical surface area *(93),* presumably resulting from decreased expansion of the radial glia pool. Other perturbations can also reduce the cortical surface area. Mutations that cause precocious neuronal differentiation may shorten the period of progenitor pool expansion, thereby decreasing the number of radial glia. Consistent with this hypothesis, *Id4* mutant mice exhibit premature neuronal differentiation in association with reduced cortical surface area at maturity *(77). Id1/Id3* double-mutant mice also exhibit precocious neurogenesis and early reduction of cortical surface area, with death by E13.5 *(92).* Similarly, *Hes1* mutants *(90)* and *Hes1/Hes5* double-mutants *(91)* demonstrate precocious neuronal differentiation, with severe hypoplasia of the cortex and other parts of the central nervous system as well as death *in utero.* Conversely, when early cortical progenitors are forced to maintain proliferative activity and expand the progenitor pool beyond normal limits (by transgenic overexpression of stabilized β-catenin), the cortical surface area is vastly increased, and the cortical mantle is thinned *(35).*

The neuronal output of each radial unit depends on the number of progenitor self-renewal cycles, the ratio of neurogenesis via direct and indirect pathways, the duration of the neurogenic period, and cell cycle kinetics. Accordingly, a thin cortex can result from many different kinds of perturbations to the system. Precocious neuronal differentiation can reduce cortical thickness by decreasing the number of progenitor self-renewal cycles, as occurs in *Id1/Id3, Hes1,* and *Hes1/Hes5* mutants *(90–92).* On the other hand, delayed neuronal differentiation can also reduce cortical thickness by decreasing the number of mitotic cycles devoted to neuron production, as in stabilized β-catenin transgenic mice *(35).* A shift away from indirect neurogenesis in favor of direct neurogenesis can also be predicted to decrease the cortical thickness, because the direct pathway produces only one neuron, whereas the indirect pathway produces two to four neurons. However, this type of change has not yet been documented.

An abnormally thick cortex may also follow from specific perturbations. An increase of indirect neurogenesis at the expense of direct neurogenesis should increase the neuronal output from each radial unit, as may occur in *Id4* mutant mice *(77).* Acceleration of the cell cycle can result in a thicker cortex by increasing the number of mitotic cycles during the neurogenic period, as reported in nestin/insulin-like growth factor-I transgenic mice *(94,95).* Although precocious neurogenesis might predictably increase the neuronal output of radial units, the opposite generally occurs, because accelerated neuronal commitment decreases the number of progenitor self-renewal cycles, as noted earlier (e. g., *Hes1*$^{-/-}$ mutants *[90]*).

Implications for Human Neurological Disorders, Repair, and Regeneration

Malformations of cortical development are a major public health problem responsible for many cases of epilepsy, cerebral palsy, and severe cognitive disability *(96).* Additionally, subtle defects of cortical development without obvious malformation may be an equally important problem, causing poorly understood, complex cognitive disorders such as mental retardation, autism, and schizophrenia. In some cases, complex cognitive disorders may even be

directly linked to mutations in genes for TFs. For example, human psychological deficits in executive and social cognition have been linked to heterozygous *PAX6* mutation with subtle structural abnormalities *(97).* The current updated view of cortical development provides a framework for investigating such disorders.

New approaches to the treatment of neurological disorders will also be impacted by research on cortical development. The hope that neural circuits may be regenerated using endogenous or transplanted stem cells is based on the belief that new neurons can be induced to migrate to the desired location, differentiate correctly, and make appropriate dendritic and axonal connections. The research on cortical development reveals that for these processes to occur effectively, neurons must express the right combination of TFs to impart regional, areal, and neuronal subtype information. Therefore, neural stem cells may have to be genetically engineered to express the right molecular profile for the location of the desired repair. TF genes may be particularly useful in this regard, because they control overall genetic programs pertaining to differentiation and connections.

Acknowledgments

I thank Magdalena Götz, John Rubenstein, and Chris Englund for critical reading of the manuscript. Supported by the National Institute of Neurological Disorders and Stroke (K02 NS045018), the Edward S. Mallinckrodt, Jr. Foundation, and the C. -M. Shaw Assistant Professorship in Investigative Neuropathology.

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