Neuronal subtype specification in the cerebral cortex

Bradley J. Molyneaux[*¶], *Paola Arlotta*[*‡¶], *Joao R. L. Menezes*[*§] and *Jeffrey D. Macklis*[*]

Abstract | In recent years, tremendous progress has been made in understanding the mechanisms underlying the specification of projection neurons within the mammalian neocortex. New experimental approaches have made it possible to identify progenitors and study the lineage relationships of different neocortical projection neurons. An expanding set of genes with layer and neuronal subtype specificity have been identified within the neocortex, and their function during projection neuron development is starting to be elucidated. Here, we assess recent data regarding the nature of neocortical progenitors, review the roles of individual genes in projection neuron specification and discuss the implications for progenitor plasticity.

*MGH-HMS Center for Nervous System Repair, Departments of Neurosurgery and Neurology, Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital; and Harvard Stem Cell Institute, Harvard University, Boston, Massachusetts 02114, USA.
‡Current address: Center for Regenerative Medicine, Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, USA.
§Laboratório de Neuroanatomia Celular, Departamento de Anatomia, Instituto de Ciências Biomédicas, Programa em Ciências Morfológicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
Correspondence to J.D.M.
e-mail: jeffrey_macklis@hms.harvard.edu
¶These authors contributed equally to this work.
doi:10.1038/nrn2151

The mammalian neocortex is a complex, highly organized, six-layered structure that contains hundreds of different neuronal cell types and a diverse range of glia[1,2]. It is the region of the brain responsible for cognitive function, sensory perception and consciousness, and as such it has undergone pronounced expansion and development during evolution[3]. There are two broad classes of cortical neurons: interneurons, which make local connections; and projection neurons, which extend axons to distant intracortical, subcortical and subcerebral targets. Projection neurons are glutamatergic neurons characterized by a typical pyramidal morphology that transmit information between different regions of the neocortex and to other regions of the brain. During development, they are generated from progenitors of the neocortical germinal zone located in the dorsolateral wall of the telencephalon[4–8]. By contrast, GABA (γ-aminobutyric acid)-containing interneurons and Cajal–Retzius cells are generated primarily from progenitors in the ventral telencephalon and cortical hem, respectively, and migrate long distances to their final locations within the neocortex[9] (BOX 1). In this manner, multiple progenitor zones contribute to the rich variety of neuronal types found in the neocortex.

Within the mature neocortex, distinct populations of projection neurons are located in different cortical layers and areas, have unique morphological features, express different complements of transcription factors, and ultimately serve different functions. The complexity and diversity of projection neuron subtypes makes any classification scheme difficult, but the most accurate system probably extends beyond hodology (anatomical projections) to include a combination of morphology, electrophysiological properties and patterns of gene expression[1,10]. Nevertheless, the most basic schema is based on hodology, which has proved useful during the initial investigation of neuronal subtype development (BOX 2).

How are these various projection neuron subtypes generated during corticogenesis? Insights from years of study in the spinal cord and retina provide models for how a diversity of neuronal types can be generated[11,12]. Within the neocortex, some of the basic mechanisms that control general neuronal specification, migration and connectivity during development have been identified[13–15]. More recently, the discovery of genes that have layer and neuronal subtype specificity within the neocortex has made it possible to begin to investigate the mechanisms underlying the specification of individual projection neuron subtypes.

Here we review the development of the rodent neocortex in the context of recent data regarding the role of individual genes in controlling the specification and development of distinct projection neuron subtypes. First, we describe the diversity of progenitors that give rise to the projection neurons of the neocortex. Next, we discuss the molecular programmes that instruct the early steps of progenitor specification, and extensively review the genes that define neuronal subtype- and layer-specific identity. Finally, we focus on subcerebral projection neurons as a prototypical population and describe recent advances in understanding the sequence of signals that control the generation of this developmentally and clinically important neuronal type.

Box 1 | **Distant progenitor zones contribute to the neuronal diversity of the neocortex**

A wealth of evidence has accumulated indicating that neocortical GABA (γ-aminobutyric acid)-containing interneurons are derived from germinal zones outside the neocortex and migrate long distances to their final locations[9]. The majority of neocortical interneurons originate in the medial and caudal ganglionic eminences in the ventral telencephalon, whereas smaller populations might be produced in the lateral ganglionic eminence and septal area[9]. Each progenitor domain contributes distinct sets of interneurons to the neocortex, indicating an evolutionary mechanism for expanding the diversity of neuronal types within the cortex[123–125]. Like interneurons, different subtypes of layer I Cajal–Retzius cells are derived from at least three regions outside the neocortex: the caudomedial cortical hem[61,126–128], the pallial–subpallial boundary[129] and the septum[129]; the bulk of Cajal–Retzius cells originate from the cortical hem[128]. Interestingly, there seems to be a difference in humans, in that a subpopulation of interneurons within the human neocortex are derived from progenitors that undergo at least their final mitosis in the dorsal telencephalon[130]. However, it is not yet known if these progenitors observed in humans represent a new class of dorsal progenitor, or if they are ventrally derived cells that migrate into the dorsal ventricular zone and subventricular zone before producing interneurons locally.

Neocortical progenitors

During early development, there is a dramatic expansion of the neuroepithelium in the dorsolateral wall of the rostral neural tube that will give rise to neocortical projection neurons. The layer immediately adjacent to the ventricle is termed the ventricular zone (VZ). As neurogenesis proceeds, an additional proliferative layer known as the subventricular zone (SVZ) forms above the VZ[16,17]. Progenitors residing in the VZ and SVZ produce the projection neurons of the different neocortical layers in a tightly controlled temporal order from embryonic day (E) 11.5 to E17.5 in the mouse[18–20], and postmitotic neurons position themselves in the developing neocortex through defined modes of radial and tangential migration[6,21–23]. The earliest born neurons appear around E10.5 in the mouse and form a layered structure termed the preplate, which is later split into the more superficial marginal zone and the deeply located subplate. The cortical plate, which will give rise to the multilayered neocortex, begins to develop in between these two layers[17], such that later born neurons arriving at the cortical plate migrate past earlier born neurons[18,20] (FIG. 1).

The precise relationships among progenitors and the identities of the individual progenitor populations that give rise to each projection neuron type are still largely unknown. Lineage tracing of clonally related populations indicates that, at the earliest stages of cortical neurogenesis (approximately E11.5 in the mouse), individual progenitors are able to give rise to pyramidal neurons across layers II–VI[7,24]. As development progresses, progenitors become progressively restricted in their competence states. Early cortical progenitors normally fated to form deep-layer neurons are multipotent and can generate later born neurons of upper layers when transplanted into the niche of late progenitors[25]. Progenitors of the upper layers have less plasticity[26,27] but can be induced to generate earlier fates under the appropriate conditions[28,29].

There are at least three basic types of neurogenic progenitors within the developing neocortex: neuroepithelial cells, radial glia and intermediate progenitors[30]. Initially, there is a single sheet of pseudostratified neuroepithelial cells undergoing symmetric cell divisions to expand the pool of multipotent progenitors as well as a smaller percentage of asymmetric cell divisions to generate the earliest born neurons[30–32]. As neurogenesis progresses, they transform into radial glia, which share some but not all antigenicity with the early neuroepithelial cells[33,34]. Possessing long processes that extend from the ventricular wall to the pial surface, radial glia have long been known to have crucial roles in guiding neurons to their final locations in the cortical plate by serving as a migratory scaffolding[6,23]. More recently, several studies have provided direct evidence that at least some radial glia also function as progenitors that make major contributions to cortical neurogenesis[22,33–38] by generating pyramidal neurons either directly through mitoses at the apical surface of the VZ, or indirectly through the production of proliferating intermediate progenitors[39]. In addition to the full-length radial glia, other neuron-producing precursors have been described in the VZ[30,38,40,41]. One study observed a subpopulation of progenitors that can be distinguished from radial glial cells by the absence of a full-length pial process and by the ability to drive the *Tα1* α-tubulin promoter and named them 'short neural precursors'[40]. Importantly, this study also found that all progenitors within the VZ are labelled with markers that had been considered radial glia specific (for example, RC2 and GLAST)[40]. Further study is needed to clarify the extent of diversity and the relationship of progenitors within the VZ.

Intermediate progenitors (also known as basal progenitors) are the other major type of neuron-producing progenitor and are located in the SVZ, and in the basal VZ early in neurogenesis before the formation of the SVZ. The SVZ starts to form at E13.5 in the mouse and expands significantly during late corticogenesis[17,42]. It has been proposed that the SVZ might be a site of neurogenesis for upper-layer neurons[42], but solid evidence supporting this hypothesis was lacking. Instead, cell divisions in the SVZ were thought to primarily contribute to gliogenesis, not neurogenesis[43]. More recently, elegant studies in slice culture have shown that radial glia frequently undergo an asymmetric division to generate an intermediate progenitor, which then migrates into the SVZ before pausing and undergoing a symmetric round of cell division to produce two neurons[39,44]. Using *Tis21*-green fluorescent protein (GFP) knock-in mice that only express nuclear GFP in cells undergoing a neuron-producing division, progenitors dividing at the basal side of the VZ (the developing SVZ) were observed to undergo symmetric cell divisions

Subcortical targets
Structures located ventral to the cortex, including the thalamus, brainstem and spinal cord.

Subcerebral targets
Structures located ventral to the cerebrum (telencephalon/diencephalon), including the brainstem and spinal cord.

Cajal–Retzius cells
Early-born neurons of cortical layer I that express reelin.

Competence state
The intrinsic molecular state of a cell that determines its differentiation potential.

Niche
Specific anatomical, cellular and molecular environment of a cell or population of cells.

Symmetric cell division
A mode of cell division that gives rise to two daughter cells of the same type.

Asymmetric cell division
A mode of cell division that gives rise to two different daughter cells.

Box 2 | **Major subtypes of projection neuron within the neocortex**

Classified by hodology, there are three basic classes of cortical projection neuron: associative, commissural and corticofugal. Below are some principal subtypes:

Commissural

Callosal projection neurons. Projection neurons of small to medium pyramidal size that are primarily located in layers II/III, V and VI, and extend an axon across the corpus callosum (CC) (panel **a**). At least three major types of callosal neuron can be classified. These maintain: single projections to the contralateral cortex (black); dual projections to the contralateral cortex and ipsilateral or contralateral striatum (blue); and dual projections to the contralateral cortex and ipsilateral frontal cortex (green). These never project axons to targets outside the telencephalon. Str, striatum.

Corticofugal (subcortical)

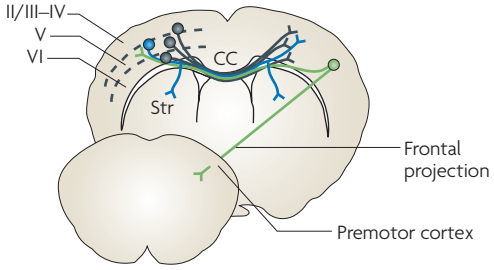
Corticothalamic neurons. Projection neurons primarily located in cortical layer VI, with a smaller population in layer V, that project subcortically to different nuclei of the thalamus (Th) (panel **b**).

Subcerebral projection neurons. Also referred to as type I layer V projection neurons (panel **c**). These include pyramidal neurons of the largest size, which are located in deep-layer V and extend projections to the brainstem and spinal cord. They can be even further subdivided into several distinct projection neuron subtypes. Among them:

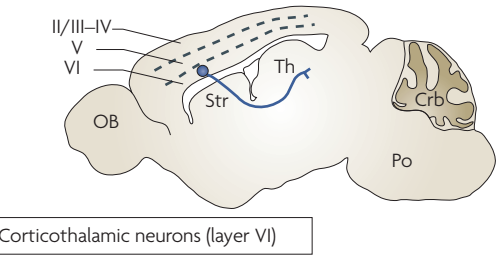
- Corticotectal neurons (orange) are located in the visual area of the cortex and maintain primary projections to the superior colliculus , with secondary collateral projections to the rostral pons (Po).
- Corticopontine neurons (pink) maintain primary projections to the pons.
- Corticospinal motor neurons (purple) are located in the sensorimotor area of the cortex and maintain primary projections to the spinal cord, with secondary collaterals to the striatum, red nucleus, caudal pons and medulla.

Many other subtypes of subcerebral projection neuron exist that send axons to different areas of the brainstem or have different combinations of collaterals, but are not depicted here for simplicity. Crb, cerebellum; OB, olfactory bulb; SC, spinal cord.

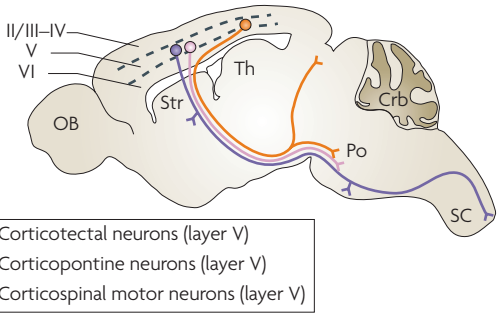
a Commissural; callosal projection neurons



b Corticofugal; corticothalamic neurons



c Corticofugal; subcerebral projection neurons



Associative projection neurons
Neurons that extend axonal projections within a single cerebral hemisphere.

Commissural projection neurons
Neurons that extend axonal projections within the cortex to the opposite hemisphere via the corpus callosum or the anterior commissure.

Corticofugal projection neurons
Neurons that extend axonal projections 'away' from the cortex. These include subcerebral projection neurons and corticothalamic neurons.

giving rise to two postmitotic neurons[45] , confirming and extending the slice culture results on a global scale.

Further evidence that this observed neurogenesis in the SVZ contributes to the generation of upper-layer neurons came from the identification of several markers that are expressed in the SVZ during upper-layer neurogenesis and are also expressed in upper-layer postmitotic neurons. For example, subventricular-expressed transcript 1 (*Svet1*) and cut-like 2 (*Cux2*, also known as *Cutl2*) are expressed in a subset of dividing cells in the SVZ during the generation of upper-layer neurons and postnatally in some neurons of layers II–IV, suggesting that *Svet1* and *Cux2* might be markers for upper-layer progenitors within the SVZ[46–48]. Interestingly, *Cux2* expression is detected in the basal VZ starting at E11.5 in mice, suggesting that progenitors committed to the generation of upper-layer neurons might be present early in cortical neurogenesis, before the formation of the SVZ and several days before the production of upper-layer neurons[46,48], although more definitive fate mapping experiments are needed to explore this possibility. A separate set of *in vivo* fate mapping experiments used the promoter regions of the gene neurogenic differentiation 6 (*Nex*, also known as *Neurod6*) to obtain *Cre* recombinase expression in progenitors of the SVZ combined with a floxed reporter delivered by adenovirus at E14 to label progenitors during the generation of upper-layer neurons. GFP reporter expression was mapped to neurons residing in the upper layers, providing more definitive support for the SVZ origin of upper-layer neurons[49]. Studies of the evolution of the mammalian cortex suggest that upper-layer neurons are a recent evolutionary addition, whereas layer V and VI projection neurons might be related to pyramidal neurons of a primitive cortex[50–52].

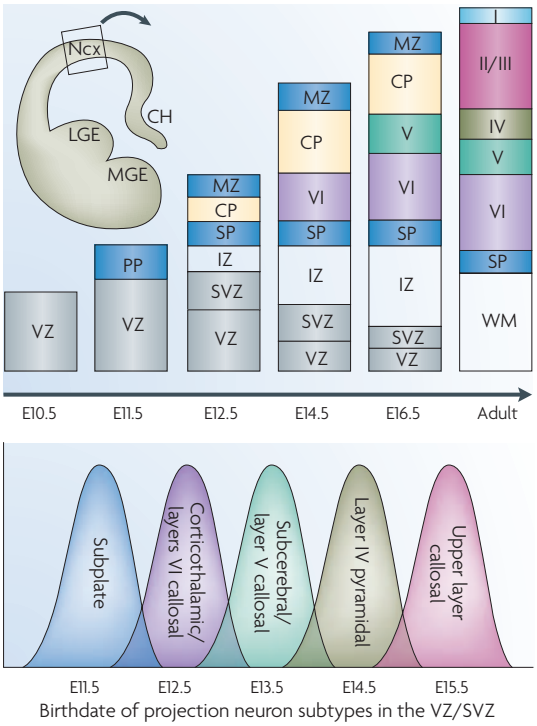


Figure 1 | **Schematic depicting how progenitors residing in the VZ and SVZ in mice produce projection neurons in an 'inside-out' fashion.** The earliest born neurons form the preplate (PP), which is later split into the more superficial marginal zone (MZ) and the deeply located subplate (SP). The cortical plate (CP), which will give rise to the multilayered neocortex, develops in between these two layers, such that later born neurons arriving at the cortical plate migrate past earlier born neurons. Different classes of projection neuron are born in overlapping temporal waves. All times listed are approximations given the neurogenic gradients that exist across the cortex, where caudomedial neurogenesis lags behind rostrolateral neurogenesis[17]. CH, cortical hem; E, embryonic day; Ncx, neocortex; IZ, intermediate zone; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; SVZ, subventricular zone; VZ, ventricular zone; WM, white matter. Modified, with permission, from REF. 131 © (2002) Elsevier Science.

Thus, the expansion of the SVZ might represent an evolutionary mechanism to increase the number of neurons within the neocortex, especially during the generation of neurons of upper layers[53,54].

Given the heterogeneity of progenitor types and their different locations in the VZ and SVZ, it is not clear how the balance of extrinsic and intrinsic signals combines to determine neuronal fate. Early experiments that tested the effect of cell–cell contact and the ventricular niche in controlling the fate of early progenitors indicate that cell–cell contact and/or extrinsic signals are required for early progenitors to give rise to deep-layer neurons upon transplantation[55]. So far, beyond a recent study demonstrating a role for brain-derived neurotrophic factor (BDNF) as an extrinsic signal in the VZ and SVZ that is important for laminar fate determination, few extrinsic signals have been identified[28]. Interestingly, time-lapse microscopy of cortical progenitors cultured at clonal density revealed that a progenitor can continue to divide *in vitro* and produce neurons that express laminar markers after the same number of cell divisions as their *in vivo* counterparts. Although it will be important to further investigate whether these cells possess a full cortical phenotype in light of prior work showing that culture manipulations can modify phenotypic acquistion by cortical progenitors[56], these data suggest that the timing and progression to produce a given subtype of projection neuron is at least partially intrinsic to progenitors[57]. This could be due to the initiation of an intrinsic signalling cascade within a multipotent progenitor several days before its postmitotic neurons are generated. Unfortunately, these *in vitro* studies did not test whether single clones could sequentially generate layer VI neurons, followed by layer V neurons, followed by upper-layer neurons. Additional experiments are necessary to investigate the clonal relationships among neuronal subtypes of different layers, and to determine whether progenitors can progress through the generation of different subtypes of pyramidal neurons while *in vitro*. Despite the existence of this intrinsic programme, it is important to note that the transplantation of early progenitors into later environments indicates that extracellular signals can alter this programme as long as the environmental signals are changed before the S phase of the cell cycle[25,58].

Progressive specification of projection neurons

Specification of neocortical progenitors. Upon induction of the telencephalon by gradients of extracellular signalling molecules such as sonic hedgehog, fibroblast growth factors and bone morphogenetic proteins[59], a number of genes that direct neocortical neurogenesis are expressed across the dorsolateral wall of the telencephalon. These include LIM homeobox 2 (*Lhx2*), forkhead box G1 (*Foxg1*), empty spiracles homologue 2 (*Emx2*) and paired box 6 (*Pax6*), each of which has crucial roles in specifying the progenitors that give rise to the projection neurons of the neocortex. Together, these four genes establish the neocortical progenitor domain by repressing dorsal midline (*Lhx2* and *Foxg1*) and ventral (*Emx2* and *Pax6*) fates (FIG. 2).

In the absence of *Lhx2*, most of the neocortical VZ is absent and there is a dramatic expansion of structures normally limited to the dorsal midline[60–62]. Similarly, in the absence of *Foxg1*, neocortical progenitors are not specified, whereas progenitors of the archicortex (which gives rise to the hippocampus) and the cortical hem (one major source of Cajal–Retzius cells) are expanded[63,64]. Remarkably, *Foxg1* removal as late as E13.5 from progenitors that already have a neocortical identity results in the production of cells with characteristics of Cajal–Retzius cells[57,65], indicating that the persistent expression of *Foxg1* throughout neurogenesis is required for the maintenance of neocortical progenitor identity. This suggests that progenitors, although seemingly progressively fate-restricted, retain a tremendous degree of plasticity.

Emx2 and *Pax6*, which are expressed in opposite and overlapping gradients in the dorsal telencephalon and are key determinants of the proper development of

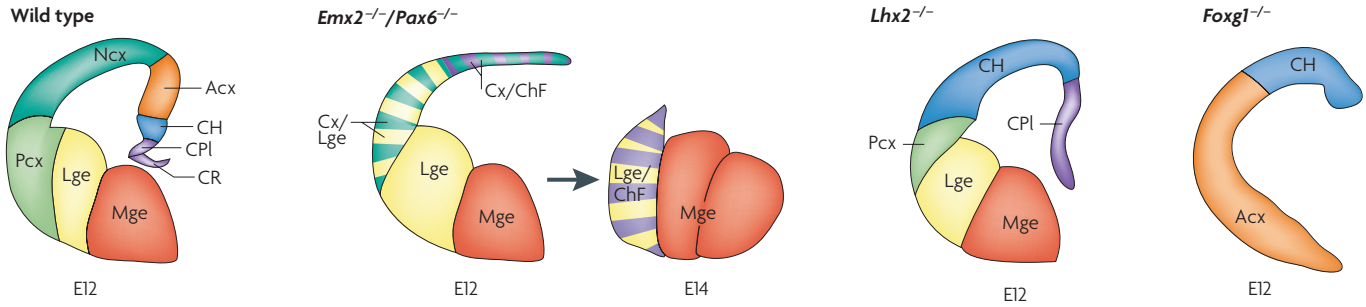


Figure 2 | ***Emx2*, *Pax6*, *Lhx2* and *Foxg1* have crucial roles in the specification of neocortical progenitors.** Loss of both empty spiracles homologue 2 (*Emx2*) and paired box 6 (*Pax6*) results in ventralization of cortical progenitors and the loss of the neocortical domain (Ncx), archicortex (Acx), cortical hem (CH) and choroid plexus (CPl) by embryonic day (E) 14 (REFS 67,132). Loss of LIM homeobox 2 (*Lhx2*) results in the expansion of the CPl and CH medial domains and the elimination of progenitors with neocortical identity[60–62]. Similarly, loss of forkhead box G1 (*Foxg1*) causes agenesis of the basal ganglia, elimination of neocortical progenitor domains and expansion of the CH and the Acx[63,64]. CR, choroidal roof; ChF, choroid field (choroid plexus and choroidal roof); Cx, cortex; Lge, lateral ganglionic eminence; Mge, medial ganglionic eminence; Pcx, paleocortex. Modified, with permission, from REF. 133 (2003) Oxford Univ. Press.

cortical areas (for a review, see REF. 66), are also required for establishing the identity of dorsal progenitors[67]. In the absence of both genes, the cortex does not form and ventral progenitor domains expand across the entire dorsal telencephalon[67]. The absence of *Pax6* alone results in the expression of markers of ventral progenitors, such as *Mash1*, *Gsh2* and *Dlx2*, and abnormalities in the production of projection neurons that are most pronounced in the rostral cortex[67–71]. This is not due to the migration of ventral cells into cortical territory[72], but rather to a cell autonomous failure to repress ventral genes in the absence of *Pax6* (REF. 73). This is most pronounced during the generation of upper-layer neurons [68–72].

Pax6, along with *Nr2e1* (also known as *Tlx*), also controls the proliferation of VZ progenitors during the establishment and expansion of the SVZ. In both *Pax6* mutants and *Nr2e1* mutants, deep-layer neurons are generated normally whereas the superficial cortical layers are decreased in thickness[46–48,68,74,75]. There is a global decrease in upper-layer neurons in both the *Pax6* and *Nr2e1* single mutants, including a reduction in the number of *Cux1*-, *Cux2*- and *Svet1*-expressing neurons in the *Pax6* mutant[46,47], with an even more severe phenotype in the double mutant[68]. It will be important to examine these mutants with additional subtype-specific markers of upper layers to elucidate whether specific populations are selectively affected, or if the absence of these genes has a more global impact on the generation of all upper-layer pyramidal neuron subtypes.

Investigations into the mechanisms underlying the decrease in upper-layer neuron number in the *Pax6* and *Nr2e1* mutants indicate that these genes control the kinetics of cell division of VZ progenitors, and the decision of a progenitor to divide symmetrically or asymmetrically. Therefore, they appear to regulate the expansion of the SVZ and the number of pyramidal neurons in the upper layers. In *Nr2e1* mutants, the SVZ is decreased in size, progenitors proliferate less, and they undergo premature differentiation, producing the most superficial layers of the neocortex 1–2 days early[75,76]. Similarly, in *Pax6* mutants, there is an increase in the proportion of progenitors undergoing asymmetrical cell division between E12.5 and E15.5 (REF. 77), with a premature decrease in the number of *Cux2*-expressing cells in the SVZ of *Pax6* mutants[46], despite an apparent expansion of the SVZ in the mutants owing to the defective migration of late-born cells[74]. Recent work has begun to offer some insight into the temporal sequence of gene expression that controls these decisions. For example, *Pax6* is expressed at high levels in progenitors dividing at the ventricular surface, whereas it is largely excluded from intermediate progenitors in the SVZ. The progressive loss of *Pax6* as cells migrate into the SVZ is associated with the initiation of *Tbr2* (also known as *Eomes*) expression, identifying the transition to intermediate progenitor cells[78]. This observation, combined with the recent finding that there is a reduction in the number of *Tbr2* intermediate progenitors in the absence of *Pax6* (REFS 73,78), suggests that *Pax6* regulates the formation and expansion of the SVZ, further supporting a role for this factor in regulating the development of the upper layers. In the future, it will be important to further investigate the specific role of *Pax6* at different stages of corticogenesis through the use of conditional knockout models as analysis of the null mutant suggests multiple roles for *Pax6* throughout development.

Subtype and laminar specification in the neocortex. Although genes that identify neocortical progenitors as a global population (for example, *Pax6* and *Tbr2*) have been discovered, so far there are no markers to distinguish among progenitors that generate different projection neuron subtypes. Therefore, much less is known about the genes that control the progressive commitment of progenitors to give rise to distinct subtypes of postmitotic projection neurons. Recently, tremendous advances have been made in the identification of laminar- and subtype-specific markers through large-scale *in situ* hybridization projects[79–82], the creation of transgenic mouse lines expressing GFP under the control of promoters of lineage- or layer-restricted genes[83], gene expression studies comparing microdissected regions of

neocortex[84,85], and the comparison of purified neuronal subtypes by microarray analysis[86,87]. These projects have dramatically expanded the number of known layer- and subtype-specific genes (FIG. 3; Supplementary information S1 (table)).

Examples of layer-specific genes include, among many others: *Cux1*, *Cux2* and *Lhx2*, markers of layers II/III to IV[46,48,88,89]; *Brn2* (also known as *Pou3f2*), a marker of layer II/III and V[90,91]; *Rorβ*, a marker of layer IV[92]; *6430573F11Rik* and encephalopsin (also known as *Opn3*), markers of layer V[81,86]; and *Foxp2*, a marker of layer VI[93]. Some of these genes have been described as being expressed in one specific neuronal type within a layer or across layers, including a large number of genes that exhibit varying degrees of restricted expression in corticospinal motor neurons[86]. Genes with expression known to be restricted to individual subtypes include: B-cell leukaemia/lymphoma 11B (*Ctip2*, also known as *Bcl11b*), which is expressed at high levels in subcerebral neurons of layer V and at much lower levels in corticothalamic neurons of layer VI[86]; *Scip* (also known as *Pou3f1*), which is primarily expressed in the subcerebral projection neurons of layer V, in addition to lower levels of expression in neurons of layers II/III[94]; orthodenticle homeobox 1 (*Otx1*), which is expressed in 40–50% of subcerebral neurons as well as a number of cells in layer VI[95]; *Er81*, which is expressed in cortico-cortical and subcerebral projection neurons of layer V[96]; *Nfh* (also known as *Nefh*), which is expressed in subcerebral projection neurons of layer V[97]; and *Lmo4*, which is expressed in callosal neurons of layers II/III and V[86,88]. Further, careful investigation of the neuronal subtypes expressing each of the other laminar-specific genes depicted in FIG. 3 is needed.

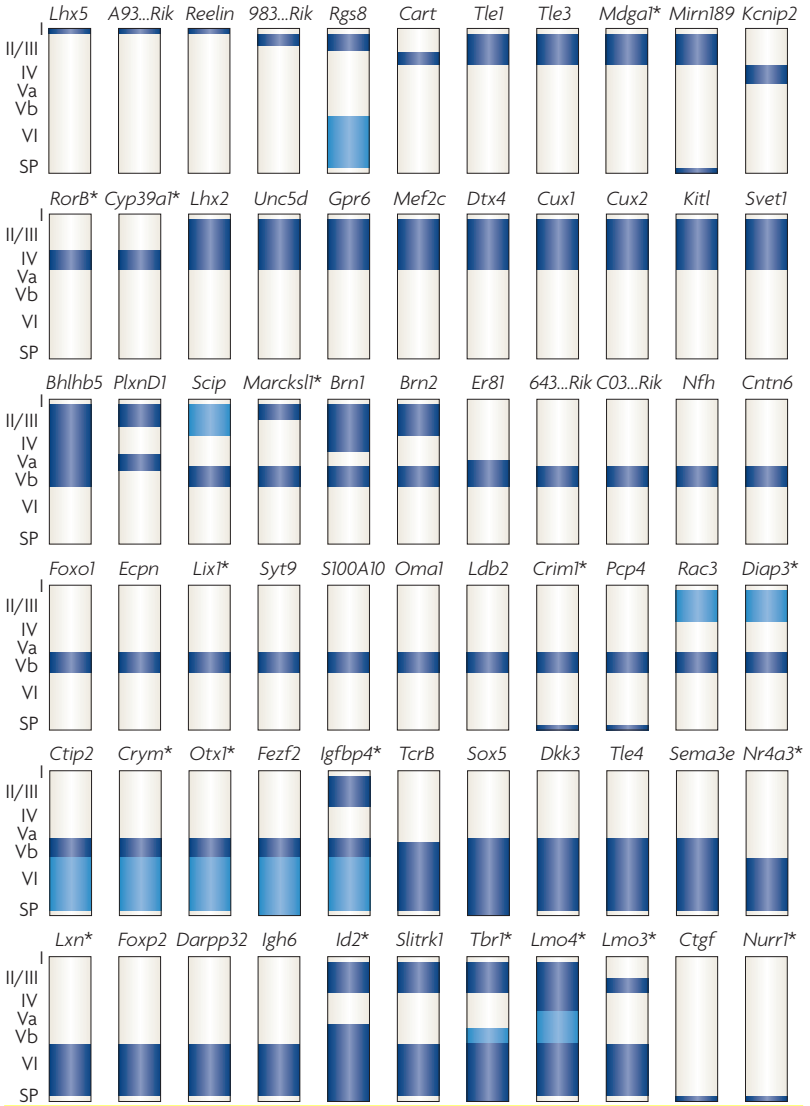


Figure 3 | **Laminar- and subtype-specific genes in the mouse neocortex.** Schematic of cortical layers depicting the laminar-specific expression of 66 genes within the neocortex. Dark blue and light blue indicate higher and lower relative levels of expression, respectively. Genes for which laminar or subtype expression varies by area within the neocortex are indicated by an asterisk. Dynamic patterns of expression during development are seen for many of these genes and should always be considered when using laminar or subtype-specific markers. For example, B-cell leukaemia/lymphoma 11B (*Ctip2*) is initially expressed at much higher levels in layer V than layer VI, but is equivalent in the two layers by postnatal day 14. Consult references for details on expression patterns and changes in expression during development. SP, subplate. Full names, Entrez Gene ID numbers, and references for each of the genes are listed in Supplementary information S1 (table).

Although great progress has been made in identifying markers of postmitotic neurons once they have reached the cortical plate, it is not clear whether the same markers can be used to identify progenitors of each neuronal subtype, or whether such lineage-committed progenitors even exist. A number of neuronal subtype-specific genes are expressed in what appear to be subpopulations of neurons in the VZ and SVZ, where they might label progenitors or early postmitotic neurons of that same neuronal subtype. For example, both Fez family zinc finger 2 (*Fezf2*, also known as *Fezl*) and *Otx1* are expressed in the VZ prior to and during the generation of layers V and VI, and are later expressed in postmitotic neurons of these layers. However, it is important to be extremely cautious when inferring that a gene has a role in the specification of subtypes at the progenitor level on the basis of restricted expression that is later observed in a particular neuronal subtype; it is entirely possible that the gene has two independent functions during development[98]. This is best illustrated by considering *Lhx2*, which is expressed in the VZ and SVZ prior to and during the generation of upper layers and is also expressed in postmitotic neurons of the upper layers. The finding that the loss of *Lhx2* results in the absence of neurons of all layers[60,61] suggests that *Lhx2* probably has two functions during development: in the VZ, it is required to establish the neocortical identity of progenitors of all layers, whereas later in development it might control more specific aspects of upper-layer differentiation. Yet, as discussed above, there is better evidence that genes like *Cux2* and *Svet1* might be identifiers of SVZ progenitors destined to give rise to an upper-layer pyramidal neuron. For each of these genes, further study is required to define the relationship between progenitors and postmitotic neurons expressing the same genes.

Investigations into the function of layer- and subtype-restricted genes are starting to provide insight into how neuronal subtypes are specified in the neocortex. *Brn1*

(also known as *Pou3f3*) and *Brn2*, which are expressed primarily in neurons of layers II–V[90,91,96], are involved in directing the differentiation and migration of neurons within these layers. The *Brn1*/*Brn2* double knockouts have decreased numbers of neurons in layers II–V, and those that are born exhibit abnormalities in migration, arresting in the VZ/SVZ[90,91]. Additionally, some markers of upper-layer neurons are expressed in these mutants, whereas others (for example, *SorLa*, also known as *Sorl1*) are absent, suggesting abnormalities in differentiation. By contrast, *Tle4*- and T-box brain gene 1 (*Tbr1*)-expressing neurons of layer VI seem to form and migrate normally into the cortical plate in the absence of *Brn1* and *Brn2* (REFS 90,91). Further analysis of *Brn1*/*Brn2* mutants with recently identified markers is needed to illuminate precisely which subtypes of neurons are affected in the absence of *Brn1* and *Brn2*. Like *Brn1* and *Brn2*, *Tbr1* is expressed by multiple types of cortical neurons, and its absence has a broad impact on projection neuron differentiation[99,100]. In the absence of *Tbr1* there are abnormalities in projection neuron migration, and defects in axon extension by subplate, corticothalamic, subcerebral and cortico-cortical projection neurons[100].

Although it has been proposed that neurogenin 1 and neurogenin 2 have key roles in regulating deep-layer neurogenesis[68,101], the majority of *Er81*-positive and *Tbr1*-positive neurons of layers V and VI are clearly generated in their absence[68,102]. Neurogenins probably function to maintain dorsal glutamatergic fate within deep-layer neurons[68], instead of having a primary role in specifying laminar or projection neuron subtype fate. *Mdga1*, a recently identified cell adhesion molecule, is required for layer II/III projection neurons to migrate to their appropriate position in the neocortex[103]. Knockdown of *Mdga1* by RNA interference results in migrational arrest in the intermediate zone and deep layers of the neocortex[103]. Similar investigations for each of the other subtype-specific genes are needed in order to delineate the programmes of gene expression that direct subtype-specific differentiation. As more evidence accumulates regarding the functional roles played by the many subtype-specific genes that are being discovered, we will probably witness rapid progress in understanding the programmes of gene expression that direct neuronal subtype differentiation in the neocortex.

Subcerebral projection neuron specification. Among the different types of cortical projection neurons, subcerebral projection neurons are an ideal model population for studying subtype specification in the neocortex. They are a discrete, readily identifiable, prototypical projection neuron population, located within layer Vb of the neocortex. They are defined by possessing axons that project below the cerebrum to targets in the spinal cord or brainstem, including the tectum, red nucleus and pons[86,104–108]. After being born in the germinal zone, all subcerebral projection neurons migrate to layer Vb and extend a primary axon through the internal capsule, cerebral peduncle and pyramidal tract towards the spinal cord. Secondary collaterals sprout from the primary axon only after it has passed other targets such as the superior colliculus and pons[109]. Inappropriate connections are later eliminated, leaving subcerebral projection neurons in the sensorimotor cortex projecting to the caudal pons and spinal cord, whereas those in the visual cortex maintain projections to the rostral pons and superior colliculus[107,109,110]. Given this common pattern of initial development, many of the genes controlling early specification and differentiation are likely to be shared among the different types of subcerebral projection neuron[86].

The most well-studied subtype of subcerebral projection neuron is the corticospinal motor neurons (CSMNs), a developmentally and clinically important population. CSMNs are of great interest because they form the basis of voluntary movement in humans, they degenerate in degenerative motor neuron diseases including amyotrophic lateral sclerosis (ALS), and their injury contributes to the loss of motor function after spinal cord injury. The precise point in development at which CSMNs begin to differ molecularly from other subcerebral projection neurons, and the mechanisms that initiate this change, are unresolved questions. Recently, the identification of a large number of subcerebral and CSMN-specific genes has enabled an expanding effort to decipher the programmes controlling CSMN development[86]. The expression patterns of these genes indicate that the fate specification and differentiation of subcerebral projection neurons in general, and CSMNs in particular, are probably directed by a combinatorial code of transcription factors and other molecules. These molecules are expressed in a pattern that together uniquely identifies CSMNs. For example, a small number of CSMN genes seem to be restricted to the sensorimotor cortex (for example, *Diap3*, *Igfbp4* and *Crim1*), suggesting that they distinguish CSMNs from other subcerebral projection neurons of layer V[86]. Other genes are expressed across the full extent of layer V (for example, *Ctip2*, encephalopsin, *Fezf2*, *Clim1*, *Pcp4* and *S100a10*), suggestive of restriction to most subcerebral projection neurons[86]. Thus far, the functions of only a few of these genes have been reported, but these studies are already revealing key roles for these genes in subcerebral specification and differentiation[29,86,95,111,112].

Fezf2, a putative transcription factor that is expressed in all subcerebral projection neurons from early stages of development through adulthood[86,113], was recently found to be required for the specification of all subcerebral projection neurons[29,111,112]. In the absence of *Fezf2* function in null mutant mice, the entire population of subcerebral projection neurons is absent, and there are no projections from the cerebral cortex to either the spinal cord or the brainstem[29,111]. Layer VI neurons and subplate neurons, which express *Fezf2* at lower levels than subcerebral projection neurons, exhibit disorganization and abnormalities in gene expression, but are less affected[29,111,114]. By contrast, upper-layer pyramidal neurons are born correctly and seem to be normal[29,111]. Importantly, without *Fezf2*, neocortical progenitors still produce similar numbers of layer V neurons[29,111], but morphologically they appear to be an expansion of layer VI, instead of exhibiting the distinctive appearance of layer V subcerebral projection neurons. Additionally, alkaline phosphatase expression from the *Fezf2* locus in

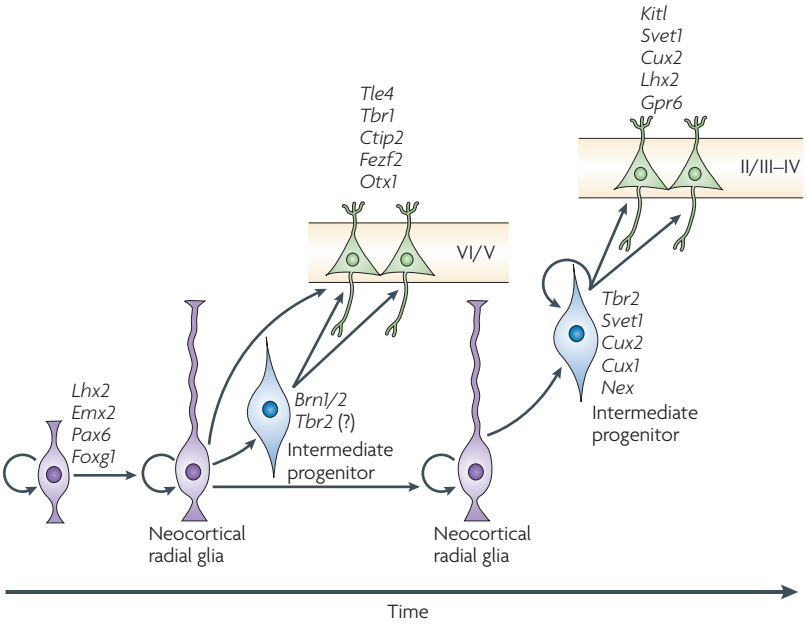


Figure 4 | **Schematic representation of a potential model for the generation of projection neuron subtypes from progenitors.** According to this model, the concerted action of genes such as forkhead box G1 (*FoxG1*), LIM homeobox 2 (*Lhx2*), paired box 6 (*Pax6*) and empty spiracles homologue 2 (*Emx2*) induce neuroepithelial cells to give rise to radial glial progenitors with neocortical potential. As development proceeds, radial glial progenitors located in the ventricular zone generate intermediate progenitors in the subventricular zone, which, under the influence of layer and neuron-type-specific genes, generate deep-layer and upper-layer projection neurons. A selection of the genes with either known stage-specific functions or restricted expression are listed. See main text and FIG. 3 for additional genes that are involved.

null mutants labels an enlarged anterior commissure, further suggesting that a different type of projection neuron is generated in place of subcerebral projection neurons[111]. Thus, *Fezf2* does not seem to affect the ability of progenitors to generate glutamatergic neurons that position themselves in layer V; it probably acts to direct the next step in the programme of specification, defining the characteristics of a subcerebral projection neuron. Additional support for *Fezf2* directing subcerebral projection neuron specification comes from evidence that the overexpression of *Fezf2* is sufficient to induce the birth of entirely new deep-layer projection neurons that express *Ctip2* and *Tbr1*, and extend axons through the internal capsule[29,112].

A second set of genes has been identified that control later aspects of subcerebral projection neuron development, probably acting downstream of factors such as *Fezf2*. In the absence of *Ctip2*, subcerebral projection neuron axons exhibit defects in fasciculation, outgrowth and pathfinding, with decreased numbers of axons reaching the brainstem[86]. In addition, reduced *Ctip2* expression in *Ctip2*-heterozygous mice results in abnormal developmental pruning of corticospinal axons[86]. These experiments identified *Ctip2* as a crucial regulator of subcerebral axon extension and of the refinement of collaterals as these neurons mature. Another key transcription factor known to have a role in the target choice of subcerebral projection neurons is *Otx1*. This protein is expressed in putative deep-layer progenitors in the VZ, exhibiting decreasing levels of expression in the VZ during the generation of upper-layer neurons[95,113]. As deep-layer projection neurons mature, localization of OTX1 shifts from the cytoplasm to the nucleus, indicating a fine regulation of the activity of this protein[95,115]. Postnatally, within layer V, *Otx1* is expressed in 40–50% of subcerebral neurons, primarily those within the visual cortex, whereas it is not expressed in callosal neurons[95]. Mice lacking the gene for OTX1 have defects in the development of corticotectal projection neurons. Without *Otx1*, corticotectal projection neurons maintain an axon that projects to the spinal cord and caudal pontine nuclei, collaterals that are only appropriate for CSMNs (normally eliminated by corticotectal projection neurons)[95]. This indicates that *Otx1* might have a later role in subcerebral projection neuron development than *Fezf2* and *Ctip2*, perhaps by controlling the refinement and pruning of axonal collaterals. Additional axon outgrowth and guidance molecules, such as insulin-like growth factor 1 and RYK, have been implicated in the extension and guidance of subcerebral projection neuron axons to targets in the brainstem and spinal cord[116–118].

Although a comprehensive understanding of the function of additional subcerebral projection-neuron-specific genes still awaits substantial experimental work *in vivo*, on the basis of the data available thus far, a possible model for the generation of subcerebral projection neurons can be put forward that requires sequential steps of progressive differentiation (FIG. 4). We propose that the concerted function of *Foxg1*, *Lhx2*, *Pax6* and *Emx2* first gives progenitors neocortical potential, setting the stage for the generation of multiple classes of glutamatergic projection neurons.

Studies of motor neuron development in *Drosophila melanogaster* have provided insight into how cortical progenitors might then progressively generate distinct subtypes of pyramidal neurons over time. In this system, progenitors express a sequential series of transcription factors during neurogenesis, followed by the maintained expression of these same transcription factors in the postmitotic progeny that were born during the window of expression of each gene in the progenitors[119]. Similarly, it is conceivable that in the mammalian neocortex radial glial progenitors might express a sequential series of transcription factors that are maintained in intermediate progenitors and postmitotic neurons, imparting subtype identity. Thus, during the generation of subcerebral projection neurons, genes such as *Brn1* and *Brn2* might act on partially specified progenitors to determine aspects of laminar identity as individual subtypes of pyramidal neurons are generated. *Fezf2* could then specify the subcerebral projection neuron lineage within a layer (that is, layer V), enabling the development of the molecular, morphological and anatomical projection properties of subcerebral projection neurons. Finally, once this cascade is initiated, the expression of genes such as *Ctip2* and *Otx1*, which govern subcerebral axonal outgrowth and target selection, could act to establish the precise connectivity and later morphological features of subcerebral projection neurons. The direct relationships between these transcription factors and the many

functionally uncharacterized genes that act in the cascade of subcerebral projection neuron development remain to be determined. Together, these molecules comprise the first elements of the molecular programme that drives the anatomical model of subcerebral projection neuron development described more than a decade ago[107].

Cortical plasticity

In defining a model of progenitor specification and neuronal subtype development, important insight into cortical plasticity is likely to be gained. For example, can late progenitors that are normally fated to generate upper-layer neurons still be induced to generate early fates? In *D. melanogaster*, expression of *hunchback*, which is normally expressed in early progenitors and their neuronal progeny, is sufficient to allow later progenitors to generate neurons with an early phenotype. However, this plasticity decreases over time such that progenitors at advanced stages of development are resistant to *hunchback* expression and do not revert to an earlier phenotype[119,120]. In an analogous fashion in the mammalian neocortex, the transcription factor *Fezf2* is expressed in the VZ during the generation of deep-layer neurons, and its expression is maintained in postmitotic neurons of layers V and VI. As development progresses, the expression of *Fezf2* in progenitors decreases, and disappears by the time upper layer neurons are generated[29,113,114]. Overexpression of *Fezf2* in progenitors soon after the generation of layers V and VI is completed (that is, in progenitors that give rise to layer IV neurons) is sufficient to at least partly override this restriction and induce later-stage progenitors to produce neurons with some molecular and anatomical features of earlier born neurons[29] in a manner reminiscent of the *hunchback* misexpression experiments in *D. melanogaster*. Further analysis of *Fezf2*-transfected neurons with additional positive and negative markers of subcerebral projection neurons is needed to determine the extent of the effect of *Fezf2* on neuronal phenotype. Interestingly, in contrast to the more restricted window of *hunchback* effect, *Fezf2* seems, at least in part, to affect progenitor plasticity late in development, as suggested by the fact that forced expression of *Fezf2* in E17 progenitors results in the generation of upper-layer neurons that inappropriately express *Tbr1* at a higher frequency than is normally observed in upper-layer neurons, and extend axonal projections to the pons (a feature of deep-layer neurons)[112]. In agreement with the limitations of plasticity seen with *hunchback* in *D. melanogaster*, *Fezf2*-overexpressing neurons are still able to migrate to the layer appropriate for this late birth date instead of layer V. Although it remains to be elucidated to what extent these late born neurons change their identity in response to *Fezf2*-overexpression, together these experiments indicate that cortical progenitors might be more plastic than previously suspected, even late in neurogenesis, if manipulated by the appropriate control molecules. In agreement with these findings, recent experiments have revealed that the manipulation of extracellular signals can also alter the fate of neurons born late in cortical neurogenesis. BDNF delivered to progenitors during the birth of later born neurons shifts them to the laminar fate of earlier born neurons if progenitors are exposed to BDNF before the S phase of the cell cycle[28]. In the future, it might be possible to manipulate neocortical progenitor plasticity much later in development or even during adulthood. In support of this idea, previous experiments have shown that with the appropriate stimuli, progenitors in the adult mouse neocortex can be induced to generate new projection neurons[121,122].

Conclusion

During the last few years there has been remarkable progress in the discovery of genes that identify distinct neuronal subtypes and control neuronal subtype development. A progressive set of mechanisms is being identified by which early progenitors that have acquired neocortical identity later progress through a series of additional specification steps controlled by genes that instruct the finer aspects of laminar and neuronal subtype-specific identity. As future research examines this model of progressive specification, detailed programmes of genes (rather than individual genes) that orchestrate the generation of the astonishing variety of projection neuron subtypes are likely to be identified. This will help elucidate the relationships between related classes of projection neurons, and contribute to defining the time points at which individual lineages branch from each other. Recombinase-based fate mapping experiments will be extremely informative in this effort to determine the relationships among different progenitors, and the sequences of changes in gene expression from progenitor to lineage-committed postmitotic projection neuron. A new era of plasticity assessment by the manipulation of programmes of genes specifying individual subtypes of neurons might soon determine the true boundaries of progenitor plasticity within the developing and adult cerebral cortex.

1. Peters, A. & Jones, E. G. *Cellular Components of the Cerebral Cortex* (Plenum, New York, 1984).
2. Ramón y Cajal, S. *Histology of the Nervous System of Man and Vertebrates* (Oxford Univ. Press, New York, 1995).
3. Finlay, B. L. & Darlington, R. B. Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578–1584 (1995).
4. Anderson, S. A., Kaznowski, C. E., Horn, C., Rubenstein, J. L. & McConnell, S. K. Distinct origins of neocortical projection neurons and interneurons *in vivo*. *Cereb. Cortex* **12**, 702–709 (2002).
5. Gorski, J. A. *et al.* Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J. Neurosci.* **22**, 6309–6314 (2002).
6. Rakic, P. Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* **145**, 61–83 (1972).
7. Tan, S. S. *et al.* Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. *Neuron* **21**, 295–304 (1998).
8. Ware, M. L., Tavazoie, S. F., Reid, C. B. & Walsh, C. A. Coexistence of widespread clones and large radial clones in early embryonic ferret cortex. *Cereb. Cortex* **9**, 636–645 (1999).
9. Wonders, C. P. & Anderson, S. A. The origin and specification of cortical interneurons. *Nature Rev. Neurosci.* **7**, 687–696 (2006).
10. Migliore, M. & Shepherd, G. M. An integrated approach to classifying neuronal phenotypes. *Nature Rev. Neurosci.* **6**, 810–818 (2005).
11. Jessell, T. M. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nature Rev. Genet.* **1**, 20–29 (2000).
12. Livesey, F. J. & Cepko, C. L. Vertebrate neural cell-fate determination: lessons from the retina. *Nature Rev. Neurosci.* **2**, 109–118 (2001).
13. Bertrand, N., Castro, D. S. & Guillemot, F. Proneural genes and the specification of neural cell types. *Nature Rev. Neurosci.* **3**, 517–530 (2002).
14. Guillemot, F. Cellular and molecular control of neurogenesis in the mammalian telencephalon. *Curr. Opin. Cell Biol.* **17**, 639–647 (2005).
15. Marin, O. & Rubenstein, J. L. Cell migration in the forebrain. *Annu. Rev. Neurosci.* **26**, 441–483 (2003).

16. Embryonic vertebrate central nervous system: revised terminology. The Boulder Committee. *Anat. Rec.* **166**, 257–261 (1970).
17. Bayer, S. A. & Altman, J. (eds) in *Neocortical Development* 255 (Raven, New York, 1991).
18. Angevine, J. B. Jr & Sidman, R. L. Autoradiographic study of cell migration during histogenesis of cerebral cortex in mouse. *Nature* **192**, 766–768 (1961).
19. Caviness, V. S. Jr & Takahashi, T. Proliferative events in the cerebral ventricular zone. *Brain Dev.* **17**, 159–163 (1995).
20. Rakic, P. Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science* **183**, 425–427 (1974).
21. Britanova, O. *et al.* A novel mode of tangential migration of cortical projection neurons. *Dev. Biol.* **298**, 299–311 (2006).
22. Noctor, S. C., Flint, A. C., Weissman, T. A., Dammerman, R. S. & Kriegstein, A. R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714–720 (2001).
23. Rakic, P. Developmental and evolutionary adaptations of cortical radial glia. *Cereb. Cortex* **13**, 541–549 (2003).
24. Reid, C. B. & Walsh, C. A. Evidence of common progenitors and patterns of dispersion in rat striatum and cerebral cortex. *J. Neurosci.* **22**, 4002–4014 (2002).
25. McConnell, S. K. & Kaznowski, C. E. Cell cycle dependence of laminar determination in developing neocortex. *Science* **254**, 282–285 (1991).
26. Frantz, G. D. & McConnell, S. K. Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron* **17**, 55–61 (1996).
One paper from a series of experiments by McConnell and colleagues investigating the fate potential of neocortical progenitors at different stages of cortical neurogenesis. Here, the authors demonstrate that late progenitors are unable to generate deep-layer neurons when transplanted into the niche that normally generates deep-layer neurons.
27. Mizutani, K. & Saito, T. Progenitors resume generating neurons after temporary inhibition of neurogenesis by Notch activation in the mammalian cerebral cortex. *Development* **132**, 1295–1304 (2005).
28. Fukumitsu, H. *et al.* Brain-derived neurotrophic factor participates in determination of neuronal laminar fate in the developing mouse cerebral cortex. *J. Neurosci.* **26**, 13218–13230 (2006).
29. Molyneaux, B. J., Arlotta, P., Hirata, T., Hibi, M. & Macklis, J. D. *Fezl* is required for the birth and specification of corticospinal motor neurons. *Neuron* **47**, 817–831 (2005).
***Fezl* was the first transcription factor found to be necessary for the generation of one neuronal population (subcerebral projection neurons) within the cortex.**
30. Gotz, M. & Huttner, W. B. The cell biology of neurogenesis. *Nature Rev. Mol. Cell Biol.* **6**, 777–788 (2005).
31. Chenn, A. & McConnell, S. K. Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. *Cell* **82**, 631–641 (1995).
32. Smart, I. H. Proliferative characteristics of the ependymal layer during the early development of the mouse neocortex: a pilot study based on recording the number, location and plane of cleavage of mitotic figures. *J. Anat.* **116**, 67–91 (1973).
33. Hartfuss, E., Galli, R., Heins, N. & Gotz, M. Characterization of CNS precursor subtypes and radial glia. *Dev. Biol.* **229**, 15–30 (2001).
34. Malatesta, P. *et al.* Neuronal or glial progeny: regional differences in radial glia fate. *Neuron* **37**, 751–764 (2003).
35. Anthony, T. E., Klein, C., Fishell, G. & Heintz, N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* **41**, 881–890 (2004).
36. Heins, N. *et al.* Glial cells generate neurons: the role of the transcription factor Pax6. *Nature Neurosci.* **5**, 308–315 (2002).
37. Malatesta, P., Hartfuss, E. & Gotz, M. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**, 5253–5263 (2000).
38. Mo, Z. *et al.* Human cortical neurons originate from radial glia and neuron-restricted progenitors. *J. Neurosci.* **27**, 4132–4145 (2007).
39. Noctor, S. C., Martinez-Cerdeno, V., Ivic, L. & Kriegstein, A. R. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neurosci.* **7**, 136–144 (2004).
Using low-titre retroviral infection and *in vitro* time-lapse imaging, the authors demonstrate that radial glia give rise to neurons through two different mechanisms: either by an asymmetric cell division in the VZ that produces a neuron and a radial glia cell or through the generation of an intermediate progenitor that migrates into the SVZ before dividing symmetrically to produce two neurons.
40. Gal, J. S. *et al.* Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. *J. Neurosci.* **26**, 1045–1056 (2006).
41. Hinds, J. W. & Ruffett, T. L. Cell proliferation in the neural tube: an electron microscopic and golgi analysis in the mouse cerebral vesicle. *Z. Zellforsch. Mikrosk. Anat.* **115**, 226–264 (1971).
42. Smart, I. H. & McSherry, G. M. Growth patterns in the lateral wall of the mouse telencephalon. II. Histological changes during and subsequent to the period of isocortical neuron production. *J. Anat.* **134**, 415–442 (1982).
43. Takahashi, T., Nowakowski, R. S. & Caviness, V. S. Jr. Early ontogeny of the secondary proliferative population of the embryonic murine cerebral wall. *J. Neurosci.* **15**, 6058–6068 (1995).
44. Miyata, T. *et al.* Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development* **131**, 3133–3145 (2004).
45. Haubensak, W., Attardo, A., Denk, W. & Huttner, W. B. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc. Natl Acad. Sci. USA* **101**, 3196–3201 (2004).
46. Nieto, M. *et al.* Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II–IV of the cerebral cortex. *J. Comp. Neurol.* **479**, 168–180 (2004).
47. Tarabykin, V., Stoykova, A., Usman, N. & Gruss, P. Cortical upper layer neurons derive from the subventricular zone as indicated by *Svet1* gene expression. *Development* **128**, 1983–1993 (2001).
48. Zimmer, C., Tiveron, M. C., Bodmer, R. & Cremer, H. Dynamics of *Cux2* expression suggests that an early pool of SVZ precursors is fated to become upper cortical layer neurons. *Cereb. Cortex* **14**, 1408–1420 (2004).
49. Wu, S. X. *et al.* Pyramidal neurons of upper cortical layers generated by NEX-positive progenitor cells in the subventricular zone. *Proc. Natl Acad. Sci. USA* **102**, 17172–17177 (2005).
50. Aboitiz, F., Morales, D. & Montiel, J. The evolutionary origin of the mammalian isocortex: towards an integrated developmental and functional approach. *Behav. Brain Sci.* **26**, 535–552; discussion 552–585 (2003).
51. Marin-Padilla, M. Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: a unifying theory. *J. Comp. Neurol.* **321**, 223–240 (1992).
52. Reiner, A. A comparison of neurotransmitter-specific and neuropeptide-specific neuronal cell types present in the dorsal cortex in turtles with those present in the isocortex in mammals: implications for the evolution of isocortex. *Brain Behav. Evol.* **38**, 53–91 (1991).
53. Kriegstein, A., Noctor, S. & Martinez-Cerdeno, V. Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. *Nature Rev. Neurosci.* **7**, 883–890 (2006).
54. Smart, I. H., Dehay, C., Giroud, P., Berland, M. & Kennedy, H. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* **12**, 37–53 (2002).
55. Bohner, A. P., Akers, R. M. & McConnell, S. K. Induction of deep layer cortical neurons *in vitro*. *Development* **124**, 915–923 (1997).
56. Hack, M. A., Sugimori, M., Lundberg, C., Nakafuku, M. & Gotz, M. Regionalization and fate specification in neurospheres: the role of Olig2 and Pax6. *Mol. Cell. Neurosci.* **25**, 664–678 (2004).
57. Shen, Q. *et al.* The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nature Neurosci.* **9**, 743–751 (2006).
58. Nguyen, L., Besson, A., Roberts, J. M. & Guillemot, F. Coupling cell cycle exit, neuronal differentiation and migration in cortical neurogenesis. *Cell Cycle* **5**, 2314–2318 (2006).
59. Rallu, M., Corbin, J. G. & Fishell, G. Parsing the prosencephalon. *Nature Rev. Neurosci.* **3**, 943–951 (2002).
60. Bulchand, S., Grove, E. A., Porter, F. D. & Tole, S. LIM-homeodomain gene *Lhx2* regulates the formation of the cortical hem. *Mech. Dev.* **100**, 165–175 (2001).
The authors report that *Lhx2* is required for the formation of the neocortical progenitor domain, and find that the neocortex of *Lhx2*-mutant mice is almost entirely replaced by an expanded cortical hem.
61. Monuki, E. S., Porter, F. D. & Walsh, C. A. Patterning of the dorsal telencephalon and cerebral cortex by a roof plate–lhx2 pathway. *Neuron* **32**, 591–604 (2001).
The authors find that *Lhx2* expression is partly regulated by bone morphogenetic protein signalling from the roof plate at the dorsal midline. Like reference 60, this paper reports that *Lhx2* is required for the formation of the neocortical progenitor domain and that the neocortex of *Lhx2*-mutant mice is almost entirely replaced by an expanded cortical hem and choroid plexus.
62. Vyas, A., Saha, B., Lai, E. & Tole, S. Paleocortex is specified in mice in which dorsal telencephalic patterning is severely disrupted. *J. Comp. Neurol.* **466**, 545–553 (2003).
63. Dou, C. L., Li, S. & Lai, E. Dual role of brain factor-1 in regulating growth and patterning of the cerebral hemispheres. *Cereb. Cortex* **9**, 543–550 (1999).
64. Muzio, L. & Mallamaci, A. Foxg1 confines Cajal–Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *J. Neurosci.* **25**, 4435–4441 (2005).
The authors demonstrate that *Foxg1* is required for the specification of neocortical progenitors. In its absence, the neocortex is replaced by an expanded cortical hem and an expanded archicortex.
65. Hanashima, C., Li, S. C., Shen, L., Lai, E. & Fishell, G. *Foxg1* suppresses early cortical cell fate. *Science* **303**, 56–59 (2004).
The authors find that the absence of *Foxg1* results in the generation of increased numbers of Cajal–Retzius cells instead of neocortical projection neurons. Remarkably, inactivation of *Foxg1* midway through the generation of projection neurons results in the production of additional Cajal–Retzius cells.
66. Mallamaci, A. & Stoykova, A. Gene networks controlling early cerebral cortex arealization. *Eur. J. Neurosci.* **23**, 847–856 (2006).
67. Muzio, L. *et al.* Conversion of cerebral cortex into basal ganglia in *Emx2*$^{-/-}$/*Pax6*$^{Sey/Sey}$ double-mutant mice. *Nature Neurosci.* **5**, 737–745 (2002).
The authors find that without at least one allele of *Emx2* or *Pax6*, no neocortex is generated.
68. Schuurmans, C. *et al.* Sequential phases of cortical specification involve *Neurogenin*-dependent and independent pathways. *EMBO J.* **23**, 2892–2902 (2004).
69. Stoykova, A., Treichel, D., Hallonet, M. & Gruss, P. Pax6 modulates the dorsoventral patterning of the mammalian telencephalon. *J. Neurosci.* **20**, 8042–8050 (2000).
70. Toresson, H., Potter, S. S. & Campbell, K. Genetic control of dorsal–ventral identity in the telencephalon: opposing roles for Pax6 and Gsh2. *Development* **127**, 4361–4371 (2000).
71. Yun, K., Potter, S. & Rubenstein, J. L. Gsh2 and Pax6 play complementary roles in dorsoventral patterning of the mammalian telencephalon. *Development* **128**, 193–205 (2001).
72. Kroll, T. T. & O'Leary, D. D. Ventralized dorsal telencephalic progenitors in Pax6 mutant mice generate GABA interneurons of a lateral ganglionic eminence fate. *Proc. Natl Acad. Sci. USA* **102**, 7374–7379 (2005).
73. Quinn, J. C. *et al.* Pax6 controls cerebral cortical cell number by regulating exit from the cell cycle and specifies cortical cell identity by a cell autonomous mechanism. *Dev. Biol.* **302**, 50–65 (2007).
74. Caric, D., Gooday, D., Hill, R. E., McConnell, S. K. & Price, D. J. Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. *Development* **124**, 5087–5096 (1997).
75. Land, P. W. & Monaghan, A. P. Expression of the transcription factor, tailless, is required for formation of superficial cortical layers. *Cereb. Cortex* **13**, 921–931 (2003).
76. Roy, K. *et al.* The *Tlx* gene regulates the timing of neurogenesis in the cortex. *J. Neurosci.* **24**, 8333–8345 (2004).
77. Estivill-Torrus, G., Pearson, H., van Heyningen, V., Price, D. J. & Rashbass, P. Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. *Development* **129**, 455–466 (2002).
78. Englund, C. *et al. Pax6*, *Tbr2*, and *Tbr1* are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J. Neurosci.* **25**, 247–251 (2005).

79. Gray, P. A. *et al.* Mouse brain organization revealed through direct genome-scale TF expression analysis. *Science* **306**, 2255–2257 (2004).
80. Lein, E. S. *et al.* Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**, 168–176 (2007).
81. Magdaleno, S. *et al.* BGEM: an *in situ* hybridization database of gene expression in the embryonic and adult mouse nervous system. *PLoS Biol.* **4**, e86 (2006).
82. Visel, A., Thaller, C. & Eichele, G. GenePaint.org: an atlas of gene expression patterns in the mouse embryo. *Nucleic Acids Res.* **32**, D552–D556 (2004).
83. Gong, S. *et al.* A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**, 917–925 (2003).
84. Liu, Q., Dwyer, N. D. & O'Leary, D. D. Differential expression of *COUP-TFI*, *CHL1*, and two novel genes in developing neocortex identified by differential display PCR. *J. Neurosci.* **20**, 7682–7690 (2000).
85. Zhong, Y. *et al.* Identification of the genes that are expressed in the upper layers of the neocortex. *Cereb. Cortex* **14**, 1144–1152 (2004).
86. Arlotta, P. *et al.* Neuronal subtype-specific genes that control corticospinal motor neuron development *in vivo*. *Neuron* **45**, 207–221 (2005).
The first paper reporting on the identification of genes that are specific to corticospinal motor neurons and that in combination identify and control development of this neuron type *in vivo*.
87. Sugino, K. *et al.* Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nature Neurosci.* **9**, 99–107 (2006).
The authors define the global expression profile of 12 neuronal populations chosen from different regions of the adult forebrain and use the gene expression data to propose a taxonomic classification of neuron types on the basis of their molecular similarities.
88. Bulchand, S., Subramanian, L. & Tole, S. Dynamic spatiotemporal expression of LIM genes and cofactors in the embryonic and postnatal cerebral cortex. *Dev. Dyn.* **226**, 460–469 (2003).
89. Nakagawa, Y., Johnson, J. E. & O'Leary, D. D. Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input. *J. Neurosci.* **19**, 10877–10885 (1999).
90. McEvilly, R. J., de Diaz, M. O., Schonemann, M. D., Hooshmand, F. & Rosenfeld, M. G. Transcriptional regulation of cortical neuron migration by POU domain factors. *Science* **295**, 1528–1532 (2002).
The authors show that *Brn1* and *Brn2* are necessary for proper cortical lamination, such that in the absence of both these genes neurons of upper layers II/III and layer V fail to migrate and position below the subplate, whereas layer VI neurons are unaffected, resulting in an inverted cortex.
91. Sugitani, Y. *et al.* Brn-1 and Brn-2 share crucial roles in the production and positioning of mouse neocortical neurons. *Genes Dev.* **16**, 1760–1765 (2002).
92. Schaeren-Wiemers, N., Andre, E., Kapfhammer, J. P. & Becker-Andre, M. The expression pattern of the orphan nuclear receptor RORβ in the developing and adult rat nervous system suggests a role in the processing of sensory information and in circadian rhythm. *Eur. J. Neurosci.* **9**, 2687–2701 (1997).
93. Ferland, R. J., Cherry, T. J., Preware, P. O., Morrisey, E. E. & Walsh, C. A. Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. *J. Comp. Neurol.* **460**, 266–279 (2003).
94. Frantz, G. D., Bohner, A. P., Akers, R. M. & McConnell, S. K. Regulation of the POU domain gene *SCIP* during cerebral cortical development. *J. Neurosci.* **14**, 472–485 (1994).
95. Weimann, J. M. *et al.* Cortical neurons require Otx1 for the refinement of exuberant axonal projections to subcortical targets. *Neuron* **24**, 819–831 (1999).
96. Hevner, R. F. *et al.* Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons. *Dev. Neurosci.* **25**, 139–151 (2003).
97. Voelker, C. C. *et al.* Selective neurofilament (SMI-32, FNP-7 and N200) expression in subpopulations of layer V pyramidal neurons *in vivo* and *in vitro*. *Cereb. Cortex* **14**, 1276–1286 (2004).
98. Alvarez-Bolado, G., Rosenfeld, M. G. & Swanson, L. W. Model of forebrain regionalization based on spatiotemporal patterns of POU-III homeobox gene expression, birthdates, and morphological features. *J. Comp. Neurol.* **355**, 237–295 (1995).
99. Bulfone, A. *et al.* T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15**, 63–78 (1995).
100. Hevner, R. F. *et al.* Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* **29**, 353–366 (2001).
The authors report that the transcription factor *Tbr1* is necessary for proper development of Cajal–Retzius cells, subplate neurons and layer VI neurons. In the absence of *Tbr1*, the preplate does not split properly, deep-layer VI neurons are located below the subplate, with disruption of cortical lamination, as well as abnormal corticothalamic, thalamocortical and cortico-cortical connectivity.
101. Guillemot, F., Molnar, Z., Tarabykin, V. & Stoykova, A. Molecular mechanisms of cortical differentiation. *Eur. J. Neurosci.* **23**, 857–868 (2006).
102. Fode, C. *et al.* A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* **14**, 67–80 (2000).
103. Takeuchi, A. & O'Leary, D. D. Radial migration of superficial layer cortical neurons controlled by novel Ig cell adhesion molecule MDGA1. *J. Neurosci.* **26**, 4460–4464 (2006).
104. Killackey, H. P., Koralek, K. A., Chiaia, N. L. & Rhodes, R. W. Laminar and areal differences in the origin of the subcortical projection neurons of the rat somatosensory cortex. *J. Comp. Neurol.* **282**, 428–445 (1989).
105. Legg, C. R., Mercier, B. & Glickstein, M. Corticopontine projection in the rat: the distribution of labelled cortical cells after large injections of horseradish peroxidase in the pontine nuclei. *J. Comp. Neurol.* **286**, 427–441 (1989).
106. Molnar, Z. & Cheung, A. F. Towards the classification of subpopulations of layer V pyramidal projection neurons. *Neurosci. Res.* **55**, 105–115 (2006).
107. O'Leary, D. D. & Koester, S. E. Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. *Neuron* **10**, 991–1006 (1993).
108. Wise, S. P. & Jones, E. G. Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. *J. Comp. Neurol.* **175**, 129–157 (1977).
109. O'Leary, D. D. & Terashima, T. Cortical axons branch to multiple subcortical targets by interstitial axon budding: implications for target recognition and 'waiting periods'. *Neuron* **1**, 901–910 (1988).
110. Schreyer, D. J. & Jones, E. G. Axon elimination in the developing corticospinal tract of the rat. *Brain Res.* **466**, 103–119 (1988).
111. Chen, B., Schaevitz, L. R. & McConnell, S. K. Fezl regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. *Proc. Natl Acad. Sci. USA* **102**, 17184–17189 (2005).
Similarly to reference 29, this reports that *Fez2f* regulates the differentiation of layer V neurons and their subcerebral projections.
112. Chen, J. G., Rasin, M. R., Kwan, K. Y. & Sestan, N. Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex. *Proc. Natl Acad. Sci. USA* **102**, 17792–17797 (2005).
The authors knocked down *Fez2f* expression in cortical neurons via small interfering RNA and showed that reduced levels of this transcription factor results in abnormal connectivity by layer V and VI neurons to subcortical targets and in abnormal neuronal subtype-specific dendritic differentiation.
113. Inoue, K., Terashima, T., Nishikawa, T. & Takumi, T. Fez1 is layer-specifically expressed in the adult mouse neocortex. *Eur. J. Neurosci.* **20**, 2909–2916 (2004).
114. Hirata, T. *et al.* Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons. *Dev. Dyn.* **230**, 546–556 (2004).
115. Frantz, G. D., Weimann, J. M., Levin, M. E. & McConnell, S. K. Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. *J. Neurosci.* **14**, 5725–5740 (1994).
116. Harel, N. Y. & Strittmatter, S. M. Can regenerating axons recapitulate developmental guidance during recovery from spinal cord injury? *Nature Rev. Neurosci.* **7**, 603–616 (2006).
117. Liu, Y. *et al.* Ryk-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract. *Nature Neurosci.* **8**, 1151–1159 (2005).
118. Ozdinler, P. H. & Macklis, J. D. IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nature Neurosci.* **9**, 1371–1381 (2006).
119. Isshiki, T., Pearson, B., Holbrook, S. & Doe, C. Q. *Drosophila* neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* **106**, 511–521 (2001).
120. Pearson, B. J. & Doe, C. Q. Regulation of neuroblast competence in *Drosophila*. *Nature* **425**, 624–628 (2003).
121. Chen, J., Magavi, S. S. & Macklis, J. D. Neurogenesis of corticospinal motor neurons extending spinal projections in adult mice. *Proc. Natl Acad. Sci. USA* **101**, 16357–16362 (2004).
122. Magavi, S. S., Leavitt, B. R. & Macklis, J. D. Induction of neurogenesis in the neocortex of adult mice. *Nature* **405**, 951–955 (2000).
123. Butt, S. J. *et al.* The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* **48**, 591–604 (2005).
124. Lopez-Bendito, G. *et al.* Preferential origin and layer destination of GAD65–GFP cortical interneurons. *Cereb. Cortex* **14**, 1122–1133 (2004).
125. Wichterle, H., Turnbull, D. H., Nery, S., Fishell, G. & Alvarez-Buylla, A. *In utero* fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development* **128**, 3759–3771 (2001).
126. Meyer, G., Perez-Garcia, C. G., Abraham, H. & Caput, D. Expression of p73 and reelin in the developing human cortex. *J. Neurosci.* **22**, 4973–4986 (2002).
127. Takiguchi-Hayashi, K. *et al.* Generation of reelin-positive marginal zone cells from the caudomedial wall of telencephalic vesicles. *J. Neurosci.* **24**, 2286–2295 (2004).
128. Yoshida, M., Assimacopoulos, S., Jones, K. R. & Grove, E. A. Massive loss of Cajal–Retzius cells does not disrupt neocortical layer order. *Development* **133**, 537–545 (2006).
129. Bielle, F. *et al.* Multiple origins of Cajal–Retzius cells at the borders of the developing pallium. *Nature Neurosci.* **8**, 1002–1012 (2005).
130. Letinic, K., Zoncu, R. & Rakic, P. Origin of GABAergic neurons in the human neocortex. *Nature* **417**, 645–649 (2002).
131. O'Leary, D. D. & Nakagawa, Y. Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. *Curr. Opin. Neurobiol.* **12**, 14–25 (2002).
132. Kimura, J. *et al. Emx2* and *Pax6* function in cooperation with *Otx2* and *Otx1* to develop caudal forebrain primordium that includes future archipallium. *J. Neurosci.* **25**, 5097–5108 (2005).
133. Muzio, L. & Mallamaci, A. *Emx1*, *Emx2* and *Pax6* in specification, regionalization and arealization of the cerebral cortex. *Cereb. Cortex* **13**, 641–647 (2003).

Acknowledgements
This work was partially supported by grants from the National Institutes of Health (NS45523, NS49553, NS41590), the Harvard Stem Cell Institute, the Spastic Paraplegia Foundation and the ALS Association to J.D.M. P.A. was partially supported by a Claflin Distinguished Scholar Award, the Harvard Stem Cell Institute, the Spastic Paraplegia Foundation and a grant from the ALS Association. B.J.M. was supported by the Harvard M.S.T.P. and the United Sydney Association.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Cux1 | *Cux2* | *Emx2* | *Fezf2* | *Foxg1* | *hunchback* | *Lhx2* | *Otx1* | *Pax6* | *Svet1* | *Tbr1*

FURTHER INFORMATION
Jeffrey Macklis's laboratory: http://macklis.mgh.harvard.edu
Allen Brain Atlas: http://www.brain-map.org
BGEM at St. Jude Children's Hospital: http://www.stjudebgem.org
Functional Genomic Atlas of the Mouse Brain: http://mahoney.chip.org/mahoney
Gene Paint: http://www.genepaint.org
GENSAT: http://www.gensat.org
International Mouse Strain Resource (IMSR): http://www.informatics.jax.org/imsr

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
Access to this links box is available online.