Fate specification of embryonic neocortical neuronal progenitor cells and the generation of projection neuron diversity.

Biomedical Sciences Master Essay

Author: Koen Bokern, S1826158.
Date: 02-12-2017
Supervisor: Dr. J.T.M.L. Paridaen
Research group: Asymmetric Cell Division and Ageing, ERIBA (UMCG/RUG).

university of groningen

Abstract

The neocortex in the mammalian brain exhibits an incredible diversity of projection neurons, despite these cells being embryonically derived from a small number of neuronal progenitor cells in one germinal zone. Projection neuron diversity is observed in morphology, function, electrophysiological properties, axonal projections, molecular identity and genome-wide expression profiles. Complex molecular and cellular processes regulate cell fate specification of neocortical neuronal progenitor cells during mammalian embryonic development. Distinct types of neuronal progenitor cells are present during different phases of development, locate to different regions of the neocortical germinal zone and in specific cases generate progeny with different characteristics. In recent years the dynamics of progenitor competence have become a controversial topic and two scientific models can describe it: progressive restriction of fate potential, which was first explored in Drosophila, and fate-restricted lineages. Further understanding of neocortical neuronal fate specification is potentially useful for research on brain development and disease, and the design of brain repair therapies.

Table of contents

1. Introduction: Projection neuron diversity……..3
2. Embryonic development of the neocortical germinal zone and the neocortex……..4
3. Additional neuronal progenitor cells: short neural precursors and outer radial glial cells……..6
4. Models of neuronal progenitor cell competence I: progressive restriction of fate potential……8
5. Models of neuronal progenitor cell competence II: fate-restricted lineages……10
6. Neuronal progenitor cell heterogeneity and progenitor type fate……13
7. Conclusion……15
8. References……16

Glossary

AP	apical progenitor	**PN**	projection neuron
CNS	central nervous system	**RGC**	radial glial cell
E	mouse embryonic day	**scRNA-Seq**	single cell RNA sequencing
IPC	intermediate progenitor cell	**SNP**	short neural precursor
NEC	neuroepithelial cell	**SVZ**	subventricular zone
NPC	neuronal progenitor cell	**TF**	transcription factor
oRG	outer radial glia	**VZ**	ventricular zone
oSVZ	outer subventricular zone		

1. Introduction: Projection neuron diversity.

The brain is the most complex organ of the body. Multiple factors attribute to this feature: the brain's large number of neurons (one of the two broad classes of cells in the brain), the incredible interconnectivity of neurons, and the brain's ability to control complex behaviour. Another broad class of brain cells called glial cells support neurons and, among other cell types include oligodendrocytes and astrocytes. In conjunction with the spinal cord the brain is the central controller of the body of vertebrates and most invertebrates. The neocortex is the part of the brain that is committed to perform complex tasks related to sensory perception, motor control and higher-order cognitive functions. The neocortex forms intricate neural networks of interconnected neurons. Neurons are electrically excitable postmitotic cells that communicate signals along axonal cell extensions via rapid shifts of cell membrane potential. These action potentials crossover from axon ends to other neurons via neurotransmitters, released in the synapse between neurons, that bind receptors on the target neuron. Neurons are divided in two main classes: projection neurons (PNs) and interneurons, of which PNs are the most abundant since they make up roughly 70-80% of all neurons of the cerebral cortex (Markram et al., 2004). PNs are excitatory neurons that extend long-range axons to different areas of the central nervous system (CNS) and communicate via the neurotransmitter glutamate. PNs generally have a pyramidal morphology with many branched dendrites and one branched axon. PNs are generated by a relatively small number of neuronal progenitor cells (NPCs) that originate in one germinal zone in the dorsal telencephalon. Interneurons are inhibitory neurons that make local connections communicate via the neurotransmitters γ-aminobutyric acid (GABA) or glycine (Lodato et al., 2011). Interneurons arise from progenitors that originate from the medial and caudal ganglionic eminences of the ventral telencephalon (Anderson, 2002; de Carlos, López-Mascaraque, & Valverde, 1996; Tamamaki, Fujimori, & Takauji, 1997).The neocortex consists of six horizontal layers of PNs that are born in an inside-out order. The layers are numbered I to VI, of which layer I is the top layer. The lower layers VI and V, those in closer proximity to the ventricular surface, are made up of early born neurons (Jackson, Peduzzi, & Hickey, 1989; Marla B. Luskin & Shatz, 1985; Rakic, 1974). The upper-layers IV, III and II, nearer to the pial surface, consist of late born neurons. Neurons of the same layer generally share similar morphology, function, and patterns of both axonal projections and gene expression (Kwan, Sestan, & Anton, 2012).

Another factor contributing to the brain's complexity is the diversity of neurons beyond the main classes of projection neurons and interneurons. PNs are classically categorized by their laminar position, their hodological features, their morphology or their electrophysiological properties. Hodology, the study of axonal targeting, is used to classify PNs as associative, commissural or corticofugal PNs. Associative PNs target neurons of the same neocortical hemisphere. Commissural PNs target neurons of the contralateral hemisphere and include callosal PNs (CPNs) that cross the midline through the corpus callosum and PNs that extend axons through the anterior commissure. CPNs primarily locate to layer II and III. Corticofugal PNs target neurons outside the cortex and include subcerebral PNs in layer V and corticothalamic PNs in layer VI. Another class of PNs projects to multiple targets (Greig, Woodworth, Galazo, Padmanabhan, & Macklis, 2013). Molecular identity is also used to categorize PNs. Molecular markers label subsets of classical classes of PNs, which indicates further diversity.

Single-cell RNA sequencing (scRNA-seq) studies showed more evidence of molecular diversity of projection neurons. Zeisel et al. did scRNA-seq on the mouse somatosensory cortex (S1) and hippocampus. Using quantitative clustering they identified 47 molecularly distinct subclasses in the S1 and found seven layer-specific subclasses of S1 pyramidal cells. S1 layers II and III showed single clusters, layer V two clusters, and layer VI and VIb showed two specific single clusters and one common clusters with deep-layer markers (Zeisel et al., 2015). Tasic et al. did scRNA-seq on the mouse primary visual cortex and identified 49 transcriptomic types including 19 glutamergic types divided in six major classes L2/3, L4, L5a, L5b, L6a, L6b on basis of a layer-specific expression of marker genes and these six classes are further divided into subclasses (Tasic et al., 2016). Tasic et al. noted that it is unclear whether a transcriptomic type represents a distinct cell type, or a transient cell state. Lake et al. did single nucleus RNA-seq on six specific cortical regions of post-mortem human brain tissue. They identified 17 clusters, including eight clusters of projection neurons, and the clusters showed expression profiles alike those found by Zeisel et al. in mice. The clusters expressed known markers associated with laminar positioning. Interneurons also make up a diverse cell population (Johnson & Walsh, 2017; Lodato & Arlotta, 2015; Miyoshi et al., 2010).

This essay covers how complex molecular and cellular processes regulate the neuronal fate potential of NPCs during mammalian embryonic development for these fate specification mechanisms contribute to PN diversity. To get acquainted with neocortical neurogenesis and the various distinct NPCs the following sections will discuss the embryonic development of the neocortical germinal zone and the neocortex. Different types of NPCs and additional heterogeneity within NPC types will be discussed as well as their potential contribution to projection neuron diversity. The dynamics of progenitor competence have become a controversial topic in recent years. Two different scientific models of progenitor competence will be discussed: progressive restriction of fate potential and fate-restricted lineages**.** Molecular factors exist that control fate specification post mitotically, but they will not be focused on here (Greig et al., 2013). Further understanding of neuronal fate specification and embryonic neocortical development is potentially useful for research on diseases related to aberrant cortical function or development, and the design of therapies to repair the damaged brain, possibly via the direction of neuronal progenitors to a specific neuronal fate.

2. Embryonic development of the neocortical germinal zone and the neocortex.

Neuroepithelial cells

Studies in rodents established the general principles of embryonic neocortical development. All neocortical PNs derive from neural stem cells of the anterior neuroectoderm called neuroepithelial cells (NECs). NECs exhibit epithelial characteristics, such as apical-basal cell polarity, intercellular connections via tight and adherens junctions, and the expression of genes coding for epithelial markers including *Nestin* and *Sox2* (Franco & Müller, 2013; Götz & Huttner, 2005). Around mouse embryonic day (E) 8, the NEC population rapidly proliferates via symmetric division. During this period of expansion, the neural plate folds, invaginates and eventually closes by E9, thereby forming the anterior neural tube and the lateral ventricles. NECs line the lateral ventricular surface with their apical cell membranes facing the

lumen, whereas their basal cell membranes face the outer, or pial, surface. At the pial surface, NECs anchor to the basal lamina via integrins.

Radial glial cells

In mice, neurogenesis starts between E9 and E10, when NECs start to transform into a type of NPC called radial glial cells (RGCs), although small populations of post-mitotic cells are generated from NECs directly (Franco & Müller, 2013). During this transformation, the cells lose certain epithelial characteristics, including the anchorage via tight junctions. RGCs characteristically express the astroglial genes brain-lipid-binding protein (BLBP) and astrocyte-specific glutamate transporter (GLAST). The embryonic brain region in which layers of NECs and RGCs are located is called the ventricular zone (VZ). RGCs remain in contact with the ventricular and pial surface, which gives rise to their radial morphology (Franco & Müller, 2013). NECs divide symmetrically, retract and regrow their basal process contacting the pial surface, while RGCs undergo vertical cleavage and retain basal contact (Subramanian, Bershteyn, Paredes, & Kriegstein, 2017). Asymmetric division of RGCs leads to self-renewal (generation of another RGC) and generation of a post mitotic neuron (in 10-20% of divisions) or another type of progenitor cell called an intermediate progenitor cell (IPC) (Haubensak, Attardo, Denk, & Huttner, 2004; Noctor et al., 2004). The peak phase of neurogenesis occurs between E12 and E18 (Pinto & Götz, 2007). Immunostaining for the antigens RC2 (encoded by *Nestin*), GLAST and BLBP and expression profiling identified distinct RGC subsets (Hartfuss, Galli, Heins, & Götz, 2001; Park et al., 2009). During neurogenesis, almost all progenitors express RC2, but subsets exist that additionally express GLAST, BLBP or both. Another subset expressing GLAST and BLBP only exists outside the VZ near the end of neurogenesis. Of early occurring subsets only the RC2/GLAST/BLBP subset persists until late neurogenesis. The heterogenic expression of BLBP and GLAST in RGCs and its effects on neocortical development are further described in section 5.

Intermediate progenitor cells

90% of mice IPCs undergo symmetric division leading to the production of two neurons, while 10% of IPCs undergo up to three further symmetric divisions that produce IPCs before terminal division into neurons (Franco & Müller, 2013; Noctor et al., 2004). IPCs express the transcription factor (TF) TBR2 (encoded by *Eome*s) and downregulate paired box protein 6 (PAX6) in comparison to RGCs. Shortly after birth, IPCs lose their apical process contacting the ventricular surface (Miyata et al., 2004). IPCs migrate to a more basal position and obtain a multipolar morphology before further division. The region these migrated and dividing IPCs take up is called the subventricular zone (SVZ). IPCs go through a lengthened G1 phase of the cell cycle compared to RGCs in the dorsal VZ/SVZ. Proliferating IPCs experience a longer S-phase than RGCs and neurogenic IPCs, which may be related to their high amplification and therefore increased importance of DNA repair processes (Arai et al., 2011). Some early born IPCs complete their final mitosis during late neurogenesis to form upper-layer neurons (W A Tyler, Medalla, Guillamon-Vivancos, Luebke, & Haydar, 2015).

3. Additional neuronal progenitor cells: short neural precursors and outer radial glial cells.

Short neural precursors

A study characterizing cells of the mouse VZ revealed a second type of apical progenitors (APs) in addition to RGCs named short neural precursors (SNPs) (Gal et al., 2006). SNPs are morphologically, ultrastructurally and molecularly distinct from other progenitor cell types. SNPs have ventricular endfeet and a basal process that retracts during division. SNPs have a higher expression of the tubulin α-1 (Tα1) promoter and the differentiation marker βIII-tubulin (TUJ1) and do not express GLAST and BLBP like RGCs or TBR2 like IPCs. In contrast with RGCs, SNPs do not show activation of the Notch signalling pathway. (Gal et al., 2006; Stancik, Navarro-Quiroga, Sellke, & Haydar, 2010; William A. Tyler & Haydar, 2013). Stancik et al. showed that SNPs differ from RGCs in their cell cycle kinetics and the phenotype of their progeny (Stancik et al., 2010). SNPs divide once or twice to generate neurons directly, while RGCs divide multiple times to generate IPCs. *In vivo* genetic fate mapping (the establishment of lineage relationships via cell- or tissue-specific expression of a site-specific recombinase in combination with a reporter transgene) of E14.5 mice SNPs showed that SNPs generate neurons that locate to layer IV of the neocortex, while RGCs generate neurons for upper-layers II and III (Stancik et al., 2010; Woodworth, Girskis, & Walsh, 2017). SNPs usually generate postmitotic neurons directly without the production of intermediate progenitors. Stancik et al. deduced that a consequence of these differences between RGCs and SNPs is that SNPs generate neurons faster than RGCs, but the number of neurons SNPs generate is limited by the lack of amplification via IPCs. The lack of Notch pathway activation may explain why SNPs are less proliferative.

Outer radial glial cells

Hansen et al. studied the outer region of the human SVZ (oSVZ) and found that, in addition to IPCs, it harbours radial glia-like cells called outer radial glial cells (oRGs) (Hansen, Lui, Parker, & Kriegstein, 2010). Fietz et al. reported similar results in both humans and ferrets (Fietz et al., 2010). oRGs contact the pial surface via a long basal process that is asymmetrically inherited during division. Unlike RGCs, oRGs do not make ventricular contact. oRGs undergo both proliferative and neurogenic divisions, of which the latter occasionally occur indirectly via intermediate progenitors (Hansen et al., 2010). On the contrary, both Wang et al. and Betizeau et al. found that oRGs form neurons directly (Betizeau et al., 2013; Wang, Tsai, LaMonica, & Kriegstein, 2011). Hansen et al. showed that inhibition of Notch signalling in oRGs induces their differentiation to neurons, which suggests Notch signalling maintains oRG identity. Based on studies in macaque, ferret and rat, Martínez-Cerdeño et al. suggest the existence of two subpopulations of oRGs; a neurogenic population that is negative for oligodendrocyte transcription factor 2 (OLIG2) that is most prevalent during neurogenesis and a gliogenic $OLIG2^+$ population that appears at the end of neurogenesis and generates astrocytes (Martínez-Cerdeño et al., 2012). Immunostaining showed that oRGs are $PAX6^+$ and $TBR2^-$ (Reillo, De Juan Romero, García-Cabezas, & Borrell, 2011). In summary, RGCs, SNPs and oRGs are mostly $PAX6^+$/ $TBR2^-$ and IPCs are $PAX6^-$/$TBR2^+$. Pollen et al. performed *in vitro* clonal analysis of single human oRGs and reported that oRGs generated both deep- and upper-layer neurons, and glial cells (Pollen et al., 2015). In these experiments single oRGs at mid-neurogenesis could generate up to 1000 daughter cells, which Pollen et al. described as a remarkable proliferative capacity compared to the 10-100 daughter cells that mice RGCs generate. Nowakowksi et al. showed that, in parallel with the transition from deep-

layer to upper-layer neurogenesis, human RGCs lose contact with the pial surface at mid-neurogenesis. This transformation starts a discontinuous scaffold phase, wherein neurons migrate along oRG basal processes to end up in upper-layers (Nowakowski, Pollen, Sandoval-Espinosa, & Kriegstein, 2016).

Morphological heterogeneity of oRGCs.

Betizeau et al. used long-term *ex vivo* live imaging and unbiased sampling of cycling macaque oSVZ NPCs to study the morphological heterogeneity of these cells (Betizeau et al., 2013). In addition to IPCs they identified four morphotypes of oRGs; types with apical and/or basal processes and a 'transient' type that frequently alternates between stages with apical and/or basal process and no processes. All four oRG morphotypes undergo multiple proliferative divisions and generate neurons without any further intermediate progenitor. Betizeau et al. found stage-specific differences for oSVZ NPCs in both cell cycle kinetics and progeny. They observed oSVZ NPCs with a shorter cell cycle and increased division rates during late neurogenesis. oSVZ NPCs born without a process acquire a transient oRG or an IPC phenotype, while those that inherited a process are more likely to become cycling oRGs that mostly maintain their morphological phenotype. Whether these different morphotypes of oRGs generate different neuronal progeny is not known.

oRGCs and the expansion of the neocortex and increased folding during evolution.

Cortical expansion and folding are thought to underlie the evolutionary changes in cognitive abilities of humans and researchers hypothesized that an increased and/or more proliferative SVZ progenitor mediates these physical changes to the neocortex (Fietz et al., 2010). Reillo et al. found that an outer SVZ region is unique to gyrencephalic mammals, which are mammals with a folded cerebral cortex, and identified oRG-like cells in gyrencephalic mammals including humans, ferrets, cats, and sheep. Surprisingly they also identified oRG-like cells in lissencephalic mammals such as mice and guinea pigs, which have a smooth brain surface (Reillo et al., 2011). Moreover, they found oRG-like cells in the human inner SVZ, which is rarely mentioned (Pollen et al., 2015)**.** In studies on mice and rats researchers found oRG-like cells in superficial layers of the SVZ (Martínez-Cerdeño et al., 2012; Shitamukai, Konno, & Matsuzaki, 2011; Wang et al., 2011). These findings lead to speculation that an increase in oRG numbers, instead of their mere existence, may contribute to cortical expansion in primates (Wang et al., 2011). The human specific *ARHGAP11B* gene potentially contributed to the evolutionary expansion of the human neocortex via increased amplification of oRGs and IPCs (M. Florio et al., 2015; Marta Florio, Namba, Pääbo, Hiller, & Huttner, 2016). Pollen et al. identified the homeodomain-only protein (HOPX) as a human-specific oRG marker (Pollen et al., 2015; Thomsen et al., 2016).

Different types of intermediate precursors and their fate potential.

While both SNPs and oRGs both contribute to neocortical development in their own way, it is interesting to note that E14.5 mice SNPs show a different laminar fate from RGCs from the same time, although RGCs from different stages of neurogenesis or other types of NPCs may generate neurons that localize to the same layers. It would be interesting to assess whether layer II and III neurons generated by SNPs are in any way different from other neurons in these layers. Tyler et al. posed the question whether the existence of three types of intermediate precursors (IPCs, SNPs, and oRGs) in the dorsal telencephalon facilitates the generation of different types of neurons or expands the neuronal output (W A Tyler et al.,

2015). They showed that TBR2 lineage progenitors (iPCs) generated layer II and III pyramidal PNs that exhibit electrophysiological and structural features distinct from non-TBR2 lineage progenitors (derived from RGCs, SNPs and oRGs).

4. Models of neuronal progenitor cell competence I: progressive restriction of fate potential

Progressive restriction

In the 1990s, before the identification of RGCs as NPCs the group of McConnel performed heterochronic transplantation studies to illuminate the dynamics of fate potential, or progenitor competence in the NPC population regarding laminar fate (Desai & McConnell, 2000; Frantz & McConnell, 1996; McConnell & Kaznowski, 1991). Back then, *in vivo* fate mapping studies via retroviral infection of mice NPCs with replication-incompetent retroviruses bearing a marker gene had already shown that single early progenitors can generate neurons in multiple layers (Maria B. Luskin, Pearlman, & Sanes, 1988). McConnel reported that early born progenitors, which generally generate deep-layer neurons, can be transplanted to older host animals, where they generate both deep- and upper-layer neurons. In contrast, transplanted late born progenitors only generate upper neurons in both old and young hosts. Based on these results she concluded that there is a sequential generation of progressively restricted types of PNs over the course of neocortical development. This scientific model of progenitor competence is called progressive restriction.

Later *in vivo* fate mapping studies on rats and ferrets showed that, in accordance with progressive restriction, late born neurons are restricted to upper-layers (C B Reid, Tavazoie, & Walsh, 1997; Christopher B. Reid, Llang, & Walsh, 1995). Progressive restriction is also supported by an *in vitro* culture study of mice NPCs that resembled the *in vivo* situation regarding the order and day of appearance of the layer-specific neurons (Shen, Wang, Dimos, & Fasano, 2006). Single progenitor cells required more divisions to express superficial neuron markers than deep neuron markers. Based on marker expression of their neuronal progeny older progenitors showed a restricted fate potential and co-culture with younger progenitors did not rescue their ability to generate deep neurons, which suggests that fate potential is intrinsically regulated.

Gao et al. investigated RGC division and neuron production by performing clonal analysis of labelled single neocortical NPCs via the Mosaic Analysis with Double Markers (MADM) technique (Gao et al., 2014; Zong, Espinosa, Su, Muzumdar, & Luo, 2005). MADM is based on CRE recombinase-mediated interchromosomal recombination and segregation of recombinant sister-chromatids into separate daughter cells, after which each daughter cell expresses either enhanced green fluorescent protein (eGFP), tdTomato, or both. Use of the tamoxifen-inducible *Emx1-CreER^{T2}* transgene allows for temporally restricted and sparse labelling of single NPCs. Gao et al. quantified symmetric proliferative and asymmetric neurogenic divisions of RGCs by observing an equal number of progeny in each colour or a majority and a minority population of different colour, respectively. They found that the transition from symmetric proliferative division to asymmetric neurogenic division mostly occurs at E11-E12. Tamoxifen injection between E10-E12 showed

that single RGCs produce an average of 8-9 neurons. The use of an inducible *Nestin-CreER^{T2}* transgene lead to similar results. Gao et al. concluded that RGCs have a defined non-random unitary output in neuronal production after they exit the proliferative phase and enter the neurogenic phase. Gao et al. also investigated the neuronal progeny of RGCs and found that throughout development most clones generated neurons located to both deep- and upper-layers and individual clones co-expressed both deep- and upper-layer markers. Induction of MADM labelling at E15 led to clones containing only upper-layer neurons, which is consistent with progressive restriction.

Gil-Sanz et al. commented on the MADM study by pointing out that the MADM technique relies on interchromosomal mitotic recombination during the G2 phase of the cell cycle, which makes the recombination efficiency cell cycle length dependent (Gil-Sanz et al., 2015). This would lead to an unequal chance of labelling for different progenitor types with different proliferation rates, with a preference of labelling for slowly proliferating cells. They were also surprised by the number of neurons (8-9) generated from asymmetrically dividing RGCs since IPCs generate more than 16 cells in a *Tbr2-Cre* mouse line (Vasistha et al., 2015). They noted that it is remarkable that single RGCs would generate fewer neurons than IPCs, unless the technique labelled a specific subpopulation of RGCs.

Temporal identity specification: from Drosophila to humans.

Further investigation into progressive restriction requires the study of temporal identity specification, which describes how progenitor competence is influenced by time-dependent, or temporal, factors such as birth order, and the sequential expression of TFs within a lineage. Studies in Drosophila first explored the concept of sequential expression of TFs as a cause of progressive restriction (Kohwi & Doe, 2013). Drosophila embryos harbour 30 NPCs, called neuroblasts, in the ventral CNS that divide asymmetrically to generate a small ganglion mother cell, which then divides symmetrically to produce two neurons or glia. The type of neuronal subtype generated changes over time in parallel with the sequential expression of TFs Hunchback (Hb), Krupple (Kr), Pou-homeodomain proteins 1 and 2 (Pdm) and Castor (Cas) (Isshiki, Pearson, Holbrook, & Doe, 2001). Hunchback and Krupple are necessary and sufficient for first- and second-born cell fate, respectively. The roles of Pdm and Cas are less clear (Kohwi & Doe, 2013). The expression of temporal identity factors may be intrinsically regulated and independent of cell cycle progression, because dissociated and cell-cycle arrested neuroblasts express the same temporal factors in order (Grosskortenhaus, Pearson, Marusich, & Doe, 2005). The loss of the early-born fate may be mediated by the movement of the *hb* genomic locus to the repressive nuclear periphery, which is dependent on the downregulation of Distal antenna (Dan) protein (Kohwi, Lupton, Lai, Miller, & Doe, 2013). Larval type I and II neuroblasts and the latter's intermediate progenitor progeny also show transitions in temporal fate identity that contribute to neuronal diversity (Kohwi & Doe, 2013).

The concept that the sequential expression of temporal identity factors lies at the basis of progressive restriction of progenitor competence could potentially translate to mammals. IKAROS is a mammalian Hunchback homologue, and potential mammalian temporal identity factor, that specifies early born neurons in the mouse retina and shows peak expression during early neurogenesis (Alsiö et al., 2013). Transgenic mice overexpressing IKAROS show an extended period of early-born neuron generation in the cortex and delayed upper-neuron generation without alterations in progenitor numbers or rate of proliferation. Introduction of IKAROS during mid-late stages of neurogenesis was not sufficient to

generate deep-layer neurons. These results indicate that, like Hunchback in embryonic neuroblasts of Drosophila, expression of IKAROS in NPCs of mice specifies early-born fate and that the progenitors lose the ability to respond to IKAROS.

Intrinsic regulation of temporal progression of progenitor identity.

The study of the temporal progression of AP identity is complex. Okamoto et al. described the molecular temporal progression of AP identity as "a superposition of various time-dependent components" that include both gene expression dynamics related to self-renewal and differentiation and unrelated changes such as those influencing the laminar fate of the progeny (Okamoto et al., 2016). When Okamoto et al. studied the coupling between these two components using genome-wide transcriptome profiling of single cells, APs and IPCs from E10 to E16, and statistical methods to resolve the components they found that cell-cycle progression is not necessary for transitions in temporal gene expression and the laminar fate of APs. Changes to global gene expression occurred at E12 that likely associate with the change from a proliferative state to a neurogenic and IPCs inherit these changes in APs. An earlier study by Mizutani and Saito in which spatiotemporal activation of the Notch pathway in the mouse cortex between E13.5 and E15.5 maintained proliferating RGC identity and inhibited neurogenesis showed that upon continuation of neurogenesis RGCs skipped the generation of deep-layer neurons (Mizutani & Saito, 2005). Okamoto et al. propose that based on these studies the temporal progression of AP competence is intrinsically regulated and tuned by extrinsic cues.

5. Models of neuronal progenitor cell competence II: fate-restricted lineages

An alternative scientific model to explain the generation of PN diversity is the existence of multiple subsets of progenitors with different progenitor competence. For example, Franco and Müller noticed that the lineage trees of the clonal cultures of the earlier mentioned study by Shen et al. in section 4 showed exclusive generation of deep or upper neurons. Therefore, another interpretation of the results of this study is that the different clonal lineages derive from two different fate-restricted progenitor types and that these types generate neurons at different times (Franco & Müller, 2013; Shen et al., 2006).

The controversy surrounding Cux2 and upper-layer neuron fate specification.

Franco et al. postulated the existence of a subpopulation of RGCs that is intrinsically specified to produce upper-layer neurons (Franco et al., 2012). Prior research showed that expression of the TF cut-like homeobox 2 (CUX2) is specific to neurons of layers II to IV. CUX2 is also expressed by some IPCs in the SVZ during embryonic development (Nieto et al., 2004; Zimmer, Tiveron, Bodmer, & Cremer, 2004). These findings lead to the hypothesis that $CUX2^+$ IPCs form a fate-restricted subpopulation of NPCs. Franco et al. used *in vivo* genetic fate mapping in mice expressing CRE recombinase from the *Cux2* locus and a reporter gene to test this hypothesis. They found that RGCs show *Cux2* mRNA expression in the VZ in a salt-and-pepper manner. Most cells (76%) derived from $Cux2^+$ RGCs located to upper-layers II to IV of the cerebral cortex. Small percentages, 17 and 7% of the progeny, located to deep-layers V and VI, respectively. Franco et al. also performed genetic fate mapping of RGCs via a CRE reporter plasmid introduced to *Cux2-Cre* embryos at E12.5 that changes expression of tandem dimer Tomato (tdTomato) red fluorescent protein to GFP upon CRE-mediated recombination. They showed that at post-natal day

(P) 10 83% of GFP$^+$ neurons locate to upper-layers II to IV. In contrast, only 7% of tdTomato$^+$/GFP$^-$ cells locate to the upper-layers. *In vitro* clonal analyses of E13.5 *Cux2-Cre;Ai9* cells showed that most *Cux2*$^+$ progenitors divide symmetrically to generate two RGCs, whereas *Cux2*$^-$ progenitors undergo neurogenic divisions. So, during early neurogenesis *Cux2*$^+$ progenitors preferably proliferate, while *Cux2*$^-$ progenitors generate deep-layer neurons. Indeed, the percentage of *Cux2*$^+$ RGCs increased over duration of neurogenesis. Inducible genetic fate mapping of *Cux2*$^+$ cells using a tamoxifen-inducible *CreER*^{T2} gene knocked into the endogenous *Cux2* locus and crossing with a CRE-reporter strain *Ai9* allowed for labelling of *Cux2*$^+$ cells in a 24-hour window. Tamoxifen injection at E10.5 showed that early *Cux2*$^+$ RGCs are already specified to generate upper-layer neurons at early neurogenesis. Franco et al. concluded that predominant targeting to the upper-layers by *Cux2*$^+$ RGCs is independent of niche and birthdate.

In response to the work of Franco et al., Guo et al. investigated whether deep layer PNs originate from progenitors expressing the deep layer neuron marker Fez family zinc finger (FEZF2), a TF that is critical for specification of subcerebral PNs (Guo et al., 2013). FEZF2 is required to specify the early-born, deep-layer neuron fate in the neocortex (Chen et al., 2008). FEZF2 represses the chromatin remodelling protein SATB2, while SATB2 promotes upper-layer fate by repressing CTIP2 (Alcamo et al., 2008; Britanova et al., 2008). Using *in vivo* genetic fate mapping Guo et al. showed that *Fezf2*-expressing RGCs exist throughout cortical development and sequentially generate all major PN subtypes, including deep- and upper-layer neurons. Guo et al. concluded that *Fezf2* expression is not indicative of specification of laminar fate.

After immunostaining for CUX2, Guo et al. found that 72% of upper-layer neurons generated by *Fezf2*$^+$ RGCs expressed CUX2. Under the assumption that *Fezf2*$^+$ RGCs express *Cux2,* this result would be remarkable if indeed *Cux2* expression by RGCs specifies for an upper-layer neuron fate since that would not comply with the finding that *Fezf2*$^+$ RGCs generate both upper- and deep-layer neurons. Additional genetic fate mapping experiments combined with CUX2 immunostaining showed that during early to mid-neurogenesis *Fezf2*$^+$ RGCs rarely expressed CUX2 protein in the VZ/SVZ and, surprisingly, the CUX2$^+$ cells present in the VZ/SVZ during this period are predominantly (94-98%) interneurons originating from the ventral telencephalon. This led Guo et al. to attempt to replicate the results of Franco et al. by using the same mouse lines: *Cux2-Cre* and *Cux2-CreER*^{T2}. Guo et al. reported that immunohistochemistry experiments showed no CUX2 or CRE in the fate mapped cells, suggesting that the CRE-reporter system is more sensitive than immunohistochemistry for low expression levels of *Cux2*. They also showed that *Cux2*$^+$ RGCs generate both deep- and upper-layer PNs. Therefore, they could not confirm the findings of Franco et al. regarding the role of *Cux2* in neuronal fate specification.

Gil-Sanz et al. stated that the inability of Guo et al. to replicate the results of Franco et al. was possibly caused by drifted transgene expression patterns in the transgenic animals due to a different genetic background and different breeding strategies that may cause epigenetic changes at the transgene locus (Gil-Sanz et al., 2015). This would lead to different patterns of CRE expression, recombination and fluorescence. Specifically, repeated sibling interbreedings over 10 generations could contribute to inconsistency between results via differential Cre expression. Gil-Sanz et al. repeated the experiments performed by Franco et al. with continuous maintenance of the genetic background of the animals by breeding heterozygous *Cux2-Cre* mice with C57BL/6 wild-type mice to maintain CRE expression as for the endogenous *Cux2* gene as determined by *in situ* hybridization. These experiments confirmed the earlier

findings by Franco et al. and showed that the majority of *Cux2*$^{+}$ neurons express SATB2 and not CTIP2, which is in line with the hypothesis that *Cux2*$^{+}$ progenitors generate upper-layer neurons. Gil-Sanz et al. could detect CRE protein in VZ progenitors and it's recombinational activity in PAX6^{+} RGCs, which indicates *Cux2* is expressed by RGCs of the VZ. Gil-Sanz et al. stress that IPCs must express *Cux2*, because Cubelos et al. showed that mice lacking *Cux2* show more proliferating IPCs.

In response to Gil-Sanz et al., Eckler et al. performed two clonal analysis studies on E10.5 neocortical NPCs from *Cux2-CreER*^{T2} and *Fezf2-CreER*^{T2} transgenic mice, respectively. To remove interneurons from the analysis they did not include individual, sparsely labelled cells. They found that that *Cux2*$^{+}$ and *Fezf2*$^{+}$ RGCs generate diverse PN subtypes located throughout layers II-VI. Based on their results, they reaffirm the conclusion of Guo et al., which suggests that most, if not all, *Cux2*$^{+}$ and *Fezf2*$^{+}$ RGCs generate diverse PN subtypes throughout the layers of the neocortex (Eckler et al., 2015). In critique of Franco et al. and Gil-Sanz et al., Eckler et al. state that the *Cux2-Cre* allele is not a suitable genetic tool to trace the lineage of *Cux2*$^{+}$ RGCs, because this constitutive form of CRE in combination with a CRE reporter permanently labels cells with transient *Cux2* expression and post-mitotic cells that only start to express *Cux2* after terminal division. This may explain the different conclusions the two labs formed. The preferential post-mitotic expression of *Cux2* in upper-layer neurons, and not the derivation from a fate-restricted lineage, could cause the widespread reporter expression among upper-layer neurons in the studies by Franco et al. and Gil-Sanz et al. Based on the contradicting findings related to *Cux2* and *Fezf2* it remains unclear whether these genes specify fate-restricted lineages during neurogenesis and these studies do not exclude that other fate-restricted lineages exist (Eckler et al., 2015; Franco & Müller, 2013).

Tbr2 and Otx1 as determinants for laminar fate?

Following these reports others investigated other potential fate-determinants. Studies showed that mice deficient for the IPC marker TBR2 have thinner neocortical upper-layers (Arnold et al., 2008; Sessa, Mao, Hadjantonakis, Klein, & Broccoli, 2008). Therefore, Mihalas et al. investigated whether *Tbr2* specifies for an upper-layer neuron fate (Mihalas et al., 2016). Inducible genetic fate mapping experiments using *Tbr2-CreER;A14* transgenic mice administered with tamoxifen between E11.5-E16.5 showed that, instead, *Tbr2*$^{+}$ IPCs are progressively restricted in the ability to form neurons with different laminar fate so that upper-layer neurons are generated throughout neurogenesis, but deep-layer neurons are only generated during early and mid-neurogenesis. Mihalas et al. also did experiments on *Tbr2*-deficient mice and showed that *Tbr2* regulates the tempo of laminar fate implementation for all layers and is not required for IPC generation, but is required for differentiation to PNs. They concluded that *Tbr2* does not specify for an upper-layer neuron fate. This study further supports progressive restriction.

Gao et al. considered *Otx1* a prospective fate-restricting gene in NPCs, because it is selectively expressed in a subpopulation of layer V and VI neurons and in VZ progenitors during deep-layer neurogenesis. In their MADM study described in section 4, they also introduced an *Otx1* knockout allele into the *Emx1-CreER*^{T2} MADM system and injected tamoxifen at E11 or E12 to investigate the effect of *Otx1* on neocortical neurogenesis. Gao et al. concluded that *Otx1*-knockout decreased neurogenic capacity of RGCs and had no effect on neurogenic capacity of IPCs. There was no sign of layer-specific reduction of neurogenic capacity. Therefore, they concluded that *Otx1* does not specify for a specific laminar fate.

6. Neuronal progenitor cell heterogeneity and progenitor type fate.

Throughout this essay some examples have been discussed of NPC heterogeneity that plays a role in the regulation of neocortical development, but does not control neuronal fate potential. This section discusses other such studies to give a complete overview of different types of NPC heterogeneity and whether they control neuronal fate potential.

Restricted neurogenic and gliogenic lineages or spatiotemporal expression patterns?

A study by Malatesta et al. suggested the existence of restricted lineages regarding neurogenic and gliogenic fate potential. Malatesta et al. did experiments to study the progeny of RGCs. In primates, RGCs express glial fibrillary acidic protein (GFAP) after the transformation from NECs. Since RGCs of rodents hardly express GFAP Malatesta et al. used a transgenic mouse line expressing GFP under the human *GFAP* promoter, applied fluorescence-activated cell sorting to isolate E14, E16 and E18 cortical RGCs, cultured them at clonal density to analyse the progeny (Malatesta, Hartfuss, & Götz, 2000; Sancho-Tello, Vallés, Montoliu, Renau-Piqueras, & Guerri, 1995). They found that about two thirds of E14 and E16 GFP^+ mice RGCs had a strict neurogenic fate potential and about one third of the cells had a strict gliogenic fate potential. The balance shifted in clones from E18 RGCs where two thirds of the RGCs had a strict gliogenic fate potential and one third had a strict neurogenic fate potential. At each timepoint about 5% of RGCs formed mixed clones. These findings go against the more recent *in vivo* MADM study by Gao et al., which identified neurogenic and multipotent E10-E13 RGC lineages, but no strict gliogenic lineages. A similar study was done for the RGC marker BLBP. Since BLBP is expressed in different subsets of RGCs, but not all, Anthony et al. investigated the influence of BLBP on fate potential (Anthony, Klein, Fishell, & Heintz, 2004; Hartfuss et al., 2001). *In vitro* culture of isolated E11.5 and E14.5 neocortical RGCs from transgenic mice expressing BLBP-GFP and clonal analysis showed 79% and 63% of RGCs, respectively formed mixed clones of neurons and glia. For the pure clones the balance shifted from neuronal to glial cells when comparing the E14.5 RGC clones to the E11.5 RGC clones. Anthony et al. proposed that the heterogeneity in BLBP expression in RGCs throughout embryonic development does not represent distinct lineages, but reflects the variety of temporal changes between regions. They support this idea by showing that all RGCs are $BLBP^+$ by the end of neurogenesis. They also found that changes in GLAST expression follow the same spatiotemporal pattern which shows parallels with the start of neurogenesis. Although inducible *in vivo* genetic fate mapping studies of both these genes could be enlightening, these studies have not been done.

Gene expression oscillation in APs controls neurogenic versus gliogenic fate.

Systematic analysis of gene expression profiles of mid-embryonic (E14) mouse NPCs identified four subgroups of APs characterized by their differential expression of *Hes5*, *Ascl1* and *Dll1* (Kawaguchi et al., 2008). Furthermore, genome-wide gene expression profiles of APs show a higher variability in expression patterns for primarily Notch signalling components, and to a lesser degree for FGF and Hedgehog pathways compared to basal progenitors. Kawaguchi et al. hypothesized that this difference could explain the variety of AP division modes. They deemed it unclear whether this variety is inherited through lineages or whether these are transient states of a common population of APs. The lab of Kageyama did a series of studies on gene expression oscillation in NPCs of the embryonic mouse brain and found that oscillation of

protein levels of basic helix-loop-helix transcription factors ASCL1, OLIG2 and HES1 maintains NPC identity, while sustained expression of one of these single genes induces neurogenic or gliogenic differentiation or NPC maintenance, respectively (Imayoshi et al., 2013; Shimojo, Ohtsuka, & Kageyama, 2008). Therefore, this form of transcriptional heterogeneity in NPCs does regulate brain development, but does not mediate the generation of projection neuron diversity.

High or low GFAP expression in RGCs controls proliferative versus neurogenic fate.

Pinto et al. studied the heterogeneity of RGCs in relation to neurogenesis (Pinto et al., 2008). Via fluorescence-activated cell sorting they selected for both high and low expression of GFAP in a population of E14 RGCs from cortices of *hGFAP-eGFP* transgenic mice and identified two co-existing subsets of RGCs. To selectively isolate apical RGCs and not their progeny they stained for the apical surface marker prominin. Prominin^{-} cells were found to express genes known to be expressed in IPCs and neurons such as *Tbr2*, *Ngn*2 and *Neurod4*. Pinto et al. cultured the prominin^{+}/GFP^{low} and the prominin^{+}/GFP^{high} *in vitro* at clonal density using a mouse-rat co-culture system, after which they performed clonal analysis of the progeny. The prominin^{+}/GFP^{low} subset consisted of neurogenic, self-renewing RGCs. RGCs that produce two TBR2^{+} IPCs made up the prominin^{+}/GFP^{high} subset. These results show that neuronal progenitors with low expression of hGFAP-EGFP preferably undergo neurogenic divisions and progenitors with high expression undergo proliferative divisions.

Microarray analysis on RNA samples from the two prominin^{+} subsets showed significant differences in the transcriptome**.** The prominin^{+}/GFP^{low} subset shows higher expression of the canonical *Pax6* splice variant, which lacks exon 5a. The prominin^{+}/GFP^{high} shows higher expression of the *Pax6* splice variant containing exon 5a. Pinto et al. suggest this adds further evidence to the theory that the *Pax6* canonical isoform has a neurogenic role and they propose that the splice variant promotes IPC fate. *Tcfap2c*, which encodes for a TF called activating enhancer binding protein 2γ (AP2γ) was enriched in the prominin^{+}/GFP^{high} subset. In mice *Tcfap2c* expression is selectively specified to APs of the cerebral cortex around E12 with peak expression during mid-neurogenesis (Pinto et al., 2009). Experiments with *AP2γ*$^{-/-}$ mice showed that a loss of *Tcfap2c* affects APs through a decreased expression of IPC fate determinants, including *Math3* (*Neurod4*) and *Tbr2,* and causes misspecification of IPCs at mid-neurogenesis and a reduction in the number of upper-layer neurons in the occipital cortex. Since *AP2γ*$^{-/-}$ mice did not show an increase in numbers of other neuronal subtypes and cell death markers showed these abnormal IPCs experienced an increase in cell death compared to the wildtype, Pinto et al. concluded that, in the occipital cortex, *AP2γ* regulates neuron production via IPC fate specification of APs, but does not regulate a neuronal fate choice (Pinto et al., 2009). Since IPCs are generated across the neocortex and throughout neocortical development this spatiotemporal of *AP2γ* is unlikely to act on a bigger scale during neocortical development (Franco & Müller, 2013; Haubensak et al., 2004).

7. Conclusion

Molecular and cellular processes that regulate cell fate specification of NPCs contribute to the diversity of neocortical projection neurons. The palette of neuronal progenitor cells mostly drives general neocortical development, but there is evidence for the existence of progenitor types that generate distinct neuronal subtypes; $TBR2^{+}$ IPCs generate layer II and III pyramidal neurons that exhibit electrophysiological and structural features different from the neuronal progeny of $TBR2^{-}$ RGCs, SNPs and oRGs (W A Tyler et al., 2015). The dynamics of progenitor competence remain a controversial topic and can be described by two scientific models: progressive restriction and restricted lineages. Multiple studies support the progressive restriction of progenitor competence, which is potentially mediated by the sequential expression of temporal identity factors as first described in Drosophila (Kohwi & Doe, 2013). IKAROS is a potential mammalian temporal identity factor (Alsiö et al., 2013). A select few studies adhere to the existence of fate-restricted lineages such as *Cux2*$^{+}$ RGCs, which are claimed to generate upper-layer neurons in these studies(Franco et al., 2012; Gil-Sanz et al., 2015). These are ingenious *in vivo* genetic fate mapping studies, but specific weaknesses in the methodological approach may have been missed. Others declared that *Cux2*$^{+}$ RGCs generate both upper- and deep-layer neurons (Eckler et al., 2015; Guo et al., 2013). Whether this one potential restricted lineage exists or not does not exclude the possibility that other such lineages exist. Further understanding of neocortical development and cell fate specification of NPCs may be valuable to brain development and disease research, and the design of brain repair therapies.

8. References

Alcamo, E. A., Chirivella, L., Dautzenberg, M., Dobreva, G., Fariñas, I., Grosschedl, R., & McConnell, S. K. (2008). Satb2 Regulates Callosal Projection Neuron Identity in the Developing Cerebral Cortex. *Neuron*, *57*(3), 364–377. http://doi.org/10.1016/j.neuron.2007.12.012

Alsiö, J. M., Tarchini, B., Cayouette, M., Livesey, F. J., Alsio, J. M., Tarchini, B., … Livesey, F. J. (2013). Ikaros promotes early-born neuronal fates in the cerebral cortex. *Proceedings of the National Academy of Sciences*, *110*(8), E716–E725. http://doi.org/10.1073/pnas.1215707110

Anderson, S. A. (2002). Distinct Origins of Neocortical Projection Neurons and Interneurons In Vivo. *Cerebral Cortex*, *12*(7), 702–709. http://doi.org/10.1093/cercor/12.7.702

Anthony, T. E., Klein, C., Fishell, G., & Heintz, N. (2004). Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron*, *41*(6), 881–890. http://doi.org/10.1016/S0896-6273(04)00140-0

Arai, Y., Pulvers, J. N., Haffner, C., Schilling, B., Nüsslein, I., Calegari, F., & Huttner, W. B. (2011). Neural stem and progenitor cells shorten S-phase on commitment to neuron production. *Nature Communications*, *2*(1), 154. http://doi.org/10.1038/ncomms1155

Arnold, S. J., Huang, G.-J., Cheung, A. F. P., Era, T., Nishikawa, S.-I., Bikoff, E. K., … Groszer, M. (2008). The T-box transcription factor Eomes/Tbr2 regulates neurogenesis in the cortical subventricular zone. *Genes & Development*, *22*(18), 2479–84. http://doi.org/10.1101/gad.475408

Betizeau, M., Cortay, V., Patti, D., Pfister, S., Gautier, E., Bellemin-M??nard, A., … Dehay, C. (2013). Precursor Diversity and Complexity of Lineage Relationships in the Outer Subventricular Zone of the Primate. *Neuron*, *80*(2), 442–457. http://doi.org/10.1016/j.neuron.2013.09.032

Britanova, O., de Juan Romero, C., Cheung, A., Kwan, K. Y., Schwark, M., Gyorgy, A., … Tarabykin, V. (2008). Satb2 Is a Postmitotic Determinant for Upper-Layer Neuron Specification in the Neocortex. *Neuron*, *57*(3), 378–392. http://doi.org/10.1016/j.neuron.2007.12.028

Chen, B., Wang, S. S., Hattox, A. M., Rayburn, H., Nelson, S. B., & McConnell, S. K. (2008). The Fezf2-Ctip2 genetic pathway regulates the fate choice of subcortical projection neurons in the developing cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(32), 11382–11387. http://doi.org/10.1073/pnas.0804918105

de Carlos, J. a, López-Mascaraque, L., & Valverde, F. (1996). Dynamics of cell migration from the lateral ganglionic eminence in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(19), 6146–6156.

Desai, A. R., & McConnell, S. K. (2000). Progressive restriction in fate potential by neural progenitors during cerebral cortical development. *Development*, *127*(13), 2863–2872.

Eckler, M. J., Nguyen, T. D., McKenna, W. L., Fastow, B. L., Guo, C., Rubenstein, J. L. R., & Chen, B. (2015). Cux2-Positive Radial Glial Cells Generate Diverse Subtypes of Neocortical Projection Neurons and Macroglia. *Neuron*, *86*(4), 1100–1108. http://doi.org/10.1016/j.neuron.2015.04.020

Fietz, S. a, Kelava, I., Vogt, J., Wilsch-Bräuninger, M., Stenzel, D., Fish, J. L., … Huttner, W. B. (2010). OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. *Nature Neuroscience*, *13*(6), 690–699. http://doi.org/10.1038/nn.2553

Florio, M., Albert, M., Taverna, E., Namba, T., Brandl, H., Lewitus, E., … Huttner, W. B. (2015). Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. *Science*, *347*(6229), 1465–70. http://doi.org/10.1126/science.aaa1975

Florio, M., Namba, T., Pääbo, S., Hiller, M., & Huttner, W. B. (2016). A single splice site mutation in human-specific ARHGAP11B causes basal progenitor amplification. *Science Advances*, *2*(12), e1601941–e1601941. http://doi.org/10.1126/sciadv.1601941

Franco, S. J., Gil-Sanz, C., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S. R., Ramos, C., & Müller, U. (2012). Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science (New York, N.Y.)*, *337*(6095), 746–9. http://doi.org/10.1126/science.1223616

Franco, S. J., & Müller, U. (2013). Shaping Our Minds: Stem and Progenitor Cell Diversity in the Mammalian Neocortex. *Neuron*, *77*(1), 19–34. http://doi.org/10.1016/j.neuron.2012.12.022

Frantz, G. D., & McConnell, S. K. (1996). Restriction of late cerebral cortical progenitors to an upper-layer fate. *Neuron*, *17*(1), 55–61. http://doi.org/10.1016/S0896-6273(00)80280-9

Gal, J. S., Morozov, Y. M., Ayoub, A. E., Chatterjee, M., Rakic, P., & Haydar, T. F. (2006). Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(3), 1045–56. http://doi.org/10.1523/JNEUROSCI.4499-05.2006

Gao, P., Postiglione, M. P., Krieger, T. G., Hernandez, L., Wang, C., Han, Z., … Shi, S. H. (2014). Deterministic progenitor behavior and unitary production of neurons in the neocortex. *Cell*, *159*(4), 775–788. http://doi.org/10.1016/j.cell.2014.10.027

Gil-Sanz, C., Espinosa, A., Fregoso, S. P., Bluske, K. K., Cunningham, C. L., Martinez-Garay, I., … Müller, U. (2015). Lineage Tracing Using Cux2-Cre and Cux2-CreERT2 Mice. *Neuron*, *86*(4), 1091–1099. http://doi.org/10.1016/j.neuron.2015.04.019

Götz, M., & Huttner, W. B. (2005). The cell biology of neurogenesis. *Nature Reviews. Molecular Cell Biology*, *6*(10), 777–88. http://doi.org/10.1038/nrm1739

Greig, L. C., Woodworth, M. B., Galazo, M. J., Padmanabhan, H., & Macklis, J. D. (2013). Molecular logic of neocortical projection neuron specification, development and diversity. *Nature Reviews. Neuroscience*, *14*(11), 755–69. http://doi.org/10.1038/nrn3586

Grosskortenhaus, R., Pearson, B. J., Marusich, A., & Doe, C. Q. (2005). Regulation of temporal identity transitions in drosophila neuroblasts. *Developmental Cell*, *8*(2), 193–202. http://doi.org/10.1016/j.devcel.2004.11.019

Guo, C., Eckler, M. J., McKenna, W. L., McKinsey, G. L., Rubenstein, J. L. R., & Chen, B. (2013). Fezf2 Expression Identifies a Multipotent Progenitor for Neocortical Projection Neurons, Astrocytes, and Oligodendrocytes. *Neuron*, *80*(5), 1167–1174. http://doi.org/10.1016/j.neuron.2013.09.037

Hansen, D. V, Lui, J. H., Parker, P. R. L., & Kriegstein, A. R. (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature*, *464*(7288), 554–561. http://doi.org/10.1038/nature08845

Hartfuss, E., Galli, R., Heins, N., & Götz, M. (2001). Characterization of CNS Precursor Subtypes and Radial Glia. *Developmental Biology*, *229*(1), 15–30. http://doi.org/10.1006/dbio.2000.9962

Haubensak, W., Attardo, A., Denk, W., & Huttner, W. B. (2004). Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(9), 3196–201. http://doi.org/10.1073/pnas.0308600100

Imayoshi, I., Isomura, A., Harima, Y., Kawaguchi, K., Kori, H., Miyachi, H., … Kageyama, R. (2013). Oscillatory Control of Factors Determining Multipotency and Fate in Mouse Neural Progenitors. *Science*, *342*(6163), 1203–1208. http://doi.org/10.1126/science.1242366

Isshiki, T., Pearson, B., Holbrook, S., & Doe, C. Q. (2001). Drosophila neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell*, *106*(4), 511–521. http://doi.org/10.1016/S0092-8674(01)00465-2

Jackson, C. A., Peduzzi, J. D., & Hickey, T. L. (1989). Visual cortex development in the ferret. I. Genesis and migration of visual cortical neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *9*(4), 1242–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2703875

Johnson, M. B., & Walsh, C. A. (2017). Cerebral cortical neuron diversity and development at single-cell resolution. *Current Opinion in Neurobiology*, *42*, 9–16. http://doi.org/10.1016/j.conb.2016.11.001

Kawaguchi, A., Ikawa, T., Kasukawa, T., Ueda, H. R., Kurimoto, K., Saitou, M., & Matsuzaki, F. (2008). Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. *Development*, *135*(18), 3113–3124. http://doi.org/10.1242/dev.022616

Kohwi, M., & Doe, C. Q. (2013). Temporal fate specification and neural progenitor competence during development. *Nature Reviews Neuroscience*, *14*(12), 823–838. http://doi.org/10.1038/nrn3618

Kohwi, M., Lupton, J. R., Lai, S. L., Miller, M. R., & Doe, C. Q. (2013). Developmentally regulated subnuclear genome reorganization restricts neural progenitor competence in Drosophila. *Cell*, *152*(1–2), 97–108. http://doi.org/10.1016/j.cell.2012.11.049

Kwan, K. Y., Sestan, N., & Anton, E. S. (2012). Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development*, *139*(9), 1535–1546. http://doi.org/10.1242/dev.069963

Lodato, S., & Arlotta, P. (2015). Generating Neuronal Diversity in the Mammalian Cerebral Cortex. *Annual Review of Cell and Developmental Biology*, *31*(1), 699–720. http://doi.org/10.1146/annurev-cellbio-100814-125353

Lodato, S., Rouaux, C., Quast, K. B., Jantrachotechatchawan, C., Studer, M., Hensch, T. K., & Arlotta, P. (2011). Excitatory Projection Neuron Subtypes Control the Distribution of Local Inhibitory Interneurons in the Cerebral Cortex. *Neuron*, *69*(4), 763–779. http://doi.org/10.1016/j.neuron.2011.01.015

Luskin, M. B., Pearlman, A. L., & Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a Recombinant Retrovirus. *Neuron*, *1*(8), 635–647. http://doi.org/10.1016/0896-6273(88)90163-8

Luskin, M. B., & Shatz, C. J. (1985). Neurogenesis of the cat's primary visual cortex. *The Journal of Comparative Neurology*, *242*(4), 611–631. http://doi.org/10.1002/cne.902420409

Malatesta, P., Hartfuss, E., & Götz, M. (2000). Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development (Cambridge, England)*, *127*(24), 5253–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11076748

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., & Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nature Reviews. Neuroscience*, *5*(10), 793–807. http://doi.org/10.1038/nrn1519

Martínez-Cerdeño, V., Cunningham, C. L., Camacho, J., Antczak, J. L., Prakash, A. N., Cziep, M. E., … Noctor, S. C. (2012). Comparative analysis of the subventricular zone in rat, ferret and macaque: Evidence for an outer subventricular zone in rodents. *PLoS ONE*, *7*(1). http://doi.org/10.1371/journal.pone.0030178

McConnell, S. K., & Kaznowski, C. E. (1991). Cell cycle dependence of laminar determination in developing neocortex. *Science (New York, N.Y.)*, *254*(5029), 282–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1925583

Mihalas, A. B., Elsen, G. E., Bedogni, F., Daza, R. A. M., Ramos-Laguna, K. A., Arnold, S. J., … Hevner, R. F. (2016). Intermediate Progenitor Cohorts Differentially Generate Cortical Layers and Require Tbr2 for Timely Acquisition of Neuronal Subtype Identity. *Cell Reports*, *16*(1), 92–105. http://doi.org/10.1016/j.celrep.2016.05.072

Miyata, T., Kawaguchi, A., Saito, K., Kawano, M., Muto, T., & Ogawa, M. (2004). Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development*, *131*(13), 3133–3145.

http://doi.org/10.1242/dev.01173

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V. H., Butt, S. J. B., Battiste, J., … Fishell, G. (2010). Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(5), 1582–94. http://doi.org/10.1523/JNEUROSCI.4515-09.2010

Mizutani, K. -i., & Saito, T. (2005). Progenitors resume generating neurons after temporary inhibition of neurogenesis by Notch activation in the mammalian cerebral cortex. *Development*, *132*(6), 1295–1304. http://doi.org/10.1242/dev.01693

Nieto, M., Monuki, E. S., Tang, H., Imitola, J., Haubst, N., Khoury, S. J., … Walsh, C. A. (2004). Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. *The Journal of Comparative Neurology*, *479*(2), 168–180. http://doi.org/10.1002/cne.20322

Noctor, S. C., Martínez-Cerdeño, V., Ivic, L., Kriegstein, A. R., Martinez-Cerdeno, V., Ivic, L., & Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci*, *7*(2), 136–144. http://doi.org/10.1038/nn1172

Nowakowski, T. J., Pollen, A. A., Sandoval-Espinosa, C., & Kriegstein, A. R. (2016). Transformation of the Radial Glia Scaffold Demarcates Two Stages of Human Cerebral Cortex Development. *Neuron*, *91*(6), 1219–1227. http://doi.org/10.1016/j.neuron.2016.09.005

Okamoto, M., Miyata, T., Konno, D., Ueda, H. R., Kasukawa, T., Hashimoto, M., … Kawaguchi, A. (2016). Cell-cycle-independent transitions in temporal identity of mammalian neural progenitor cells. *Nature Communications*, *7*, 11349. http://doi.org/10.1038/ncomms11349

Park, D., Xiang, A. P., Zhang, L., Mao, F. F., Walton, N. M., Choi, S. S., & Lahn, B. T. (2009). The radial glia antibody RC2 recognizes a protein encoded by Nestin. *Biochemical and Biophysical Research Communications*, *382*(3), 588–592. http://doi.org/10.1016/j.bbrc.2009.03.074

Pinto, L., Drechsel, D., Schmid, M.-T., Ninkovic, J., Irmler, M., Brill, M. S., … Götz, M. (2009). AP2γ regulates basal progenitor fate in a region- and layer-specific manner in the developing cortex. *Nature Neuroscience*, *12*(10), 1229–1237. http://doi.org/10.1038/nn.2399

Pinto, L., & Götz, M. (2007). Radial glial cell heterogeneity--the source of diverse progeny in the CNS. *Progress in Neurobiology*, *83*(1), 2–23. http://doi.org/10.1016/j.pneurobio.2007.02.010

Pinto, L., Mader, M. T., Irmler, M., Gentilini, M., Santoni, F., Drechsel, D., … Götz, M. (2008). Prospective isolation of functionally distinct radial glial subtypes-Lineage and transcriptome analysis. *Molecular and Cellular Neuroscience*, *38*(1), 15–42. http://doi.org/10.1016/j.mcn.2008.01.012

Pollen, A. A., Nowakowski, T. J., Chen, J., Retallack, H., Sandoval-Espinosa, C., Nicholas, C. R., … Kriegstein, A. R. (2015). Molecular Identity of Human Outer Radial Glia during Cortical Development. *Cell*, *163*(1), 55–67. http://doi.org/10.1016/j.cell.2015.09.004

Rakic, P. (1974). Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science (New York, N.Y.)*, *183*(4123), 425–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4203022

Reid, C. B., Llang, I., & Walsh, C. (1995). Systematic widespread clonal organization in cerebral cortex. *Neuron*, *15*(2), 299–310. http://doi.org/10.1016/0896-6273(95)90035-7

Reid, C. B., Tavazoie, S. F., & Walsh, C. a. (1997). Clonal dispersion and evidence for asymmetric cell division in ferret cortex. *Development*, *124*(12), 2441–2450.

Reillo, I., De Juan Romero, C., García-Cabezas, M. Á., & Borrell, V. (2011). A Role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex. *Cerebral Cortex*, *21*(7), 1674–1694.

http://doi.org/10.1093/cercor/bhq238

Sancho-Tello, M., Vallés, S., Montoliu, C., Renau-Piqueras, J., & Guerri, C. (1995). Developmental pattern of GFAP and vimentin gene expression in rat brain and in radial glial cultures. *Glia*, *15*(2), 157–166. http://doi.org/10.1002/glia.440150208

Sessa, A., Mao, C.-A., Hadjantonakis, A.-K., Klein, W. H., & Broccoli, V. (2008). Tbr2 directs conversion of radial glia into basal precursors and guides neuronal amplification by indirect neurogenesis in the developing neocortex. *Neuron*, *60*(1), 56–69. http://doi.org/10.1016/j.neuron.2008.09.028

Shen, Q., Wang, Y., Dimos, J. T., & Fasano, C. A. (2006). The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. *Nature*, *9*(6), 743–751. http://doi.org/10.1038/nn1694

Shimojo, H., Ohtsuka, T., & Kageyama, R. (2008). Oscillations in Notch Signaling Regulate Maintenance of Neural Progenitors. *Neuron*, *58*(1), 52–64. http://doi.org/10.1016/j.neuron.2008.02.014

Shitamukai, A., Konno, D., & Matsuzaki, F. (2011). Oblique Radial Glial Divisions in the Developing Mouse Neocortex Induce Self-Renewing Progenitors outside the Germinal Zone That Resemble Primate Outer Subventricular Zone Progenitors. *Journal of Neuroscience*, *31*(10), 3683–3695. http://doi.org/10.1523/JNEUROSCI.4773-10.2011

Stancik, E. K., Navarro-Quiroga, I., Sellke, R., & Haydar, T. F. (2010). Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(20), 7028–36. http://doi.org/10.1523/JNEUROSCI.6131-09.2010

Subramanian, L., Bershteyn, M., Paredes, M. F., & Kriegstein, A. R. (2017). Dynamic behaviour of human neuroepithelial cells in the developing forebrain. *Nature Communications*, *8*, 14167. http://doi.org/10.1038/ncomms14167

Tamamaki, N., Fujimori, K. E., & Takauji, R. (1997). Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(21), 8313–8323.

Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., … Zeng, H. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature Neuroscience*, *19*(2), 335–46. http://doi.org/10.1038/nn.4216

Thomsen, E. R., Mich, J. K., Yao, Z., Hodge, R. D., Doyle, A. M., Jang, S., … Ramanathan, S. (2016). Fixed single-cell transcriptomic characterization of human radial glial diversity. *Nature Methods*, *13*(1), 87–93. http://doi.org/10.1038/nmeth.3629

Tyler, W. A., & Haydar, T. F. (2013). Multiplex genetic fate mapping reveals a novel route of neocortical neurogenesis, which is altered in the Ts65Dn mouse model of down syndrome. *Annals of Internal Medicine*, *158*(6), 5106–5119. http://doi.org/10.1523/JNEUROSCI.5380-12.2013

Tyler, W. A., Medalla, M., Guillamon-Vivancos, T., Luebke, J. I., & Haydar, T. F. (2015). Neural Precursor Lineages Specify Distinct Neocortical Pyramidal Neuron Types. *Journal of Neuroscience*, *35*(15), 6142–6152. http://doi.org/10.1523/JNEUROSCI.0335-15.2015

Vasistha, N. A., García-Moreno, F., Arora, S., Cheung, A. F. P., Arnold, S. J., Robertson, E. J., & Molnár, Z. (2015). Cortical and Clonal Contribution of Tbr2 Expressing Progenitors in the Developing Mouse Brain. *Cerebral Cortex*, *25*(10), 3290–3302. http://doi.org/10.1093/cercor/bhu125

Wang, X., Tsai, J.-W., LaMonica, B., & Kriegstein, A. R. (2011). A new subtype of progenitor cell in the mouse embryonic neocortex. *Nature Neuroscience*, *14*(5), 555–61. http://doi.org/10.1038/nn.2807

Woodworth, M. B., Girskis, K. M., & Walsh, C. A. (2017). Building a lineage from single cells: Genetic techniques for

cell lineage tracking. *Nature Reviews Genetics*, *18*(4), 230–244. http://doi.org/10.1038/nrg.2016.159

Zeisel, A., Manchado, A. B. M., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, A., … Linnarsson, S. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, *347*(6226), 1138–42. http://doi.org/10.1126/science.aaa1934

Zimmer, C., Tiveron, M.-C., Bodmer, R., & Cremer, H. (2004). Dynamics of Cux2 Expression Suggests that an Early Pool of SVZ Precursors is Fated to Become Upper Cortical Layer Neurons. *Cerebral Cortex*, *14*(12), 1408–1420. http://doi.org/10.1093/cercor/bhh102

Zong, H., Espinosa, J. S., Su, H. H., Muzumdar, M. D., & Luo, L. (2005). Mosaic Analysis with Double Markers in Mice. *Cell*, *121*(3), 479–492. http://doi.org/10.1016/j.cell.2005.02.012