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# Orchestrated freedom: new insights into cortical neurogenesis

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In mammals, the construction of the cerebral cortex involves the coordinated output of large populations of apical progenitor cells. Cortical progenitor cells use intrinsic molecular programs and complex regulatory mechanisms to generate a large diversity of excitatory projection neurons in appropriate numbers. In this review, we summarize recent findings regarding the neurogenic behavior of cortical progenitors during neurogenesis. We describe alternative models explaining the generation of neuronal diversity among excitatory projection neurons and the role of intrinsic and extrinsic signals in the modulation of the individual output of apical progenitor cells.

## Addresses

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## Introduction

The mammalian neocortex contains an extraordinary diversity of neuronal types organized in six layers. The different types of neurons are characterized by distinctive — although rarely unique — molecular identities, projection patterns and morphologies [1–5]. The origin of this diversity is the dynamic and complex neurogenic behavior of progenitor cells during embryonic development. This behavior is under the control of highly sophisticated regulatory mechanisms that orchestrate cortical neurogenesis and ensure the production of appropriate numbers of each of the neuronal elements that are required for the assembly of cortical circuits.

There are two main classes of cortical neurons, glutamatergic projection neurons and GABAergic interneurons.

Both classes of neurons originate from different progenitor regions in the embryo: glutamatergic neurons derive from progenitor cells in the pallium — the roof — of the telencephalon, while GABAergic cells derived from different progenitor pools in the subpallium. This review focuses on recent studies that have contributed to increase our understanding of the mechanisms regulating the neurogenesis of glutamatergic projection neurons (PNs) in the developing neocortex.

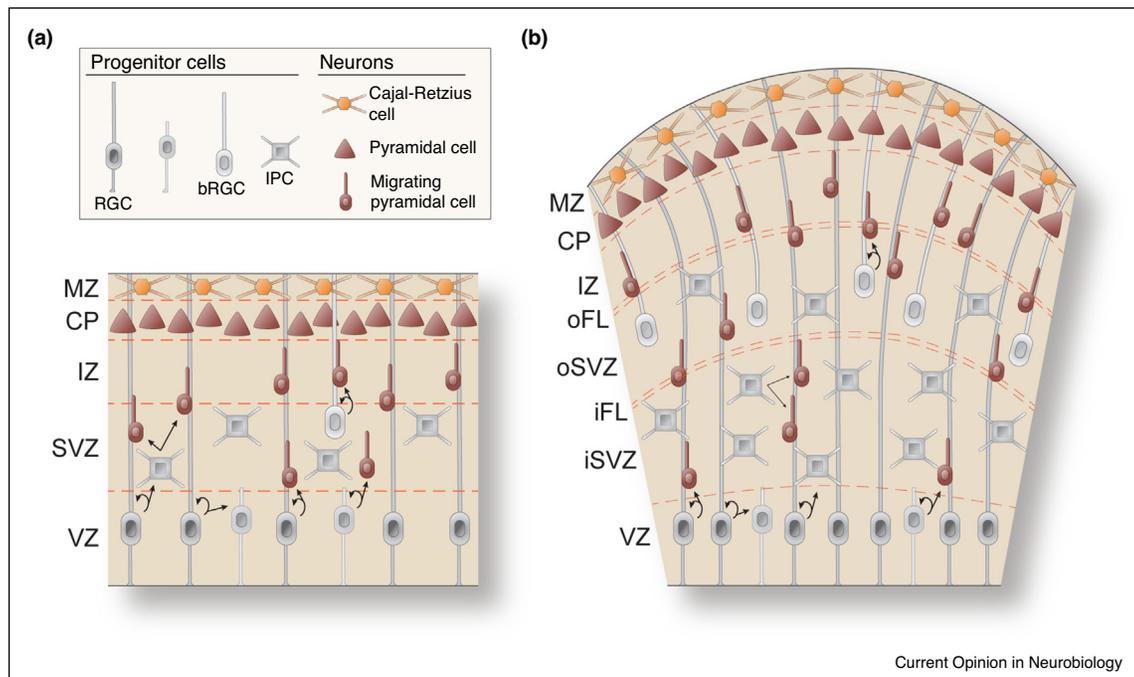
## Progenitor cells in the developing cortex

During early embryogenesis the pallium consists in a monolayer of proliferating stem cells in contact with the lumen of the lateral ventricles known as neuroepithelial cells (NECs). These cells divide first symmetrically multiple times to expand the progenitor pool. Subsequently, NECs experience remarkable phenotypic changes, acquiring glial features. From this point, these apical progenitor cells are known as radial glial cells (RGCs) [6,7]. RGCs exhibit a characteristic bipolar morphology, with an apical process in contact with the ventricle and a basal process that extends to the pial surface (Figure 1a), and express distinctive transcription factors such as Pax6, Emx1 and Sox9. The transition from NEC to RGC identity marks the start of neurogenesis. During this period, each RGC divides multiple times to generate PNs while self-renewing. Nascent neurons use the basal processes of RGCs to migrate towards the pial surface. This migratory behavior leads to the emergence of an inside-out pattern of cortical lamination that roughly correlates with neuronal birthdate: early-born PNs end up in deep layers of the neocortex, while late-born PNs occupy progressively more superficial layers [2,3,8–10]. At the end of neurogenesis, some RGCs become gliogenic and undergo additional rounds of division to produce astrocytes.

RGCs self-renew and generate either a post-mitotic neuron or an intermediate progenitor cell (IPC) in each division. The majority of IPCs express the transcription factor Tbr2, are multipolar and quickly migrate to a more basal germinal layer, the subventricular zone (SVZ). Once there, they typically divide once to generate two post-mitotic neurons, consequently expanding the neuronal pool [11–14] (Figure 1a).

Short neural precursors (SNPs) constitute a second population of IPCs which remain in an apical position. These cells have a distinctive morphology, extending an apical process in contact with the ventricular surface and a short

Figure 1



Several populations of progenitor cells build the mammalian cerebral cortex. **(a)** Cortical neurogenesis in lissencephalic mammals. Apical radial glial cells (RGCs) divide in the embryonic ventricular zone (VZ) to generate short neural precursors (SNP), intermediate progenitor cells (IPCs) and neurons. SNPs divide few times generating postmitotic neurons. IPCs divide terminally in the embryonic subventricular zone (SVZ) generating two postmitotic neurons. Few apical RGC divisions generate basal radial glial cells (bRGCs). These cells perform self-renewing divisions in the SVZ generating large cohorts of neurons. Newborn neurons migrate radially following the basal processes of RGCs to reach the cortical plate (CP). **(b)** Cortical neurogenesis in gyrencephalic mammals. These species have an enlarged SVZ, which can be divided into inner and outer regions (iSVZ and oSVZ, respectively), separated by the inner fiber layer (iFL). The growth of the SVZ correlates with a prominent increase in the number of bRGCs, which mainly populate the oSVZ. MZ, marginal zone; IZ, intermediate zone. oFL, outer fiber layer.

basal process that does not reach the pia [15] (Figure 1a). Unlike RGCs, these cells are thought to primarily generate neurons, thus generating a small neuronal output [16,17]. Interestingly, SNPs seem to generate neurons that occupy slightly deeper strata than coetaneous neurons derived from RGCs [16]. SNPs arise from RGCs, and the molecular mechanisms instructing their fate are just beginning to be understood [18].

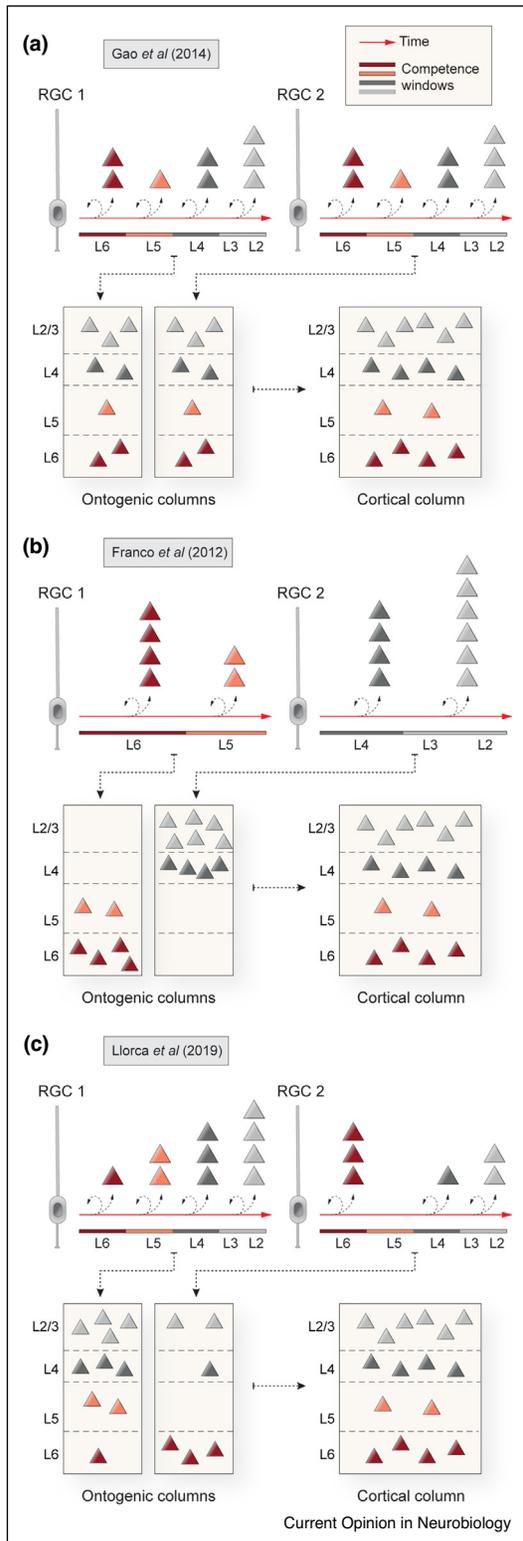
In addition to IPCs, the SVZ contains a second type of basal progenitor, known as basal radial glial cells (bRGCs). These cells have a long basal process that contacts the pial surface, but lack an apical process in contact with the ventricular surface [19–21]. Similar to their apical counterparts, bRGCs have self-renewing capacity and can generate both neurons and IPCs [22–24]. bRGCs are relatively rare in the rodent cortex [25,26] but they are very abundant in gyrencephalic mammals, in which they are thought to be responsible of the evolutionary expansion of superficial cortical layers. In these species, the embryonic SVZ has grown so large that can be divided into superficial (outer) and deep (inner) sublaminae, with the outer SVZ containing the highest density of bRGCs [27–29] (Figure 1b). Interestingly, bRGCs are

particularly abundant in certain regions of the murine cortex, where they contribute to an increased generation of superficial layer neurons [30].

### Deterministic and stochastic models of cortical neurogenesis

Cortical progenitor cells generate many different types of PNs in a temporally organized manner. Consistently, the outcome of multiple neurogenic RGCs reflects the entire diversity of excitatory neurons that populate the adult neocortex in mice [31]. The most commonly held view of this process postulates that RGCs are multipotent and progress through a sequence of competence windows as neurogenesis proceeds. During each of these windows RGCs are competent to generate a specific type of PC. The strictest interpretation of this mechanism proposes that individual RGCs would generate a column of radially organized cells containing all the different types of PNs. This column would constitute a fundamental building block of cortical cytoarchitecture, known as the ‘radial unit’. According to this view, the construction of cortical circuits would rely on multiple repeats of the basic ‘radial unit’ [32] (Figure 2a). Consistent with this idea, fate-mapping experiments in the murine

Figure 2



Models of cortical neurogenesis. **(a)** Deterministic model. Radial glial cells (RGCs) generate unitary outputs, similar in size and composition. Cortical circuits are constructed by multiple repeats of these unitary outputs. **(b)** Fate restriction model. Different types of RGCs specialize in generating specific types of neurons. Cortical circuits are

cortex have revealed that RGCs are multipotent and produce diverse neuronal fates distributed across all cortical layers containing PNs [33–35]. These observations led to the conclusion that the neurogenic behavior of RGCs is deterministic, with all RGCs producing ‘unitary outputs’ of similar size and composition [33].

The temporal progression of RGCs is thought to be driven by intrinsic molecular programs [36], as observed in multiple invertebrate neural lineages [37]. Consistently, cortical progenitor cells isolated *in vitro* seem capable to recapitulate the sequence of neuronal identities that are normally generated *in vivo* [38,39]. In *Drosophila*, temporal transcription factors are sequentially expressed in embryonic neuroblasts and instruct the progression of these stem cells through different competence windows [37]. Although similar principles are believed to govern the temporal transition of RGCs through equivalent competence windows, the identification of specific temporal transcription factors in these cells has been difficult and is still a matter of active investigation [40]. A recent study from the Jabaudon lab has shed light into this issue. Telley *et al.* used single cell transcriptomics to identify highly dynamic patterns of gene expression in both RGCs and early postmitotic neurons, thereby revealing clear molecular signatures of temporal progression in the mammalian cortex [41<sup>••</sup>]. Work from the Novitsch lab has identified FoxP1 as one of the transcription factors involved in this process. FoxP1 is exclusively expressed during early neurogenesis and promotes the genesis of deep layer neurons [42]. Epigenetic regulation of gene expression has also been implicated in the temporal progression of cortical progenitor cells [43–45]. For example, it has been suggested that the temporally organized expression of certain miRNAs during development may contribute to instruct the identity of nascent neurons [46<sup>•</sup>]. In addition, recent work from the Miller lab indicates that translational regulation may be another mechanism controlling the temporal progression of RGCs. They observed that RGCs contain mRNAs of many genes known to specify different neuronal identities. However, selective translation of some of these transcripts both in RGCs and newborn neurons directs the specification of neuronal identity [47<sup>•</sup>]. Finally, in addition to temporally controlled molecular programs, recent evidence indicate that the membrane potential of RGCs also regulates temporal progression [48<sup>•</sup>].

constructed by the combination of modules arising from different pools of progenitor cells. **(c)** Stochastic model. Most RGCs follow similar molecular programs, but make stochastic ‘decisions’ along development and generate diverse outcomes. Cortical circuits are constructed by collective assembly of the individual output of progenitor cells. Schemas represent the neurogenic fate of individual RGCs. Time is represented as the relative progression of individual RGCs through successive divisions. Hence RGC1 and RGC2 in each example generate different cohorts of cells in subsequent divisions, but the divisions of the two RGCs are not necessarily synchronous in time (i.e. in (b), RGC1 would mostly divide during early neurogenesis, while RGC2 would mostly divide during late neurogenesis).

In spite of the evidence supporting the multipotency of cortical progenitors, some studies have suggested the existence of different pools of progenitors with restricted potential to generate specific types of PNs [49–51] (Figure 2b). These observations are, however, compatible with the temporal progression of RGCs. A recent study from our lab has provided evidence that may reconcile both sets of results. Llorca *et al.* used a combination of lineage tracing methods to map the neuronal output of individual RGCs in the mouse cortex. They observed that individual lineages are extremely heterogeneous: while a majority of RGCs generate multiple neuronal fates, a small fraction produce progenies that are restricted to some cortical layers [52\*\*] (Figure 2c). These findings suggest that while the behavior of individual progenitors is highly unpredictable, the net sum of their output consistently leads to the formation of a relatively invariant structure. In other words, stochastic — probabilistic — rules seem to govern cortical neurogenesis, as has been previously suggested for the mammalian retina [53,54]. Of note, these results also suggest certain constraints in the fate of RGCs, since universal stochastic rules are able to explain most but not all the observed RGC outputs. The regulation of the probabilistic behavior of RGCs by extrinsic cues may confer an important degree of flexibility and robustness in the development of the cerebral cortex [55].

### Extrinsic control of cortical progenitor behavior

Classic transplantation experiments in the ferret first revealed that extrinsic cues influence the neurogenic fate of cortical progenitor cells [56]. These studies also led to the suggestion that the potential of cortical progenitor cells is progressively reduced over time. However, recent work in the developing mouse cortex indicates that this might not be the case for all types of progenitor cells. In transplantation experiments in which they study the fate of RGCs and IPCs separately, Oberst *et al.* found that transplanted RGCs adapt their neurogenic fate to the corresponding host age [57\*\*]. In contrast, IPCs are not prone to be reprogrammed following heterochronic transplantation, and their potential seems to be locked to the stage in which they are generated. The discrepancy between these two studies is likely due to the fact that Desai and McConnell transplants were enriched in IPCs, although species-specific differences cannot be ruled out.

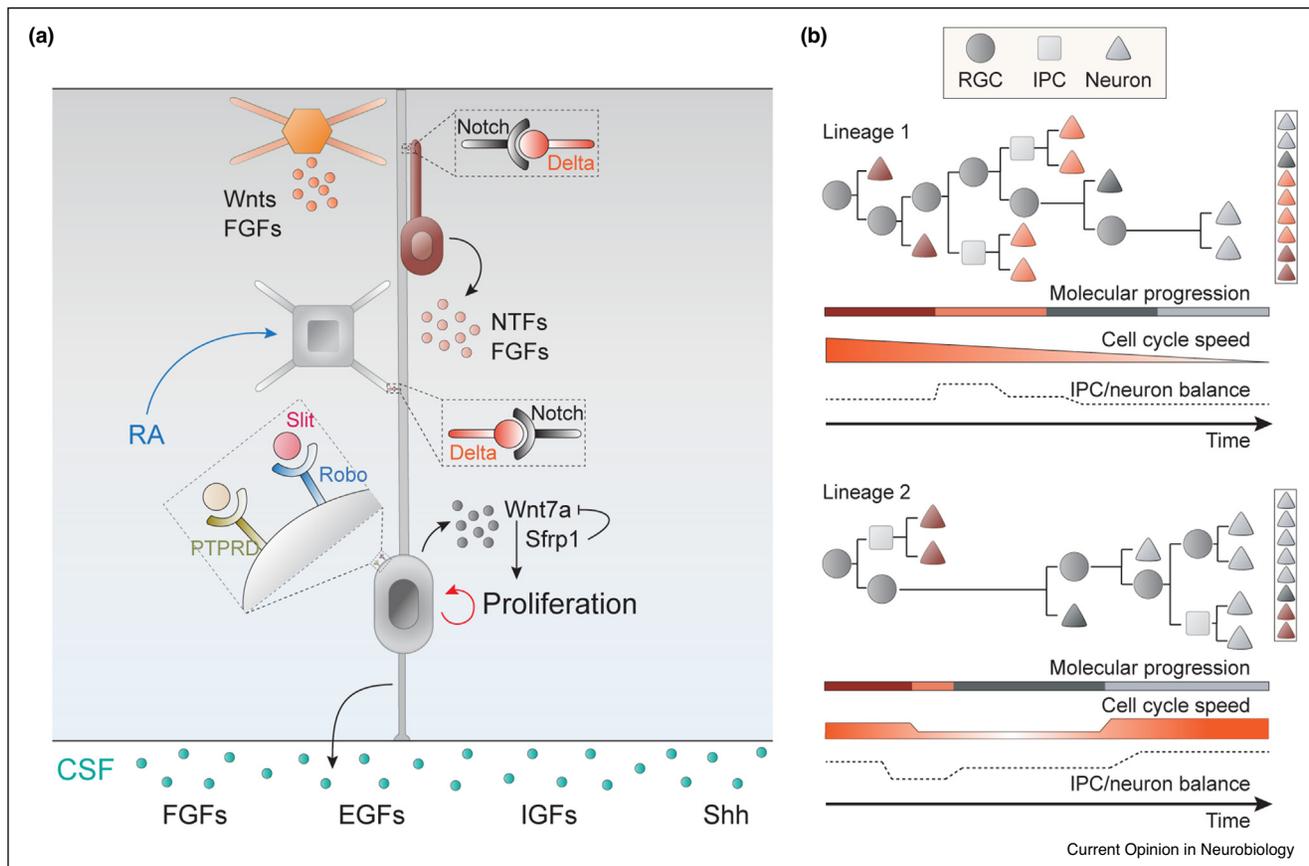
It is now well established that cortical progenitor cells are under the influence of multiple extrinsic factors [58] (Figure 3a). Consistent with this idea, both progenitor cells and newborn neurons become progressively more sensitive to external cues as neurogenesis proceeds [41\*\*]. External signals can interact with cell-autonomous mechanisms to modulate multiple aspects of RGC behavior. For instance, environmental cues may influence intrinsic

molecular pathways to regulate progression through the temporal sequence of competence states. This could occur by modulating the length of the different competence windows, making RGCs more or less likely to produce certain types of PNs (Figure 3b). Interestingly, various neuronal fates seem to be simultaneously produced during early neurogenesis [59\*]. Also, RGCs dividing at the same time are able to generate neurons with different laminar identities [52\*\*]. These observations suggest a certain degree of asynchrony or heterogeneity in the intrinsic programs of cortical progenitor cells. Such heterogeneity may be a landmark of extrinsic regulation, as extrinsic signals may differentially affect each individual RGC. Remarkably, the temporal progression of RGCs is severely impaired in cultured conditions that prevent contact with nascent progenies [60\*\*]. This suggests a possible role of feedback signaling in their temporal progression, as proposed for the retina [61]. Indeed, contact-mediated activation of notch signaling in RGCs via different delta-like ligands expressed by nascent neurons and IPCs has been reported [62]. Several other cues have also been implicated in this feedback mechanism, including neurotrophins and fibroblast growth factors (FGFs) [63]. In addition, it has been suggested that RGCs plasticity relies on their ability to sense dynamic changes in extracellular Wnt (Oberst *et al.* [40]). Indeed, the progressive hyperpolarization of stem cells regulates temporal progression by modulating their sensitivity to extrinsic Wnt signaling [48\*]. Shh signaling also seems to influence the molecular progression of RGCs, promoting the switch from neurogenesis to gliogenesis [64].

The neuronal output of RGCs can also be controlled via regulation of progenitor cell division. While a common molecular sequence runs inside progenitor cells, environmental cues may facilitate or prevent their division during each competence window. The modulation of proliferation would, therefore, determine the ultimate composition of individual lineages (Figure 3b). Importantly, the temporal progression of cortical RGCs seems to be cell cycle independent. Consistent with this idea, Okamoto *et al.* reported that temporal changes in gene expression can occur in cell cycle arrested progenitors, so that when progenitors are released from cell cycle arrest they continue to generate temporally appropriate neuronal fates [60\*\*].

Interestingly, local regulation of cell cycle kinetics has been previously linked to the genesis of region-specific cortical cytoarchitectures [65]. For instance, signals secreted by Cajal-Retzius cells contribute to neocortical arealization via region-specific regulation of progenitor proliferation [66]. Thalamic axons also known to influence the proliferation of RGCs [21,58]. Moreover, several diffusive cues regulate RGC division. For example, Wnt7 and secreted frizzled-related protein 1 (Sfrp1) play opposite roles in modulating cortical progenitor proliferation [67]. Moreover, the cerebrospinal fluid contains multiple

Figure 3



Extrinsic influences modulate the activity of cortical progenitor cells. **(a)** Multiple adhesive and diffusive signals influence the neurogenic behavior of radial glial cells (RGCs). **(b)** These influences modulate the temporal progression, cell cycle speed, and mode of division of cortical progenitor cells. The modulation of these parameters influences the neurogenic output of individual RGCs, which is, therefore, heterogeneous and largely unpredictable. CSF, cerebrospinal fluid; EGF, epithelial growth factor; FGF, fibroblast growth factor; IGF, insulin growth factor; IPC, intermediate progenitor cell; NTF, neurotrophin; PTPRD, protein tyrosine phosphatase receptor delta; RA, retinoic acid; SHH, Sonic hedgehog; Wnt, wingless.

signals known to influence neurogenesis [68,69]. Most of these signals have been implicated in the regulation of RGC division, including FGFs [70–72], epithelial growth factors (EGFs) [70], insulin growth factors (IGFs) [72,73], and Sonic hedgehog (Shh). Interestingly, some of these cues, such as IGF1 and IGF2, are synthesized by RGCs themselves, and therefore function in a paracrine manner [70,72]. Feedback signaling from newborn neurons has also been proposed to regulate the division of RGCs. FGFs and Notch signaling induce the initiation of calcium waves in postmitotic layers of the developing cortex. Such waves propagate from the basal processes to the cell bodies of RGCs [74] and influence proliferation [75]. The neurotransmitters glutamate and GABA, released from newborn excitatory cells and migrating interneurons respectively, are also able to influence the cell cycle of cortical progenitors [76,77].

A final key aspect of neurogenesis that is regulated by extrinsic signals is the mode of cell division. RGCs

could be directed to preferentially generate neurons or IPCs during specific competence windows, ultimately affecting the organization of individual lineages (Figure 3b). Indeed, an increase in SVZ mitoses has been linked to the evolutionary expansion of superficial cortical layers in some mammals. Several studies have highlighted the importance of extrinsic signaling in this process. For instance, Robo signaling through its downstream effector delta-like 1 (Dll1) regulates the balance between direct and indirect neurogenesis in the developing mouse cortex [68]. This signaling mechanism has been recently associated with the evolutionary increase in indirect neurogenesis that characterizes neurogenesis in mammals [78\*\*]. Similarly, the protein tyrosine phosphatase receptor delta (PTPRD), a transmembrane receptor involved in the transduction of extracellular signals, regulates the production of IPCs [79]. Retinoic acid is another diffusive signal known to promote indirect neurogenesis in the developing neocortex [80].

IPCs are able to divide multiple times in gyrencephalic mammals [81]. This feature has also been observed in rodents [82,83], although is thought to be rare in these species [27,84]. Several signaling pathways are thought to be responsible for the increased proliferative capacities of IPCs. Notch signaling through the human-specific variant NOTCH2N seems to promote maintenance of an IPC fate and leads to increased proliferation [85–87]. Also, IPCs in the human cortex express a membrane-bound form of Palmdelphin (PALMD-Caax) that is absent in the murine cortex. This molecule promotes the growth of IPC processes, which allows these cells to receive increased integrin-mediated signaling and promotes their division [88\*]. Finally, bRGC genesis and proliferation in gyrencephalic species are also prone to modulation via extrinsic cues such as Shh, FGFs and PDGFs [89].

### Conclusions and perspectives

In this review, we provide a brief snapshot of several models of mammalian cortical neurogenesis and its multiple modulatory mechanisms, with special emphasis on recent discoveries. Such mechanisms are extraordinarily complex and diverse, extending far beyond the limits of this review and the limits of our current knowledge. The combination of regulatory mechanisms is critical for the generation of appropriate neuronal numbers and the construction of the neuronal circuits underlying cortical function. Immerse in this complex regulatory logic, cortical progenitor cells likely adapt their neuronal output in response to mechanisms orchestrating neurogenesis at the population level. From this perspective it is not entirely surprising that their individual outcomes exhibit great levels of variability, including progenies confined to certain cortical layers in some cases. This view may help to reconcile previous observations, which provided conflicting results on the fate of RGCs in the developing mammalian cortex. The fine modulation of RGC behavior, driven by the local environment, could indeed be a critical mechanism for the generation of the region-specific cellular architectures that characterize different cortical areas. This is an interesting possibility, but new studies would be required to test this hypothesis. Such studies should provide a better understanding of how local differences in modulatory signals may influence the behavior of progenitor cells in different regions of the developing cortex.

### Conflict of interest statement

Nothing declared.

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### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bakken TE, Jorstad NL, Hu Q, Lake BB, Tian W, Kalmbach BE, Hodge RD, Krienen FM, Sorensen SA, Eggermont J *et al.*: **Evolution of cellular diversity in primary motor cortex of human, marmoset monkey and mouse.** *bioRxiv* 2020 <http://dx.doi.org/10.1101/2020.03.31.016972>.
2. Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD: **Molecular logic of neocortical projection neuron specification, development and diversity.** *Nat Rev Neurosci* 2013, **14**:755-769.
3. Lodato S, Arlotta P: **Generating neuronal diversity in the mammalian cerebral cortex.** *Annu Rev Cell Dev Biol* 2015, **31**:699-720.
4. Wang Y, Ye M, Kuang X, Li Y, Hu S: **A simplified morphological classification scheme for pyramidal cells in six layers of primary somatosensory cortex of juvenile rats.** *IBRO Rep* 2018, **5**:74-90.
5. Lim L, Mi D, Llorca A, Marín O: **Development and functional diversification of cortical interneurons.** *Neuron* 2018, **100**:294-313.
6. Götz M, Huttner WB: **The cell biology of neurogenesis.** *Nat Rev Mol Cell Biol* 2005, **6**:777-788.
7. Taverna E, Götz M, Huttner WB: **The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex.** *Annu Rev Cell Dev Biol* 2014, **30**:465-502.
8. Fairén A, Cobas A, Fonseca M: **Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex.** *J Comp Neurol* 1986, **251**:67-83.
9. Rakic P: **Neurons in rhesus monkey visual cortex systematic relation between time of origin and eventual disposition.** *Science (80-)* 1974, **83**:425-427.
10. Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD: **Neuronal subtype specification in the cerebral cortex.** *Nat Rev Neurosci* 2007, **8**:427-437.
11. Englund C, Fink A, Lau C, Pham D, Daza RAM, Bulfone A, Kowalczyk T, Hevner RF: **Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex.** *J Neurosci* 2005, **25**:247-251.
12. Haubensak W, Attardo A, Denk W, Huttner WB: **Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis.** *Proc Natl Acad Sci U S A* 2004, **101**:3196-3201.
13. Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR: **Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases.** *Nat Neurosci* 2004, **7**:136-144.
14. Pontious A, Kowalczyk T, Englund C, Hevner RF: **Role of intermediate progenitor cells in cerebral cortex development.** *Dev Neurosci* 2008, **30**:24-32.
15. Gal JS, Morzov YM, Ayoub AE, Chatterjee M, Rakic P, Haydar TF: **Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones.** *J Neurosci* 2006, **26**:1045-1056.
16. Stancik EK, Navarro-Quiroga I, Sellke R, Haydar TF: **Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex.** *J Neurosci* 2010, **30**:7028-7036.
17. Tyler WA, Haydar TF: **Multiplex genetic fate mapping reveals a novel route of neocortical neurogenesis, which is altered in the Ts65Dn mouse model of down syndrome.** *J Neurosci* 2013, **33**:5106-5119.

18. Ramos SI, Makeyev EV, Salierno M, Kodama T, Kawakami Y, Sahara S: **Tuba8 drives differentiation of cortical radial glia into apical intermediate progenitors by tuning modifications of tubulin C termini.** *Dev Cell* 2020, **52**:477-491.e8.
19. Fietz SA, Kelava I, Vogt J, Wilsch-Bräuninger M, Stenzel D, Fish JL, Corbeil D, Riehn A, Distler W, Nitsch R *et al.*: **OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling.** *Nat Neurosci* 2010, **13**:690-699.
20. Hansen DV, Lui JH, Parker PRL, Kriegstein AR: **Neurogenic radial glia in the outer subventricular zone of human neocortex.** *Nature* 2010, **464**:554-561.
21. Reillo I, de Juan Romero C, García-Cabezas MÁ, Borrell V: **A role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex.** *Cereb Cortex* 2011, **21**:1674-1694.
22. Gertz CC, Lui JH, LaMonica BE, Wang X, Kriegstein AR: **Diverse behaviors of outer radial glia in developing ferret and human cortex.** *J Neurosci* 2014, **34**:2559-2570.
23. LaMonica BE, Lui JH, Hansen DV, Kriegstein AR: **Mitotic spindle orientation predicts outer radial glial cell generation in human neocortex.** *Nat Commun* 2013, **4**.
24. Martínez-Martínez MÁ, De Juan Romero C, Fernández V, Cárdenas A, Götz M, Borrell V: **A restricted period for formation of outer subventricular zone defined by Cdh1 and Trnp1 levels.** *Nat Commun* 2016, **7**.
25. Shitamukai A, Konno D, Matsuzaki F: **Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer subventricular zone progenitors.** *J Neurosci* 2011, **31**:3683-3695.
26. Wang X, Tsai J-W, LaMonica B, Kriegstein AR: **A new subtype of progenitor cell in the mouse embryonic neocortex.** *Nat Neurosci* 2011, **14**:555-561.
27. Lui JH, Hansen DV, Kriegstein AR: **Development and evolution of the human neocortex.** *Cell* 2011, **146**:18-36.
28. Smart IHM, 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* 2002, **12**:37-53.
29. Zecevic N, Chen Y, Filipovic R: **Contributions of cortical subventricular zone to the development of the human cerebral cortex.** *J Comp Neurol* 2005, **491**:109-122.
30. Vaid S, Camp JG, Hersemann L, Oegema CE, Heninger AK, Winkler S, Brandl H, Sarov M, Treutlein B, Huttner WB *et al.*: **A novel population of Hopx-dependent basal radial glial cells in the developing mouse neocortex.** *Development* 2018, **145**.  
This study reveals that the mouse medial cortex contains many more basal radial glial cells than the lateral cortex. Gene expression in these progenitor cells is similar to that observed in human outer radial glial cells. The enlargement of this population of basal radial glial progenitors in the medial cortex is attributed to an increased expression in Hopx.
31. Cadwell CR, Scala F, Fahey PG, Kobak D, Mulherkar S, Sinz FH, Papadopoulos S, Tan ZH, Johnsson P, Hartmanis L *et al.*: **Cell type composition and circuit organization of clonally related excitatory neurons in the juvenile mouse neocortex.** *eLife* 2020, **9**.
32. Rakic P: **Specification of cerebral cortical areas.** *Science (80-)* 1988, **241**:170-176.
33. Gao P, Postiglione MP, Krieger TG, Hernandez L, Wang C, Han Z, Streicher C, Papusheva E, Insolera R, Chugh K *et al.*: **Deterministic progenitor behavior and unitary production of neurons in the neocortex.** *Cell* 2014, **159**:775-788.
34. Guo C, Eckler MJ, McKenna WL, McKinsey GL, Rubenstein JLR, Chen B: **Fezf2 expression identifies a multipotent progenitor for neocortical projection neurons, astrocytes, and oligodendrocytes.** *Neuron* 2013, **80**:1167-1174.
35. Kaplan ES, Ramos-Laguna KA, Mihalas AB, Daza RAM, Hevner RF: **Neocortical Sox9+ radial glia generate glutamatergic neurons for all layers, but lack discernible evidence of early laminar fate restriction.** *Neural Dev* 2017, **12**:1-10.
36. Leone DP, Srinivasan K, Chen B, Alcamo E, McConnell SK: **The determination of projection neuron identity in the developing cerebral cortex.** *Curr Opin Neurobiol* 2008, **18**:28-35.
37. Doe CQ: **Temporal patterning in the Drosophila CNS.** *Annu Rev Cell Dev Biol* 2017, **33**:219-240.
38. Gaspard N, Bouschet T, Hourez R, Dimidschstein J, Naeije G, van den Aemele J, Espuny-Camacho I, Herpoel A, Passante L, Schiffmann SN *et al.*: **An intrinsic mechanism of corticogenesis from embryonic stem cells.** *Nature* 2008, **455**:351-357.
39. Shen Q, Wang Y, Dimos JT, Fasano CA, Phoenix TN, Lemischka IR, Ivanova NB, Stifani S, Morrisey EE, Temple S: **The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells.** *Nat Neurosci* 2006, **9**:743-751.
40. Oberst P, Agirman G, Jabaudon D: **Principles of progenitor temporal patterning in the developing invertebrate and vertebrate nervous system.** *Curr Opin Neurobiol* 2019, **56**:185-193.
41. Telley L, Agirman G, Prados J, Amberg N, Fièvre S, Oberst P, Bartolini G, Vitali I, Cadilhac C, Hippenmeyer S *et al.*: **Temporal patterning of apical progenitors and their daughter neurons in the developing neocortex.** *Science (80-)* 2019, **364**.  
This study used single cell transcriptomics to analyze precisely timed progenitor cells and nascent postmitotic neurons. The authors report a core set of temporally regulated genes in progenitor cells. Genes involved in extracellular signalling seem to be more prominent in late neurogenesis, suggesting that these cells are progressively more sensitive to external signals.
42. Pearson CA, Moore DM, Tucker HO, Dekker JD, Hu H, Miquelajáuregui A, Novitsch BG: **Foxp1 regulates neural stem cell self-renewal and bias toward deep layer cortical fates.** *Cell Rep* 2020, **30**:1964-1981.
43. Albert M, Kalebic N, Florio M, Lakshmanaperumal N, Haffner C, Brandl H, Henry I, Huttner WB: **Epigenome profiling and editing of neocortical progenitor cells during development.** *EMBO J* 2017, **36**:2642-2658.
44. Albert M, Huttner WB: **Epigenetic and transcriptional pre-patterning—an emerging theme in cortical neurogenesis.** *Front Neurosci* 2018, **12**:1-9.
45. Yoon KJ, Vissers C, Ming Gli, Song H: **Epigenetics and epitranscriptomics in temporal patterning of cortical neural progenitor competence.** *J Cell Biol* 2018, **217**:1901-1914.
46. Shu P, Wu C, Ruan X, Liu W, Hou L, Fu H, Wang M, Liu C, Zeng Y, Chen P *et al.*: **Opposing gradients of microRNA expression temporally pattern layer formation in the developing neocortex.** *Dev Cell* 2019, **49**:764-785.e4.  
This study reports the temporally regulated expression of three microRNAs in the mouse cortex. Using a combination of gain and loss of function experiments, the authors find that these microRNAs play essential roles in instructing the temporal progression of cortical progenitor cells.
47. Zahr SK, Yang G, Kazan H, Borrett MJ, Yuzwa SA, Voronova A, Kaplan DR, Miller FD: **A translational repression complex in developing mammalian neural stem cells that regulates neuronal specification.** *Neuron* 2018, **97**:520-537.e6.  
This study reports that cortical progenitor cells contain mRNAs coding for factors implicated in the specification of different neuronal identities. Selective translational repression of some of these transcripts ensures the temporally organized expression of these genes and the appropriate specification of neuronal fates.
48. Vitali I, Fievre S, Telley L, Oberst P, Bariselli S, Frangeul L, Baumann N, McMahon JJ, Klingler E, Bocchi R *et al.*: **Progenitor hyperpolarization regulates the sequential generation of neuronal subtypes in the developing neocortex.** *Cell* 2018, **174**:1264-1276.  
This study proposes that the progressive hyperpolarization of cortical progenitor cells regulates their temporal progression. Hyperpolarization seems particularly critical for the inhibition of extrinsic Wnt signaling, which regulates progenitor progression through the different competence windows.

49. Franco SJ, Gil-Sanz C, Martínez-Garay I, Espinosa A, Harkins-Perry SR, Ramos C, Muller U: **Fate-restricted neural progenitors in the mammalian cerebral cortex.** *Science* (80-) 2012, **337**:746-749.
50. García-Moreno F, Molnár Z: **Subset of early radial glial progenitors that contribute to the development of callosal neurons is absent from avian brain.** *Proc Natl Acad Sci U S A* 2015, **112**:E5058-E5067.
51. Ellender TJ, Avery SV, Mahfooz K, Scaber J, von Klemperer A, Nixon SL, Buchan MJ, van Rheede JJ, Gatti A, Waites C *et al.*: **Embryonic progenitor pools generate diversity in fine-scale excitatory cortical subnetworks.** *Nat Commun* 2019, **10**.
52. Llorca A, Ciceri G, Beattie R, Wong FK, Diana G, Serafeimidou-Pouliou E, Fernández-Otero M, Streicher C, Arnold SJ, Meyer M *et al.*: **A stochastic framework of neurogenesis underlies the assembly of neocortical cytoarchitecture.** *eLife* 2019, **8**:1-27.
- This study reports that the neuronal output of cortical radial glial cells in the mouse cortex is extremely heterogeneous. Such diversity of outcomes supports a stochastic model of cortical neurogenesis. In this model, multipotent progenitor cells take a number of 'decisions' as they progress through a common sequence of competence states, which ultimately determines the composition of their progenies.
53. Gomes FLAF, Zhang G, Carbonell F, Correa JA, Harris WA, Simons BD, Cayouette M: **Reconstruction of rat retinal progenitor cell lineages in vitro reveals a surprising degree of stochasticity in cell fate decisions.** *Development* 2010, **138**:227-235.
54. He J, Zhang G, Almeida AD, Cayouette M, Simons BD, Harris WA: **How variable clones build an invariant retina.** *Neuron* 2012, **75**:786-798.
55. Johnston RJ Jr, Desplan C: **Stochastic mechanisms of cell fate specification that yield random or robust outcomes.** *Annu Rev Cell Dev Biol* 2010, **26**:689-719.
56. Desai AR, McConnell SK: **Progressive restriction in fate potential by neural progenitors during cerebral cortical development.** *Development* 2000, **127**:2863-2872.
57. Oberst P, Fièvre S, Baumann N, Concetti C, Bartolini G, Jabaudon D: **Temporal plasticity of apical progenitors in the developing mouse neocortex.** *Nature* 2019, **573**:370-374.
- This study reports that heterochronic transplantation of radial glial cells in the mouse cortex is able to reprogram their neurogenic fate. These observations challenge previous observations obtained in the ferret cortex and indicates that apical progenitor cells are plastic to return to previous competence windows after progressing to later ones.
58. Reillo I, de Juan Romero C, Cárdenas A, Clascá F, Martínez-Martínez MÁ, Borrell V: **A complex code of extrinsic influences on cortical progenitor cells of higher mammals.** *Cereb Cortex* 2017, **27**:4586-4606.
59. Magrinelli E, Wagener RJ, Jabaudon D: **Simultaneous production of diverse neuronal subtypes during early corticogenesis.** *bioRxiv* 2018 <http://dx.doi.org/10.1101/369678>.
- This is a preprint describing experiments that suggests that multiple neuronal fates are simultaneously specified during early neurogenesis. Cells of different laminar position, marker expression and projection patterns are produced in coetaneous mitosis of cortical radial glial cells.
60. Okamoto M, Miyata T, Konno D, Ueda HR, Kasukawa T, Hashimoto M, Matsuzaki F, Kawaguchi A: **Cell-cycle-independent transitions in temporal identity of mammalian neural progenitor cells.** *Nat Commun* 2016, **7**.
- This study pioneered the identification of temporal changes in gene expression in cortical progenitor cells. The authors also reveal that cell cycle arrest does not prevent temporal progression in progenitor cells, which indicates that such progression is independent of mitotic activity. Temporal changes in gene expression partially rely on extrinsic signaling, since they are compromised when progenitor cells are isolated in culture.
61. Belliveau MJ, Cepko CL: **Extrinsic and intrinsic factors control the genesis of amacrine and cone cells in the rat retina.** *Development* 1999, **126**:555-566.
62. Nelson BR, Hodge RD, Bedogni F, Hevner RF: **Dynamic interactions between intermediate neurogenic progenitors and radial glia in embryonic mouse neocortex: potential role in Dll1-notch signaling.** *J Neurosci* 2013, **33**:9122-9139.
63. Seuntjens E, Nityanandam A, Miquelajauregui A, Debruyjn J, Stryjewska A, Goebbels S, Nave K-A, Huylebroeck D, Tarabykin V: **Sip1 regulates sequential fate decisions by feedback signaling from postmitotic neurons to progenitors.** *Nat Neurosci* 2009, **12**:1373-1380.
64. Zhang Y, Liu G, Guo T, Liang XG, Du H, Yang L, Bhaduri A, Li X, Xu Z, Zhang Z *et al.*: **Cortical neural stem cell lineage progression is regulated by extrinsic signaling molecule sonic hedgehog.** *Cell Rep* 2020, **30**:4490-4504.e4.
65. Dehay C, Kennedy H: **Cell-cycle control and cortical development.** *Nat Rev Neurosci* 2007, **8**:438-450.
66. Griveau A, Borello U, Causeret F, Tissir F, Boggetto N, Karaz S, Pierani A: **A novel role for Dbx1-derived Cajal-Retzius cells in early regionalization of the cerebral cortical neuroepithelium.** *PLoS Biol* 2010, **8**.
67. Miao N, Bian S, Lee T, Mubarak T, Huang S, Wen Z, Hussain G, Sun T: **Opposite roles of Wnt7a and Sfrp1 in modulating proper development of neural progenitors in the mouse cerebral cortex.** *Front Mol Neurosci* 2018, **11**.
68. Borrell V, Cárdenas A, Ciceri G, Galcerán J, Flames N, Pla R, Nóbrega-Pereira S, García-Frigola C, Peregrín S, Zhao Z *et al.*: **Slit/Robo signaling modulates the proliferation of central nervous system progenitors.** *Neuron* 2012, **76**:338-352.
69. Lehtinen MK, Walsh CA: **Neurogenesis at the brain-cerebrospinal fluid interface.** *Annu Rev Cell Dev Biol* 2011, **27**:653-679.
70. Lamus F, Martín C, Carnicero E, Moro JA, Fernández JMF, Mano A, Gato, Alonso MI: **FGF2/EGF contributes to brain neuroepithelial precursor proliferation and neurogenesis in rat embryos: the involvement of embryonic cerebrospinal fluid.** *Dev Dyn* 2020, **249**:141-153.
71. Lukaszewicz A, Savatier P, Cortay V, Kennedy H, Dehay C: **Contrasting effects of basic fibroblast growth factor and neurotrophin 3 on cell cycle kinetics of mouse cortical stem cells.** *J Neurosci* 2002, **22**:6610-6622.
72. Lehtinen MK, Zappaterra MW, Chen X, Yang YJ, Hill AD, Lun M, Maynard T, Gonzalez D, Kim S, Ye P *et al.*: **The cerebrospinal fluid provides a proliferative niche for neural progenitor cells.** *Neuron* 2011, **69**:893-905.
73. Mairet-Coello G, Tury A, DiCicco-Bloom E: **Insulin-like growth factor-1 promotes G1/S cell cycle progression through bidirectional regulation of cyclins and cyclin-dependent kinase inhibitors via the phosphatidylinositol 3-kinase/Akt pathway in developing rat cerebral cortex.** *J Neurosci* 2009, **29**:775-788.
74. Rash BG, Ackman JB, Rakic P: **Bidirectional radial Ca<sup>2+</sup> activity regulates neurogenesis and migration during early cortical column formation.** *Sci Adv* 2016, **2**:e1501733.
75. Weissman TA, Riquelme PA, Ivic L, Flint AC, Kriegstein AR: **Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex.** *Neuron* 2004, **43**:647-661.
76. LoTurco JJ, Owens DF, Heath MJS, Davis MBE, Kriegstein AR: **GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis.** *Neuron* 1995, **15**:1287-1298.
77. Wang DD, Kriegstein AR: **Defining the role of GABA in cortical development.** *J Physiol* 2009, **587**:1873-1879.
78. Cárdenas A, Villalba A, de Juan Romero C, Picó E, Kyrrousi C, Tzika AC, Tessier-Lavigne M, Ma L, Drukker M, Cappello S *et al.*: **Evolution of cortical neurogenesis in amniotes controlled by Robo signaling levels.** *Cell* 2018, **174**:590-606.e21.
- This study investigates Robo signaling as a mechanism that regulates the balance between direct and indirect neurogenesis in the developing cortex. The authors describe that the progressive increase in indirect neurogenesis observed during amniote evolution is linked with an attenuation of Robo signaling.
79. Tomita H, Cornejo F, Aranda-Pino B, Woodard CL, Rioseco CC, Neel BG, Alvarez AR, Kaplan DR, Miller FD, Canciano GI: **The protein tyrosine phosphatase receptor delta regulates developmental neurogenesis.** *Cell Rep* 2020, **30**:215-228.e5.

80. Haushalter C, Asselin L, Fraulob V, Dollé P, Rhinn M: **Retinoic acid controls early neurogenesis in the developing mouse cerebral cortex.** *Dev Biol* 2017, **430**:129-141.
81. Sawada K: **Follow-up study of subventricular zone progenitors with multiple rounds of cell division during sulcogyrogenesis in the ferret cerebral cortex.** *IBRO Rep* 2019, **7**:42-51.
82. Mihalas AB, Hevner RF: **Clonal analysis reveals laminar fate multipotency and daughter cell apoptosis of mouse cortical intermediate progenitors.** *Development* 2018, **145**:dev164335-7.
83. Vasistha NA, García-Moreno F, Arora S, Cheung AFP, Arnold SJ, Robertson EJ, Molnár Z: **Cortical and clonal contribution of Tbr2 expressing progenitors in the developing mouse brain.** *Cereb Cortex* 2015, **25**:3290-3302.
84. Florio M, Huttner WB: **Neural progenitors, neurogenesis and the evolution of the neocortex.** *Development* 2014, **141**:2182-2194.
85. Fiddes IT, Lodewijk GA, Mooring M, Bosworth CM, Ewing AD, Mantalas GL, Novak AM, van den Bout A, Bishara A, Rosenkrantz JL *et al.*: **Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis.** *Cell* 2018, **173**:1356-1369.e22.
86. Suzuki IK, Gacquer D, Van Heurck R, Kumar D, Wojno M, Bilheu A, Herpoel A, Lambert N, Cheron J, Polleux F *et al.*: **Human-specific NOTCH2NL genes expand cortical neurogenesis through delta/notch regulation.** *Cell* 2018, **173**:1370-1384.
87. Florio M, Heide M, Pinson A, Brandl H, Albert M, Winkler S, Wimberger P, Huttner WB, Hiller M: **Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex.** *eLife* 2018, **2018**:7.
88. Kalebic N, Gilardi C, Stepien B, Wilsch-Bräuninger M, Long KR, Namba T, Florio M, Langen B, Lombardot B, Shevchenko A *et al.*: **Neocortical expansion due to increased proliferation of basal progenitors is linked to changes in their morphology.** *Cell Stem Cell* 2019, **24**:535-550.e9.

This study reports that pro-proliferative integrin-based signaling accounts for the increased proliferative capacity of intermediate progenitor cells in the cerebral cortex of gyrencephalic species. Intermediate progenitors with long, extensive processes such as those observed in humans, are more likely to receive integrin signaling and thus undergo multiple rounds of division.

89. Penisson M, Ladewig J, Belvindrah R, Francis F: **Genes and mechanisms involved in the generation and amplification of basal radial glial cells.** *Front Cell Neurosci* 2019, **13**.