Deciphering inhibitory neuron development: the paths to diversity

Rachel C. Bandler[1,2] and Christian Mayer[2*]

[1]Yale University, Department of Psychiatry, New Haven, CT 06510, USA
[2]Max Planck Institute for Biological Intelligence, Martinsried, 82152, Germany
[*]christian.mayer@bi.mpg.de

Submitted: December 28th, 2022

Abstract

The regulation of fate decisions in progenitor cells lays the foundation for the generation of neuronal diversity and the formation of specialized circuits with remarkable processing capacity. Since the discovery more than 20 years ago that inhibitory (GABAergic) neurons originate from progenitors in the ventral part of the embryonic brain, numerous details about their development and function have been unveiled. GABAergic neurons are an extremely heterogeneous group, comprising many specialized subtypes of local interneurons and long-range projection neurons. Clearly distinguishable types emerge during postmitotic maturation, at a time when precursors migrate, morphologically mature, and establish synaptic connections. Yet, differentiation begins at an earlier stage within their progenitor domains, where a combination of birthdate and place of origin are key drivers. This review explains how new insights from single-cell sequencing inform our current understanding of how GABAergic neuron diversity emerges.

Introduction

Brain computation relies on dynamic interactions between excitatory and inhibitory neuronal circuits (1). Inhibitory neurons, which produce the neurotransmitter Gamma-aminobutyric acid (GABA), are highly diverse and can be identified by morphological features, subcellular targets, neurochemical markers, firing patterns, and gene expression profiles (2). In the visual cortex of mice alone, more than two dozen different types have been identified based on a combination of morphological, electrophysiological, and transcriptomic features (3). Inhibitory neurons can act locally, or they can extend long-range axons to remote cortical and subcortical areas (4). In the cerebral cortex, a majority of inhibitory neurons are interneurons that are an integral part of the cortical circuit, as they reciprocally connect to other cortical neurons. This local inhibition orchestrates both spontaneous and sensory-driven activity in the cerebral cortex(1), and for example allows neurons to synchronize their firing, giving rise to rhythmic oscillations of activity (5). GABAergic projection neurons primarily populate subcortical regions such as the striatum, globus pallidus, and the amygdalar complex, and they often work as an integrating hub. Spiny projection neurons of the striatum, for example, receive glutamatergic inputs from different cortical and thalamic areas, and send GABAergic projections to neighboring basal ganglia nuclei (6). These projections are thought to contribute to motivated behavior, reward learning and decision-making (7; 8).

GABAergic neurons are developmentally derived from proliferative zones in the ventral telencephalon: the medial ganglionic eminence (MGE), the caudal ganglionic eminence (CGE), the lateral ganglionic eminence (LGE) and the preoptic area (9; 10; 11) (Figure 1). Because of the large number of different cell types they each produce, the ganglionic eminences (GEs) represent a fascinating study object for how cellular diversity emerges. For example, the MGE and CGE produce many distinct types of interneurons in the cortex, striatum, and hippocampus (12; 13; 14).

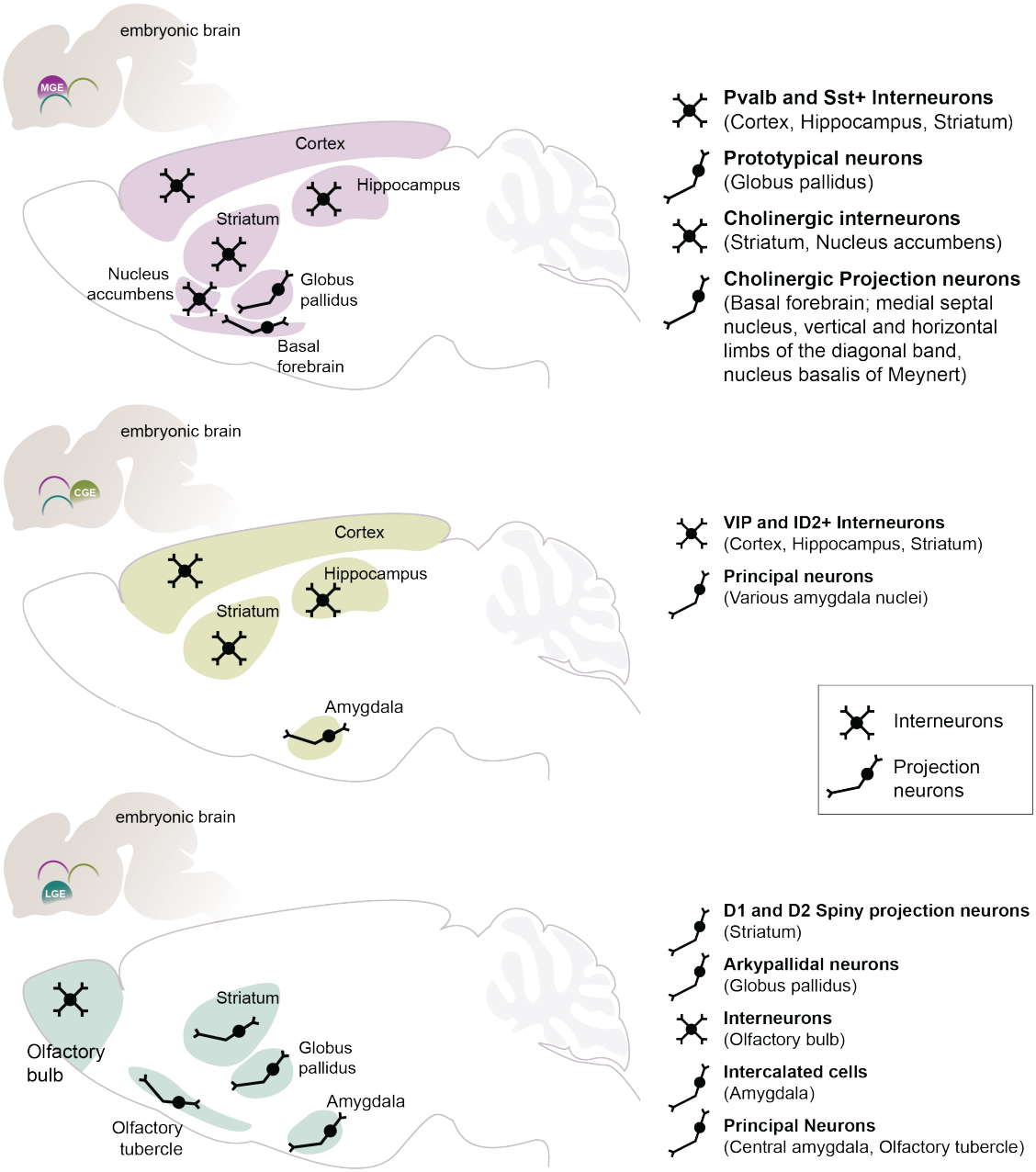

Figure 1: Origins of inhibitory cell types. Inhibitory projection neurons and interneurons are born in the ganglionic eminences. Schematic demonstrating the cell types that the medial, caudal and lateral ganglionic eminences (MGE, CGE and LGE, respectively) produce and the brain structures they occupy.

In addition, the MGE generates prototypic neurons of the globus pallidus (15), basal forebrain cholinergic neurons (16), and the CGE contributes to numerous amygdala nuclei (17). The LGE generates direct and indirect spiny projection neurons of the striatum (18), arkypallidal neurons of the globus pallidus(15), olfactory bulb interneurons(18), as well as neurons of the olfactory tubercle and amygdala (19) (Figure 1). Although most neurons that originate from the GEs are GABAergic, the GEs also generate cholinergic neurons as well as glia (20; 21; 22). Intensive efforts have been made to link properties of progenitors within the GEs to cell fate of their progeny. These include mode of cell-division (23; 24), time of cell-cycle exit (25; 26; 24)), cell-cycle length (27; 28; 29), progenitor heterogeneity (30), spatial subdomains and transcription factors that transduce patterning signals (31; 32; 33; 13; 34; 9; 35; 20; 36; 37). All these factors seem to act concurrently to produce neuronal heterogeneity. This review focuses on recent work that has employed single-cell omic methods to shed new light on the sequence of events that lead to the emergence of GABAergic diversity.

Developmental diversification of cortical interneurons

Cell type heterogeneity arises through a series of cell proliferation and differentiation events. An early mitotic phase generates a neuroproliferative layer surrounding the ventricles of the neural tube. This layer contains the so called radial-glia progenitors and intermediate progenitors that undergo their final rounds of cell-division, which generates postmitotic neurons, astrocytes, and oligodendrocytes (38; 39). Postmitotic precursors migrate to their final settling positions (40; 41), where they grow specific morphologies (42) and integrate into circuits. Single-cell RNA sequencing (scRNA-seq), a technique that allows the study of quantitative relationships between cell types, has been used to explore when during development the identities of GABAergic types emerge. Some of the gene expression signatures of differentiated cell types have already been found in neurons

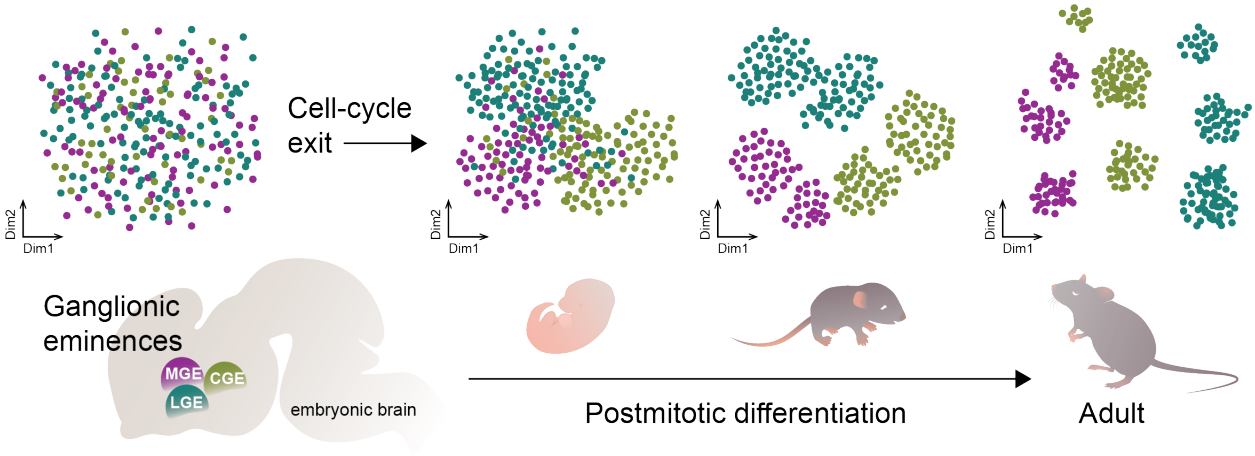

Figure 2: Developmental diversification of inhibitory neurons. Schematic of a two-dimensional representation of cell type heterogeneity in single-cell gene expression experiments. Characterization of gene expression in single cells allows quantification of cell type heterogeneity at different time points. Cells are colored by site of origin (MGE purple, CGE green, LGE blue). Different cell types become increasingly distinct from each other during development; as a result, clearly distinguishable clusters emerge in single-cell transcription experiments. Clusters that unambiguously group cells according to a cell's site of origin do not form until shortly before birth.

that have just exited the cell cycle in the scRNA-seq studies. For instance, all major classes of cortical interneurons were distinguishable in postmitotic neurons of scRNA-seq datasets of the GE at embryonic day 13. Furthermore, signatures of GABAergic projection neurons, and cholinergic neurons that mature to form pallidal structures, were also distinguishable (43; 44). However, refinement of clusters within theses broad classes unfolded over a lengthy period of time (Fig.2) and temporally coincided with developmental processes such as migration (45), morphological maturation (42), synaptogenesis (46) and the emergence of sensory-driven network activity (47). Surprisingly, using highly variable genes for feature selection, as is commonly used in scRNA-seq analysis, did not separate proliferating cells in a way that could be linked to adult cell types. And similarly, diffusion map analysis of E13 MGE scATAC–seq datasets showed that interneuron type-specific opening of chromatin at distal elements was first seen when cells exited the cell cycle (48). Only through the use of a curated approach, which combined enhancer-based cell labeling and

transcription factor-anchored scRNA-seq clustering, was it possible to increase the resolution of regional and developmental populations in mitotic cells to a level where spatially defined subtypes of MGE-derived GABAergic projection neurons and interneurons could be distinguished (49).

Trajectory estimation in the GEs

Cluster analysis methods aim to assess heterogeneity in a tissue by categorizing cells into groups. Embryonic brain tissue, however, typically consists of a continuum of cell states along maturation and differentiation axes, rather than discrete states. Many analysis tools have been developed that can provide high-resolution descriptions of cell trajectories as they transition between states (50). These tools have been used to explore scRNA-seq (43; 51; 52), single-nucleus (53) and single-cell ATAC-seq datasets of the GEs, both in mouse (48) and human (54) (Figure 3). In the GEs, the progression along pseudotime largely recapitulates known maturation markers, from Nestin and Ccnd2 expressing cycling progenitors to Dcx, Gad1 and Dlx6 expressing postmitotic neurons (43; 48; 51; 52). Consistent with cluster-based approaches, fate bifurcations along the maturation trajectory occurred when cells became postmitotic (Figure 3). These early bifurcations, or ”precursor states”, already exhibited a clear transcriptomic signature linking them to adult cell types. When precursor states from each GE were compared to one another, they displayed similar gene expression profiles (43) (Figure 3). This was very surprising because in the adult brain, the MGE, CGE and LGE produce distinct non-overlapping cell types. Why then would the GEs utilize common precursor states along their maturation trajectories? Each GE produces both locally projecting interneurons as well as long-range projection neurons. The common precursor states present in the GEs might initially act as a conserved regulatory program for generating these very different neuron classes. Only later in development, once precursors have embarked on a path towards differentiated neurons, do eminence-specific factors become a major source of heterogeneity (43).

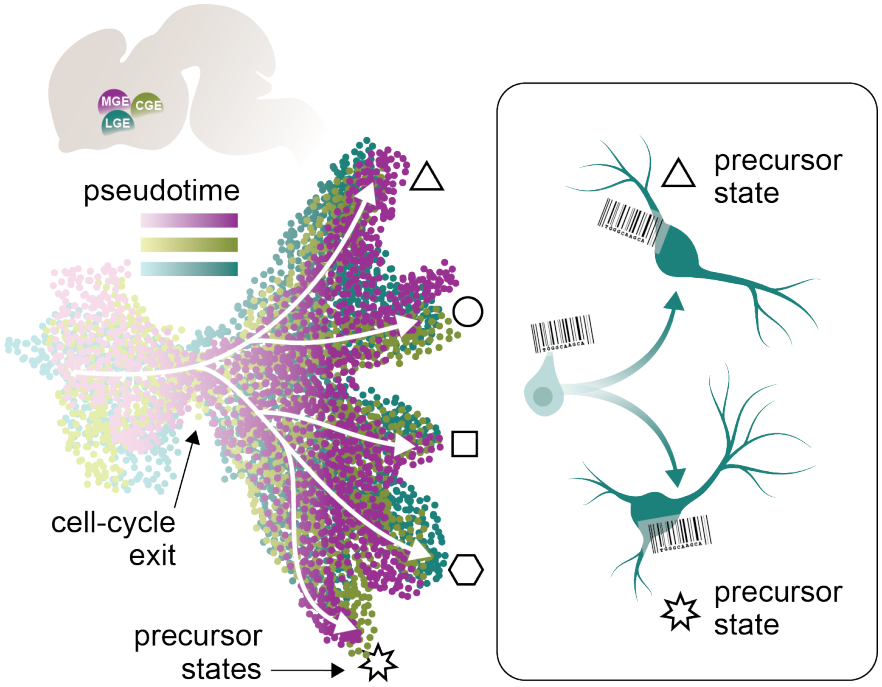

Figure 3: A trajectory describing the transition between cell states. Schematic of developmental trajectories of single-cell transcriptomes from the GEs. Fate bifurcations occur when progenitors exit the cell cycle. Different shapes (triangle, circle, square, hexagon, star) denote different precursor states (or branches), which initially show similarities between the GEs. Lineage barcoding methods have revealed that a single progenitor can produce daughter cells occupying different precursor states.

While trajectory estimation offers a population-level view of differentiation, it does not directly reveal relationships between individual cells. Do the progeny of a single progenitor enter the same precursor state, or disperse across multiple states? A way to link a mitotic progenitor and its progeny is with lineage tracing, by labelling an individual progenitor at an early time point and tracking the cell-states their clonal progeny differentiated into at a later time point. A recent study using synthetic oligonucleotides to tag progenitors and their descendants in the GEs found that, in about one-third of cases, clones disperse into different precursor states when they leave the cell-cycle. For example, a single LGE progenitor produced olfactory bulb interneurons, striatal spiny projection neurons, and intercalated cells of the amygdala. Thus, at least a subset of GE progenitors are multipotent, generating transcriptomically and anatomically disparate cell types (51). Whether the sequential production of different types involves stochastic events that occur during cell-cycle exit, or follows a stereotypic sequence, remains unknown.

Comparisons between species

The major cell classes of inhibitory neurons classified in mice have been identified in reptiles (55), nonhuman primates (56) and humans (57). Recently, however, a number of differences have come to light. For example, several primate-specific cell types have been discovered, such as the ”rosehip” interneurons in the cortex (Boldog et al 2018), a TAC3-expressing population of interneurons in the striatum (58), and TH-expressing neurons of the striatum laureatum (56). Other features, like the laminar positioning of cell types, the proportions of cell types, gene expression profiles (58), and even lineage relationships (59) have been found to be different in primates. In development, many gene regulatory networks and transcription factors are the same in mouse and human inhibitory neurons (60; 54; 61; 62; 63). As in mice, the fates of inhibitory neurons, such as GABAergic interneurons and different projection neurons, are established already in fetal human ganglionic

eminences (54; 62). In the future, a more detailed comparison of gene regulatory relationships between mice and primates should provide insight into previously unknown regulatory processes driving the diversification of neurons. This has great potential to provide valuable insights into the development and function of these neurons, as well as their role in neurological disorders and diseases.

Summary/Conclusions

In this review, we explore the processes underlying inhibitory neuron diversification, with a focus on recent studies employing single-cell omic methods. Different GABAergic cell types arise from regional differences in the specification of GE progenitors, which are initially established by morphogenic molecules such as retinoic acid (64), fibroblast growth factors and sonic hedgehog (65; 66; 67; 68). While single cell omic techniques have offered valuable insights into cell maturation and differentiation, they have not explained the underlying mechanisms by which spatial and temporal signals influence cell fate decisions. Transcription factor activity can be highly context-dependent. TALE transcription factors, for example, which are expressed in the ganglionic eminences, have been shown to act as broad activators of homeobox genes (69) and to interact with other transcription factors such as PBX, HOX, TBX, and Pax6 to promote differentiation in limbs, heart, lens, hindbrain, and olfactory bulb development (70; 69; 71; 72; 73). It is possible that such factors interact with spatial cues to selectively activate enhancers in different parts of the ganglionic eminences. To better understand the role of spatial selective enhancer activation in the early determination of GABAergic identities, additional methods such as reporter assays may provide a more detailed and mechanistic understanding of how early spatial signals shape cell fate decisions. Moreover, methods that map protein-DNA interactions, such as CUT&RUN, could be used to study where transcription factors bind within select groups of GE progenitors, and how differential access to transcriptional regulatory

142 elements is controlled during development.

143 Single-cell omics experiments have shown that the vast majority of cell type-specific heterogeneity
144 in gene expression occurs during a prolonged period of postmitotic development, toward the end of
145 embryogenesis and after birth (43; 44; 74). This coincides with the developmental stage at which
146 morphological maturation, synaptogenesis, and the specification of electrophysiological properties
147 occur. This raises the important question as to what extent the manifestation of heterogeneity follows
148 a prescribed unfolding of programs initiated in embryonic progenitor zones, and what role, in
149 contrast, environmental interactions play. Mounting experimental evidence shows that environmental
150 stimuli play an important role. For example, Lim et al. (2018) (45) showed that environmental cues
151 can influence the migration and differentiation of neural stem cells during development. Similarly,
152 De-Marco-Garcia et al. (2011) (42) found that electrical inputs can modulate gene expression in
153 developing neurons. However, many questions remain about the relative importance of intrinsic
154 and extrinsic processes in shaping gene expression patterns, and the mechanisms through which
155 these processes interact. It is not yet clear whether certain subtypes are more influenced by extrinsic
156 signals than others, or how extrinsic signals interact with evolving gene regulatory networks. Further
157 experimentation is needed to fully understand these complex processes and how they contribute to
158 the development of cell-types. Single-cell omics, with their quantitative nature, provide a unique
159 opportunity to explore the effect of environmental influences on the development of interneurons.
160 As major inhibitory neuron types can already be identified within the GEs before migration or
161 synaptic wiring, the initial formation of these cell types is likely a cell intrinsic process. Using
162 genetic approaches to manipulate environmental factors (e.g. early brain network activity or sensory
163 inputs) in a cell type-specific manner and analyzing the effect with single-cell omics would allow for
164 the precise determination of how, when, and to what extent different subtypes of GABAergic neurons
165 require environmental influences for their maturation. Such experiments may provide insights into

the complex process of interneuron differentiation and how it is shaped by environmental factors during development.

We have summarized the processes that lay the foundation for cell type heterogeneity proliferating cells. These processes range from spatial gradients and temporal signals to clonal dispersion of individual progenitors. Together, these processes could form a combinatorial framework that facilitates the emergence of neuronal diversity. It is not clear whether these processes work together, like the cogs of a clock, or whether they run in parallel and largely independently of each other. A hint is provided by the clonal dispersion into precursor states upon the cell-cycle exit: similar precursor states emerge in all GEs, differing only in a relatively small amount of domain-specific gene expression. Thus, a universal mechanism might generate precursor states in all GEs, to which a local identity is imbued by region-specific signals. This is an interesting possibility, but new studies are needed to test these hypotheses. Such studies should provide a comprehensive picture of how the different processes cooperate to ultimately establish cellular identity and connectivity in the adult brain.

Acknowledgements

We thank members of the Mayer laboratory for feedback and discussion and Julia Kuhl (somedonkey.com) for illustrations. This work was supported by the Max-Planck Society, the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation program (ERC-2018-STG, grant agreement no. 803984)

Short title

Inhibitory neuron development: the paths to diversity

References

[1] J. S. Isaacson and M. Scanziani, "How inhibition shapes cortical activity," *Neuron*, vol. 72, no. 2, pp. 231–43, Oct 2011.

[2] A. Kepecs and G. Fishell, "Interneuron cell types are fit to function," *Nature*, vol. 505, no. 7483, pp. 318–26, Jan 2014.

[3] N. W. Gouwens, S. A. Sorensen, F. Baftizadeh, A. Budzillo, B. R. Lee, T. Jarsky, L. Alfiler, K. Baker, E. Barkan, K. Berry, D. Bertagnolli, K. Bickley, J. Bomben, T. Braun, *et al.*, "Integrated morphoelectric and transcriptomic classification of cortical gabaergic cells," *Cell*, vol. 183, no. 4, pp. 935–953.e19, 11 2020.

[4] A. Caputi, S. Melzer, M. Michael, and H. Monyer, "The long and short of gabaergic neurons," *Curr Opin Neurobiol*, vol. 23, no. 2, pp. 179–86, Apr 2013.

[5] S. Royer, B. V. Zemelman, A. Losonczy, J. Kim, F. Chance, J. C. Magee, and G. Buzsáki, "Control of timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition," *Nat Neurosci*, vol. 15, no. 5, pp. 769–75, Mar 2012.

[6] N. R. Wall, M. De La Parra, E. M. Callaway, and A. C. Kreitzer, "Differential innervation of direct- and indirect-pathway striatal projection neurons," *Neuron*, vol. 79, no. 2, pp. 347–60, Jul 2013.

[7] A. C. Bostan and P. L. Strick, "The basal ganglia and the cerebellum: nodes in an integrated network," *Nat Rev Neurosci*, vol. 19, no. 6, pp. 338–350, 06 2018.

[8] J. Cox and I. B. Witten, "Striatal circuits for reward learning and decision-making," *Nat Rev Neurosci*, vol. 20, no. 8, pp. 482–494, 08 2019.

[9] C. P. Wonders and S. A. Anderson, "The origin and specification of cortical interneurons," *Nat Rev Neurosci*, vol. 7, no. 9, pp. 687–96, Sep 2006.

[10] D. Gelman, A. Griveau, N. Dehorter, A. Teissier, C. Varela, R. Pla, A. Pierani, and O. Marín, "A wide diversity of cortical gabaergic interneurons derives from the embryonic preoptic area," *J Neurosci*, vol. 31, no. 46, pp. 16 570–80, Nov 2011.

[11] S. A. Anderson, O. Marín, C. Horn, K. Jennings, and J. L. Rubenstein, "Distinct cortical migrations from the medial and lateral ganglionic eminences," *Development*, vol. 128, no. 3, pp. 353–63, Feb 2001.

[12] S. J. B. Butt, M. Fuccillo, S. Nery, S. Noctor, A. Kriegstein, J. G. Corbin, and G. Fishell, "The temporal and spatial origins of cortical interneurons predict their physiological subtype," *Neuron*, vol. 48, no. 4, pp. 591–604, Nov 2005.

[13] S. Nery, G. Fishell, and J. G. Corbin, "The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations," *Nat Neurosci*, vol. 5, no. 12, pp. 1279–87, Dec 2002.

[14] G. Miyoshi, J. Hjerling-Leffler, T. Karayannis, V. H. Sousa, S. J. B. Butt, J. Battiste, J. E. Johnson, R. P. Machold, and G. Fishell, "Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons," *J Neurosci*, vol. 30, no. 5, pp. 1582–94, Feb 2010.

[15] P. D. Dodson, J. T. Larvin, J. M. Duffell, F. N. Garas, N. M. Doig, N. Kessaris, I. C. Duguid, R. Bogacz, S. J. B. Butt, and P. J. Magill, "Distinct developmental origins manifest in the specialized encoding of movement by adult neurons of the external globus pallidus," *Neuron*, vol. 86, no. 2, pp. 501–13, Apr 2015.

[16] K. C. Allaway and R. Machold, "Developmental specification of forebrain cholinergic neurons," *Dev Biol*, vol. 421, no. 1, pp. 1–7, Jan 2017.

[17] K. Tang, J. L. R. Rubenstein, S. Y. Tsai, and M.-J. Tsai, "Coup-tfii controls amygdala patterning by regulating neuropilin expression," *Development*, vol. 139, no. 9, pp. 1630–9, May 2012.

[18] K. Yun, S. Garel, S. Fischman, and J. L. R. Rubenstein, "Patterning of the lateral ganglionic eminence by the gsh1 and gsh2 homeobox genes regulates striatal and olfactory bulb histogenesis and the growth of axons through the basal ganglia," *J Comp Neurol*, vol. 461, no. 2, pp. 151–65, Jun 2003.

[19] H.-A. Ko, S.-Y. Chen, H.-Y. Chen, H.-J. Hao, and F.-C. Liu, "Cell type-selective expression of the zinc finger-containing gene nolz-1/zfp503 in the developing mouse striatum," *Neurosci Lett*, vol. 548, pp. 44–9, Aug 2013.

[20] A. Fragkouli, N. V. van Wijk, R. Lopes, N. Kessaris, and V. Pachnis, "Lim homeodomain transcription factor-dependent specification of bipotential mge progenitors into cholinergic and gabaergic striatal interneurons," *Development*, vol. 136, no. 22, pp. 3841–51, Nov 2009.

[21] N. Kessaris, M. Fogarty, P. Iannarelli, M. Grist, M. Wegner, and W. D. Richardson, "Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage," *Nat Neurosci*, vol. 9, no. 2, pp. 173–9, Feb 2006.

[22] M. A. Petryniak, G. B. Potter, D. H. Rowitch, and J. L. R. Rubenstein, "Dlx1 and dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain," *Neuron*, vol. 55, no. 3, pp. 417–33, Aug 2007.

[23] T. J. Petros, R. S. Bultje, M. E. Ross, G. Fishell, and S. A. Anderson, "Apical versus basal neurogenesis directs cortical interneuron subclass fate," *Cell Rep*, vol. 13, no. 6, pp. 1090–1095, Nov 2015.

[24] S. M. Kelly, R. Raudales, M. He, J. H. Lee, Y. Kim, L. G. Gibb, P. Wu, K. Matho, P. Osten, A. M. Graybiel, and Z. J. Huang, "Radial glial lineage progression and differential intermediate progenitor amplification underlie striatal compartments and circuit organization," *Neuron*, vol. 99, no. 2, pp. 345–361.e4, 07 2018.

[25] M. Inan, J. Welagen, and S. A. Anderson, "Spatial and temporal bias in the mitotic origins of somatostatin- and parvalbumin-expressing interneuron subgroups and the chandelier subtype in the medial ganglionic eminence," *Cereb Cortex*, vol. 22, no. 4, pp. 820–7, Apr 2012.

[26] G. Miyoshi and G. Fishell, "Gabaergic interneuron lineages selectively sort into specific cortical layers during early postnatal development," *Cereb Cortex*, vol. 21, no. 4, pp. 845–52, Apr 2011.

[27] S. B. Glickstein, H. Moore, B. Slowinska, J. Racchumi, M. Suh, N. Chuhma, and M. E. Ross, "Selective cortical interneuron and gaba deficits in cyclin d2-null mice," *Development*, vol. 134, no. 22, pp. 4083–93, Nov 2007.

[28] S. Lodato, G. S. Tomassy, E. De Leonibus, Y. G. Uzcategui, G. Andolfi, M. Armentano, A. Touzot, J. M. Gaztelu, P. Arlotta, L. Menendez de la Prida, and M. Studer, "Loss of coup-tfi alters the balance between caudal ganglionic eminence- and medial ganglionic eminence-derived cortical interneurons and results in resistance to epilepsy," *J Neurosci*, vol. 31, no. 12, pp. 4650–62, Mar 2011.

[29] N. Zong, M. Wang, Y. Fu, D. Shen, and Y.-C. Yu, "Cell-cycle length of medial ganglionic eminence progenitors contributes to interneuron fate," *Protein Cell*, vol. 13, no. 2, pp. 141–147, 02 2022.

* Used a triple thymidine analog labeling method to label progenitors in the MGE, found that average cell cycle length gradually increases and progenitors with different cell cycle lengths are biased towards generating specific interneuron types. Progenitors undergoing longer cell cycles more likely to become Pvalb-expressing interneurons, vs. shorter cell cycles more likely Sst-expressing interneurons.

[30] F. van Heusden, A. Macey-Dare, J. Gordon, R. Krajeski, A. Sharott, and T. Ellender, "Diversity in striatal synaptic circuits arises from distinct embryonic progenitor pools in the ventral telencephalon," *Cell Rep*, vol. 35, no. 4, p. 109041, 04 2021.

* Used in utero labeling to explore how distinct embryonic neural progenitor pools in the LGE contribute to neuronal diversity and synaptic circuit connectivity in the mouse striatum. Found that spiny projection neurons derived from apical intermediate progenitors preferentially received excitatory inputs from medial prefrontal cortex, whereas SPNs derived from other progenitors preferentially receive inputs from visual cortex. Strength of local SPN inputs controlled by birthdate, not progenitor type.

[31] J. L. Rubenstein and L. Puelles, "Homeobox gene expression during development of the vertebrate brain," *Curr Top Dev Biol*, vol. 29, pp. 1–63, 1994.

[32] K. Shimamura, D. J. Hartigan, S. Martinez, L. Puelles, and J. L. Rubenstein, "Longitudinal organization of the anterior neural plate and neural tube," *Development*, vol. 121, no. 12, pp. 3923–33, Dec 1995.

[33] H. Wichterle, D. H. Turnbull, S. Nery, G. Fishell, and A. Alvarez-Buylla, "In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain," *Development*, vol. 128, no. 19, pp. 3759–71, Oct 2001.

[34] Q. Xu, I. Cobos, E. De La Cruz, J. L. Rubenstein, and S. A. Anderson, "Origins of cortical interneuron subtypes," *J Neurosci*, vol. 24, no. 11, pp. 2612–22, Mar 2004.

[35] N. Flames, R. Pla, D. M. Gelman, J. L. R. Rubenstein, L. Puelles, and O. Marín, "Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes," *J Neurosci*, vol. 27, no. 36, pp. 9682–95, Sep 2007.

[36] P. Flandin, S. Kimura, and J. L. R. Rubenstein, "The progenitor zone of the ventral medial ganglionic eminence requires nkx2-1 to generate most of the globus pallidus but few neocortical interneurons," *J Neurosci*, vol. 30, no. 8, pp. 2812–23, Feb 2010.

[37] M. Sandberg, P. Flandin, S. Silberberg, L. Su-Feher, J. D. Price, J. S. Hu, C. Kim, A. Visel, A. S. Nord, and J. L. R. Rubenstein, "Transcriptional networks controlled by nkx2-1 in the development of forebrain gabaergic neurons," *Neuron*, vol. 91, no. 6, pp. 1260–1275, Sep 2016.

[38] S. C. Noctor, A. C. Flint, T. A. Weissman, R. S. Dammerman, and A. R. Kriegstein, "Neurons derived from radial glial cells establish radial units in neocortex," *Nature*, vol. 409, no. 6821, pp. 714–20, Feb 2001.

[39] X. Zhang, C. V. Mennicke, G. Xiao, R. Beattie, M. A. Haider, S. Hippenmeyer, and H. T. Ghashghaei, "Clonal analysis of gliogenesis in the cerebral cortex reveals stochastic expansion of glia and cell autonomous responses to egfr dosage," *Cells*, vol. 9, no. 12, 12 2020.

[40] O. Marín and J. L. Rubenstein, "A long, remarkable journey: tangential migration in the telencephalon," *Nat Rev Neurosci*, vol. 2, no. 11, pp. 780–90, Nov 2001.

[41] J. G. Corbin, S. Nery, and G. Fishell, "Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain," *Nat Neurosci*, vol. 4 Suppl, pp. 1177–82, Nov 2001.

[42] N. V. De Marco García, T. Karayannis, and G. Fishell, "Neuronal activity is required for the development of specific cortical interneuron subtypes," *Nature*, vol. 472, no. 7343, pp. 351–5, Apr 2011.

[43] C. Mayer, C. Hafemeister, R. C. Bandler, R. Machold, R. Batista Brito, X. Jaglin, K. Allaway, A. Butler, G. Fishell, and R. Satija, "Developmental diversification of cortical inhibitory interneurons," *Nature*, vol. 555, no. 7697, pp. 457–462, 03 2018.

[44] D. Mi, Z. Li, L. Lim, M. Li, M. Moissidis, Y. Yang, T. Gao, T. X. Hu, T. Pratt, D. J. Price, N. Sestan, and O. Marín, "Early emergence of cortical interneuron diversity in the mouse embryo," *Science*, vol. 360, no. 6384, pp. 81–85, 04 2018.

[45] L. Lim, J. M. P. Pakan, M. M. Selten, A. Marques-Smith, A. Llorca, S. E. Bae, N. L. Rochefort, and O. Marín, "Optimization of interneuron function by direct coupling of cell migration and axonal targeting," *Nat Neurosci*, vol. 21, no. 7, pp. 920–931, 07 2018.

[46] J. De Felipe, P. Marco, A. Fairén, and E. G. Jones, "Inhibitory synaptogenesis in mouse somatosensory cortex," *Cereb Cortex*, vol. 7, no. 7, pp. 619–34, 1997.

[47] L. C. Katz and C. J. Shatz, "Synaptic activity and the construction of cortical circuits," *Science*, vol. 274, no. 5290, pp. 1133–8, Nov 1996.

[48] K. C. Allaway, M. I. Gabitto, O. Wapinski, G. Saldi, C.-Y. Wang, R. C. Bandler, S. J. Wu, R. Bonneau, and G. Fishell, "Genetic and epigenetic coordination of cortical interneuron development," *Nature*, vol. 597, no. 7878, pp. 693–697, 09 2021.

** Performed single-cell multi-omic (RNA and ATAC) sequencing on the MGE at embryonic day 13 (E13). Diffusion map analysis showed promoter accessibility corresponded with RNA expression of maturation genes, and interneuron-specific opening of chromatin at distal elements when cells exited the cell cycle. Also constructed cell type specific gene regulatory networks for Pvalb and Sst interneurons after settling in the cortex.

[49] L. Su-Feher, A. N. Rubin, S. N. Silberberg, R. Catta-Preta, K. J. Lim, A. R. Ypsilanti, I. Zdilar, C. S. McGinnis, G. L. McKinsey, T. E. Rubino, Jr, M. J. Hawrylycz, C. Thompson, Z. J. Gartner, L. Puelles, *et al.*, "Single cell enhancer activity distinguishes gabaergic and cholinergic lineages in embryonic mouse basal ganglia," *Proc Natl Acad Sci U S A*, vol. 119, no. 15, p. e2108760119, 04 2022.

** Combined scRNA-seq with enhancer based transgenic reporter mouse lines within the E11.5 GEs. This curated approach increase the resolution of regional and developmental populations in mitotic cells to a level where spatially defined subtypes of MGE-derived GABAergic projection neurons and interneurons could be distinguished. The paper highlights the specificity and complexity of developmental enhancer activity during regional and cell type specification.

[50] W. Saelens, R. Cannoodt, H. Todorov, and Y. Saeys, "A comparison of single-cell trajectory inference methods," *Nat Biotechnol*, vol. 37, no. 5, pp. 547–554, 05 2019.

[51] R. C. Bandler, I. Vitali, R. N. Delgado, M. C. Ho, E. Dvoretskova, J. S. Ibarra Molinas, P. W. Frazel, M. Mohammadkhani, R. Machold, S. Maedler, S. A. Liddelow, T. J. Nowakowski, G. Fishell, and C. Mayer, "Single-cell delineation of lineage and genetic identity in the mouse brain," *Nature*, vol. 601, no. 7893, pp. 404–409, 01 2022.

** Combined synthetic oligo barcodes and scRNA-seq to trace lineage relationships in the GE. Found that diverse types of GABAergic neurons are clonally related, and that GABAergic clones diverge upon cell-cycle exit within the ganglionic eminences.

[52] D. R. Lee, C. Rhodes, A. Mitra, Y. Zhang, D. Maric, R. K. Dale, and T. J. Petros, "Transcriptional heterogeneity of ventricular zone cells in the ganglionic eminences of the mouse forebrain," *Elife*, vol. 11, 02 2022.

* Utilized the Nes-dVenus mouse to enrich for ventricular zone mitotic GE progenitors, as well as to differentiate between apical progenitors and basal progenitors. ScRNA-seq revealed spatial transcriptional diversity throughout ventricular zone layers, however, did not directly link mitotic progenitors to mature inhibitory neuron types.

[53] C. T. Rhodes, J. J. Thompson, A. Mitra, D. Asokumar, D. R. Lee, D. J. Lee, Y. Zhang, E. Jason, R. K. Dale, P. P. Rocha, and T. J. Petros, "An epigenome atlas of neural progenitors within the embryonic mouse forebrain," *Nat Commun*, vol. 13, no. 1, p. 4196, 07 2022.

[54] Y. Yu, Z. Zeng, D. Xie, R. Chen, Y. Sha, S. Huang, W. Cai, W. Chen, W. Li, R. Ke, and T. Sun, "Interneuron origin and molecular diversity in the human fetal brain," *Nat Neurosci*, vol. 24, no. 12, pp. 1745–1756, 12 2021.

[55] M. A. Tosches, T. M. Yamawaki, R. K. Naumann, A. A. Jacobi, G. Tushev, and G. Laurent, "Evolution of pallium, hippocampus, and cortical cell types revealed by single-cell transcriptomics in reptiles," *Science*, vol. 360, no. 6391, pp. 881–888, 05 2018.

[56] M. T. Schmitz, K. Sandoval, C. P. Chen, M. A. Mostajo-Radji, W. W. Seeley, T. J. Nowakowski, C. J. Ye, M. F. Paredes, and A. A. Pollen, "The development and evolution of inhibitory neurons in primate cerebrum," *Nature*, vol. 603, no. 7903, pp. 871–877, 03 2022.

** Reconstruct gene expression trajectories specifying INs generated throughout the neurogenic period in macaques and mice. Found that the initial classes of INs generated prenatally are largely conserved among mammals. And that classes of olfactory bulb bound precursors are redirected to expanded primate white matter and striatum.

[57] R. D. Hodge, T. E. Bakken, J. A. Miller, K. A. Smith, E. R. Barkan, L. T. Graybuck, J. L. Close, B. Long, N. Johansen, O. Penn, Z. Yao, J. Eggermont, T. Höllt, B. P. Levi, *et al.*, "Conserved cell types with divergent features in human versus mouse cortex," *Nature*, vol. 573, no. 7772, pp. 61–68, 09 2019.

[58] F. M. Krienen, M. Goldman, Q. Zhang, R. C H Del Rosario, M. Florio, R. Machold, A. Saunders, K. Levandowski, H. Zaniewski, B. Schuman, C. Wu, A. Lutservitz, C. D. Mullally, N. Reed, *et al.*, "Innovations present in the primate interneuron repertoire," *Nature*, vol. 586, no. 7828, pp. 262–269, 10 2020.

** Used single-nucleus RNA sequencing to profile RNA expression in interneurons across homologous brain regions from three primates (human, macaque, and marmoset), a rodent (mouse) and a weasel (ferret). Discovered a LAMP5+ LHX6+ cell type present in mouse hippocampus to be widespread throughout the primate cortex. Also found a primate-specific population of TAC3-expressing striatal interneurons.

[59] R. N. Delgado, D. E. Allen, M. G. Keefe, W. R. Mancia Leon, R. S. Ziffra, E. E. Crouch, A. Alvarez-Buylla, and T. J. Nowakowski, "Individual human cortical progenitors can produce excitatory and inhibitory neurons," *Nature*, vol. 601, no. 7893, pp. 397–403, 01 2022.

** Developed a lentiviral lineage barcoding method called STICR (scRNA-seq-compatible tracer for identifying clonal relationships). The paper found clonally related GABAergic and glutamatergic neurons in primary human cell cultures from the cortical germinal zone using this method.

[60] L. Ma, Y. Du, Y. Hui, N. Li, B. Fan, X. Zhang, X. Li, W. Hong, Z. Wu, S. Zhang, S. Zhou, X. Xu, Z. Zhou, C. Jiang, *et al.*, "Developmental programming and lineage branching of early human telencephalon," *EMBO J*, vol. 40, no. 21, p. e107277, 11 2021.

[61] Z. Zhao, D. Zhang, F. Yang, M. Xu, S. Zhao, T. Pan, C. Liu, Y. Liu, Q. Wu, Q. Tu, P. Zhou, R. Li, J. Kang, L. Zhu, *et al.*, "Evolutionarily conservative and non-conservative regulatory networks during primate interneuron development revealed by single-cell rna and atac sequencing," *Cell Res*, vol. 32, no. 5, pp. 425–436, 05 2022.

[62] Y. Shi, M. Wang, D. Mi, T. Lu, B. Wang, H. Dong, S. Zhong, Y. Chen, L. Sun, X. Zhou, Q. Ma, Z. Liu, W. Wang, J. Zhang, *et al.*, "Mouse and human share conserved transcriptional programs for interneuron development," *Science*, vol. 374, no. 6573, p. eabj6641, Dec 2021.

** Used single-cell RNA sequencing to characterize the emergence of cell diversity in the human ganglionic eminences. Showed that GABAergic cells are specified within the human ganglionic eminences by transcriptional programs similar to those previously identified in rodents.

[63] V. D. Bocchi, P. Conforti, E. Vezzoli, D. Besusso, C. Cappadona, T. Lischetti, M. Galimberti, V. Ranzani, R. J. P. Bonnal, M. De Simone, G. Rossetti, X. He, K. Kamimoto, I. Espuny-Camacho, *et al.*, "The coding and long noncoding single-cell atlas of the developing human fetal striatum," *Science*, vol. 372, no. 6542, 05 2021.

[64] C. Chatzi, T. Brade, and G. Duester, "Retinoic acid functions as a key gabaergic differentiation signal in the basal ganglia," *PLoS Biol*, vol. 9, no. 4, p. e1000609, Apr 2011.

[65] E. E. Storm, S. Garel, U. Borello, J. M. Hebert, S. Martinez, S. K. McConnell, G. R. Martin, and J. L. R. Rubenstein, "Dose-dependent functions of fgf8 in regulating telencephalic

patterning centers," *Development*, vol. 133, no. 9, pp. 1831–44, May 2006.

[66] N. Molotkova, A. Molotkov, and G. Duester, "Role of retinoic acid during forebrain development begins late when raldh3 generates retinoic acid in the ventral subventricular zone," *Dev Biol*, vol. 303, no. 2, pp. 601–10, Mar 2007.

[67] U. Borello, I. Cobos, J. E. Long, J. R. McWhirter, C. Murre, and J. L. R. Rubenstein, "Fgf15 promotes neurogenesis and opposes fgf8 function during neocortical development," *Neural Dev*, vol. 3, p. 17, Jul 2008.

[68] B. Shohayeb, Z. Muzar, and H. M. Cooper, "Conservation of neural progenitor identity and the emergence of neocortical neuronal diversity," *Semin Cell Dev Biol*, vol. 118, pp. 4–13, Oct 2021.

[69] L. Bridoux, P. Zarrineh, J. Mallen, M. Phuycharoen, V. Latorre, F. Ladam, M. Losa, S. M. Baker, C. Sagerstrom, K. A. Mace, M. Rattray, and N. Bobola, "Hox paralogs selectively convert binding of ubiquitous transcription factors into tissue-specific patterns of enhancer activation," *PLoS Genet*, vol. 16, no. 12, p. e1009162, 12 2020.

[70] D. Schulte and D. Geerts, "Meis transcription factors in development and disease," *Development*, vol. 146, no. 16, 08 2019.

[71] I. Delgado, G. Giovinazzo, S. Temiño, Y. Gauthier, A. Balsalobre, J. Drouin, and M. Torres, "Control of mouse limb initiation and antero-posterior patterning by meis transcription factors," *Nat Commun*, vol. 12, no. 1, p. 3086, 05 2021.

[72] L. Selleri, V. Zappavigna, and E. Ferretti, "'building a perfect body': control of vertebrate organogenesis by pbx-dependent regulatory networks," *Genes Dev*, vol. 33, no. 5-6, pp. 258–275, 03 2019.

[73] Z. Agoston, P. Heine, M. S. Brill, B. M. Grebbin, A.-C. Hau, W. Kallenborn-Gerhardt, J. Schramm, M. Götz, and D. Schulte, "Meis2 is a pax6 co-factor in neurogenesis and dopaminergic periglomerular fate specification in the adult olfactory bulb," *Development*, vol. 141, no. 1, pp. 28–38, Jan 2014.

[74] E. Favuzzi, R. Deogracias, A. Marques-Smith, P. Maeso, J. Jezequel, D. Exposito-Alonso, M. Balia, T. Kroon, A. J. Hinojosa, E. F Maraver, and B. Rico, "Distinct molecular programs regulate synapse specificity in cortical inhibitory circuits," *Science*, vol. 363, no. 6425, pp. 413–417, Jan 2019.

Papers with special interest (*) or outstanding interest (**) for this review were selected and annotated.