Laminar fate specification in the cerebral cortex

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Abstract

The cerebral cortex is composed of hundreds of different types of neurons, which underlie its ability to perform highly complex neural processes. How this astonishing cell diversity is generated during development constitutes a major challenge in developmental neurosciences, with important implications for neurological diseases. Here we review some recent and exciting advances in this field, from the description of the cellular processes at the origin of cortical neuron diversity, to the dissection of the molecular logic underlying fate selection in cortical neurons.

Introduction

The cerebral cortex is arguably the most complex structure in the mammalian brain, consisting of hundreds of distinct neuronal subtypes, each connected to a specific part of the brain. Neuronal diversity is at the core of cortical function and underlies its most sophisticated tasks that set us apart as higher mammals, such as language, reasoning, and memory. Understanding the mechanisms underlying this diversity may one day allow us to repair the damage wrought to the cerebral cortex by aging and trauma.

Neurons of the cerebral cortex belong to two broad classes: excitatory pyramidal neurons and inhibitory interneurons. Pyramidal neurons, named after their triangular-shaped cell body, can be categorized further into dozens of subtypes, each characterized by specific morphology, electrophysiology, and connectivity [1]. Cortical neurons are not arranged randomly in space; the location of a pyramidal neuron in a specific cortical area and layer broadly predicts its participation in a modality-specific neuronal network. The surface of the cortex consists of areas of neurons that are specialized in particular functions, such as vision or language. In addition, each area is divided through its thickness into six different layers (laminae), which contain specific subtypes of neurons. The laminar position of a neuron is correlated with its

pattern of connectivity (Figure 1). Corticofugal neurons are located in the deep layers of the cortex (layers 5 and 6) and mainly send their axons to subcortical structures (such as the basal ganglia, thalamus, brainstem, and spinal cord) whereas corticocortical-projecting neurons, which connect one region of the cortex to another, and especially callosal-projecting neurons, whose axons project into the corpus callosum, reside mostly in the upper layers 2 and 3. Layer 4 neurons on the other hand mainly receive input from the rest of brain.

The six-layer organization is central to cortical function and is highly conserved in all mammals despite the fact that the cortex has grown in complexity throughout recent mammalian evolution. This leads us to question the developmental mechanisms involved that link early embryonic events with later phases of patterning of connectivity. In addition, the identification of the factors that can (re)specify the identity of cortical neurons, and thereby their patterns of connectivity, could have major implications for future therapeutic strategies aimed at repairing the cortex following injury or degeneration.

Here, we will review recent and exciting advances in the understanding of the mechanisms that control the generation of pyramidal neuron diversity and their relation to laminar patterns of neuronal fate.

**Figure 1. Laminar organization and pattern of cortical projections**
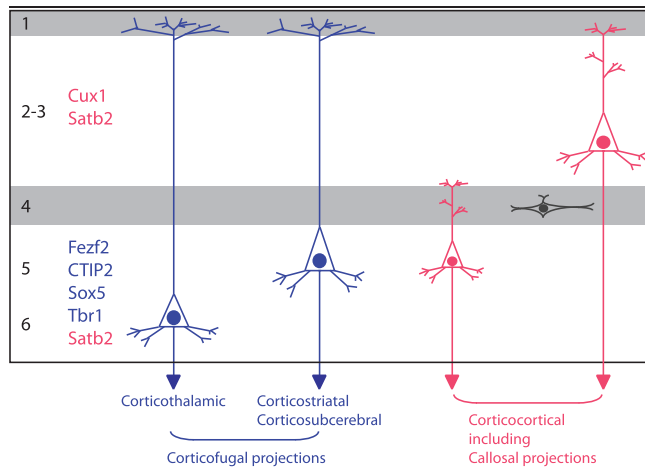
Simplified scheme depicting the laminar organization of the cortex in terms of gene expression and axonal projections. Neurons sending corticofugal projections (in blue) reside exclusively in the deep layers 5 and 6 of the cortex, while those sending projections within the cortex (in red), including callosal projections to the contralateral side, reside primarily in the upper layers, with a small contingent of callosal-projecting neurons in layer 5. Each subtype expresses specific combinations of transcription factors (in blue and red).

Diversity starts in cortical progenitors

The entire pyramidal neuronal population arises from cortical progenitor cells in the proliferative zones of the dorsal forebrain. These progenitors constitute a diverse population of cells with distinct molecular and cellular properties that scientific research has only just begun to unveil (Figure 2).

Among the cortical progenitors are the radial glial cells, which constitute a major subtype (reviewed in [2,3]). They are characterized by their unique morphology, consisting of a contact with the ventricular surface and a radial projection stretching from the ventricular zone (the most apical cell layer that lines the ventricle) to the outer, or basal, surface of the cortex. Radial glial cells undergo stereotypical patterns of symmetric and asymmetric cell divisions, thereby enabling the generation of diverse types of neurons while maintaining a pool of progenitors, thus following stem cell-like behavior [4,5].

In addition to radial glial cells, several other types of progenitors have been identified that are likely to contribute to neuronal diversity [4,6,7]. Of special interest among these are basal progenitors, which are also called intermediate progenitor cells [4,8]. Newly generated intermediate progenitor cells migrate to the upper part of the ventricular zone to create an additional proliferative zone above it, called the subventricular zone. Unlike radial glial cells, intermediate progenitor cells divide

Figure 2. Diversity of cortical progenitors
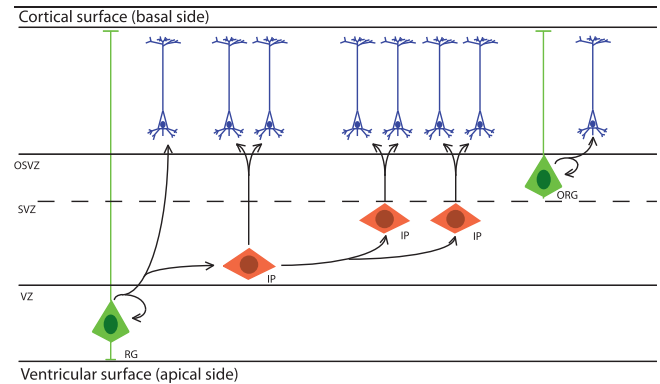
Several types of cortical progenitors and their modes of division towards neurons (in blue) are depicted, including radial glial cells (RG), intermediate progenitors (IP), and outer radial glial cells (ORG), with their specific location in ventricular (VZ), subventricular (SVZ) or outer subventricular (OSVZ) zones.

symmetrically only once or twice before generating neurons and thus act as transit amplifying cells. As there seems to be a correspondence in the expression of markers between subventricular-zone intermediate progenitor cells and upper-layer neurons [9,10], it was proposed that upper-layer neurons arise mainly from intermediate progenitor cells. This was recently confirmed in mice through the analysis of the function of various genes, such as *Tbr2*, *Ap2γ*, and *Insm1*, that were shown to be required for IP-cell specification and amplification, as well as upper-layer neuron specification [11-15]. Interestingly, some of these studies also revealed that intermediate progenitor cells are involved in the generation of some deep-layer neurons as well, so there is no unequivocal link between one type of progenitor and one type of cortical neuron.

The diversity of progenitors has also been proposed to contribute to the evolution and complexification of the cerebral cortex. As intermediate progenitor cells are progressively more abundant in higher mammals and primates, it was proposed that the observed increase in the relative number of upper-layer neurons in higher mammals may also be due to an expansion of the subventricular zone or altered properties of intermediate progenitor cells [16,17]. However, another major type of progenitor has now been described within a specialized compartment of the human developing cortex called the outer subventricular zone [18,19]. These so-called "outer" radial glial cells share many features with regular radial glial cells, including the potential for self-renewal, but they lack any apical projection (Figure 2). As these cells have only been found so far in human and ferret cortex, and not in any smaller-sized and/or simpler

cortex, they might constitute a species-specific feature that could underlie the expansion or complexification of the cortex in higher mammals.

Acquisition of laminar fate through temporal patterning

In vivo lineage-tracing analyses have revealed that cortical progenitors generate radial clones that consist of neurons arranged in multiple layers. Interestingly, a prominent feature of this process is its time dependence; that is, cortical progenitors generate different layers of neurons at distinct developmental stages. As corticogenesis proceeds, newly generated neurons migrate radially past the ones generated earlier to progressively create the six cortical layers in which neurons are located, according to their birth date, in an inside-out fashion. Early-generated neurons thus reside in deep layers and late-generated neurons in the upper layers [20]. It remains unknown how cortical progenitors use time-dependent intrinsic and extrinsic cues to generate distinct types of neurons [21]. Somewhat surprisingly, in vitro studies using dissociated cortical progenitors [22] or even cortical progenitors derived from embryonic stem cells [23] have revealed that the temporal sequence of cell division and neuron specification is also conserved within clones of cortical cells arising from a single progenitor. This suggests that, at least in vitro, some progenitors are multipotent and are capable of generating multiple types of neurons in a lineage-intrinsic pattern by changing their competence (i.e., their capacity to respond to intrinsic and extrinsic differentiation cues) over time. The molecular mechanisms underlying this intriguing process remain largely unknown, although it is reminiscent of similar time-dependent clonal neurogenesis described in the fly embryo [24,25].

Towards a molecular logic of specification of cortical neurons

While the data reviewed above have provided an important framework to understand the generation of neuronal diversity in the cortex, they do not explain how neuronal fate choices are instructed in the cortex, particularly in relation to layer identity and patterns of connectivity. This important issue has begun to be addressed recently through the discovery of genes that are expressed in neuron subtype-specific patterns at the time of their differentiation. Several of these genes were identified through a connectivity-based screening strategy, where different subtypes of cortical neurons were first isolated on the basis of their patterns of axonal projections, followed by the analysis of their transcriptome [26].

Among the genes identified in this screen, *Fezf2* was found to be expressed in corticospinal projection neurons [26,27]. In *Fezf2*-mutant mice, deep-layer neurons are generated normally but fail to mature and to extend axons to their subcortical targets such as spinal cord [27-30]. Most strikingly, the development of corticofugal neurons is not only blocked in these mutants but is also partially switched to other fates (Figure 3), as they display many properties of callosal-projecting neurons, such as sending their axons to the contralateral cortex [27,31]. Conversely, overexpression of *Fezf2* in late progenitors leads to the generation of neurons displaying corticofugal instead of callosal projections [27,29,31]. Although this fate switch is incomplete [28,29,31], the data suggest that *Fezf2* is one of the master genes for the specification of corticofugal neurons. This was strikingly illustrated by a recent study showing that *Fezf2* overexpression in progenitors of the ventral forebrain, normally fated to generate striatal neurons, resulted in their respecification into corticofugal neurons [32].

Fezf2 was thus isolated as a gene that is selectively expressed among distinct neuronal subtypes, but it seems to act mainly within progenitors. While this confirms that patterns of cortical neuron diversity emerge in cortical progenitors, recent data indicate that this diversity also builds up within differentiating neurons themselves, through genes acting downstream of *Fezf2* within different types of cortical neurons to instruct them to acquire specific patterns of identity and connectivity (Figure 3). One such gene is *Ctip2*, which is downregulated in *Fezf2*-mutants [27,28,31]. It is one of the first genes found to be expressed specifically among corticofugal neurons [26]. *Ctip2* is expressed by these neurons during development and in the postnatal period until adulthood, and is especially enriched in layer 5 corticospinal-projecting neurons [26]. In *Ctip2*-mutant mice, corticospinal axons do not reach their normal targets, a similar picture to that seen in *Fezf2*-mutants. Overexpression of *Ctip2* is able to rescue the axonal projection phenotype in *Fezf2*-mutant mice and to instruct some upper-layer neurons to extend aberrant subcortical projections in normal mice, suggesting that it acts as a major effector of *Fezf2* in the development of subcortical projections [31].

Another interesting gene identified in corticofugal neurons is *Sox5* [33,34]. In *Sox5*-mutant mice, it is not only the final fate of corticofugal neurons that is altered but also the timing of their generation and laminar fate [33,34]. Corticothalamic and corticospinal neurons fail to segregate in their specific layers and the corticospinal tract, although present, is abnormal [34]. Interestingly, the additional loss of *Ctip2* reverses some of the defects observed, suggesting that the normal role of *Sox5* is to repress the early expression of *Ctip2* to correctly specify early-generated cortical neurons. Gain-of-function experiments in upper-layer neurons suggest that, at least at

Figure 3. From genes to neuronal fates
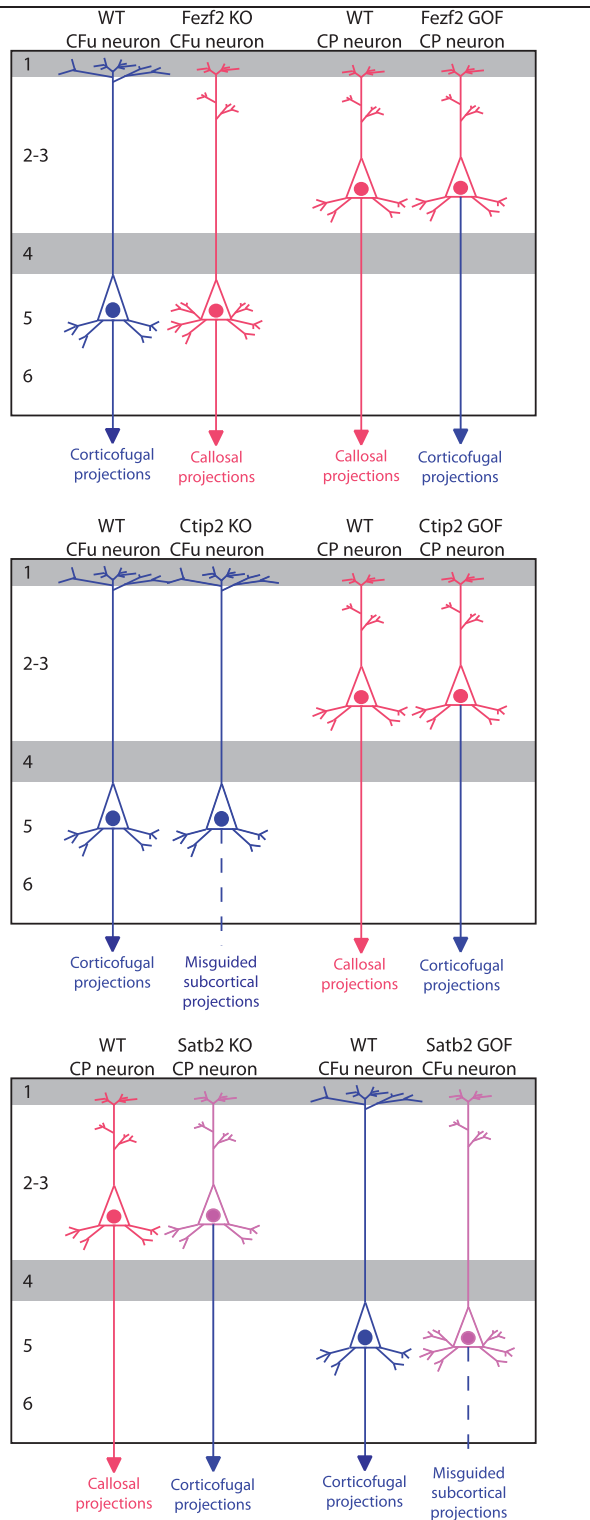
Scheme depicting the axonal projections of cortical neurons in normal (wild-type) conditions (WT) or following knockout (KO) or gain-of-function (GOF) of three major transcription factors involved in fate specification: Fezf2, Ctip2, and Satb2. See text for further explanation.

certain time points, *Sox5* is able to inhibit the growth of callosal-projecting neurons and promote corticofugal projections [33,34].

Besides the neuronal genes that "switch on" subcortical-projecting neuron fate, others seem to promote the acquisition of upper-layer or callosal-projecting neuron fate. Among these, *Satb2* is normally expressed in upper-layer neurons and in callosal-projecting neurons of layer 5 [35,36]. In *Satb2*-mutant mice, upper-layer neurons ectopically express *Ctip2*, but not *Fezf2*, and contribute to the corticospinal tract in place of the corpus callosum, which is absent [35,36]. Importantly, *Satb2* was found to be able to repress *Ctip2* expression, probably through direct chromatin modifications in the Ctip2 gene [35,36]. Conversely, while it is not clear whether *Ctip2* can actively repress *Satb2*, *Fezf2* disruption results in ectopic expression of *Satb2* among corticofugal-projection neurons [31]. Thus, *Satb2*, *Ctip2*, and *Fezf2* take part in a genetic network enabling selection of neuronal fate, at least at the level of connectivity. In callosal-projecting neurons, *Fezf2* is absent, so expression of *Satb2* is possible, which in turn represses the expression of *Ctip2* and leads to the development of callosal projections. In corticofugal neurons, *Fezf2* is present and represses the expression of *Satb2*, while it induces the expression of *Ctip2* and the development of corticofugal projections.

Overall, these data converge to suggest a model whereby the combinatorial expression of transcription factors leads to the precise specification of the different subtypes of deep-layer and corticofugal neurons on the one hand and callosal-projecting and upper-layer neurons on the other hand. This model remains fragmentary but constitutes a solid foundation to start unraveling the mechanisms of generation of cortical neuron diversity.

Where to go next

Recent advances have helped us to understand how the astonishing cellular diversity of the cerebral cortex arises, but many questions remain. Firstly, the extent of the diversity of the neural progenitors probably remains underestimated, and it will be crucial to determine the full range of this diversity, how it is established, and how it contributes to neuronal diversity. Another mystery to be solved is the molecular mechanism(s) that allows cortical progenitors to change competence in a time-dependent fashion, thereby generating even more diversity. Finally, how are the various features of the identity of a cortical neuron coordinated to achieve a proper match between laminar position and connectivity, and how is this related to the differentiation of cortical areas? Indeed, while recent work has also allowed us to gain insights into the mechanisms of areal specification, including the

contribution of graded morphogens and transcription factors (reviewed in [37,38]), it is striking to note that a number of genes involved in this process may also control laminar fate [39-41]. This molecular link between areal and laminar patterning may provide the first hints as to how different areas are composed of the same six layers, but in distinct proportions.

Clearly, much more work will be needed to address all these complex questions. But together with the recent description of how diverse types of cortical neurons are generated from embryonic stem cells [42,43], a better knowledge of neuronal fate specification may provide unprecedented opportunities for the rational design of specific types of cortical neurons, which could be used to model specific types of cortical neuron diseases or pave the (still long) way towards cortical repair with replacement therapies.

Competing interests

The authors declare they have no competing interests.

Acknowledgements

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